Received: 30 November 2021 | Accepted: 6 April 2022
DOI: 10.1111/2041-210X.13944

Methods in Ecology and Evolution
BRITISH ECOLOGICAL SOCIETY

REVIEW

Temporal design for aquatic organismal sampling across the National Ecological Observatory Network

Stephanie M. Parker[1] | Ryan M. Utz[1,2]

[1]National Ecological Observatory Network, Battelle, Boulder, CO, USA
[2]Falk School of Sustainability & Environment, Chatham University, Pittsburgh, PA, USA

Correspondence
Stephanie M. Parker
Email: sparker@battelleecology.org

Funding information
National Science Foundation

Handling Editor: Aaron Ellison

Abstract

1. The National Ecological Observatory Network (NEON) is a continental-scale research platform designed to assess the impacts of climate change, land-use change and invasive species on ecosystem structure and function at field sites distributed across 20 ecoclimatic domains (or regions) from Alaska to Puerto Rico. Aquatic sites within the NEON network include 24 streams, 7 lakes and 3 rivers among 19 domains. A significant challenge to this effort is defining standardized methodology for sampling at sites with substantially variable geomorphology and hydrology. The aquatic temporal sampling design provides timing windows for seasonal sampling to best assess organismal diversity and abundance.
2. The need to establish a rule set for temporal sampling was addressed via site-specific seasonal sampling windows, defined using a suite of environmental variables collected from publicly available meteorological data. Variables integrated into the design include stream flow, growing degree days and riparian phenology. Thresholds for these variables were determined using published literature and used to create three sampling windows for each sample site. Sampling windows target biological community diversity and seasonal changes in abundance, and roughly align with spring, mid-summer and autumn.
3. NEON-generated organismal data from 2014 to 2021 were analysed for inter- and intra-annual variability to quantify community-scale variability among seasonal sampling windows and years at each site. Algae, macroinvertebrate and zooplankton community structure was significantly different within sites across sampling windows, supporting the sampling design of three separate windows per year. Moreover, 93% of sampling windows were completed from 2014 to 2021. An analysis of macroinvertebrate data at 14 sites shows that seasonal β-diversity represents an important attribute of the stream community, even if interannual trends among sites may differ.
4. We conclude that the temporal sampling design for NEON aquatic biological sampling is justified in having three sampling windows per site and is achievable at most NEON aquatic sites. Although sampling windows have been adjusted at a select number of sites, the original sampling windows are used successfully at the

majority of NEON aquatic sites. Sampling windows will be updated with NEON continuous sensor data from each site once sufficient (>3 years) data are available.

KEYWORDS
freshwater biology, lake, long-term monitoring, National Ecological Observatory Network, open-access data, river, sample timing, stream

1 | INTRODUCTION

The National Ecological Observatory Network (NEON) is the first continental-scale ecological data observatory in North America enabling detection and forecasting of the impacts of climate change, land use change, and invasive species on natural resources and biodiversity (Goodman et al., 2014; Kao et al., 2012). Data generated by NEON include sensor-based streams in air, soil and water; organismal sampling; and airborne remote sensing (see Musinsky et al., 2022; Sturtevant et al., 2022, this issue). Terrestrial and aquatic sites are coupled to facilitate measurements of nutrient and energy subsidization across ecosystem boundaries. NEON domain boundaries, which divide North America into 20 ecological zones to ensure that sites reflect ecological heterogeneity at the continental scale, were delineated using clustering of nine climatic variables into ecoregions (Keller et al., 2008). The variables used for clustering included variations of air temperature, precipitation, soil water-holding capacity and solar insolation. NEON organismal data from aquatic sites includes microbe, algae, aquatic plants, macroinvertebrates, zooplankton (lakes only) and fish collections, all of which are intensively quality-checked and freely available on the NEON data portal. Aquatic sites within the NEON network include 34 streams, lakes and rivers within the continental U.S., Puerto Rico and Alaska. Data are collected at a set of core (wildland sites representative of each ecoclimatic domain) and gradient (representing local ecological gradients) sites across the network for 30 years.

One fundamental challenge to ecological observatories such as NEON is standardizing methodology and instrumentation across the network to ensure data comparability among sites representing highly variable ecological and climatic regions (Goodman et al., 2014). Determining sample timing across broadly distributed NEON aquatic sites requires a consistently applicable approach for every site that also allows for flexibility to account for variation in seasonality, latitude, altitude and logistics. Environmental factors such as flooding and scouring, water level, light and nutrient availability strongly structure aquatic primary producers and consumer communities (Allan & Castillo, 2007; Biggs et al., 1999; Hynes, 2001; Peterson & Stevenson, 1992). Consequently, such factors could be included in decision-making pertaining to standardized organismal sampling timing for long-term monitoring. Variables selected as being most important to aquatic community phenology across the NEON network include metrics that quantify discharge in flowing waters (Poff et al., 1997), ice on/ice off in lakes (Shuter et al., 2012), water temperature (Woods et al., 2021) and phenological changes to riparian vegetation (Singh et al., 2014). The NEON aquatic organismal temporal sampling design therefore integrates attributes from these three variables: stream flow, air temperature (representative of ice cover and water temperature) and changes to riparian vegetation.

Flow regime ranks among the most important factors affecting stream benthic communities (Biggs et al., 1999; Bornette & Puijalon, 2010; Clausen & Biggs, 1997; Poff & Ward, 1989; Porter et al., 1993) and is fundamentally connected to precipitation and snowmelt inputs (Flotemersch et al., 2000). In lakes, increased precipitation affects phytoplankton communities by causing variations in light intensity (Figueredo & Giani, 2001), wave activity and water level influence lake benthic communities by limiting the behaviour of mobile organisms (Gabel et al., 2011; Keddy, 1985). Within lotic ecosystems, high flow events and channel drying (Lenat & Barbour, 1994) most significantly affect stream invertebrates (Cuffney et al., 1993; Minshall, 1988; Poff & Ward, 1989; Resh et al., 1988; Vinson & Hawkins, 1998). Key flow variables structuring communities and diversity include intermittency, flood frequency, flood predictability and flow predictability (Poff & Ward, 1989). Relatively stable flow conditions allow organisms to recolonize substrate and mature benthic communities to develop (Porter et al., 1993) but inevitable disturbances induced by flow ensure that taxa specializing in post-flood conditions persist (Lake, 2000). Therefore, stable flows combined with intermediate disturbances maintain diversity in benthic communities (Connell, 1978; Huston, 1979). Consequently, routine sampling of aquatic organisms must consider hydrologic phenology to ensure data comparability among sites and/or temporal intervals.

Phenological flow events among different ecological settings may disparately inform sampling protocols for aquatic organisms. For example, streams with snowmelt-dominated flow regimes cannot be sampled during spring snowmelt periods because flow is too high to sample safely and benthic organisms are likely drifting at high frequencies (Allan, 1987). Intermittent streams, such as desert streams that experience periods of severe drying (e.g. Sycamore Creek, AZ), streams that freeze solid during winter (e.g. Oksrukuyik Creek, AK), or lakes that experience low-water levels (e.g. Prairie Lake, ND) also support communities structured by severe but seasonally predictable physical events (Feminella, 1996; Grimm & Fisher, 1989; Parker & Huryn, 2011). Consequently, benthic organisms should be sampled during relatively low to normal flows that span periods before or after such events, both to ensure safe wading and site access, and because communities tend to be more diverse at stable flows (Cuffney et al., 1993; Thorp & Covich, 2001).

Water temperature also represents an important environmental variable to consider for sampling benthic communities as a fundamental driver of organismal growth and seasonal succession. Water temperature directly affects microbial, algal and invertebrate community structure within a single year because species possess heterogeneous growth temperature optima (Fierro et al., 2021; Letelier et al., 1993; Sun et al., 2017), thus incorporating seasonal temperature variability into the sampling design may capture populations reflecting heterogeneity in optimal temperatures for growth, especially when thermal regimes vary substantially (Haidekker & Hering, 2008; Korhonen et al., 2010; Larned, 2010). Due to a lack of NEON sensor-collected water temperature data during the establishment of the Observatory, air temperature data were used as a proxy for water temperature. Air temperature also indicates seasonality in adjacent terrestrial environments, such as light intensity and terrestrial leaf-out or leaf fall status. In temperate North America, late winter to early spring is often considered the best time to sample benthic invertebrates to quantify diversity in streams, as insects that have overwintered as larvae are large enough to identify and both winter and summer taxa are also likely present (Chester & Robson, 2011; Mackay & Kalff, 1969). Most aquatic invertebrate studies that target diversity suggest sampling during seasons when most insect species are at or near maturity to minimize the likelihood of collecting early instars or species in a resting stage, such as larvae in diapause, eggs or pupae (Chester & Robson, 2011; Cuffney et al., 1993; Flotemersch et al., 2000).

The NEON aquatic temporal sample design focuses on phenological metrics among vastly different sites to achieve biological sampling with consistency and target diversity among seasons. Both inter- and intra-annual differences in community are expected over lifetime of the NEON project, thus multiple sampling dates per year at each site were selected (Cook et al., 2018; Korhonen et al., 2010). Consequently, NEON sampling for periphyton, phytoplankton, aquatic plants, benthic microbes, macroinvertebrates and zooplankton communities occurs three times per year roughly aligning with the spring, summer and autumn seasons and fish are sampled twice per year (spring and autumn). Due to the interacting effects of the above environmental phenomena on aquatic organisms, the environmental hierarchy for NEON aquatic organismal sampling consists of the following rule sets:

1. Sampling for stream sites must fall within the 3× the median stream discharge (Clausen & Biggs, 1997) calculated using the previous 3–5 years of discharge transect data at a site. Sampling must also fall within guidelines for safe-wading in a stream as described in Lane and Fay (1997). Each sampling protocol requires a period of organismal recolonization after scouring stream flows that varies by taxa (e.g. 14 days of recolonization for periphyton, 5 days for macroinvertebrates). This metric is calculated by field scientists at each lotic NEON site and is not built into the seasonal sampling windows.
2. Percentage of cumulative degree days within 5%–15% (sampling window 1), 45%–55% (sampling window 2) or 85%–95% (sampling window 3) for the year with 0% on 1 January, coinciding with three sampling windows per organism category.
3. The first and third sampling windows occur within 4 weeks of riparian green-up or within 4 weeks of autumn leaf-fall. Exceptions include the 7 NEON aquatic sites where riparian ecosystems do not seasonally shed leaves because they are located in a tropical, sub-tropical or desert climates (i.e. Puerto Rico, Florida, Arizona, grassland Texas and Oklahoma, and high-elevation California sites) where sampling windows are based on #2 above.
4. Sequential sampling windows include a minimum of 14 days between the end of one sampling window and the start of the next, with the exception of Arctic Alaska (Domain 18), which has a short growing season that only allows for 10 days between sampling windows.

The objective of this work is to provide targeted sampling windows for each NEON aquatic site in a flexible, yet standardized framework, that allows NEON field staff to schedule observational sampling, allows NEON to generate and host data that are collected using a comparable rule set among sites and maximizes organismal diversity at each site by sampling during multiple seasons and years. Below, we (a) present the detailed NEON aquatic organismal temporal sampling strategy with specificity across the network, and (b) describe how the sampling design was configured prior to NEON having adequate instrument data during the earliest stages of observatory sampling. Because aquatic organisms have been collected for up to 7 years at NEON sites, we also include (c) an assessment of interannual versus seasonal variability in community structure across taxa to demonstrate how effectively the sampling design may be capturing temporal variability in biotic metrics and (d) an example analysis of β-diversity in the macroinvertebrate community at stream sites with >5 years of data as an example of the potential analyses that may be accomplished with these datasets. We hypothesize that three sampling windows per year at each NEON aquatic site significantly contribute to differences seasonally in organismal community structure. Our intention is to provide a detailed depiction of temporal attributes in the NEON aquatic organismal sampling design so that researchers using NEON data can do so with the full logic determining sample timing comprehensively described, or apply this logic to similar studies, and to provide an example of how these data may be used in future analyses by NEON data users.

1.1 | NEON aquatic sites

Aquatic sites are located in 19 of 20 NEON domains ranging from Puerto Rico to Alaska. Thirty-four NEON aquatic sites are distributed among 24 wadeable streams, 7 lakes and 3 large rivers (see neonscience.org for supporting site information). Observational sampling, including water and sediment chemistry, biological assemblages (microbes, periphyton and phytoplankton, aquatic plants, macroinvertebrates, zooplankton and fish), discharge and morphology began at a subset of NEON sites in 2014, with sites added incrementally each year until all 34 NEON aquatic sites were operational in spring 2019.

Field sites are permitted through NEON's permitting department, which supports the NEON program with various permitting and environmental compliance requirements. Permitting and regulations vary based on site host agreements among the 34 aquatic sites.

1.2 | Temporal design

NEON infrastructure and continuously monitoring sensors were not installed prior to the start of observational sampling. Therefore, publicly available data were used to construct the initial temporal sample design using streamflow, air temperature and riparian greenness (Table 1). Data used to structure the initial sampling design were accessed in 2013, prior to the beginning of NEON sampling in 2014. Designs for NEON sites that were established after 2013 (i.e. Little Rock Lake, WI, Upper Big Creek, CA and Teakettle 2 Creek, CA) used data that were accessed in later years (2016–2018). For sites where sampling proved unsuccessful during the standard sampling windows, additional data such as historic ice-cover dates or USGS gage data have been factored into the design to improve site accessibility and NEON's ability to collect samples (see Section 1.3 below).

1.2.1 | Streamflow

Stream flow data were derived from USGS discharge records for the nearest site with a similar watershed size and ≥9 continuous years of record available (Table S1; waterdata.usgs.gov/nwis). Normal flow was defined as flow falling between the 25th and 75th percentile over the course of >15 years (Helsel & Hirsch, 2002; National Research Council, 2004). As the majority of flow data are from nearby sites (see Table S1), they are not used directly in the calculations to determine sampling windows, but rather are used as a general guide for flow conditions for streams of similar size in each region. The 25th and 75th percentile lines are presented on the figures for stream sites to help determine periods most likely to have stream flows in the normal range at the NEON stream site. When available, these metrics will be replaced with NEON-generated discharge data.

1.2.2 | Air temperature

Air temperature data were obtained from the NOAA National Climatic Data Center (NCDC; www.ncdc.noaa.gov) for nearby sites with ≥10 years of continuous data. Such data were applied to determine windows of cumulative degree days (Gullan & Cranston, 2014) at 5%–15% (spring), 45%–55% (mid-summer) and 85%–95% (autumn). Degree days were considered to be zero on 1 January. Criteria for selecting suitable air temperature sites included records with ≥10 years of data and a reasonably close weather station at an elevation similar to the NEON aquatic site (Table S1). Data for two sites, Rio Cupeyes, PR and McRae Creek, OR, consisted of <10 years of data.

Canopy greenness: Canopy greenness and phenology data were obtained from MODIS satellite imagery-derived enhanced vegetation index (EVI) phenology data (modis.ornl.gov), depicting mean onset and decrease of greenness in terrestrial vegetation. The polygon of downloaded data centred on each NEON aquatic site and covered 36 km^2 wide area. Most data were accessed in 2013, and newly established sites were accessed in 2016–2018. All data represent 9 years prior to download.

Twenty-eight day (~1 month) sampling windows were generated with the publicly available data listed above (Figure 1, see also Table S1). Three sampling windows were designated per site per year: algae, microbes, plants, macroinvertebrates and zooplankton are sampled during all three sampling windows while fish are sampled during the first and third sampling windows. Plots and date Domain 16 ranges for sampling windows can be generated using the R code provided (Supplemental Code 1, Parker & Utz, 2022). Sampling window selection was made using the following rule set at most sites:

- Sampling window 1 (spring) is the 28-day average centring on 5%–15% cumulative degree days and mean onset of riparian greenness. In lakes, sampling window 1 starts after mean historic ice off, if known.
- Sampling window 2 (mid-summer) at is centred on the 45%–55% cumulative degree day period.

TABLE 1 Environmental parameters used to determine organismal sampling dates. Outliers are noted in the text

Sampling window	1	2	3
Season	Spring—targets late winter/early spring green up, warming temperatures, and increasing light levels	Summer—targets peak greenness and relatively low water levels favouring baseflow conditions	Autumn—targets cooling temperatures, decreasing light levels, and leaf litter in aquatic habitats
Hydrology (discharge)	25th to 75th percentile	25th to 75th percentile	25th to 75th percentile
Temperature (degree days)	5%–15% cumulative degree days	45%–55% cumulative degree days	85%–95% cumulative degree days
Riparian phenology	4 weeks centred on mean onset greenness	not applicable	4 weeks before mean decrease greenness

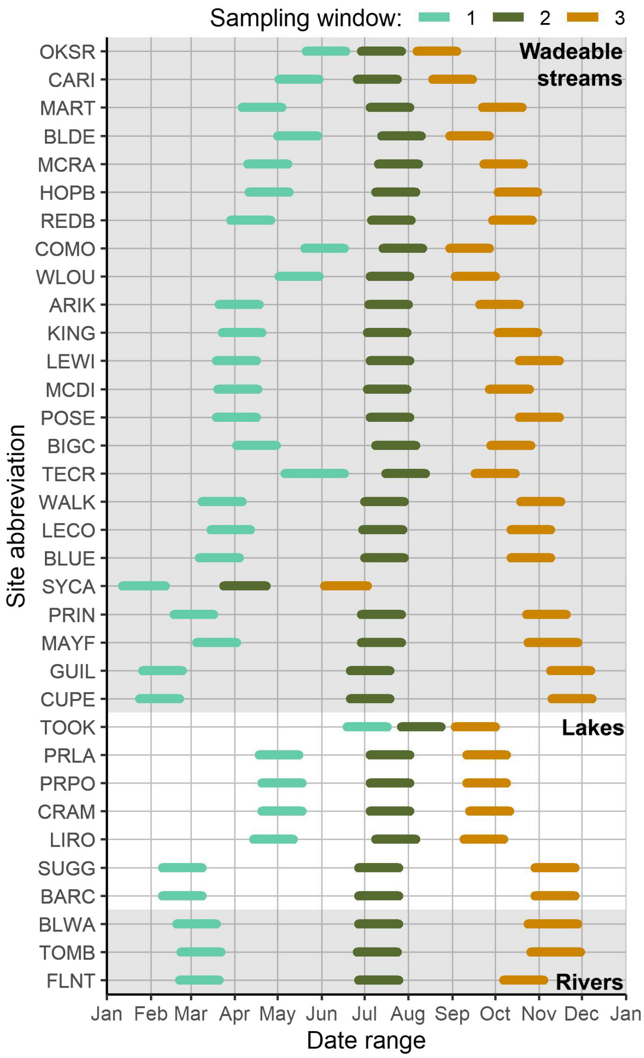

FIGURE 1 Sampling windows for each NEON aquatic site. BARC, SUGG, CUPE, GUIL, BLUE, TECR and BIGC do not use phenology data. MAYF, BLWA, TOMB, SYCA, TECR and TOOK dates reflect site-specific adjustments for logistics or local environmental factors, such as ice-off or stream drying.

- Sampling window 3 (autumn) is the 28-day average centring on the 85%–95% cumulative degree day period and mean decrease of riparian greenness. This window ends before the mean historic ice-on date in lakes, if known.
- For all sampling windows, stream sites are only sampled if discharge falls within the safe wading guidelines (Lane & Fay, 1997), using the 25th to 75th discharge percentile as a guideline.

Sampling window 1 is designated as a late winter/early spring sampling period for most sites. Such periods typically reflect when light levels reach a threshold in temperate streams, often before leaf-out, that allow primary producers to attain high growth rates following low light levels during winter (Hill et al., 2001). At sites with significant accumulation of winter snowfall, sampling window 1 coincides with spring flooding, which can cause schedule delays or inappropriate conditions for sampling, such as high water. Snowmelt anomalies will be addressed and incorporated into the design when >3 years of data from NEON in-situ sensors are available to further improve sampling windows at sites where sampling windows are frequently missed due to flooding or drying. This should also improve our ability to adjust sampling windows along with changing climate over the 30 years of the NEON project to continue to target seasonal diversity.

Sampling window 2 occurs at midsummer, a period often coinciding with peak greenness and relatively low discharge in many systems. Such conditions allow taxa that favour slower moving or warmer water to proliferate (Grimm & Fisher, 1989; Keddy, 1985; Peterson & Stevenson, 1992). In the first 7 years of NEON sampling, several streams experienced intermittent flow (Kings Creek and McDiffett Creek, KS) or complete drying (Sycamore Creek, AZ) during this sampling window. Sampling contingencies are built into protocols and implemented under these conditions, such as decreasing the number of samples collected to accommodate dry stream sections. Fish are never collected during sampling window 2.

Finally, sampling window 3 occurs in autumn, a period when water temperatures cool, light levels begin to decrease, and leaf litter and shredding macroinvertebrates exhibit rapid growth (Heino et al., 2003). Autumn also represents the end of the growing season and annual biomass accrual for fish communities. Sampling window 3 is often a period of stable flow, with the exception of sites affected by hurricanes (Rio Cupeyes and Rio Guilarte, PR, Lake Suggs and Lake Barco, FL, and Mayfield Creek, Black Warrior River, and Tombigbee River, AL). When flood waters rise at NEON sites, contingent decisions including delayed sampling may be implemented, causing sampling to occur later then the end of the sampling window (Figure 2).

1.3 | Design exceptions

Ecological and environmental heterogeneity inherent to the NEON site network inevitably produces idiosyncratic challenges related to the temporal sampling strategy outlined above. Consequently, some sites require adjustments to the basic rule sets that define the sampling windows (Table 2). The fundamental sampling strategy is minimally amended in such sites to allow for safe and effective sampling.

Additional site-specific adjustments to strategies will be made when >3 years of NEON sensor data are available. High latitude and high altitude stream sites will be targeted for the first round of sampling window adjustments to better characterize spring snowmelt and flooding dynamics and minimize missed sampling windows. Planned adjustments include using NEON sensor data to update the spring sampling windows at Como Creek and West St. Louis Creek, CO, and Blacktail Deer Creek, WY, where high elevation and late snowpack have often deterred spring sampling efforts. As designs are updated, the same rule sets used from original proxy site data will continue to be applied to NEON-generated sensor data (e.g. percentage of growing degree days, riparian senescence).

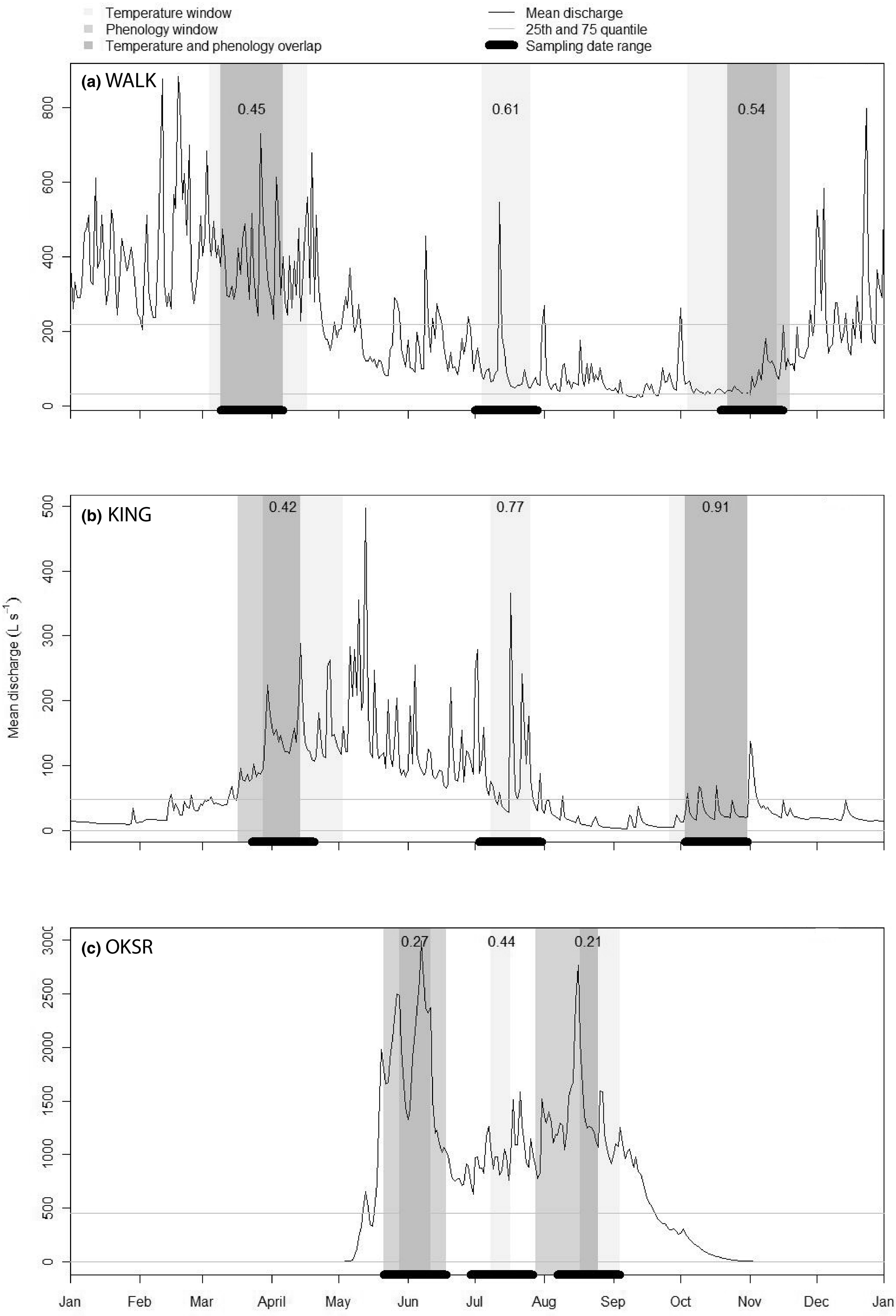
Temperature window
Phenology window
Temperature and phenology overlap
Mean discharge
25th and 75 quantile
Sampling date range
(a) WALK
0.45
0.61
0.54
(b) KING
0.42
0.77
0.91
(c) OKSR
0.27
0.44
0.21
Mean discharge (L s⁻¹)
Jan
Feb
Mar
April
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan

FIGURE 2 Examples of the NEON organismal temporal sample design visualization in three different types of streams produced by R code (Parker & Utz, 2022). The temperature window uses degree day data from air temperature, phenology window uses the MODIS green-up and brown-down data. The 'overlap' is the mean of the temperature window and the phenology window. The 25th and 75th quantile refers to USGS streamflow data from a nearby site, and the sampling date range is the final 28-day sampling window. Proportions listed at the top of the figure associated with each sampling window reflect the mean probability of discharge falling within the 25th and 75th quantiles during the period. (a) Domain 7 Walker Branch, Tennessee is a baseflow stream. (b) Domain 6 Kings Creek, Kansas is a prairie stream that experiences drying. (c) Domain 18 Oksrukuyik Creek, Alaska represents a high-latitude snowmelt-dominated system.

TABLE 2 Design exceptions for the NEON aquatic temporal sampling strategy

Design exception	Sites affected	Reason for exception	Design outcome
Riparian MODIS data not used	D03: BARC, SUGG (FL) D04: CUPE, GUIL (PR) D11: BLUE (OK) D17: BIGC (CA)	MODIS data did not show clear green-up and brown-down, likely due to lack of deciduous vegetation	Sampling windows based on percentage of cumulative degree days
Extended sampling window	D08: BLWA, MAYF, TOMB (AL)	Seasonal hunting closures and holidays fall during sampling window making scheduling logistically difficult	Autumn sampling window extended from 4 to 6 weeks
USGS flow data incorporated	D14: SYCA (AZ)	Seasonal drying prevents sampling during much of the year	All 3 sampling windows moved earlier in the year to target the typically wet period
Historic ice-off data incorporated	D18: TOOK (AK)	Ice-cover on the lake limits spring and autumn sampling	Historic ice-cover data from the Toolik Field Station (Environmental Data Center Team, 2018) were used to move spring and autumn sampling windows later to allow for ice melt; summer window was placed at the midpoint between spring and autumn windows due to the short sampling season
Multiple exceptions: MODIS data not used, extended sampling window, field observations of snow cover incorporated	D17: TECR (CA)	Remote, high-elevation site with variable annual snow cover significantly affects site accessibility; MODIS data did not show clear green-up and brown-down; spring sampling window extended to allow for rescheduling and logistics	(a) Sampling windows based on local air temperature from Teakettle Experimental Forest; (b) spring sampling window extended from 4 to 6 weeks; (c) spring window start moved 14 days later and autumn window 14 days earlier to target low snow cover for site access

1.4 | Statistical analysis

We analysed the biological communities of benthic algae (DP1.20166.001), macroinvertebrates (DP1.20120.001) and zooplankton (DP1.20219.001) using a distance-based redundancy analysis (dbRDA, Legendre & Anderson, 1999) based on Bray-Curtis dissimilarities to determine the amount of variation in community composition that could be characterized as inter-annual variation (year) versus intra-annual variation (sampling window) within each site. Each NEON site was run as a separate model using year or sampling window number as factors (Supporting Information 2). The results of total temporal variation (full model, year + sampling window) are also presented in Supporting Information 2. Data were downloaded from the NEON data portal (data.neonscience.org; NEON, 2022a, 2022b, 2022c, 2022d, 2022g, 2022h; Parker & Utz, 2022), calculations were applied per each data product's 'User Guide' document (e.g. standardizing macroinvertebrate count to individuals per m^2; Chesney et al., 2021; Parker & Scott, 2021; Parker & Vance, 2020), and data were log-transformed. At each site, we used the adjusted *R*-squared values from the dbRDA models fit to the data to quantify the variation in community composition that could be attributed to inter-annual variation (year), intra-annual variation (sampling window) and total temporal variation (year + sampling window) following methods described by Peres-Neto et al. (2006), with *p*-values associated with the adjusted *R*-squared. All analyses were conducted using the VEGAN package (Oksanen et al., 2020) in R v.4.1.0 (R Core Team, 2021).

2 | RESULTS

NEON aquatic data collections from 2014 to 2021 show that the success of sampling windows is most strongly affected by drying (Domain 06, Kansas and Domain 14, Arizona), higher than average snowpack (Domain 12 Wyoming), and late ice out at lake sites (Table 3). Hurricanes, while infrequent, have also affected sampling windows in Domain 03 (Florida), Domain 04 (Puerto Rico) and Domain 08 (Alabama). In 2020 and 2021, wildfires affected sample timing in Domain 13 (Colorado) and Domain 17 (California).

Among periphyton communities, 32 of 34 sites exhibited significant variation among the three sampling windows ($p = 0.001$–0.008; Figure 3). Domain 17 TECR lacked sufficient data prior to 2021, and Domain 3 FLNT did not show a significant difference in the algal community among the three sampling windows. Thirty-one of 34 sites in the algal community also showed significant variation from year to year (MART, MCRA and TECR lacked enough data for analysis), TECR lacked sufficient data for analysis in the macroinvertebrate community for annual variation as there is only data for 1 year available on the NEON portal (Table S2).

Macroinvertebrate communities showed significant differences among the three sampling windows for all 34 sites ($p = 0.001$–0.005;

TABLE 3 Number of biology sampling windows scheduled by the NEON project from July 2014 to October 2021 across 34 aquatic sites. Sampling windows missed due to snow were all high-elevation sites (COMO, WLOU, TECR). Sampling windows missed due to dry channels were in Kansas and Arizona (KING, SYCA). Sites missed due to rain were in Puerto Rico (CUPE, GUIL). Finally, 'other' represents permitting or funding limitations (BARC, SUGG, COMO). Partial sampling windows missed a portion of the total samples in a given sampling window due to snow or dry conditions.

	Number of sampling windows
Expected sampling windows	570
Completed sampling windows	495
Partial sampling windows	11
Entire sampling window missed: COVID-19	33
Entire sampling window missed: dry channel	9
Entire sampling window missed: high flow	10
Entire sampling window missed: snow, ice cover	5
Entire sampling window missed: wildfire	1
Entire sampling window missed: other (e.g. permitting)	6

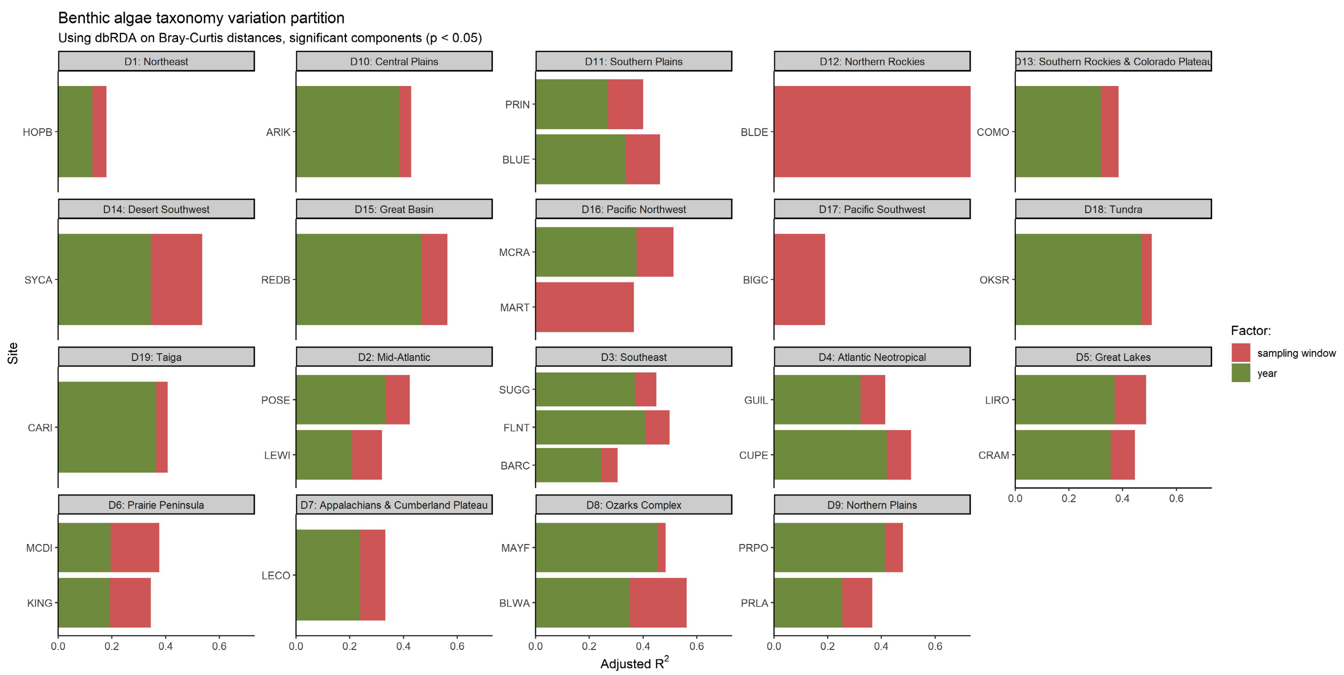

FIGURE 3 Variation partitioning for the benthic algal community showing significant differences across sites from year to year and among sampling windows. Overlap between the two factors is shown in Table S2.

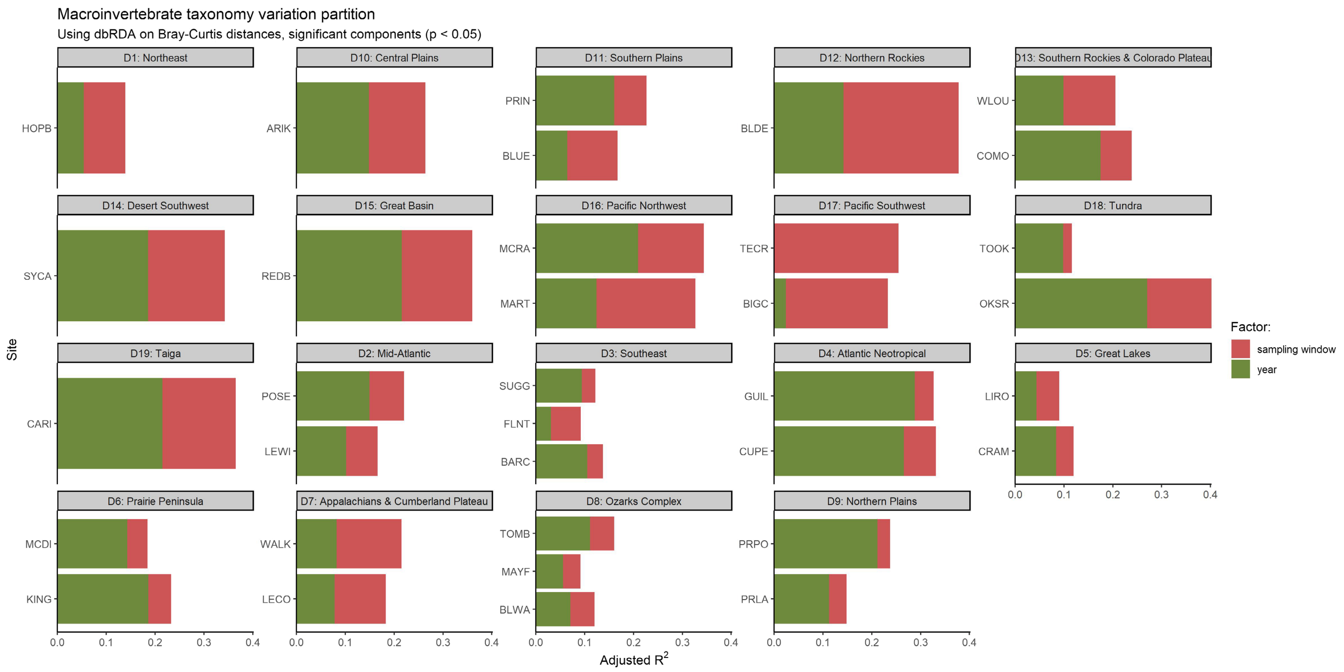

FIGURE 4 Variation partitioning for the benthic macroinvertebrate community showing significant differences across sites from year to year and among sampling windows. Overlap between the two factors is shown in Table S2.

Figure 4). Zooplankton communities showed significant community variation between sampling windows at 6 of 7 lake sites ($p = 0.001$–0.029), with the exception of Domain 3 BARC. Significant variation in annual zooplankton communities were detected among year in 6 of 7 lake sites, with the exception of Domain 5 LIRO (Figure 5).

3 | DISCUSSION

The NEON temporal sampling design for aquatic organisms was crafted to capture heterogeneity in communities within and among years while providing a standard framework for sampling. Our design targets multiple seasons to maximize community diversity observations and capture data at the beginning (spring) and end of the growing seasons (autumn) in a manner that allows for comparability among sites at vastly different locations. Some biomonitoring projects in the continental U.S. sample only in late winter/early spring to target benthic invertebrate diversity in streams, as such periods reflect overwintered insects as well as summer-emergent taxa (Chester & Robson, 2011; Mackay & Kalff, 1969). Taxa with short life cycles, such as many Chironomidae and Ephemeroptera, often have several life cycles per year (multivoltine) and should still be present on one or more of these seasonal sampling dates (Gullan & Cranston, 2014). NEON sampling, however, aims to collect data that quantify seasonality and long-term change over time.

Sampling for aquatic organisms in the NEON network began in 2014 at select sites, with all 34 aquatic sites producing data by 2019. With several years of data at a subset of NEON sites, we are now able to assess the sampling design to determine whether three sampling windows per year are, in fact, targeting inter-annual community variation within a site. Through analysis of these data, we have shown that there is significant inter-annual and intra-annual variation in the benthic algae, macroinvertebrate and zooplankton communities in existing NEON data, supporting the three sampling windows per year design to capture diversity throughout the year at each site. Similar trends have been shown by Cook et al. (2018) and Korhonen et al. (2010).

Analysis of sampling impractical records from 2014 to 2021 shows that few complete sampling windows were missed (Table 3), indicating that the a-priori sampling windows are logistically effective. The most common reasons for missed sampling windows have been due to COVID shutdowns in 2020 (33 sampling windows), followed by high stream flows (10 sampling windows) and dry channels (9 sampling windows). When we consider the sampling windows missed due to non-COVID reasons, the planned collection protocols were achieved in 93% of sampling windows from 2014–2021. Over time, sites that consistently miss the same sampling window for three consecutive years will have the sampling windows reassessed. As of 2021, three sites have had sampling window adjustments (see Section 1.3 above), but most sites and sampling windows continue to follow the initial standard design.

Maximizing the scale of community diversity data that NEON data represents also represents a fundamental goal of aquatic observational sampling. The dbRDA analysis of macroinvertebrate, periphyton and zooplankton data shows that in sites with sufficient data to run the model, the spring, summer and autumn sampling windows produce significant differences in community composition within a site (Figures 3–5), a finding consistent with other long term studies (Korhonen et al., 2010). Cumulative results among sites and across time suggest that sampling three separate sampling windows

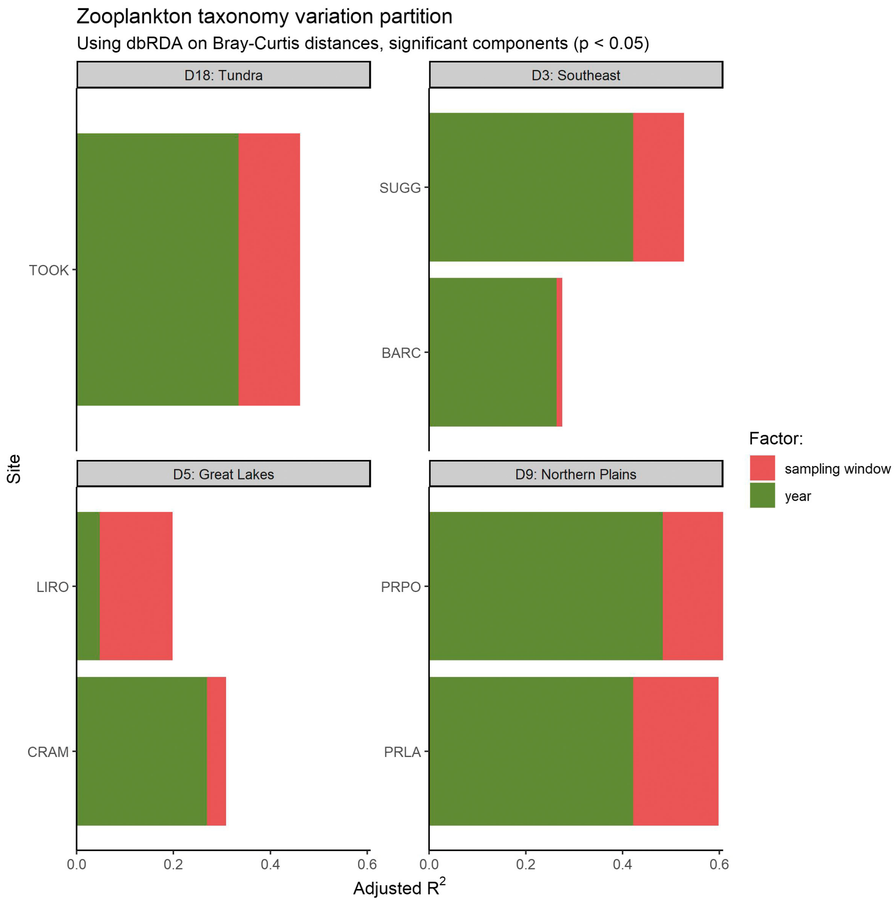

FIGURE 5 Variation partitioning for the zooplankton community showing significant differences across sites from year to year and among sampling windows. Overlap between the two factors is shown in Table S2.

is necessary to build comprehensive taxon lists and document cumulative site diversity over time. This trend of significant inter- and intra-annual variation in organismal communities allows for a number of questions to be asked using NEON publicly-available data: one such question is presented in the vignette below.

4 | NEON DATA EXPLORATION VIGNETTE

Although our fundamental purpose is to provide a detailed overview of the sampling design, we include here a brief example of how NEON aquatic organismal data enables continent-scale analyses with emphasis on temporal variability. Many aquatic organism assemblages exhibit substantial intra-annual turnover (seasonal β diversity) associated with seasonally variable trophic resource availability (Torres-Ruiz et al., 2007) or differences in life history attributes, such as differences in insect adult-stage emergence timing among species (Füreder et al., 2005; Jensen et al., 2021). However, in some cases seasonal β-diversity turnover may be minimal or absent, even when potentially limiting resources such as sunlight are highly seasonally variable (Rosemond, 1994; Tonkin et al., 2016). Phenological changes in community structure, when present, can introduce complications to biomonitoring sampling designs because samples collected at different dates in the same ecosystem yield variable biotic integrity scores (Leunda et al., 2009; Šporka et al., 2006). Furthermore, major environmental stressors such as eutrophication can drive a loss of temporal β-diversity (Cook et al., 2018; Florencio et al., 2016).

The NEON aquatic organismal sampling design, with three sampling windows per year, facilitates investigations of β-diversity variability across North America at multiple temporal scales. We explored such potential by exploring wadeable stream macroinvertebrate community data among NEON aquatic sites that have at least 5 years of available data as of 2022 ($n = 14$ sites). Figure 6 illustrates examples from four of these sites with variable patterns in stream macroinvertebrate composition among sampling windows and years (Supplemental Code 2, Parker & Utz, 2022). Two sites, Arikaree River, CO and especially Walker Branch, TN, exhibit moderate to strong seasonal turnover, with sampling windows clustering together in multivariate space. However, interannual trends between these two sites differ, with Arikaree River showing signs of long-term change while communities in Walker Branch remain relatively consistent among years. The other two sites exhibit weak or absent community composition consistency within sampling windows among years.

We next explored potential causes and consequences of seasonal β-diversity turnover. The degree of homogeneity in benthic macroinvertebrate community coherence within sampling windows among sites was quantified by generating analysis of similarity (ANOSIM) models using the VEGAN program (Oksanen et al., 2020). The ANOSIM statistic from models with sampling window as the grouping variable provides a quantitative metric that conveys how strong seasonal differences are among communities within sampling windows. We then

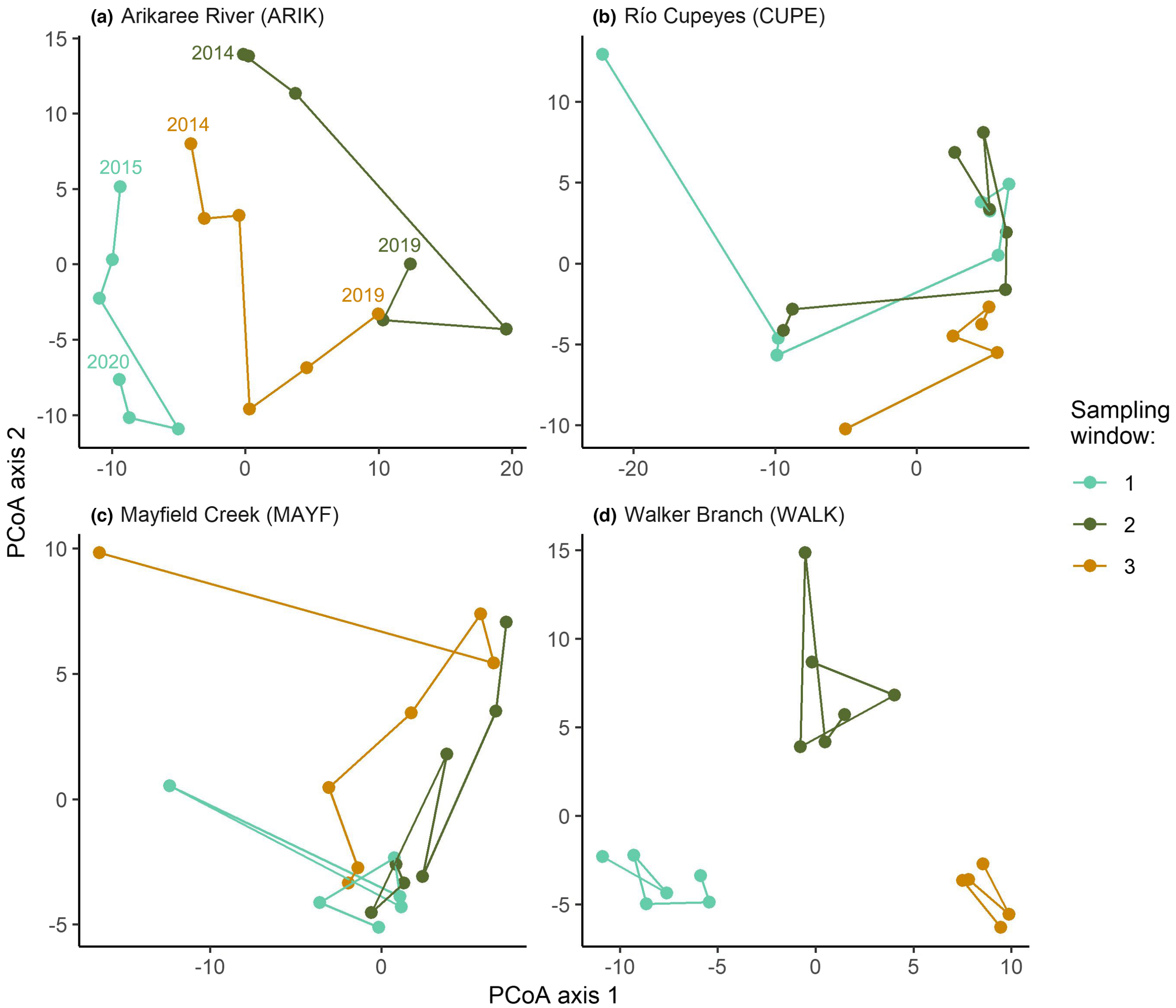

FIGURE 6 Principle coordinates analysis ordination of wadeable stream macroinvertebrate communities among sampling windows and years for (a) the Arikaree River, Domain 10 (Colorado), (b) Rio Cupeyes, Domain 4 (Puerto Rico), (c) Mayfield Creek, Domain 8 (Alabama) and (d) Walker Branch, Domain 7 (Tennessee)

explored the possibility that the strength of seasonal turnover among sampling windows is influenced by air temperature variability among sites. Although NEON collects high-frequency data of both air and water temperature at each aquatic site, we explored air temperature as a driving factor because seasonal differences in air temperature likely better reflect temporal changes in critical resources, such as sunlight. Air temperature at 30-min intervals (DP1.00002.001) for the entire record of available data for each site were averaged by date and the standard deviation of daily air temperature variability was calculated to serve as a metric of air temperature seasonality (NEON, 2022e, 2022f). Finally, we quantified annual total Shannon-Weiner biodiversity metrics at each site by aggregating data among sampling windows so that we could explore how seasonal β-diversity impacts biodiversity at a broader temporal scale.

Two basic analyses suggest that seasonal β-diversity represents a fundamentally important attribute of wadeable stream macroinvertebrate communities among NEON sites. A linear model suggests that sites with greater variability in air temperatures tend to have greater seasonal β-diversity turnover ($F_{1,13}$ = 8.1, p = 0.0137, Figure 7a). Additionally, mean annual biodiversity was positively related to the strength of seasonal β-diversity ($F_{1,13}$ = 12.8, p = 0.0034, Figure 6a), thus sites with coherent seasonal changes tend to be more biodiverse overall.

Such analyses are meant to serve as an illustration of analytical possibilities using NEON aquatic organismal data that are poised to greatly expand as the network database grows. Standardized sampling and data organization across the network enables such inquiries at the continental scale. Furthermore, the comprehensive

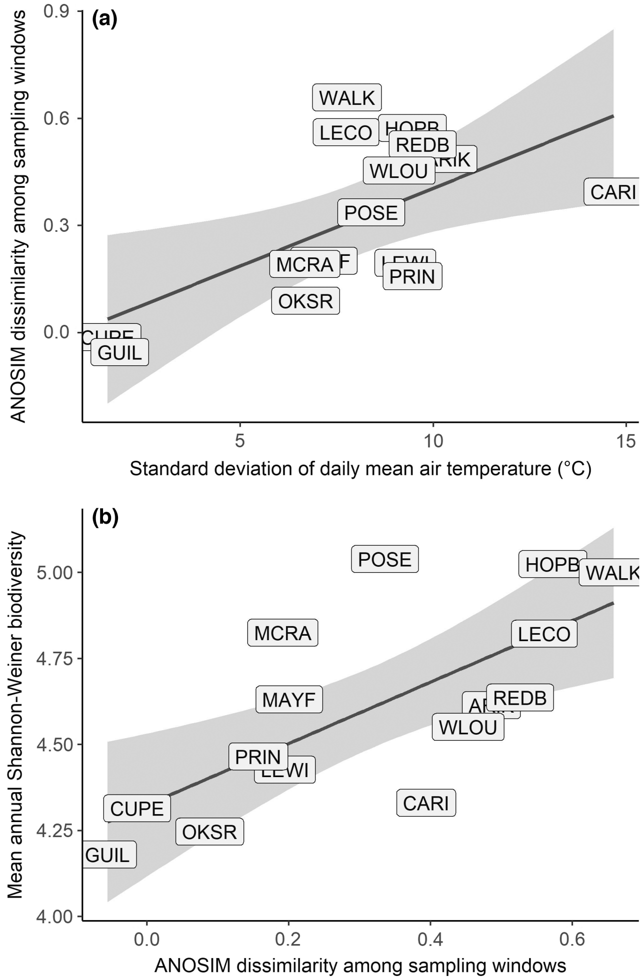

FIGURE 7 Potential causes and consequences of β-diversity among seasons in wadeable stream macroinvertebrate communities. (a) ANOSIM dissimilarity of communities among sampling windows as a function of air temperature variability among sites. (b) Mean annual biodiversity among sites as a function of ANOSIM dissimilarity of communities among sampling windows.

suite of data that NEON produces greatly facilitates analytical scope expansion. For example, hydrologic metrics often structure lotic communities, including differences in β-diversity (Dong et al., 2021; Korhonen et al., 2010), and NEON collects 1-min resolution discharge data at all flowing water sites. Our cursory analyses presented here suggests that enough organismal data already exist to explore relationships between biological, physical and chemical ecosystem attributes.

5 | CONCLUSIONS

Although defining seasonal sampling can be straightforward in temperate, boreal and arctic sites due to the dominant influence of snowmelt as a defining environmental attribute, determining optimal sampling times can be more difficult at desert, sub-tropical and tropical sites that lack clear seasonality. Therefore, we implemented a sampling design that follows a standard rule set yet is based on local, publicly available data from each region. Because the seasonality of key parameters differs greatly along a latitudinal gradient from Puerto Rico to Alaska, we selected hydrology, temperature (degree days and ice on/ice out) and riparian greenness (onset and decrease in greenness) to determine sampling windows using consistent rule sets at each site. Temporal sampling design exceptions are assessed on an as-needed basis and continue to follow standardized, traceable rules and typically use more specialized data collected in closer proximity to the NEON site than the originally processed data. As NEON instrument data become available consistently for three or more years at each aquatic site, we intend to (a) update temporal designs by adding NEON-generated discharge data to shape sampling windows, and (b) update designs for all high latitude and high altitude snowmelt-driven streams using site-specific flow data. Additionally, it is possible that the Airborne Observation Platform (AOP) could be leveraged for riparian phenology in the future (e.g. Musinsky et al., 2022). Since NEON aquatic sampling began in 2014, notable trends that affect the sampling design include stream intermittency, frequent large floods (e.g. 30 year flood events occurring annually in Blue River, OK [N. Harrison, pers. comm.]), temporally variable ice cover at high latitude lakes sites, and variable snowmelt and snowpack at high altitude sites.

The National Ecological Observatory Network provides quality controlled data collected in a standardized way across the observatory to the user community. In this analysis, we present a framework for the standardized temporal sampling strategy used for collecting aquatic organisms and analyse its efficacy after the first 7 years of data collection. We conclude that during the first 7 years of NEON aquatic sampling, the temporal sampling design employing three sampling windows per year has shown significant differences in community structure among sampling windows within a site, providing a more complete record of diversity than a single sampling event per year per site. An initial analysis using macroinvertebrate β-diversity in streams shows the importance of seasonal sampling and provides an example of the many future studies that could be accomplished by data users accessing publicly available NEON organismal and sensor data.

AUTHORS' CONTRIBUTIONS

S.M.P.: Conceptualization (lead); writing—original draft (lead); initial analysis and code development (supporting); final data analysis (equal); writing—review and editing (equal); R.M.U.: Conceptualization (supporting); writing—original draft (supporting); initial analysis and code development (lead), final data analysis (equal); writing—review and editing (equal).

ACKNOWLEDGEMENTS

The National Ecological Observatory Network is a program sponsored by the National Science Foundation and operated under cooperative agreement by Battelle. This material is based in part upon work supported by the National Science Foundation through the NEON Program. We thank our colleagues who contributed to early iterations of the temporal sampling design, including Keli Goodman, Caren Scott, Charlotte Roehm, Jenna Stewart and members of NEON's Aquatic Technical Working Group; Courtney Meier for reviewing an early draft; and Eric Sokol for providing R code templates and assistance with statistical analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/2041-210X.13944.

DATA AVAILABILITY STATEMENT

Data from the 2022 NEON data release are available at NEON (2022b, 2022d, 2022f, 2022g). Provisional data (2019–2021) are archived at the Environmental Data Initiative (Parker & Utz, 2022).

ORCID

Stephanie M. Parker https://orcid.org/0000-0002-7180-7245
Ryan M. Utz https://orcid.org/0000-0001-8036-2228

REFERENCES

Allan, J. D. (1987). Macroinvertebrate drift in a Rocky Mountain stream. *Hydrobiologia*, *144*(3), 261–268. https://doi.org/10.1007/BF00005560

Allan, J. D., & Castillo, M. M. (2007). Stream ecology. Springer Netherlands. https://doi.org/10.1007/978-1-4020-5583-6

Biggs, B. J. F., Smith, R. A., & Duncan, M. J. (1999). Velocity and sediment disturbance of periphyton in headwater streams: Biomass and metabolism. *Journal of the North American Benthological Society*, *18*(2), 222–241.

Bornette, G., & Puijalon, S. (2010). Response of aquatic plants to abiotic factors: A review. *Aquatic Sciences*, *73*(1), 1–14. https://doi.org/10.1007/s00027-010-0162-7

Chesney, T., Parker, S., & Scott, C. (2021). *NEON user guide to aquatic macroinvertebrate collection (DP1.20120.001) version B*. NEON (National Ecological Observatory Network).

Chester, E. T., & Robson, B. J. (2011). Drought refuges, spatial scale and recolonisation by invertebrates in non-perennial streams. *Freshwater Biology*, *56*(10), 2094–2104. https://doi.org/10.1111/j.1365-2427.2011.02644.x

Clausen, B., & Biggs, B. (1997). Relationships between benthic biota and hydrological indices in New Zealand streams. *Freshwater Biology*, *38*(2), 327–342. https://doi.org/10.1046/j.1365-2427.1997.00230.x

Connell, J. H. (1978). Diversity in tropical rain forests and coral reefs. *Science*, *199*(24), 1302–1310.

Cook, S. C., Housley, L., Back, J. A., & King, R. S. (2018). Freshwater eutrophication drives sharp reductions in temporal beta diversity. *Ecology*, *99*(1), 47–56.

Cuffney, T. F., Gurtz, M. E., & Meador, M. R. (1993). *Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program* (Open-File Report No. 93–406). U.S. Geological Survey.

Dong, R., Wang, Y., Lu, C., Lei, G., & Wen, L. (2021). The seasonality of macroinvertebrate β diversity along the gradient of hydrological connectivity in a dynamic river-floodplain system. *Ecological Indicators*, *121*, 107112. https://doi.org/10.1016/j.ecolind.2020.107112

Environmental Data Center Team. (2018). *Meteorological monitoring program at Toolik, Alaska*. Retrieved from https://toolik.alaska.edu/edc/journal/annual_summaries.php

Feminella, J. W. (1996). Comparison of benthic macroinvertebrate assemblages in small streams along a gradient of flow permanence. *Journal of the North American Benthological Society*, *15*(4), 651–669. https://doi.org/10.2307/1467814

Fierro, P., Hughes, R. M., & Valdovinos, C. (2021). Temporal variability of macroinvertebrate assemblages in a Mediterranean coastal stream: Implications for bioassessment. *Neotropical Entomology*, *50*(6), 873–885. https://doi.org/10.1007/s13744-021-00900-3

Figueredo, C. C., & Giani, A. (2001). Seasonal variation in the diversity and species richness of phytoplankton in a tropical eutrophic reservoir. *Hydrobiologia*, *445*, 165–174. https://doi.org/10.1023/A:1017513731393

Florencio, M., Díaz-Paniagua, C., & Serrano, L. (2016). Relationships between hydroperiod length, and seasonal and spatial patterns of beta-diversity of the microcrustacean assemblages in Mediterranean ponds. *Hydrobiologia*, *774*(1), 109–121. https://doi.org/10.1007/s10750-015-2515-7

Flotemersch, J. E., Cormier, S. M., & Autrey, B. C. (2000). *Comparisons of boating and wading methods used to assess the status of flowing waters*. U.S. Environmental Protection Agency.

Füreder, L., Wallinger, M., & Burger, R. (2005). Longitudinal and seasonal pattern of insect emergence in alpine streams. *Aquatic Ecology*, *39*(1), 67–78. https://doi.org/10.1007/s10452-004-2969-6

Gabel, F., Pusch, M. T., Breyer, P., Burmester, V., Walz, N., & Garcia, X.-F. (2011). Differential effect of wave stress on the physiology and behaviour of native versus non-native benthic invertebrates. *Biological Invasions*, *13*(8), 1843–1853. https://doi.org/10.1007/s10530-011-0003-1

Goodman, K. J., Parker, S. M., Edmonds, J. W., & Zeglin, L. H. (2014). Expanding the scale of aquatic sciences: The role of the National Ecological Observatory Network (NEON). *Freshwater Science*, *34*(1), 377–385. https://doi.org/10.1086/679459

Grimm, N. B., & Fisher, S. G. (1989). Stability of periphyton and macroinvertebrates to disturbance by flash floods in a desert stream. *Journal of the North American Benthological Society*, *8*(4), 293–307. https://doi.org/10.2307/1467493

Gullan, P. J., & Cranston, P. S. (2014). *The insects: An outline of entomology* (5th ed.). John Wiley & Sons, Inc.

Haidekker, A., & Hering, D. (2008). Relationship between benthic insects (Ephemeroptera, Plecoptera, coleoptera, Trichoptera) and temperature in small and medium-sized streams in Germany: A multivariate study. *Aquatic Ecology*, *42*(3), 463–481. https://doi.org/10.1007/s10452-007-9097-z

Heino, J., Muotka, T., & Paavola, R. (2003). Determinants of macroinvertebrate diversity in headwater streams: Regional and local influences. *Journal of Animal Ecology*, *72*(3), 425–434. https://doi.org/10.1046/j.1365-2656.2003.00711.x

Helsel, D. R., & Hirsch, R. M. (2002). *Statistical methods in water resources* (Vol. A3). Unpublished. https://doi.org/10.13140/RG.2.2.36048.10248

Hill, W. R., Mulholland, P. J., & Marzolf, E. R. (2001). Stream ecosystem responses to Forest leaf emergence in spring. *Ecology*, *82*(8), 2306. https://doi.org/10.2307/2680233

Huston, M. (1979). A general hypothesis of species diversity. *The American Naturalist*, *113*(1), 81–101. https://doi.org/10.1086/283366

Hynes, H. B. N. (2001). *The ecology of running waters*. Blackburn Press.

Jensen, M. R., Egelyng Sigsgaard, E., Agersnap, S., Jessen Rasmussen, J., Baattrup-Pedersen, A., Wiberg-Larsen, P., & Francis Thomsen, P. (2021). Seasonal turnover in community composition of stream-associated macroinvertebrates inferred from freshwater environmental DNA metabarcoding. *Environmental DNA*, *3*(4), 861–876. https://doi.org/10.1002/edn3.193

Kao, R. H., Gibson, C. M., Gallery, R. E., Meier, C. L., Barnett, D. T., Docherty, K. M., Blevins, K. K., Travers, P. D., Azuaje, E., Springer, Y. P., Thibault, K. M., McKenzie, V. J., Keller, M., Alves, L. F., Hinckley, E.-L. S., Parnell, J., & Schimel, D. (2012). NEON terrestrial field observations: Designing continental-scale, standardized sampling. *Ecosphere*, *3*(12), art115. https://doi.org/10.1890/ES12-00196.1

Keddy, P. A. (1985). Wave disturbance on lakeshores and the within-lake distribution of Ontario's Atlantic coastal pain flora. *Canadian Journal of Botany*, *63*, 656–660.

Keller, M., Schimel, D. S., Hargrove, W. W., & Hoffman, F. M. (2008). A continental strategy for the national ecological observatory network. *Frontiers in Ecology and the Environment*, *6*(5), 282–284. https://doi.org/10.1890/1540-9295(2008)6[282:ACSFTN]2.0.CO;2

Korhonen, J. I., Soininen, J., & Hillebrand, H. (2010). A quantitative analysis of temporal turnover in aquatic species assemblages across ecosystems. *Ecology*, *91*(2), 508–517. https://doi.org/10.1890/09-0392.1

Lake, P. S. (2000). Disturbance, patchiness, and diversity in streams. *Journal of the North American Benthological Society*, *19*(4), 573–592. https://doi.org/10.2307/1468118

Lane, S. L., & Fay, R. G. (1997). *Safety in field activities: U.S. Geological Survey techniques of water-resources investigations, book 9, chapter A9.*

Larned, S. T. (2010). A prospectus for periphyton: Recent and future ecological research. *Journal of the North American Benthological Society*, *29*(1), 182–206. https://doi.org/10.1899/08-063.1

Legendre, P., & Anderson, M. J. (1999). Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs*, *69*(1), 1–24. https://doi.org/10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2

Lenat, D. R., & Barbour, M. T. (1994). Using benthic macroinvertebrate community structure for rapid, cost-effective, water quality monitoring: Rapid bioassessment. In S. L. Loeb & A. Spacie (Eds.), *Biological monitoring of aquatic systems* (pp. 187–215). Lewis Publishers.

Letelier, R. M., Bidigare, R. R., Hebel, D. V., Ondrusek, M., Winn, C. D., & Karl, D. M. (1993). Temporal variability of phytoplankton community structure based on pigment analysis. *Limnology and Oceanography*, *38*(7), 1420–1437. https://doi.org/10.4319/lo.1993.38.7.1420

Leunda, P. M., Oscoz, J., Miranda, R., & Ariño, A. H. (2009). Longitudinal and seasonal variation of the benthic macroinvertebrate community and biotic indices in an undisturbed Pyrenean river. *Ecological Indicators*, *9*(1), 52–63. https://doi.org/10.1016/j.ecolind.2008.01.009

Mackay, R. J., & Kalff, J. (1969). Seasonal variation in standing crop and species diversity of insect communities in a small Quebec stream. *Ecology*, *50*(1), 101–109. https://doi.org/10.2307/1934667

Minshall, G. W. (1988). Stream ecosystem theory—A global perspective. *Journal of the North American Benthological Society*, *7*(4), 263–288.

Musinsky, J., Goulden, T., Wirth, G., Leisso, N., Krause, K., & Haynes, M. (2022). Spanning scales: The airborne spatial and temporal sampling design of the National Ecological Observatory Network. *Methods in Ecology and Evolution*. https://doi.org/10.1111/2041-210X.13942

National Research Council. (2004). *Assessing the National Streamflow Information Program*. National Academies Press. https://doi.org/10.17226/10967

NEON (National Ecological Observatory Network). (2022a). *Macroinvertebrate collection (DP1.20120.001).* Retrieved from https://data.neonscience.org

NEON (National Ecological Observatory Network). (2022b). *Macroinvertebrate collection, RELEASE-2022 (DP1.20120.001).* https://doi.org/10.48443/855x-0n27. Retrieved from https://data.neonscience.org

NEON (National Ecological Observatory Network). (2022c). *Periphyton, seston, and phytoplankton collection (DP1.20166.001).* Retrieved from https://data.neonscience.org

NEON (National Ecological Observatory Network). (2022d). *Periphyton, seston, and phytoplankton collection, RELEASE-2022 (DP1.20166.001).* https://doi.org/10.48443/3cvp-hw55. Retrieved from https://data.neonscience.org

NEON (National Ecological Observatory Network). (2022e). *Single aspirated air temperature (DP1.00002.001).* Retrieved from https://data.neonscience.org

NEON (National Ecological Observatory Network). (2022f). *Single aspirated air temperature, RELEASE-2022 (DP1.00002.001).* https://doi.org/10.48443/0tyt-ky71. Retrieved from https://data.neonscience.org

NEON (National Ecological Observatory Network). (2022g). *Zooplankton collection (DP1.20219.001).* Retrieved from https://data.neonscience.org

NEON (National Ecological Observatory Network). (2022h). *Zooplankton collection, RELEASE-2022 (DP1.20219.001).* https://doi.org/10.48443/qzr1-jr79. Retrieved from https://data.neonscience.org

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry, H., Stevens, H., Szoecs E., & Wagner, H. (2020). *Vegan: Community ecology package*. R package version 2.5-7. Retrieved from https://CRAN.R-project.org/package=vegan

Parker, S., & Scott, C. (2021). *NEON user guide to aquatic zooplankton collection (DP1.20219.001) version C*. NEON (National Ecological Observatory Network).

Parker, S., & Vance, T. (2020). *NEON user guide to periphyton and phytoplankton collection (DP1.20166.001) version C*. NEON (National Ecological Observatory Network).

Parker, S. M., & Huryn, A. D. (2011). Effects of natural disturbance on stream communities: A habitat template analysis of arctic headwater streams. *Freshwater Biology*, *56*(7), 1342–1357. https://doi.org/10.1111/j.1365-2427.2011.02573.x

Parker, S. M., & Utz, R. M. (2022). NEON provisional aquatic biology and single aspirated air temperature data 2020-2021 ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/14548323b29b30b73da9039382b91bb7

Peres-Neto, P. R., Legendre, P., Dray, S., & Borcard, D. (2006). Variation partitioning of species data matrices: Estimation and comparison of fractions. *Ecology*, *87*(10), 2614–2625. https://doi.org/10.1890/0012-9658(2006)87[2614:VPOSDM]2.0.CO;2

Peterson, C. G., & Stevenson, R. J. (1992). Resistance and resilience of lotic algal communities—Importance of disturbance timing and current. *Ecology*, *73*(4), 1445–1461.

Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, B. D., Sparks, R. E., & Stromberg, J. C. (1997). The natural flow regime. *Bioscience*, *47*(11), 769–784.

Poff, N. L., & Ward, J. V. (1989). Implications of streamflow variability and predictability for lotic community structure—A regional-analysis of streamflow patterns. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*(10), 1805–1818.

Porter, S. D., Cuffney, T. F., Gurtz, M. E., & Meador, M. R. (1993). *Methods for collecting algal samples as part of the National Water-Quality Assessment Program* (Open-File Report No. 93–409). U.S. Geological Survey.

R Core Team (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Resh, V. H., Brown, A. V., Covich, A. P., Gurtz, M. E., Li, H. W., Minshall, G. W., Reice, S. R., Sheldon, A. L., Wallace, J. B., & Wissmar, R. C. (1988). The role of disturbance in stream ecology. *Journal of the North American Benthological Society*, *7*(4), 433–455.

Rosemond, A. D. (1994). Multiple factors limit seasonal variation in periphyton in a forest stream. *Journal of the North American Benthological Society*, *13*(3), 333–344. https://doi.org/10.2307/1467363

Shuter, B. J., Finstad, A. G., Helland, I. P., Zweimüller, I., & Hölker, F. (2012). The role of winter phenology in shaping the ecology of freshwater fish and their sensitivities to climate change. *Aquatic Sciences*, *74*(4), 637–657. https://doi.org/10.1007/s00027-012-0274-3

Singh, S., Inamdar, S., Mitchell, M., & McHale, P. (2014). Seasonal pattern of dissolved organic matter (DOM) in watershed sources: Influence of hydrologic flow paths and autumn leaf fall. *Biogeochemistry*, *118*(1–3), 321–337. https://doi.org/10.1007/s10533-013-9934-1

Šporka, F., Vlek, H. E., Bulánková, E., & Krno, I. (2006). Influence of seasonal variation on bioassessment of streams using macroinvertebrates. *Hydrobiologia*, *566*(1), 543–555. https://doi.org/10.1007/s10750-006-0073-8

Sturtevant, C., DeRego, E., Metzger, S., Ayres, E., Allen, D., Burlingame, T., Catolico, N., Cawley, K., Csavina, J., Durden, D., Florian, C., Frost, S., Gaddie, R., Knapp, E., Laney, C., Lee, R., Lenz, D., Litt, G., Luo, H., … SanClements, M. (2022). A process approach to quality management doubles NEON sensor data quality. *Methods in Ecology and Evolution*. https://doi.org/10.1111/2041-210X.13943

Sun, W., Xia, C., Xu, M., Guo, J., & Sun, G. (2017). Seasonality affects the diversity and composition of bacterioplankton communities in Dongjiang River, a drinking water source of Hong Kong. *Frontiers in Microbiology*, *8*, 1644. https://doi.org/10.3389/fmicb.2017.01644

Thorp, J. H., & Covich, A. P. (Eds.). (2001). *Ecology and classification of north American freshwater invertebrates* (2nd ed.). Academic Press.

Tonkin, J. D., Arimoro, F. O., & Haase, P. (2016). Exploring stream communities in a tropical biodiversity hotspot: Biodiversity, regional occupancy, niche characteristics and environmental correlates. *Biodiversity and Conservation*, *25*(5), 975–993. https://doi.org/10.1007/s10531-016-1101-2

Torres-Ruiz, M., Wehr, J. D., & Perrone, A. A. (2007). Trophic relations in a stream food web: Importance of fatty acids for macroinvertebrate consumers. *Journal of the North American Benthological Society*, *26*(3), 509–522. https://doi.org/10.1899/06-070.1

Vinson, M. R., & Hawkins, C. P. (1998). Biodiversity of stream insects: Variation at local, basin, and regional scales. *Annual Review of Entomology*, *43*(1), 271–293. https://doi.org/10.1146/annurev.ento.43.1.271

Woods, T., Kaz, A., & Giam, X. (2021). Phenology in freshwaters: A review and recommendations for future research. *Ecography*, *2022*(6), ecog.05564. https://doi.org/10.1111/ecog.05564

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Parker, S. M., & Utz, R. M. (2022). Temporal design for aquatic organismal sampling across the National Ecological Observatory Network. *Methods in Ecology and Evolution*, *13*, 1834–1848. https://doi.org/10.1111/2041-210X.13944