AOS PROTOCOL AND PROCEDURE:
SEDIMENT CHEMISTRY SAMPLING IN WADEABLE STREAMS

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See configuration management system for approval history.

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	07/02/2014	ECO-01126	Initial release
B	11/05/2014	ECO-02271	Minor updates based on feedback from the field. Moved datasheets to NEON.DOC.002419.
C	01/22/2015	ECO-02632	Migration to new protocol template
D	02/25/2016	ECO-03503	Protocol updates based on current external lab arrangements and revisions following the technician review
E	03/09/2017	ECO-04465	CM updated with new template and changes based on feedback from FOPS. Added TOC/TC sample methods.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 OVERVIEW

1.1 Background

Sediment is a naturally occurring material that originates from the weathering and erosion of rock. It can be subsequently transported and redistributed by wind, water and ice. Sediments in aquatic environments and as defined for this protocol constitute the bed material of wadeable streams. Sediments are defined as the fraction smaller than 2 mm in grain size.

Sediments are sinks for elemental cycles in aquatic systems and are recognized as one of the largest sources of pollutants. As such, sediments provide information on various processes such as sedimentation, water dynamics, sediment contaminant interaction, sediment-organism interaction and historical indicators (IAEA, 2003). Under certain environmental conditions, sediments can be re-suspended within the water column and transported downstream or elsewhere in the water body. This mechanism allows for trace and other elements and compounds to re-enter the food chain. While this may provide essential nutrients and energy for organisms, it also potentially poses a threat to the aquatic systems.

The variation in the composition of bed surface sediments results from the inherent heterogeneity of the surrounding watershed and airshed. Many metals and other elements are most likely to be concentrated in sediments typified by fine particle size samples and high organic matter content. This association is largely dependent on the sorptive capacity of fine sediments and organic matter imparted by their surface charges. Hence, even though element concentrations in the water column may be low, suspended sediments and bed sediments can contain large elemental concentrations. Fine bed sediments are most likely to be collected from depositional zones. A depositional zone is defined as the area within a river where the energy regime is low and typically are found at the inside bend of a stream, riffle, pool lip, downstream from obstacles or simply shallow waters near the shore (USGS, 1994) (Figure 1). Depositional zones represent the net outcome of multiple processes and flows.

Sediment deposition patterns are a direct consequence of the flow characteristics of a waterbody. The appropriate season and hydrologic conditions for sampling stream bed sediment are determined by current and antecedent discharge conditions. Access to the sampling site can be limited during seasonal high-flow conditions. Unusually high flows can wash out, redistribute, or bury substantial parts of sediment deposits; therefore, sampling should be delayed following major discharge events to allow fresh sediment to deposit. When sampling for bed sediment during summer or autumn, low-flow conditions are recommended to provide maximum direct access to the stream bed and to minimize seasonal streamflow variability.

The sample strategy for sediment analysis focuses on fine-grained surficial sediments from natural depositional zones during low-flow conditions (USGS, 1994). Surface sediment is considered to range from 1 to 3 cm in depth (Golterman et al., 1983; Keith, 1991). Samples are composited from between 5-

10 depositional zones within a station in the stream reach in order to smooth local scale variability and represent average conditions of the reach. To increase the probability of detecting inorganics and to enhance the comparability of data among sites, bed-sediment samples will be sieved so that the fine grained size fraction is analyzed for the elements of interest.

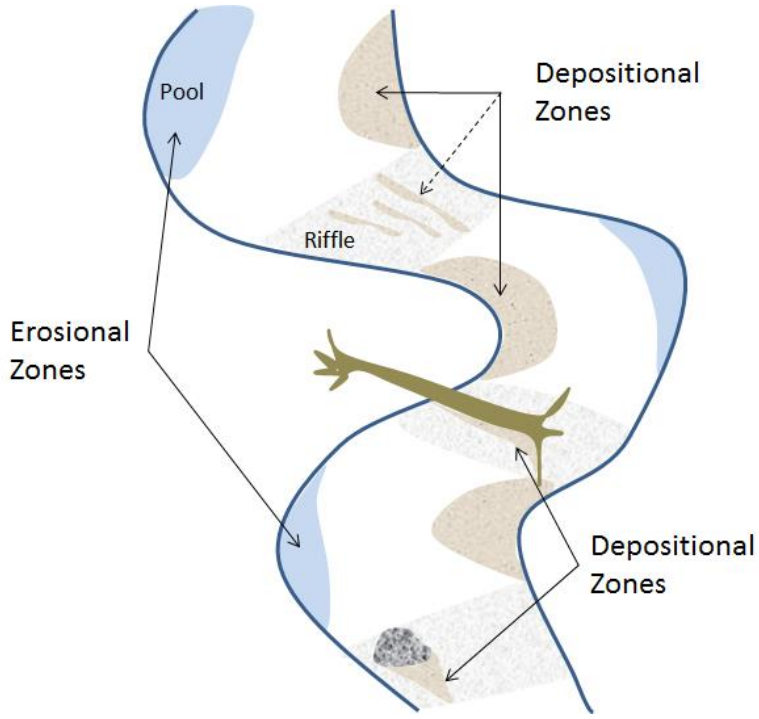

Figure 1. Examples of depositional zones in a wadeable stream.

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

This protocol is based on modified versions of the United States Geological Survey (2006), United States Geological Survey (1994), and United States Environmental Protection Agency (1994).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.014051	Field Audit Plan
AD[06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC. 002419	Datasheets for AOS Protocol and Procedure: Sediment Chemistry Sampling in Wadeable Streams
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[09]	NEON.DOC.001153	AOS Protocol and Procedure: Wadeable Stream Morphology Mapping
RD[10]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

2.3 External References

ER[01]	YSI Inc. 1998. Handheld Oxygen, Conductivity, Salinity and Temperature System, Operations Manual. ITEM # 038503, Revision E http://www.enviroequipment.com/rentals/PDF/YSI-85-Manual.pdf.

2.4 Acronyms

Acronym	Definition
cm	Centimeter
g	Gram
km^2	Square kilometer
L	Liter
µL	Microliter
µm	Micrometer
m	Meter
mm	Millimeter

2.5 Definitions

Decant: To draw off (a liquid) without disturbing the sediment or the lower liquid layers.

Depositional Zone: Area where sediments settle and accumulate on the stream bottom.

Flow Regime: A stream's naturally occurring changes in water flow through the course of a year.

Pool: A stretch of a stream in which water velocity is low and the water depth is above average.

Reach: A stretch of a stream which includes riffles, pools and runs. NEON defines this area as the permitted reach for sampling.

Riffle: A stretch of a stream denoted as shallow and coarse bedded where water flows at a higher velocity and turbulence.

Run: A stretch of a stream where water flows smoothly.

Stage: The water level above some arbitrary point in the stream.

Thalweg: Deepest part of the stream or channel, usually the line of fastest water flow.

3 METHOD

This protocol follows closely USGS (1994).

The spatial distribution of sediment characteristics depends on processes such as flow, turbulence, stream morphology, chemical precipitation and turbidity, on physical factors and on catchment characteristics such as underlying geology, the number and size of inflows and land use (Håkanson and Jansson, 1983; Ashley, 1995; Kumke et al., 2005). In wadeable streams, samples are taken from two stations, each representing composited samples from between 5-10 depositional zones (Figure 2). The sampling stations should each be approximately 25% of the reach (50% total). For example, for aquatic sites with a 1,000 m reach, sampling station 1 would represent half of one reach (250 m) and sampling station 2 would represent half of the other reach (250 m). Compositing samples allow for greater representativeness of mean concentrations for the area and results in smoothing of variability otherwise encountered between depositional areas. In a given depositional zone, samples are taken at a minimum of two to a maximum of five points and composited with samples from other depositional zones within the same station (Figure 3). The number of samples from each zone will be based on the surface area of each zone (i.e., the larger the area of the zone, the greater the number of subsamples collected). The sampling distribution should follow approximately 5 samples per square meter; however, it is usually difficult to estimate the size of depositional zones in non-wadeable streams and lakes due to water depth and turbidity. Therefore, a minimum of two locations will be sampled with up to five points composited at each station.Partially wetted zones (< 5 cm water) should only be sampled when no other sites are available, and sampling conditions should be documented in the field notes and data records as a potential outlier. Each sample consists of the surficial 1-3 cm of bed sediment. See Figure 4 and Figure 5 for a work flow chart for collecting sediment.

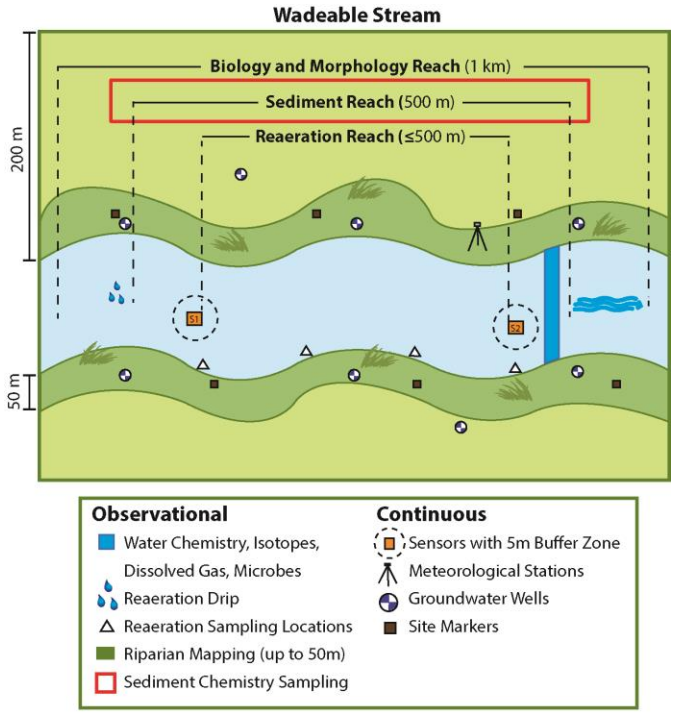

Figure 2. A generic wadeable stream site layout with sediment chemistry sampling locations

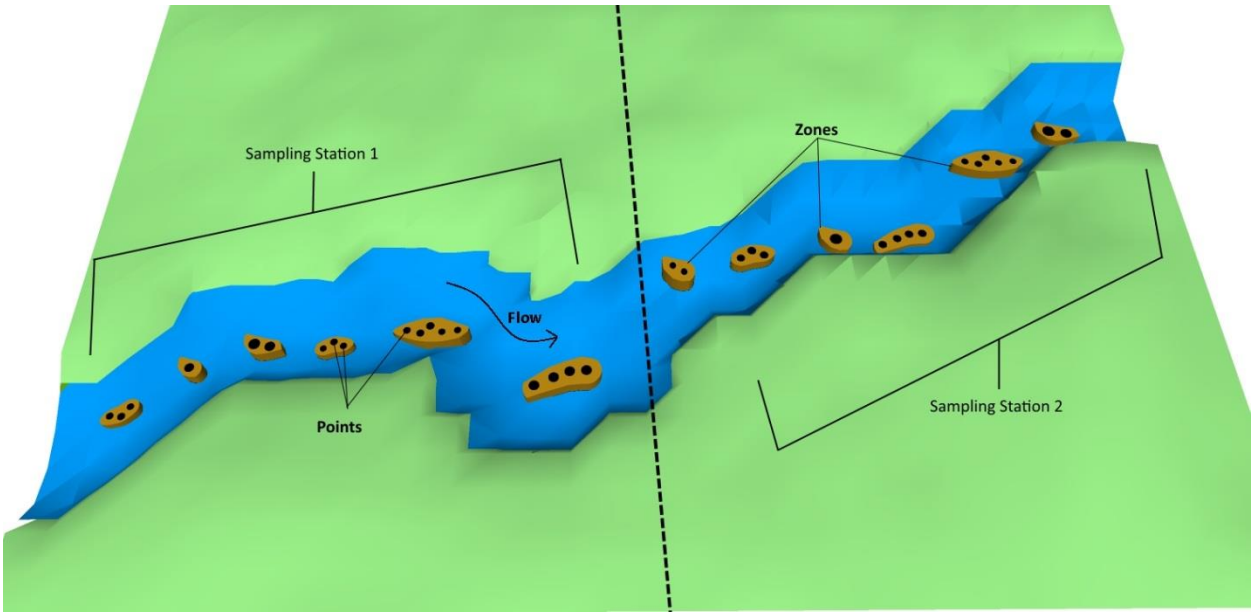

Figure 3. Identifying the location for sediment sampling in wadeable streams. The two sediment sampling stations should contain 5-10 depositional zones that represent upstream influences and various flow regimes.

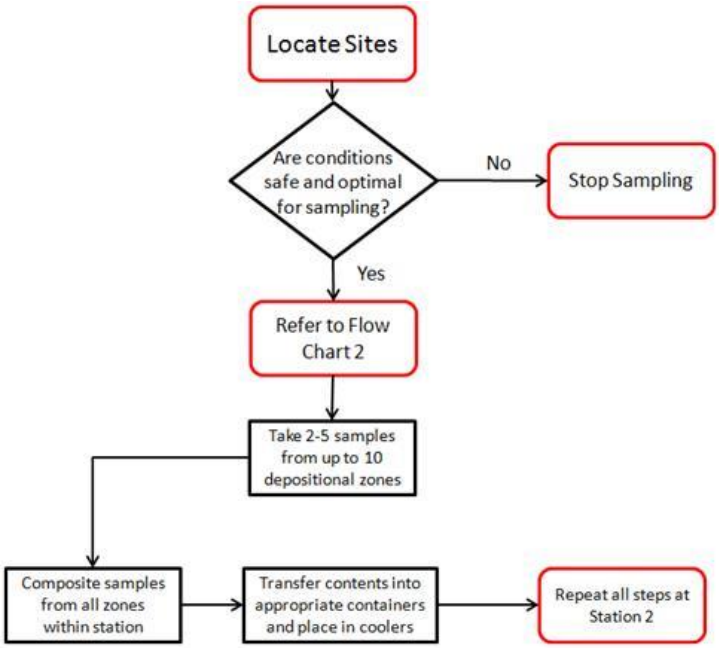

Figure 4. Decision flow chart 1 for determining the number of samples to be taken at each location

The sampling method used in wadeable streams depends on the sediment type. For softer fine sediments (< 2 mm), rich with deep organic material, a hand corer is used to extract the sediment samples with minimal impact on the surface sediments. For harder and shallow substrate with more coarse sediments (> 2 mm) a scoop sampler is used (Figure 5). Both the hand corer and the scoop can be used throughout the reach within the sampling bout. For example, if sampling in a riffle, the scoop is used to sample coarse sediment then in a stream run the hand corer is used to sample finer sediment material. Within the individual depositional zones, keep the sampler type consistent. Do not use both the hand corer and the scoop in one depositional zone. Be sure to document which tool or tools were used during sample collection. Sediment samples are collected after most of the biological sampling activities, except fish sampling in order to avoid disturbance of benthic habitats (JIRA 602).

One 8 oz. (~250 mL) round glass jar will be used to collect samples for organic analyses, one 4 oz.(~125 mL) round glass jar for Total Organic Carbon and Total Carbon (TOC/TC) analyses, and another 8 oz.(~250 mL) jar will be used to collect sediments for inorganic analyses. An additional 1.0 U.S. gallon (~3.8 L) of sediment will be collected in two U.S. gallon plastic (Ziploc) sealable bags. Each bag will be filled half way, sealed, and placed in another sealable gallon-sized Ziploc bag. This method is recommended to prevent completely filling a single gallon-size plastic bag which could open and spill during transport or sample shipping. Two stations per wadeable stream are sampled on each sampling date for a total of approximately 9.0 L of collected sediment.

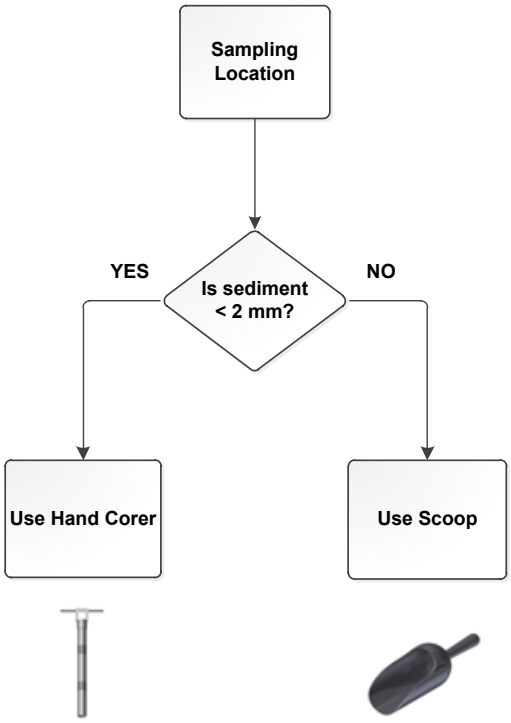

Figure 5. Decision flow chart 2 for determining appropriate sampler to use in the field

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[05]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[06]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Sampling for wadeable stream bottom sediments shall occur up to 5 times per year at the determined sampling dates. Sediments will be collected during the three biological sampling bouts at most sites. In

some cases, additional sediment samples may be collected following a large disturbance event (e.g. flood or drought) or other shift in site conditions (e.g. chemical spill). Sampling shall occur within +/- 2 weeks of the given dates. The timing of these samples shall follow the procedures outlined in the NEON Aquatic Sample Strategy Document (RD[07]).

The timing of the sampling is flow dependent in wadeable streams. Should a major event occur that disrupts sediments, wadeable stream samples should not be collected for at least 5 days following a major flow event, allowing for the settlement of suspended sediments into depositional environments. All samples should be taken at the same time each day, within, +/- 2 hours of previous sampling bout start.

4.2 Criteria for Determining Onset and Cessation of Sampling

The NEON aquatic program will be sampling stream sediments 3 times per year during the spring, summer and fall sampling bouts. Two additional sample events may be warranted following a major storm event that alters the morphology of the system. The timing of the sampling is flow dependent. Sampling bouts will occur approximately every 2 months and during base flow and/or stable flow conditions to provide maximum direct access to the stream bed and to minimize seasonal streamflow variability. Sediment samples can only be taken when velocity in the wadeable streams is below 0.93 m^2/s (USGS, 2008). The specific times will be determined using multivariate statistics and site specific historical information provided in the NEON Aquatic Sample Strategy Document (RD[07]).Criteria for Determining Sampling Dates

Sample timing should reflect the influence of chemical and biological inputs and varying chemical conditions. Hence the temporal strategy for NEON samples will include sampling dates reflective of key times of leaf – on, leaf- off and peak greenness. The timing of such samples should reflect the hydrologic nature of the stream and the temporal variability of the system. As such, sampling will occur during base flow and/or stable flow conditions to provide maximum direct access to the stream bed and to minimize seasonal streamflow variability. Should a major event occur that disrupts sediments, wadeable stream samples should not be collected for at least 5 days following a major flow event (>3 times median discharge for the preceding year) to allow time for the settlement of suspended sediments into depositional environments.

4.3 Timing for Laboratory Processing and Analysis

For the purpose of analysis the samples will be processed within 12 hours of return to the Domain lab. Ship samples to the external Laboratory within 72 hours following processing. See Appendix F for a complete list of sediment chemistry analyses, storage requirements, and hold times.

4.4 Sampling Timing Contingencies

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If sampling stirred up sediments or added chemical constituents to the stream/lake (i.e., gas additions) within the past hour, allow the water to clear and disturbance to pass, sample upstream/upwind of the disturbance.	No adverse outcome.
	Should flow rates change (>3 times median discharge for the preceding year) during sampling, stop work for the day and allow for flow conditions to stabilize. Discard all samples.	No adverse outcome.
Days	Following a major precipitation event and resulting high flow, samples will be taken at least 5 days following a major flow event.	No adverse outcome.

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

In addition, the following safety requirements are sought:

1. Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 10 ft^2/s (0.93 m^2/s).
2. When handling hazardous products (such as nitric acid) follow laboratory safety standards and have Safety Data Sheet (SDS) readily available to review prior to handling any chemicals. Wear gloves, a laboratory coat and protective eyewear.
3. Personal flotation devices (PFDs) shall be worn when wading in deep streams (per USGS, A Guide to Safe Field Operations). Depth requirements may vary in different regions.
4. In areas with alligators or other submerged wildlife dangers, extra precaution must be taken. The crew will be required to not put hands and feet in the water and to make sure a safe distance is kept from alligators.
5. At extreme cold water sites, additional safety training may be required (example Oksrukuyik Creek) and include the use of special equipment (Mustang jackets) for added safety.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Table 2. Equipment list – Field equipment list

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX108989	R	Scoop, Polyethylene	Collecting inorganic and size analysis samples	1	N
MX102978	R	Scoop, Stainless Steel	Collecting organic samples	1	N
MX100309	R	Hand Corer	Collecting samples	1	N
	R	Stainless Steel Bucket 5 gallons w/ lid	Homogenizing organic samples	1	N
	R	Plastic bucket 5 Gallons w/ lid	Homogenizing inorganic and size analysis samples	1	N
	R	60 mL syringes	Syphoning water from samples	2	N
	R	Stopcocks	Syphoning water from samples	2	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	50 cm of Tygon tubing 1/8” inner diameter	Syphoning water from samples	2	N
	R	Wash Bottle, plastic, 500mL	Rinsing sediment from samplers into buckets	1	N
	R	Wash Bottle, Teflon, 500mL	Decontaminating equipment with methanol	1	N
	R	Plastic Funnel 13 oz.	Collecting inorganic, nutrient and size analysis samples	1	N
	R	Stainless Steel Funnel 64 oz.	Collecting organic samples	1	N
	R	Plastic spatula	Collecting inorganic, nutrient and size analysis samples	1	N
	R	Stainless steel spatula	Collecting organic samples	1	N
	R	Flexible forceps, featherweight	Removing debris from samples	1	N
	R	Measuring stick, foldable, plastic	Measuring the water depth at sampling locations	1	N
	R	Field documentation forms and field books	Documenting notes in the field	3	N
	R	Brush, scrub, soft nonmetallic	Cleaning samplers	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX100514	R	Multisonde	Measuring % DO, temperature and salinity	1	N
	R	4,000 µm sieve	Sieving samples before transferring into bottles	1	N
	R	Coolers	For shipping, provided by the external lab	1	N
	R	Boots and/or hip waders	Safe wading	2	N
	R	First Aid Kit	Safety	1	N
	R	Camera	Photographing sampling observations	1	N
	R	GPS	Navigating to sampling locations	1	N
Consumable items					
	R	Ice Pack	Keeping samples cool, provided by the external lab	Multiple	N
	R	Nitrile gloves, in plastic bag	Not contaminating samples	Multiple	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Clear Boston-style round glass jar, 8 oz. (~250 mL), for organic and inorganic analyses	Sample container, provided by the external lab	4	N
	R	Clear Boston-style round glass jar, 4 oz. (~120 mL), for Total Organic Carbon/Total Carbon	Sample container, provided by the external lab	2	N
	R	Clear Ziploc-style 1-gallon plastic bag for sediment grain size analyses	Sample container, provided by the external lab	4	N
	R	Foil, aluminum, heavy duty, one roll	Storing equipment and avoiding contamination	1	N
	R	Permanent Markers	Labeling samples	3	N
	R	Bottle labels	Labeling samples	14	N
MX100351	R	Phosphate free detergent	Decontaminating equipment	1	N
	R	Nitric acid	Decontaminating equipment	1	Y
	R	Methanol	Decontaminating equipment	1	Y

R/S=Required/Suggested

6.2 Training Requirements

All technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]).

Personnel are to be trained in wadeable stream sediment chemistry measurements and safe working practices for stream work. All personnel required to wade in the streams to collect sediments shall review the USFWS Wader Safety Video is required by the NEON safety training program. The safety video is available with CSP2202-OLT Electrofishing Safety.

6.3 Specialized Skills

N/A

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

We estimate wadeable stream sediment chemistry sampling requires 2 technicians for 4-6 hours each sampling day plus travel to and from the site.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

1. **Ensure all equipment has been cleaned and stored appropriately** (see SOP B.4).
2. Mark the hand corer/liner with 1 cm increments using a waterproof marker to help define the sampling depth.
2. Check the sediment chemistry field sampling kit to make sure all supplies are packed.
3. Ensure GPS locations of sampling stations are entered into the GPS system.
4. Confirm type of sediment samples that will be collected and take the appropriate bottles and collection devices (Figure 5 and Figure 6).
5. Use NEON bottle labels; do not use labels provided by the external lab (Figure 7).
6. Use a Sharpie to fill out bottle labels (Figure 7). Labels are waterproof but should be filled out before getting wet to ensure ink will stick to the labels. Circle the correct bottle type code (Figure 7) on the labels for each bottle.

7. Complete field data sheets on the personal digital assistant (PDA) associated with the sediment chemistry protocol. When the PDA is unavailable, fill out site information on the General Field Sampling Data Sheet (metadata) (RD[06]) and the additional sample collection datasheets (RD[05]). The General Field Sampling Data Sheet only needs to be collected once per day either using the PDA or on the data sheets.
    a. Record the Date (YYYYMMDD) and the time of day (use local, military time; ex. 13:46) that samples were collected on the stream Sediment Chemistry Sampling Datasheet (RD[05]).
    b. NOTE: Use the same time for all bottles filled at the same sampling station during each sampling event (i.e., the time the sediments were collected from the stream).
    c. The Sample ID, Date (YYYYMMDD), and Time must match the sediment chemistry label (Figure 7). Station ID is either "1" (station 1) or "2" (station 2). Indicate sample type with either "I" (inorganic), "O" (organic), "C" (TOC/TC), or "SS" (sediment size).

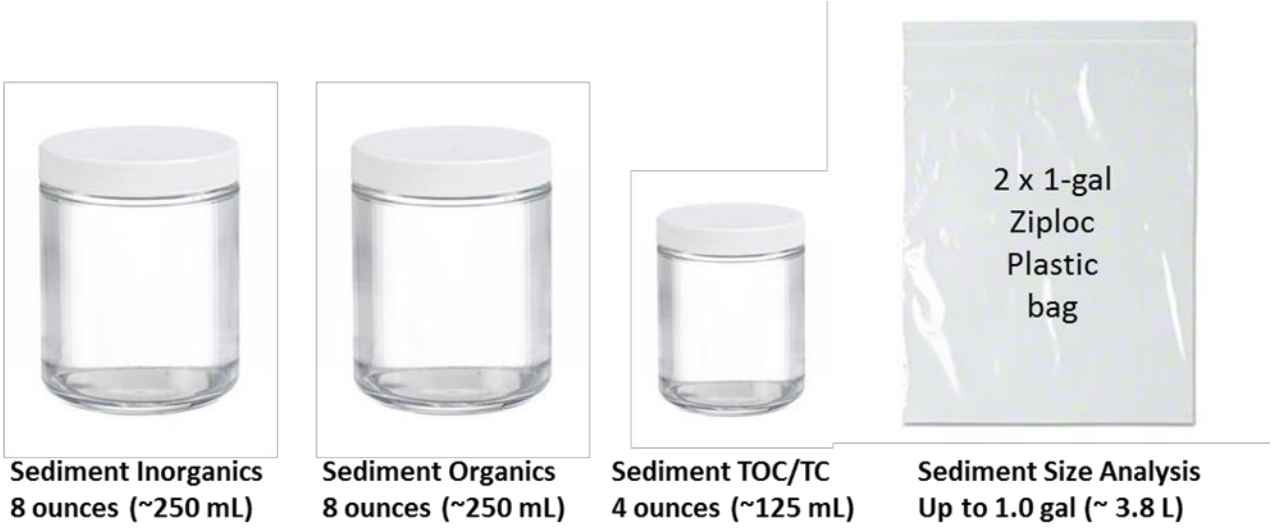

Figure 6. Example of clear Boston round glass jars with PTFE lined lid used for collecting sediment for inorganics, organics, and TOC/TC analyses. Sediments for grain size samples are collected by filling 2 1-gallon (~3.8L) plastic Ziploc bags about half-full.

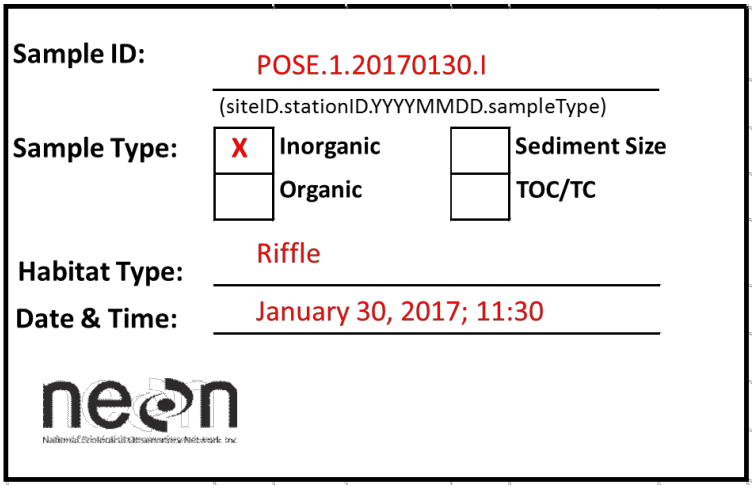

Figure 7. Example of NEON sediment chemistry bottle label. Habitat types in wadeable streams may include riffle, run, pool, or step pool.

SOP B Field Sampling

Before sampling:

1. Identify the suitable sediment sampling stations, zones, and point locations based on bathymetric and sediment geomorphology maps if available.
2. Place the equipment on the aluminum foil and bags. Be sure not to contaminate bags.
3. Insert ice/ ice packs into the cooler.
4. Line each shipping cooler with a trash bag.
5. Calibrate the multisonde for dissolved oxygen before sampling (see instructions in ER [01]).
6. When in the field, rinse all equipment three times with native water prior to use.

Stream sediment samples shall be collected from two identified sediment-sampling stations within the 500 m sediment sampling reach (Figure 2). Each station covers up to 250 m or half of the 500 m sediment reach. The location of the station divide is defined by the mid-way point between the top and bottom of the biology and morphology reach (Figure 3). At each of the 2 sampling stations, identify at least 5 wadeable depositional zones containing fine-grained particulate matter (Figure 8a). The number of depositional zones will be dependent on stream morphology (see RD[09]). Identification of depositional zones can be aided by using the site characterization stream morphology maps if available. If the site has no large depositional zones, take samples from depositional environments as per Figure 8b. Likewise, should the streambed be mostly sedimentary or organic in nature, then distribute the samples as per Figure 8c. The areal extent of each zone should be estimated and if possible quantified.

Due to the affinity of metals and other nutrients to bind to smaller sized particles (<2 mm), this size fraction found in depositional zones better represents the potential quantification of sediment chemistry. The zones should not interfere with the biological sampling locations when possible. At sites that are limited by depositional zone areas or sediment substrate size, follow the instructions in Figure 8

and Figure 9. The priority for these sites is to collect sufficient material for nutrients and particle size analyses.

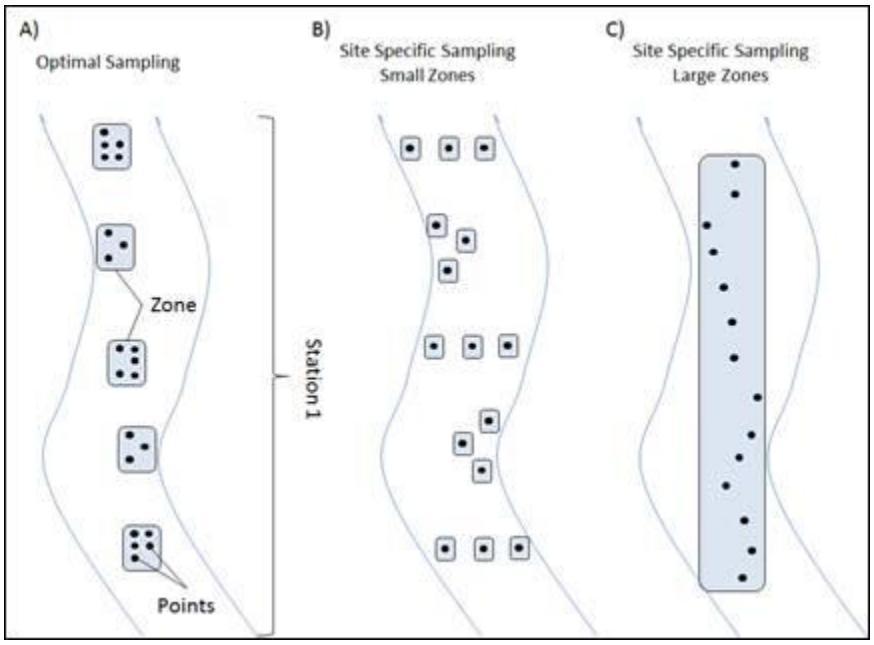

Figure 8. Diagram to exemplify determination of sampling zones based on site differences. A) Ideal sampling set up based on availability of different depositional zones (~1 m^2); B) in sites with only random small pockets of sediment; and C) in sites where most of the stream bed is characterized by sedimentary or organic substrate.

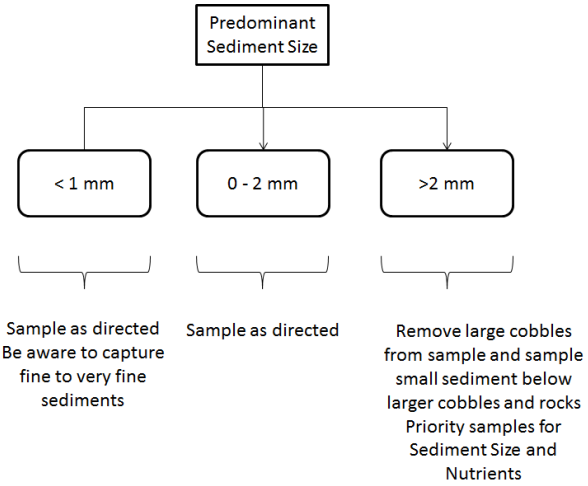

Figure 9. Decision flow chart for sampling in streams with stream bed materials that are limited in quantity or type.

B.1 Sampling in Wadeable Streams

3. Start at the most downstream station and zone working from downstream to upstream in order to minimize sediment disruption. If sediments are disrupted, wait until the area has cleared before sampling.
2. Ensure the General AQU Field Metadata Sheet (RD [06]) is completed.
3. Measure the % oxygen, temperature and specific conductivity (µS/cm) of the water about 10 cm above the sediment in each sampling zone and note data on the field sampling sheet (RD[05]). For zones in less than 10 cm water depth, measure water quality half way between the water surface and the sediment. Rinse all equipment with native water three times before use. Sediments should be collected and composited from depositional zones throughout the station for samples that are representative of the station (Figure 8). When sampling sediments that are smaller than 2 mm use a Hand Corer (SOP B.2) and use a Scoop when sampling coarser sediments greater than 2 mm (SOP B.3).
    a. Collect sediments from several points from depositional zones distributed across the sampling station (Figure 8a) and record the water quality and a GPS location on the datasheet.
    b. For depositional zones less than 5 square meters collect water quality and a GPS point from one center location (Figure 8b).
    c. For depositional zones greater than 5 square meters collect water quality and a GPS point from the downstream and upstream locations (Figure 8c).
4. Using a folding measuring stick, measure the water depth at each sampling point within each zone on the field sampling sheet (RD[05]).
5. Repeat steps 1 – 4 above at each sediment sample zone when taking samples.
6. Proceed to next station and repeat steps 3-5.

B.2 Sampling with a Hand Corer

When the bed sediment is composed of primarily smaller sediment sizes (<2 mm) use the hand corer for collecting sediments (Figure 10).

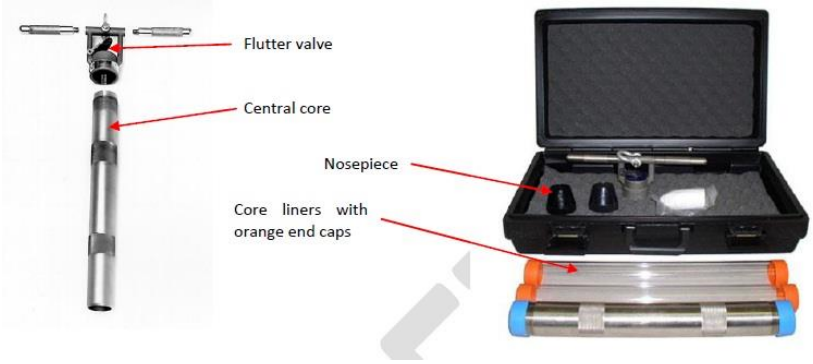

Figure 10. Hand corer for use in wadeable streams with fine sediments

1. Put on gloves (nitrile).
2. Assemble the hand coring sampler: if sampling for metals or sediment size insert a core liner; if sampling for organics do not insert a liner.
3. Collect samples from up to a maximum of 5 points per depositional zone greater than one square meter (Figure 3). If the depositional zones are less than 1 m^2, a minimum of 2 points within each depositional zone should be collected. Ensure that each depositional zone is sampled equally for inorganic, organic, TOC/TC, and sediment size samples. Up to 4.3 L of sediment will be collected at each station to run the full suite of analyses.
4. To operate the hand corer:
    a. Insert sampler into the bed up to 3 cm.
    b. Use a metal spatula for organics or a plastic spatula for inorganics to cap the end of the corer before the sampler breaks to surface of the water to prevent sediment from falling out.
    c. Only collect the top 3 cm of the bed material from the exterior of the core.
    d. Ensure the upper flutter valve is closed and lift the corer above the surface maintaining it as vertical as possible. It may require using native water to keep the flutter valve wet for a good seal. Inspect for adequate fine material; if not appropriate, discard near the bank downstream of the depositional zone and take another sample.
    e. Gently remove the nosepiece and replace with an orange core cap if sampling for inorganics. Otherwise simply cap end of corer with blue cap.
    f. Move to the processing area on the stream bank.
5. Deposit all inorganic samples into the plastic bucket and proceed with the next sample.
    a. For the organic samples, collect the sediment samples into the stainless steel bucket and use stainless steel tools (Figure 11). Use the TOC/TC equipment when collecting those composited sediments into the TOC/TC sample jar.
    b. Sediment size samples can be composited from either the inorganic or organic sample material (Figure 11).
6. Composite the collected sediments from all zones ensuring proportional amounts of material are contributed from each zone. However, the amount of material collected from a deposition zone depends on the size of the deposits (Figure 8).
    a. If fines or organics are left attached to the walls of the sampler, use a Nalgene wash bottle filled with native water to rinse down the sediments from the corer into the bucket.
7. Immediately record observations regarding the appearance of the sediment (i.e., texture, color, odor, presence of biota, presence of detritus, and the depth of sediment sampled).
8. Repeat steps 1-5. Collect samples from up to 5 points per square meter (Figure 3) or from up to 2 points if the depositional zones are less than 1 m^2. Ensure that a representative sample for organics is collected from each depositional zone.

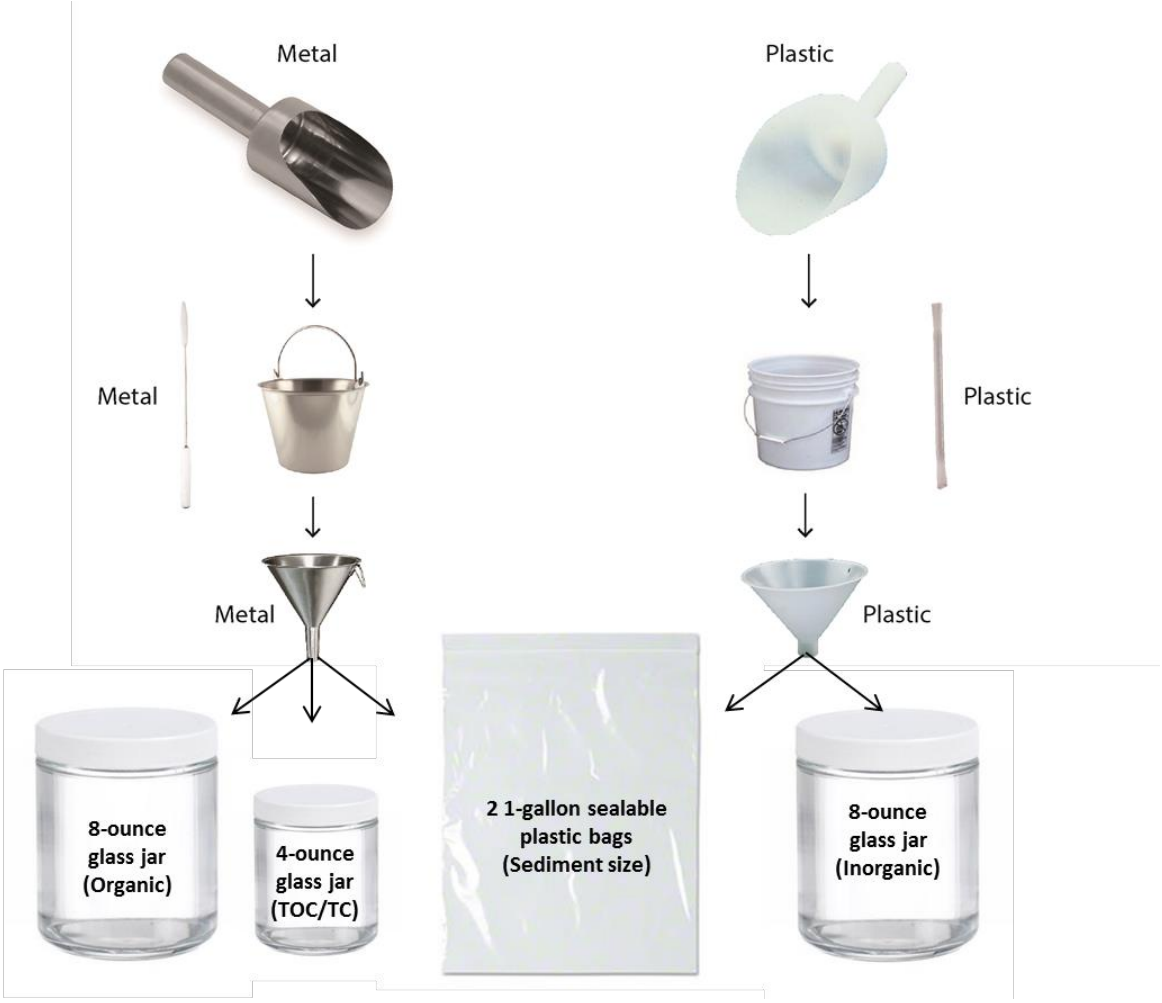

Figure 11. Sediment collection instruments by sample type

7. For light (sparse) debris > 4 mm, use forceps to remove litter from the sample.
8. If the sample contains heavy debris, many large pebbles and cobbles (> 4mm), place collected material in the bucket to composite then either pick out or sieve the debris and pebbles/cobbles out of the sample prior to transfer to the sample bottle using the US-5 (4,000 µm) mesh .). Coarse rock and debris may be discarded on the shore away from other depositional zones. The external lab will screen these composited samples to < 2 mm at their facility. It is VERY IMPORTANT that the majority of the sample (> 50%) contain sediment (not water) in order to provide the external lab with a sufficient volume of material to run all of the required analytical parameters.
9. Use the funnel to distribute the composite sample into the collection bottle for the organics.

10. Let the sample settle for up to five minutes then gently decant any excess water (> 3 cm or 100 mL) from the container by gently pouring off the liquid layer taking care to prevent disposing of the fine sediment size fraction. It may be helpful to remove the liquid layer using a syringe and tube (or just the syringe) to suction off remaining water. For samples with excessive suspended sediment, it may be necessary to move onto the next sampling station and let the sediments settle prior to decanting. Minimize the time that the plastic syringe or tubing is in contact with sediments sampled for organics.
11. Repeat steps **Error! Reference source not found.**1-7 using a plastic spatula and funnel to distribute the composite sample into the collections bottles for inorganics, nutrients and sediment size.
12. Collect a total volume of approximately 4.5 L of wet sediment per Station enough to fill one 8 oz. (~250 mL) glass jar for collecting sediment for organic analyses (including organic contaminants), one 4 oz. (~125 mL) glass jar for TOC/TC analyses, and one 8 oz. (~250 mL) glass jar for inorganic. Collect enough sediment to fill two 1-gallon (~3.8 L) plastic Ziploc bag at least halfway with sediment for grain size analyses.
13. Sediment for grain size analyses can be collected from either the plastic or metal buckets used for compositing the sample material; it is also acceptable to combine sediments from both buckets for the sediment grain size analysis sample (Figure 11).
14. Place the combined samples in a cooler with ice as soon as they are transferred to the pre-labeled bottles. DO NOT FREEZE.
15. Dispose of any excess sediment not collected for external lab analyses near the shore, away from depositional zones.
16. Clean the sampler with the scrub brush.
17. Proceed to SOP D for sample treatment and shipping preparation in the field.

NOTE: Collected sediment material from each station will include one glass jar (8 oz, 250 mL) of organic sample, one glass jar (8 oz, 250 mL) of inorganic sample, and one gallon (~3.8 L) of sediment in 2 1-gallon sized half-filled Ziploc-style plastic bags. The same number of samples and containers will be collected from the second station.

B.3 Sampling with a Scoop

When insufficient sediment depth and/or larger sediment size (>2mm) is encountered, particularly in rocky streams and rock bottomed streams, use the scoop sampler (Figure 12). Be sure that the scoop size is the same for both the metal and plastic scoops. This will enable more proportional samples within each depositional zone.

Figure 12. Scoop sampler for use in wadeable streams

1. Put on gloves (nitrile).
2. Take the 5-gallon sediment collection buckets (stainless steel and plastic) whilst sampling in the stream in order to minimize the disturbance from entering and exiting the stream.
3. Collect samples from up to a maximum of 5 points per depositional zone greater than one square meter (Figure 3). If the depositional zones are less than 1 m^2, a minimum of 2 points within each depositional zone should be collected.
   a. Remove the top layer (approximately 1-3 cm) of fine sediment carefully by gently scooping in the upstream direction.
      1) Ensure the scoop is plastic when sampling for inorganics (Figure 11).
      2) Use the metal instruments when sampling for organics.
      3) The sediment size sample can be collected with either scoop type.
   b. Slowly pour off most of the overlying water over one of the BACK corners of the scoop. Make sure that the top layer of fine sediment is not discarded.
   c. Inspect for adequate fine material; if not appropriate, discard.
4. For all inorganic samples, place sediment in the plastic bucket and proceed with the next sample. Composite samples from all zones ensuring that approximately the same amount of sediment is contributed from each zone for all sample analyses. For organics use the stainless steel bucket and utensils. If fines or organics are left attached to the walls of the scoop, use a Nalgene wash bottle filled with native water to rinse down the sediments from the scoop into the bucket. Use the glass bowl to further composite samples if needed following settlement of suspended particles.

5. Repeat steps 1-4. Collect samples from up to 5 points per square meter (Figure 3) or from up to 2 points if the depositional zones are less than 1 m^2. Ensure that a representative sample for organics is collected from each depositional zone.
6. Using forceps, remove any debris or litter from the sample.
7. If the sample contains many large pebbles and cobbles (> 4mm), place collected material in the bucket to composite then either pick out or sieve the debris and pebbles/cobbles out of the sample prior to transfer to the sample bottle using the US-5 (4,000 µm) mesh. Coarse rock and debris may be discarded on the shore away from other depositional zones.
8. Use the funnel to distribute the composite sample into the collection bottle for the organics.
9. Let the sample settle for up to five minutes then gently decant any excess water (> 3 cm or 100 mL) from the container by gently pouring off the liquid layer taking care to prevent disposing of the fine sediment size fraction. It may be helpful to remove the liquid layer using a syringe and tube (or just the syringe) to suction off remaining water. For samples with excessive suspended sediment, it may be necessary to move onto the next sampling station and let the sediments settle prior to decanting. Minimize the time that the plastic syringe or tubing is in contact with sediments sampled for organics.
10. Repeat steps 1-8 using a plastic spatula and funnel to distribute the composite sample into the collections bottles for inorganics and sediment size.
11. Collect enough sediment to fill two 1-gallon plastic Ziploc bag at least halfway for a full gallon of sediment for the grain size analysis (1.0 gallons; ~3.8 L).
12. Collect a total volume of approximately 4.5 L of wet sediment per Station using the appropriate tools for field collection and homogenization.
13. Place the combined samples in a cooler with ice as soon as they are transferred to the pre-labeled bottles. DO NOT FREEZE. Clean the sampler with the scrub brush.
14. Dispose of any excess sediment not collected for external lab analyses near the shore, away from depositional zones.
15. Proceed to SOP D for sample treatment and shipping preparation in the field.

NOTE: Collected sediment material from each station will include one glass jar (8 oz., 250 mL) of organic sample, one glass jar (8 oz., 250 mL) of inorganic sample, one glass jar (4 oz., 125 mL) for TOC/TC, and one gallon (~3.8 L) of sediment in 2 1-gallon sized half-filled Ziploc-style plastic bags. The same number of samples and containers will be collected from the second station.

B.4 Ending the Sampling Day

1. Refreshing the sampling kit
   a. Re-order sample kit (bottles and coolers) from external lab at least one week prior to the next scheduled sampling. Restock the sampling kit (shipping cooler) with new sediment chemistry sampling bottles (with new labels attached), and other equipment and consumables in Table 2.
2. Equipment maintenance, cleaning and storage
   a. Decontaminate all equipment according to NEON Aquatic Decontamination Protocol, (RD[08]) before using at another site. For equipment specific cleaning see below.
   b. Ensure all containers, tools, and equipment used for **inorganic, organic, TOC/TC, and sediment size sample collection** are cleaned prior to storage or reuse:
      1) Rinse equipment and containers to remove obvious residual sediments (dump rinsate into an appropriate container; large sediments could clog lab sink drains over time).
      2) Prepare a tub with 0.2% phosphate-free detergent.
      3) Wash containers/equipment in the detergent bath.
      4) Soak the containers/equipment for at least 30 minutes.
      5) Rinse thoroughly with de-ionized water three times using new water each time.
      6) For containers and sampling equipment intended for **inorganic** and **organic** sediment collection, see steps c and d below.
      7) For all remaining equipment, allow to air dry.
   c. Preparing equipment for **inorganic** analyses sampling (plastic sampling tools):
      1) After the detergent soak described above, rinse with the containers/equipment with 5% high purity nitric acid (HNO_3).
         a) Here is an example for making 1.0 L of 5% HNO_3 with 69.1% nitric solution. Mix 72.36 mL of HNO_3 with 927.64 mL tap water. **ALWAYS add acid to water!**
         b) VERY IMPORTANT, consult the domain specific chemical hygiene plan for disposal of acid waste or other hazardous chemicals.
      2) Rinse three times with deionized water.
      3) Allow to air dry.
      4) Store in plastic bags or within the decontaminated plastic compositing bucket and covered with plastic wrap or the plastic bucket lid. Avoid exposing inorganic sampling equipment to metal.
   d. Preparing equipment for **organic** and **organic-contaminant** sampling:
      1) After the detergent soak described above, rinse containers and equipment with methanol from the Teflon wash bottle.
      2) Allow methanol rinse to air dry
      3) Allow to air dry.

4) Store in aluminum foil or within the decontaminated metal compositing bucket and covered with foil or the metal bucket lid. Avoid exposing the organic sampling equipment to plastic materials.

e. Preparing equipment for **Total Organic Carbon** and **Total Carbon (TOC/TC)** sampling:
   1) After the detergent soak described above, rinse containers and equipment with methanol from the Teflon wash bottle.
   2) Rinse three times with deionized water. Allow to air dry.
   3) Store in aluminum foil or within the decontaminated metal compositing bucket and covered with foil or the metal bucket lid. Avoid exposing the organic sampling equipment to plastic materials and methanol. Label these tools for TOC/TC sampling.

SOP C Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

SOP D Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the CLA shipping document on CLA's NEON intranet site.

D.1 Handling Hazardous Material

N/A

D.2 Supplies/Containers and Conditions

1. Place glass bottles in individual sealable plastic bag, place the gallon-size closeable bags in a second sealable plastic bag, and place in packing material (foam sleeves) for protection from breaking.
2. Place the round glass sample jars and gallon closeable plastic bags into the 5 gallon cooler with ice packs.
3. Complete and include the shipping label, chain of custody (COC) form, and shipping inventory. Also, include a copy of the appropriate sediment shipping permit. Place the completed forms in a Ziploc bag and securely tape the bag to the cooler lid, which will help keep the forms dry. Make sure the time and date on the bottle(s) matches the time and date on the form(s) (RD[05]).
4. Tie the garbage bag; security seals across the opening of the cooler and ship to address provided by NEON.

D.3 Timelines

Ship samples to the external Laboratory within 72 hours following field processing. Ship samples “Priority Overnight".

D.4 Grouping/Splitting Samples

N/A

D.5 Return of Materials or Containers

N/A

D.6 Shipping Inventory

Include sample shipment inventory (RD[10]). Email shipping inventory to external lab contact and copy the NEON CLA contact.

D.7 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA’s NEON intranet site.

8 REFERENCES

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

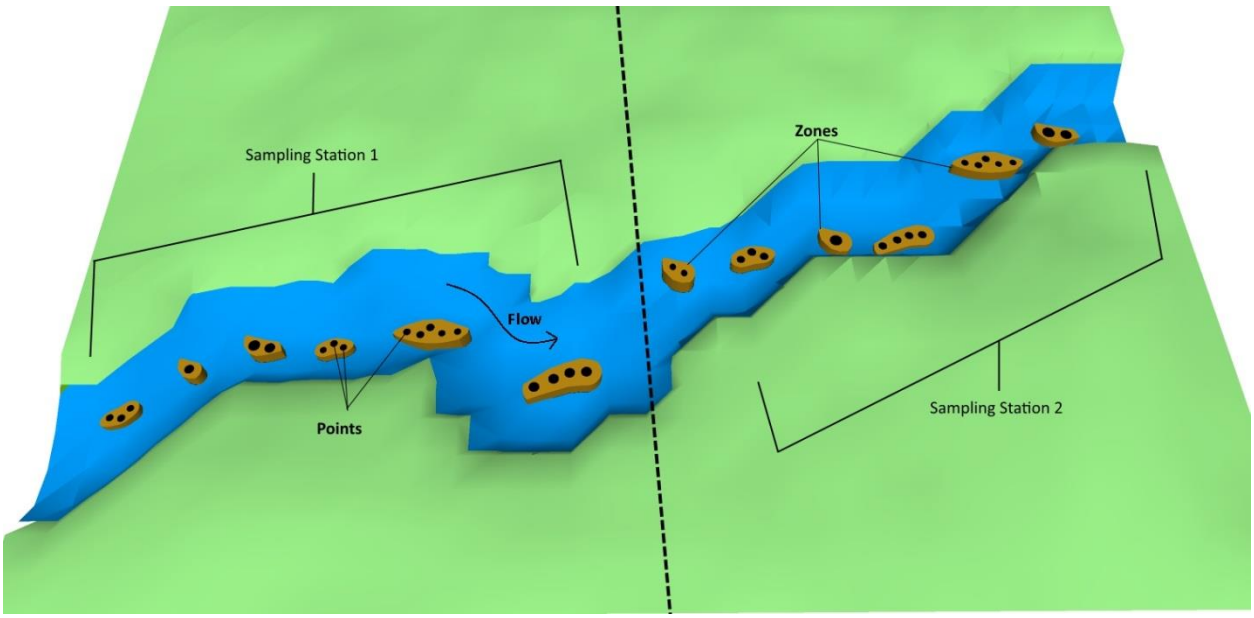
Table 3. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC. 002419	Datasheets for AOS Protocol and Procedure: Sediment Chemistry Sampling in Wadeable Streams
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

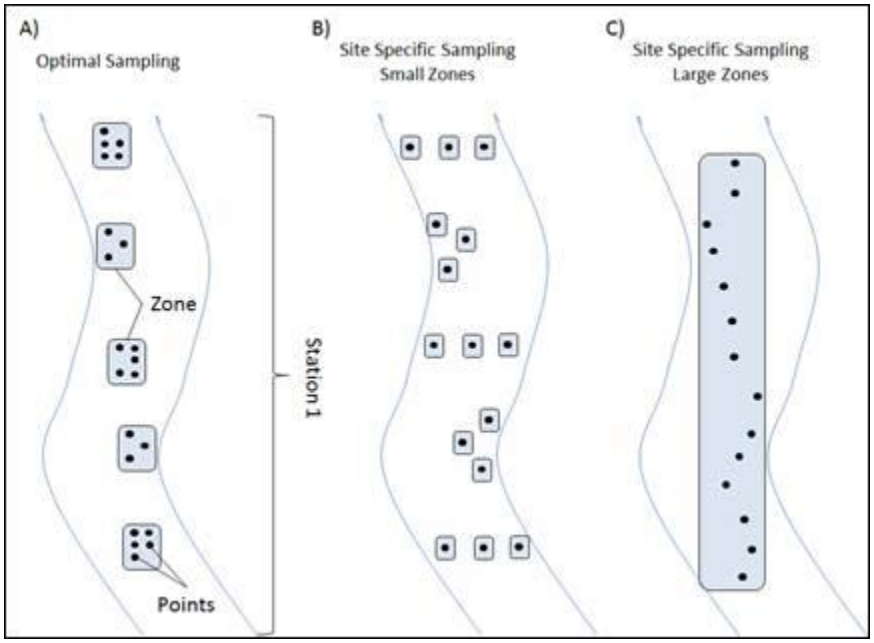
These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

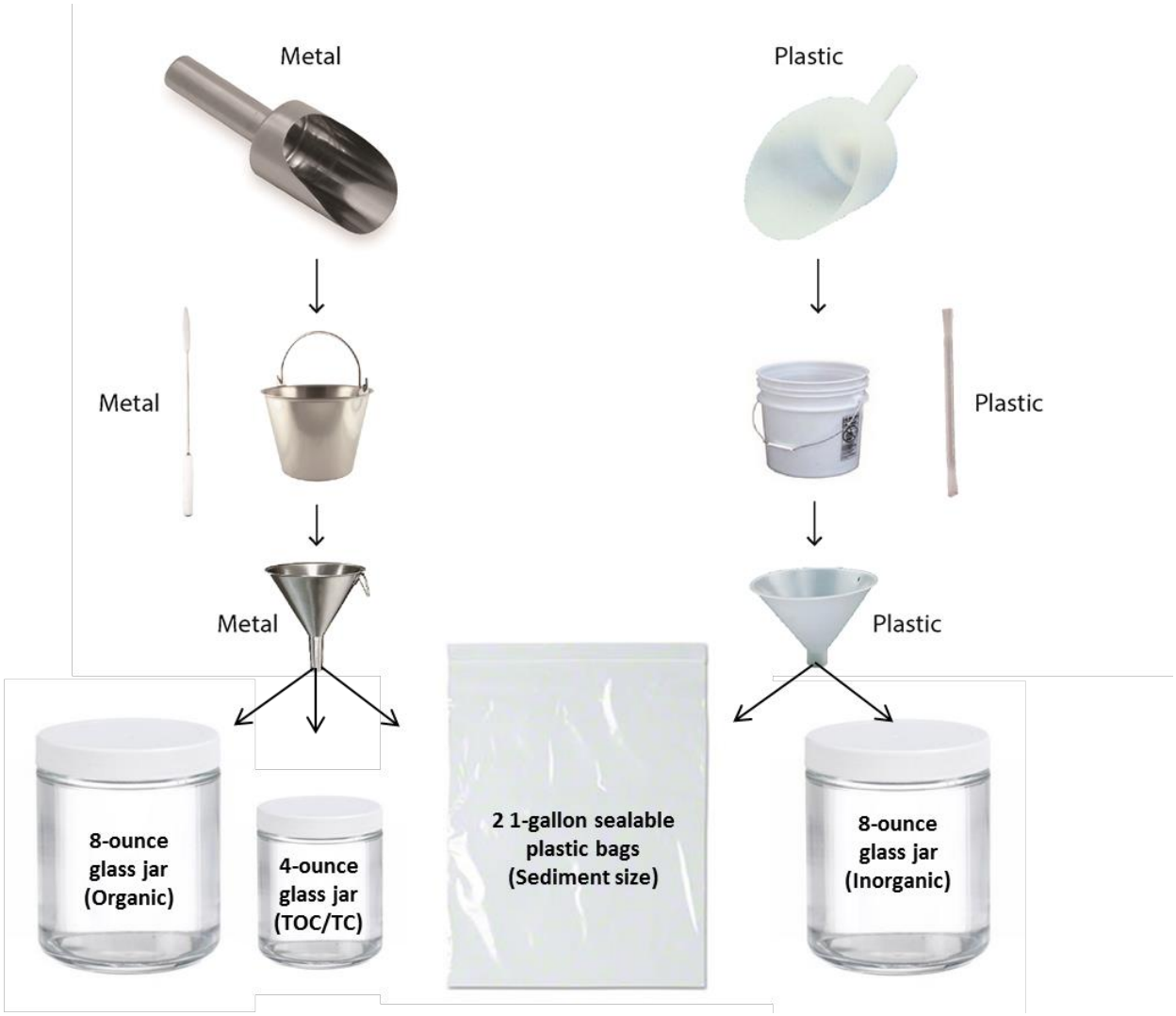
B.1 Diagrams for Identifying the Locations of Sediment Sampling



B.2 Diagram of Sample Collection Zones



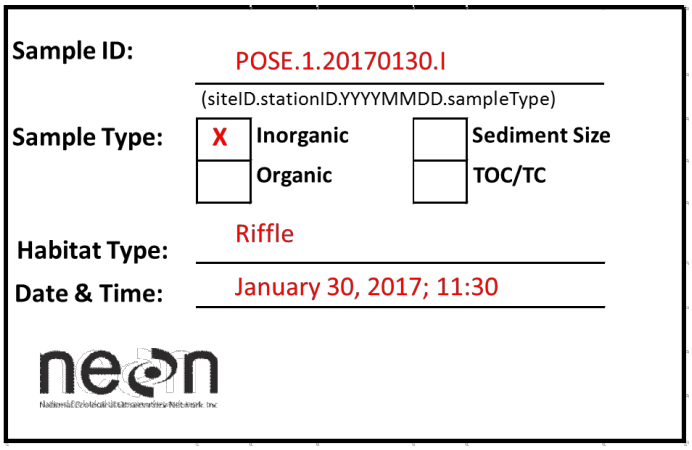
B.3 Flowchart of Sample Collection



B.4 Steps for Sediment Chemistry Sampling

Step 1 – Check the sediment chemistry field sampling kit to make sure all supplies are packed.

Step 2 – Prepare labels (2" x 4").



Step 3 – Ensure the General AQU Field Metadata Sheet (RD[06]) is completed per field site visit.

Step 4 – Begin sampling at the most downstream zone.

Step 5 – When insufficient sediment depth and larger sediment size (>2mm) is encountered, particularly in rocky streams and rock bottomed streams, use the **scoop sampler.**

Step 6 – When the bed sediment is composed of primarily smaller sediment sizes (<2 mm) use the **hand corer**.

Step 7 – Collect samples from 2 to 3 locations (or 5 per m^2) within each depositional zone.

Step 8 – For all inorganic and sediment size samples, place sediment in the plastic bucket and proceed with the next sample. Composite samples from all zones ensuring approximately the same amount of sediment is contributed from each zone.

Step 9 – For organics and organic contaminant samples use the stainless steel bucket and utensils. If fines or organics are left attached to the walls of the sampler, use a Nalgene wash bottle filled with native water to rinse down the sediments from the corer into the bucket.

Step 10 – Repeat the collection process at 5 points (or 3-5 per m^2) within each depositional zone. Ensure that a representative sample for organics is collected from each depositional zone.

Step 11 – Place the combined samples into the appropriate pre-labeled container and then place the containers into a cooler with ice. DO NOT FREEZE.

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare all equipment including labels.
- ☑ Pre-print labels on waterproof paper.
- ☑ Fill out the labels before they get wet.

Sample collection: Be sure to…

- ☑ When insufficient sediment depth and larger sediment size (>2mm) is encountered, particularly in rocky streams and rock bottomed streams, use the **scoop sampler.**
- ☑ When the bed sediment is composed of primarily smaller sediment sizes (<2 mm) use the **hand corer**.
- ☑ For samples that are to be analyzed for organics and organic contaminants, the spatula and container must not be plastic (the sample must be collected and shipped in a glass bottle).
- ☑ For samples that are to be analyzed for metals (inorganic), the spatula must not be metallic. Note: only a metal sieve is available at this time and may be used when removing coarse material even with inorganic samples.
- ☑ Do not sample anywhere you or other field technicians have walked in the reach, or locations that appear recently disturbed. Wait for disturbance to pass.
- ☑ Use caution when sampling as items can easily fall into stream while bending to sample.
- ☑ Let the sample settle for up to five minutes then gently decant any excess water (> 3 cm or 100 mL) from the container. Use a syringe and tube (or just the syringe) to suction off remaining water if helpful. For samples with excessive suspended sediment, it may be necessary to move onto the next sampling station and let the sediments settle prior to decanting. Minimize the time that the plastic syringe or tubing is in contact with sediments sampled for organics. Also, minimize the volume of water applied from plastic wash bottels when rinsing organic sample material.
- ☑ Ensure the sediments in the water have settled prior to decanting. If necessary move onto the next sampling and let the sediments settle prior to decanting.
- ☑ Place the combined samples in a cooler with ice as soon as they are transferred to the pre-labeled bottles.
- ☑ DO NOT FREEZE samples.

NOTE: Collected sediment material from each station will include one glass jar (8 oz, 250 mL) of organic sample, one glass jar (8 oz, 250 mL) of inorganic sample, and one gallon (~3.8 L) of sediment in 2 1-gallon sized half-filled Ziploc-style plastic bags. The same number of samples and containers will be collected from the second station.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

See the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

APPENDIX E SITE-SPECIFIC INFORMATION

See the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

APPENDIX F SEDIMENT CHEMISTRY ANALYSES, STORAGE CONDITIONS, AND HOLD TIMES

Sample Type	Required Analyses	Target Minimum Quantity (g dry weight)	Required Method	Storage Conditions	Hold time
Carbon (samples with ".C" suffix)	Total organic carbon	5	ASA No.9 29-2.2.4	Refrigerate 0-6 °C	28 days from collection
	Total carbon				
Organic (samples with ".O" suffix)	PAHs	90	M3540/8270C	Refrigerate 0-6 °C	14 days from collection
	PCBs		M3540/8082		
Inorganic (samples with ".I" suffix)	Aluminum, total Antimony, total Barium, total Beryllium, total Cadmium, total Calcium, total Chromium, total Copper, total Iron, total Lead, total Magnesium, total Manganese, total Molybdenum, total Nickel, total Phosphorus, total Potassium, total Sodium, total Tin, total Titanium, total Zinc, total	10	M 3050/6010b or 6020	Refrigerate 0-6 °C	Digested within 6 months of collection/analyzed within 6 months of digestion
	Mercury by Direct Combustion AA	3	M7473		28 days from collection
	Conductivity, Saturated Paste	50	SM2510B		28 days from collection
	pH, Saturated Paste		EPA 600/2-78-054 section 3.2.2		28 days from collection
	Total Alkalinity Bicarbonate as CaCO3 Carbonate as CaCO3 Hydroxide as CaCO3	50	SM2320B		Analysis within 12 days of extraction
	Total Nitrogen	5	M351.2		Analysis within 28 days of extraction
	Nitrate as N, soluble (water)	50	Calculate (NO3 NO2) – NO2		Analysis within 28 days of extraction

Sample Type	Required Analyses	Target Minimum Quantity (g dry weight)	Required Method	Storage Conditions	Hold time
	Nitrate/Nitrite as N, soluble (water)		M353.2		Analysis within 28 days of extraction
	Nitrite as N, soluble (water)		M353.2		Analysis within 28 days of extraction
	Nitrogen, ammonia (water)		M350.1		Analysis within 28 days of extraction
	Grain size	100	ASA no.9 15-4.2.2		Indefinitely
Size analysis (samples with ".SS" suffix)	Soil texture analysis	300	ASTM D 422	Room temperature	Indefinitely