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ARTICLE

Special Feature: Harnessing the NEON Data Revolution



Using large, open datasets to understand spatial and temporal patterns in lotic ecosystems: NEON case studies

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Abstract

Leveraging big, open data is the next frontier in ecology. The National Ecological Observatory Network (NEON) is a network of monitoring sites collecting ecological data from across the United States. Using a case study approach, we provide examples of how NEON data can be applied to address a few big questions in aquatic ecology. First, we examined spatial patterns in stream water chemistry, to determine whether sites tend to cluster into regions based on geographic proximity. We found that this was not the case, likely because the hydrologic, geologic, and anthropogenic factors that drive heterogeneity in stream water chemistry vary across smaller spatial scales. Second, we examined temporal variability in stream water chemistry. We determined that the majority of catchments are relatively chemostatic (i.e., discharge varies by orders of magnitude more than concentrations) and that differences between catchments are likely to shift across decades due to changes in network conductivity. Third, we tested predictions of the River Continuum Concept (RCC) along a gradient from a second-order stream to a seventh-order river. We found that longitudinal patterns in metabolism, carbon chemistry, and macroinvertebrate community composition generally follow the patterns predicted by the RCC. NEON is only in its third year of full operations, with a planned 30-year life. The studies presented here show the utility of NEON data, while only using a subset of the many data products that NEON produces. The massive amounts and types of data NEON generates, in conjunction with other national-scale datasets, will allow the research community to better understand how aquatic ecosystems function and respond to drivers of long-term change.

KEYWORDS

aquatic ecosystems, NEON, open data, river continuum, Special Feature: Harnessing the NEON Data Revolution, stream chemistry

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INTRODUCTION

Using big, open datasets is the next frontier in ecology (Durden et al., 2017; Farley et al., 2018; Hampton et al., 2013). In addition to enabling more equitable access to information (Soranno et al., 2015), these datasets can be used to answer fundamental ecological questions at large spatial and temporal scales. Large-scale questions often require methods such as meta-analyses and machine-learning approaches that utilize large datasets, typically more data than can be collected by an individual researcher.

The National Ecological Observatory Network (NEON) is a continental-scale network of 81 monitoring sites, including 24 wadeable streams, 3 rivers, and 7 lakes located throughout the contiguous United States, Alaska, and Puerto Rico (Goodman et al., 2015; McDowell, 2015). Sites are instrumented with an array of automated sensors to collect high-frequency meteorological, hydrological, and biogeochemical data (Hensley et al., 2021). Trained field ecologists perform manual sampling of riparian and benthic habitats, phytoplankton, macroinvertebrates, and fish. Data collection is standardized, allowing long-term within-site comparison over the 30-year life of the project, and cross-site comparisons at continental scales (Goodman et al., 2015; Parker & press, in review).

While terrestrial ecologists have been quick to embrace NEON, the use of NEON aquatic data has lagged behind, with only a handful of aquatic publications existing to date. The amount of data that will be generated by NEON is unprecedented, and truly harnessing its potential will take decades of work and hopefully result in hundreds of research studies that create and test innovative ecological models, as well as aid in the managing and protecting of aquatic ecosystems. Below, we highlight ways in which NEON data could be used to address important questions in aquatic ecology and provide case studies to demonstrate these uses. These case studies are intended to be brief examples, using only the first few years of data or a subset of sites.

CASE STUDIES

Identifying spatial patterns in stream water chemistry

Researchers can take a variety of approaches to evaluating large datasets to identify important trends that drive ecological structure and function or inform resource management. Regional- or national-scale studies have utilized multivariate analyses, specifically principal component analysis (PCA) and hierarchical cluster analysis (HCA), to identify relationships and evaluate large water

quality datasets (Ayeni & Soneye, 2013; Mishra, 2010; Zeinalzadeh & Rezaei, 2017). These studies found that the most common factors affecting stream surface water quality are the waterbody flow regime, catchment characteristics, and anthropogenic influences. Regardless of geographic location, studies were often able to discern the influence of agricultural land use (nonpoint source nutrients) versus sewage versus physicochemical variability on surface waters (Ayeni & Soneye, 2013; Mishra, 2010; Simeonov et al., 2003; Singh et al., 2020). In less populated areas with less agricultural land use, the influence of geologic factors (weathering and soil leaching) more strongly affected surface water quality (Ramos et al., 2016; Simeonov et al., 2003).

Although subtle changes in water chemistry (i.e., nutrients, pH, ions, and organic carbon concentration) are not often drivers of biological community composition, extremes or dramatic changes can impact biological activity and alter ecosystem functioning. For this reason, we chose to describe patterns in surface water chemistry at lotic NEON sites using a multivariate cluster analysis and examine the similarities or differences across the NEON Domains. Unlike other regional work in the published literature, NEON site selection was not intended to create replicates within ecoclimatic regions (i.e., regional Domains; Figure 1) of the United States, but rather to maximize the diversity of stream characteristics that likely influence stream processing, including stream size, geomorphology, and geology (Schimel et al., 2007). Thus, we did not necessarily expect to see similarities in water chemistry among sites close to each other geographically (i.e., spatial autocorrelation).

This analysis used the chemical properties of surface water (NEON, 2021a) data product collected from the 24 stream and 3 river sites in the NEON network to perform a PCA followed by HCA to group NEON sites based on 16 water chemistry parameters (Appendix S1: Figure S1, Tables S1 and S2). The six groups we identified through HCA (Figure 1) capture similarities in water chemistry between sites. Code for analyses completed in this case study and the following two studies is publicly available on GitHub (King et al., 2021).

Group 1 (CARI, COMO, HOPB, MAYF, and OKSR) had high concentrations of dissolved organic carbon (DOC) and transition metals and average or low values of anions, cations, and nutrients (Table 1). Both of the streams in Alaska are in Group 1, along with a first-order, high-elevation stream in Colorado, a second-order stream in Alabama, and a second-order stream in Massachusetts. Group 2 (LECO, MART, MCRA, and TECR) had low or average water chemistry concentrations of all constituents (Table 1). Group 2 streams include the two streams in the Pacific Northwest, a high gradient, second-order stream in Tennessee, and a first-order stream in the Sierra Nevada.

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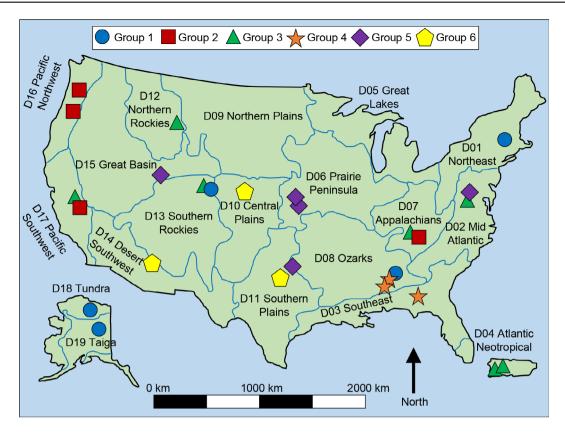


FIGURE 1 Map of NEON Domains (D01–D19). D20 is not shown because it does not contain any aquatic sites. Stream and river sites are indicated by colored symbols according to group affiliation as identified using hierarchical cluster analysis (HCA).

Groups 3 and 4 had average concentrations of most cations and anions. Group 3 (BIGC, BLDE, CUPE, GUIL, POSE, WALK, and WLOU) had higher-than-average silicate and lower-than-average ammonium concentrations (Table 1). With seven sites, Group 3 had the largest number of sites that grouped together and covered a wide latitudinal gradient from the two tropical sites in Puerto Rico to a high-elevation site in the Rocky Mountains, Colorado. Group 4 was composed of the three large rivers (BLWA, TOMB, and FLNT) and had high relative ammonium concentrations (Table 1).

Groups 5 and 6 both had high concentrations of many cations and anions. However, Group 5 (BLUE, KING, LEWI, MCDI, and REDB) had low iron concentrations, while Group 6 (ARIK, PRIN, and SYCA) had high DOC, manganese, and silica. The geographic distribution of HCA groups 1–6 indicates that, while a single pair of sites in four different groups were located close to one another, in general, sites assigned to the same HCA group were not in the same NEON Domain (Figure 1).

These patterns demonstrate that the NEON sites reflect similar water chemistry drivers as previous studies and cover a broad range of conditions within the United States making them well suited for cross-site comparison studies. The regional-scale ecoclimatic characteristics used to delineate NEON Domains, which included nine climatic variables (Hargrove & Hoffman, 2004; Keller et al., 2008), were ineffective at explaining similarities in water chemistry between groups.

Future research could test the differences in abiotic and biotic drivers across clusters. We hypothesize that local variables may be influencing the differences in water chemistry across groups, so linking NEON sites to other datasets such as soil structure, chemical weathering of bedrock, and shallow groundwater hydrology and chemistry will help predict differences in surface water chemistry behavior between groups. Anthropogenic impacts can also be further assessed. While the majority of NEON sites would traditionally be considered lower impact, that is not to say they are all unimpacted. LEWI, for example, receives wastewater effluent, while ARIK experiences very high irrigation withdrawals, explaining marked outliers in NO2 + NO3 concentrations and water yield, respectively. In addition, internal biological processing can be examined as a driver. For example, identifying the DOC sources, both autochthonous and allochthonous, and linking these to NEON periphyton and phytoplankton data, could improve global estimates of greenhouse gas emissions through its control of microbial respiration rates. Regardless of the focus, increased

TABLE 1 Surface water hierarchical clustering results showing which NEON sites grouped together, the relative principal component analysis (PCA) component loadings for each group, and which variables grouped together

Component and variables	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Component 1	Low	Low	Med	Med	High	High
Component 2	High	Med	Low	High	Med	Med
Component 3	Med	Med	Med	Med	Med	High
Component 1 variables						
DIC	_	_	ND	ND	+	+
Ca ²⁺	_	_	ND	ND	+	+
Mg^{2+}	_	_	ND	ND	+	ND
pН	_	_	ND	ND	+	ND
Na ⁺	ND	ND	ND	ND	ND	+
K^+	_	_	ND	ND	ND	+
SO_4^{2-}	ND	_	ND	ND	+	ND
Cl ⁻	ND	ND	ND	ND	ND	+
F^-	_	ND	ND	ND	+	+
TDP	_	ND	ND	ND	ND	ND
Component 2 variables						
DOC	+	_	ND	ND	ND	+
Fe ²⁺	+	ND	ND	ND	_	ND
Mn^{2+}	+	ND	ND	ND	ND	+
Component 3 variables						
$\mathrm{NH_4}^+$	ND	ND	_	+	ND	ND
$NO_3^{\ 2-} + NO_2^{\ -}$	ND	ND	ND	ND	ND	ND
Si ²⁺	_	ND	+	ND	ND	+

Note: Signs indicate whether the group mean was larger (+) or smaller (-) than the overall mean for a given variable. Dissolved inorganic carbon (DIC) through total dissolved phosphorus (TDP) are associated with Component 1, DOC through $\mathrm{Mn^{2+}}$ are associated with Component 2, and $\mathrm{NH_4^+}$ through $\mathrm{Si^{2+}}$ are associated with Component 3. Variables with an ND indicate no significant difference in group mean versus overall mean. Differences between overall means and group means denoted by a plus or minus sign were statistically significant at p < 0.001. Group 1 sites are CARI, COMO, HOPB, MAYF, and OKSR; Group 2 sites are LECO, MART, MCRA, and TECR; Group 3 sites are BIGC, BLDE, CUPE, GUIL, POSE, WALK, and WLOU; Group 4 sites are BLWA, TOMB, and FLNT; Group 5 sites are BLUE, KING, LEWI, MCDI, and REDB; and Group 6 sites are ARIK, PRIN, and SYCA. Abbreviations: DOC, dissolved organic carbon; Med, medium.

collaboration between scientists within the broader community will strengthen the creative use of products resulting from NEON data collection in combination with other data products across the United States.

In summary, the NEON clustering of water chemistry across the observatory reflects geologic, hydrologic, and anthropogenic controls. Interestingly, the spatial distribution of clustered sites does not align with the NEON Domains for many locations, indicating that a further area of research may be to determine a set of variables that define aquatic ecoregions that can be used for scaling NEON data from site level to the continental scale. NEON alone does not cover extensive gradients of geology or land use; however, our case study showed consistency with other studies. With integration of other monitoring data, the NEON aquatic sites will contribute

to future efforts to parameterize global climate models and improve our ability to understand and manage ecosystems.

Controls on temporal variability in stream chemistry

Many of the spatial patterns in stream water chemistry and their drivers are from a "snapshot" in time, with less known about how the patterns or their drivers might change over long time periods across different regions. Abbott et al. (2018) found stable spatial patterns across headwater catchments over 12 years, and similarly, Dupas et al. (2019) found temporal synchronicity across regions over a 6-year time series. These spatial patterns

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are often remarkably stable through time (Abbott et al., 2018), likely a consequence of low temporal variability within sites (i.e., relative chemostatic behavior; Godsey et al., 2009). However, over longer time periods (30+ years), the effects of a changing climate may vary across different regions. For example, changes in precipitation patterns (flooding or drought) may make hydrology the dominant driver of stream chemistry in some regions, but not have an effect in others.

Understanding the drivers of temporal variability in stream chemistry allows managers to predict seasonal changes in water quality and, in response, alter treatment plans or establish alternative sources for irrigation and drinking water if quality becomes low or aquatic biological communities are stressed. In addition, if changes in water quality persist over decades, community composition and rates of organic matter production and consumption may be fundamentally altered (new stable state), thereby impacting dissolved material transport rates to recipient systems.

While continental-scale gradients in temperature have been shown to affect rates of stream nutrient processing and export (Schaefer & Alber, 2007), climatic effects on conservative solute concentrations are less clear (White & Blum, 1995). NEON will provide a 30-year dataset to allow researchers to test the effects of climate on water chemistry through time across a broad spatial extent. These data will be essential in answering questions on long-term chemical stability in streams, including how will decadal-scale changes in precipitation patterns influence the relative importance of hydrology in controlling stream chemistry?

As a first approach to evaluating drivers of temporal patterns in stream chemistry, we examined whether concentration–discharge (C-Q) relationships within sites might vary across the HCA groups identified in the first case study. C-Q relationships have been discussed extensively in the literature (Godsey et al., 2009; Moatar et al., 2017), and climatic variation and landscape heterogeneity have been found to produce contrasting C-Q patterns across catchments (Godsey et al., 2009; Herndon et al., 2015). We predicted that C-Q relationships within sites would be stable across the 3-year time period of our data. NEON sites would not be expected to exhibit strict temporal synchronicity described in Dupas et al. (2019), because sites on opposite sides of the country do not share wet and dry seasons and individual storm events are completely independent at that spatial scale. We used NEON water chemistry data (as described in case study 1) from wadeable stream sites and corresponding discrete measures of discharge (NEON, 2021b) for the period 2017–2019. Because C-Q relationships generally follow a power function, data were log-transformed and fit with

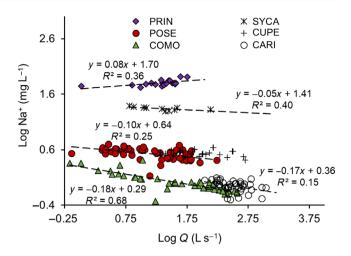


FIGURE 2 Examples of C–Q relationships. Major cations such as Na $^+$ often showed a dilution response. Note data were log-transformed. There is no regression shown for the CUPE site as the C–Q results were not significant at p < 0.05.

simple linear regressions using the glm() function in R, testing first that the assumptions of normality were met.

As a second approach to evaluating watershed hydrology and surface water chemistry, we examined temporal variability in water chemistry parameters at each site by calculating the coefficient of variation of the entire 3 years of water chemistry data for each site for each analyte, then generated a linear regression (SigmaPlot v.14.0) with our calculated water yield using 27 aquatic NEON sites. Assumptions of normality were confirmed using kurtosis, skewness, and the Shapiro-Wilk test. Annual water yield was used as a surrogate measure of the connectivity of the mainstem with its tributary network, with higher water yield indicating a more frequent and expansive connection with the terrestrial soils through tributaries. This precipitation-water yield relationship is a function of catchment geology, vegetation, and channel morphology (Prancevic & Kirchner, 2019; Tucker & Slingerland, 1997; Ward et al., 2018). We used a simple water yield calculation, where we first averaged all discharge values (Q_{avg}) through the end of the water year 2019 collected using continuous Q measurements (NEON, 2021c). From this value, we calculated a normalized discharge (q_{avg}) by dividing Q_{avg} by the area of the watershed (A_{WS}) . Normalized discharge was then divided by average annual precipitation (P_{avg}) to provide a unitless value termed water yield.

Using our first approach to explore temporal dynamics, we found individual C–Q relationships varied significantly across solutes and sites (Appendix S1: Table S3). Discharge was an extremely strong predictor of surface water chemistry for some regressions, and nearly not at all for others (R^2 values ranged from 0.99 to 0.00). Across solutes, conservative cations such as Na $^+$ (Figure 2), Ca $^{2+}$, and Mg $^{2+}$, as well as DIC, were the most likely to vary with discharge,

exhibiting a dilution response (i.e., negative slope) in roughly half to two thirds of the sites and chemostasis (i.e., slope not significantly different from 0) in the remainder. The notable exception was enrichment (i.e., positive response) of Na^+ in the two Southern Plains sites, PRIN and BLUE (Appendix S1: Table S3). Very few solutes consistently exhibited a significant positive slope indicative of an enrichment response; DOC enrichment was the most common, occurring in slightly more than half of the sites, while $\mathrm{NO_3} + \mathrm{NO_2}$ enrichment occurred in about a third. The response of $\mathrm{SO_4}^{2-}$ was the most varied; it was the only solute to exhibit statistically significant dilution in at least five sites and enrichment in at least five others.

Statistically significant slopes never reached -1, which would be indicative of a "pure dilution" response with a fixed mass flux of solute being diluted by variable volumes of water (Godsey et al., 2009). Indeed, slopes were rarely outside the range of -0.3 to 0.3, indicating that while modest dilution or enrichment was occurring, concentrations were still remarkably stable. Overall, our C-Q results reinforced the relative chemostatic nature of watersheds (Godsey et al., 2009). Even in cases where C-Q slopes were statistically nonzero (suggesting an enrichment or dilution response), within-site variation in C rarely exceeded more than an order of magnitude, despite Q variation nearly always exceeding several orders of magnitude. This temporal stability across catchments reflects findings elsewhere (e.g., Abbott et al., 2018; Dupas et al., 2019).

As a second approach to identifying controls on temporal variability, our calculations of water yield were correlated with variability in water chemistry parameters across NEON sites (measured as coefficient of variation) for just a subset of conservative ions, suggesting increasing annual water yield resulted in larger fluctuations in some conservative ions in the surface waters (Figure 3). But while increased water yield created more variability for specific compounds, higher variability was not associated with a tighter coupling between water chemistry concentrations and Q. In fact, five of the six sites with the highest number of statistically significant C-Q relationships had intermediate annual water yield below the median value, falling in the 0.2–0.4 range.

Using the *C*–*Q* and water yield relationships described above, we can begin considering decadal-scale changes in temporal variability in stream chemistry at NEON sites resulting from continued climate change. Watersheds experiencing more intense and frequent rain events due to atmospheric warming are predicted to see an increase in the areal extent of the watershed actively contributing to overland runoff and stream transport (termed network connectivity), while network connectivity will likely shrink in areas experiencing drought (Slater et al., 2019; Tucker & Slingerland, 1997). Increases in network

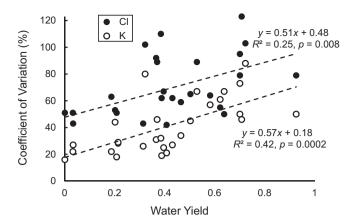


FIGURE 3 Relationship between the coefficient of variation and the catchment water yield at 27 aquatic NEON sites using all available measurements of surface water chloride (dark circles) and potassium (open circles)

connectivity create periods of low stream ion concentrations due to dilution during flooding, followed by shortened interflood periods where groundwater chemistry and instream processing dominate surface water quality, which results in frequent alternation in source areas feeding the surface waters (Covino, 2017; Zimmer & McGlynn, 2018). This frequent switching between sources could lead to increased variability in stream chemistry throughout the year, a pattern we already documented across the NEON stream sites for specific ions, but not, yet, through time at a single site (Figure 3).

Increased variability in source water chemistry due to increasing frequency and intensity of flooding (represented as a shift from the dotted line to the solid black line in Figure 4) may erode the significant C-Q relationships currently found and create chemostatic stream chemistry behavior for most ions. We also hypothesize that streams experiencing drought will interact less frequently with the terrestrial portion of the watershed as network connectivity declines, increasing surface water chemistry through evaporative concentration and lowering temporal variability in conservative ions (shift from dotted line to dashed line in Figure 4). This decreased variability would also create chemostatic behavior in C-Q relationships. Sites in PCA groups 5 and 6 (Figure 1) are predicted to follow this drying trend, and larger, temporally complete datasets such as those created by NEON will test these hypotheses in the future.

Concentrations of bioreactive elements at NEON sites, in contrast to conservative ions, did not fluctuate predictably with discharge at most sites, suggesting instream dynamics will continue to control N and P supply at NEON sites regardless of the disturbance regime, assuming anthropogenic sources do not increase. The exception was a positive *C*–*Q* relationship for DOC; thus,

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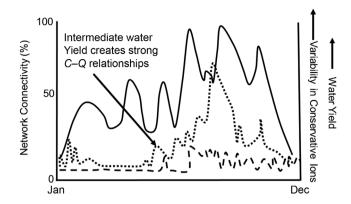


FIGURE 4 Conceptual model relating network connectivity (left-hand *y*-axis) and annual water yield (right-hand *y*-axis) to the variability in stream chemistry and coupling of *C*–*Q*. High variability in conservative ion concentrations is predicted for streams with high water yield and network connectivity (solid line), and streams with very little network connectivity or water yield (dashed line) are predicted to have low variability in conservative ion concentrations. Intermediate network connectivity and water yield (dotted line) are more likely to maintain predictable *C*–*Q* relationships.

we predict as network connectivity declines due to drought, so will the supply of allochthonous carbon to the stream. Not only could drought lower DOC delivery to the stream, it could lower the average chemical complexity of the pool of organic compounds. Alternatively, in streams experiencing increased flooding, a larger percentage of DOC will be allochthonous in origin, which would influence rates of decomposition and possibly lower light penetration through the water column due to adsorption by DOC (Harms et al., 2021; Zimmer & McGlynn, 2018).

These changes in temporal patterns of hydrology and the availability of conservative and bioreactive ions will ultimately affect stream biological communities. For example, in watersheds experiencing drought, an increase in water conductivity, particularly in shrinking pools of surface water, creates an environment selective for hardier, slowergrowing species of algae able to cope with these stressors (Larned, 2010; Ledger et al., 2008; Rolls et al., 2018). In fact, under drought conditions, high ionic concentrations in surface waters coupled with decreased temporal variability in chemistry could result in permanent changes in periphyton community composition, encouraging dominance by lowflow adapted species. Alternatively, streams with increased flooding, lower ion concentrations, and more variable chemistry throughout the year would create ideal conditions for resilient periphyton species, such as diatoms (Grimm & Fisher, 1989; Lake, 2000; Schneck et al., 2017). Overall, as NEON datasets stretch into the future, our conceptual models linking climate drivers to temporal variability in

stream chemistry will improve, sharpening our ability to predict seasonal changes in water quality and shifts in biotic community composition. In turn, managers will be able to develop withdrawal schedules that protect stream resources and avoid the extraction of water for human use when surface water chemistry is unsuitable.

Patterns and processes along a river network: Assessing the River Continuum Concept

It is somewhat remarkable that after 40 years and thousands of citations (Barmuta & Lake, 1982; Doretto et al., 2020), the River Continuum Concept (RCC) can simultaneously remain so foundational to the field of lotic ecology, yet so frequently be called into question (Doretto et al., 2020). First formalized by Vannote et al. (1980), the RCC conceptualizes a river network as a continuous gradient of physical characteristics from headwaters to mouth, which produces associated chemical and biological adjustments in response. From the beginning, empirical evidence tended to support the basic predictions of the RCC (Bott et al., 1985; Bruns & Minshall, 1985; Culp & Davies, 1982), yet numerous exceptions can be found (Perry & Schaeffer, 1987; Statzner & Higler, 1985; Winterbourn et al., 1981). Several alternatives to the RCC have been proposed, foremost among them being the Serial Discontinuity Concept (Ward & Stanford, 1983), which envisions a sequence of discrete shifts where the smooth gradients of the RCC are continually reset. Though originally focused on dams (Ward & Stanford, 1983), tributary confluences have also been recognized as potential discontinuities (Minshall et al., 1983). It is worth noting this potential resetting of the continuum was explicitly acknowledged within the original RCC framework (Vannote et al., 1980).

The NEON Ozarks Domain (D08) in the southeastern United States contains three sites along a river network, explicitly chosen to be studied within the context of the RCC. These sites are Mayfield Creek (MAYF), a secondorder stream, Black Warrior River (BLWA), a sixth-order river, and Tombigbee River (TOMB), a seventh-order river. The latter two are part of one of the most economically important navigable waterways in the southeastern United States and are impounded along nearly their entire length by a series of locks and dams. These data can be used to test several predictions of the RCC. First, longitudinal gradients in benthic light availability produce commensurate patterns in rates of stream metabolism. Second, as stream order increases, labile carbon availability increases with a gradual shift from allochthonous to autochthonous forms. Third, the benthic community will organize itself to best utilize the available forms of carbon.

Using dissolved oxygen (DO) from the NEON Water Quality data product (NEON, 2021d) for a period of relatively stable flow in August 2019, we calculated rates of gross primary production (GPP) and ecosystem respiration for each site using the streamMetabolizer model (Appling et al., 2018). The results tend to follow the predictions of the RCC (Figure 5), with sunlight-driven GPP being low in the narrow, shaded headwater streams, peaking in the wider mid-order rivers, and then perhaps beginning to decline in the largest order rivers due to greater water column light attenuation (NEON Secchi depth measurements shown in Table 2).

Water chemistry data (NEON, 2021a) showed a significant accumulation of total organic carbon (TOC) with downstream distance (Table 2). Notably, in the context of the RCC, it is primarily in the dissolved form (DOC). There was not a substantial accumulation of particulate carbon (TPC), either measured or inferred from the balance between TOC and DOC, suggesting this material is being utilized by the aquatic community. Specific ultraviolet absorbance (SUVA) at 254 and 280 nm tended to decline with downstream distance, suggesting decreasing aromaticity. This is consistent with the RCC prediction of

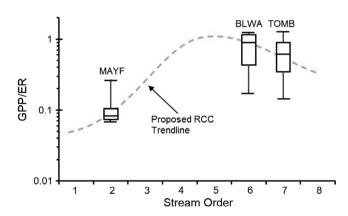


FIGURE 5 Ratio of gross primary production (GPP) to ecosystem respiration (ER) from August 2019 for NEON D08 sites ordinated by Strahler stream order. Boxes show interquartile range, while whiskers show 10th and 90th percentiles. Dashed line shows hypothetical River Continuum Concept (RCC) trend (Vannote et al., 1980).

a transition from allochthonous versus autochthonous sources of carbon. Of note, the MAYF SUVA280 appears especially high, potentially the result of iron absorption; MAYF has by far the highest Fe²⁺ of any NEON site.

Macroinvertebrate sampling (NEON, 2021e) shows that in all three sites, Diptera was the most predominant order of invertebrates, averaging around 70% of individuals across surveys. Trichoptera (caddisflies) were also relatively equally abundant in all three sites (Figure 6). However, Plecoptera (stoneflies), which were the second most abundant order in MAYF, were absent from BLWA and TOMB where Tubificida (worms) were the next most abundant order. This transition from shredders to filter feeders is also consistent with RCC predictions of ecosystem structure.

While this preliminary analysis suggests patterns of water chemistry, community composition, and ecosystem functioning along the NEON D08 sites are consistent with predictions of the RCC, the nature of at-a-point sampling makes it difficult to identify whether changes occur discretely or along a continuum (Ensign et al., 2017; Hensley et al., 2020). It is worth noting that the BLWA and TOMB sites are located 70 and 140 km downstream of dams, distances potentially sufficient for any effects to have largely dissipated (Ellis & Jones, 2013; Ward & Stanford, 1983).

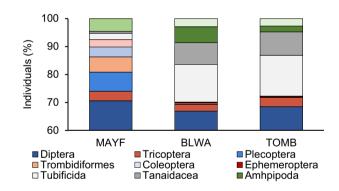


FIGURE 6 Distribution of macroinvertebrate orders across NEON D08 sites. The *y*-axis scale begins at 60% to allow visualization of groups with a smaller overall contribution to community composition (\sim 70% of individuals at all three sites were classified as Diptera).

TABLE 2 Summary of carbon chemistry and optical properties of NEON D08 stream and river sites (means ± SD)

NEON site ID	$TOC (mg L^{-1})$	TPC (mg L^{-1})	$DOC (mg L^{-1})$	$SUVA_{254}$ (L $mg^{-1} m^{-1}$)	$SUVA_{280} (L mg^{-1} m^{-1})$	Secchi (m)
MAYF	2.37 ± 0.82	0.27 ± 0.04	2.16 ± 0.67	5.03 ± 1.74	3.73 ± 1.42	-
BLWA	3.20 ± 0.47	0.29 ± 0.04	3.00 ± 0.41	2.90 ± 0.60	1.96 ± 0.52	0.70 ± 0.24
TOMB	$\textbf{5.02} \pm \textbf{0.93}$	0.30 ± 0.04	4.70 ± 0.87	3.88 ± 0.80	2.67 ± 0.72	$\textbf{0.46} \pm \textbf{0.20}$

Note: Sites are listed in order from headwater (MAYF) to furthest downstream (TOMB). MAYF has no measurable Secchi depth as it is shallow enough for light to reach the bottom.

Abbreviations: TOC, total organic chemistry; TPC, total particulate carbon; DOC, dissolved organic carbon; SUVA, specific ultraviolet absorbance; MAYF, Mayfield Creek; BLWA, Black Warrior River; TOMB, Tombigbee River.

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However, both are also only 15 km upstream of other dams. While the most focus has been on downstream impacts, it is quite likely that ecological impacts of dams extend upstream as well, especially given stage and discharge at these locations are controlled by the downstream dams. There is also a large, effluent-discharging paper mill located between the sites, which may act as a discontinuity and potentially explain some of the increase in SUVA at TOMB. Determining whether effects of discontinuities are permanent or transient and how far upstream and downstream they propagate is a potential avenue of further research.

Another consideration for future research is the role of discharge variation on the continuum, most notably flood pulses (Junk et al., 1989), something this preliminary analysis did not examine. The attenuation of light due to elevated turbidity during higher flows can reduce GPP (Hall Jr et al., 2015; Uehlinger, 2000), in effect shifting larger rivers to levels more common for small, shaded streams (Young & Huryn, 1996). High flows can also deliver large pulses of allochthonous carbon and substantially disturb the biological community (Dodds et al., 1996; Fisher et al., 1982). These are temporal dynamics that NEON datasets are designed to capture, in these D08 sites along a network, but also at continental scales across the entire observatory.

FUTURE DIRECTIONS

The case studies presented in this paper relied primarily on observational sampling (e.g., discrete grab samples of water chemistry or collection of invertebrates). NEON also collects data using an array of in situ sensors (Hensley et al., 2021). These high-frequency data, with sampling intervals on the order of seconds to minutes, will provide insight into processes varying over commensurate timescales (Kirchner et al., 2004; Rode et al., 2016). Examples include stream metabolism (Bernhardt et al., 2018), coupled nutrient assimilation (Heffernan & Cohen, 2010), and storm event *C*–*Q* hysteresis (Evans & Davies, 1998). Integration of instrument and observational sampling will allow us to better understand how short-term processes such as metabolism and nutrient cycling affect larger patterns such as community structure.

Another underutilized source of data is specimens, which NEON collects and maintains as part of their sampling protocol. Whole animal and plant specimens, tissues, and excess BioRepository samples enable researchers to investigate chemical composition, zoonosis (Lister et al., 2011), and phenotypic expression (Card et al., 2021) response to climate change impacts (Schmitt et al., 2019). These preserved archived samples and analyses could be useful in answering fundamental ecological questions about

range expansion and contraction, the emergence of new diseases, speciation, and adaptation (Bi et al., 2013; Stuart et al., 2021).

The design of NEON also facilitates the integration of the aquatic data presented here with other types of data such as atmospheric and terrestrial data. The same type of sensors is deployed with comparable configurations at aquatic and terrestrial sites (for a list of shared and complementary data products, see information on the NEON website). About two thirds of aquatic sites are within several kilometers of a terrestrial site, and some terrestrial sites are within aquatic watersheds, which will allow tracing the fate and transport of carbon, nutrients, and greenhouse gasses across ecosystems. The majority of the NEON study sites are relatively unimpacted (2 of 24 sites have watersheds with >10% high impact land use); thus, linking NEON terrestrial and aquatic sites can elucidate future changes in aquatic ecosystem structure and function as the surrounding landscape changes in response to invasive species, land use, and climate change. Groundwater also functions as a link between terrestrial and aquatic systems. The hydrologic and chemical data produced from the groundwater wells at most NEON aquatic sites could further support determining terrestrial and aquatic linkages underpinning long-term ecological change.

Finally, NEON also collects airborne remote sensing data from all of its sites as part of its Aerial Observation Platform (AOP). This includes LIDAR, an imaging spectrometer, and high-resolution digital imagery. From a scientific perspective, these data may be useful in linking catchment characteristics (e.g., topography, long-term changes in vegetative cover) with hydrologic response and biogeochemical processing. From a methodological perspective, the intensive "on-the-ground" sampling will be exceedingly useful in ground-truthing remote sensing measurements. This applies not only to the NEON AOP program but potentially also in calibrating/validating other airborne and satellite-based remote sensing platforms, which periodically pass over NEON sites.

At present, NEON is only in its third year of operations, with a planned 30-year life. The studies highlighted here use a fraction of the data NEON is expected to generate. They are presented as a starting point, not an ending point. Future work should reexamine and expound upon the analyses presented here, incorporating not only more years of data, but integrating with other types of data. Many NEON sites are also located within or in close proximity to other research sites (e.g., LTER, CZO, and universities). Linking NEON data with historic and/or contemporaneous data from these other entities, which also provide large, open datasets, "networking-of-networks," has the potential to provide an even deeper understanding of important questions in aquatic ecology,

at spatial scales ranging from watershed to continents, and temporal scales ranging from minutes to decades.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data used in this study (NEON, 2021a, 2021b, 2021c, 2021d, 2021e) are cited in the *Case studies* section.

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SUPPORTING INFORMATION

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