

Coupled Nutrient-Carbon Cycle Processes and Related Ecological-Flow Drivers of Aquatic Health

By Mark M. Dornblaser, Kimberly P. Wickland, Deborah A. Repert, Richard L. Smith, and Judson W. Harvey

Chapter B of

Knowledge Gaps and Opportunities in Water-Quality Drivers of Aquatic Ecosystem Health

Edited by Judson W. Harvey, Christopher H. Conaway, Mark M. Dornblaser, Allen C. Gellis, A. Robin Stewart, and Christopher T. Green

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Supplemental Information

Concentrations of chemical constituents in water are given in either milligrams per liter (mg/L) or micrograms per liter ($\mu\text{g/L}$).

Abbreviations

ARG	antibiotic resistance gene
BDOC	biodegradable dissolved organic carbon
CEC	contaminant of emerging concern
DNA	deoxyribonucleic acid
DNRA	dissimilatory nitrate reduction
DOC	dissolved organic carbon
EPA	U.S. Environmental Protection Agency
FDOM	fluorescent dissolved organic matter
HAB	harmful algal bloom
IWAAS	USGS WMA Integrated Watershed Availability Assessments Program
IWS	USGS Integrated Water Science
NGWOS	USGS Next Generation Water Observing Stations
OC	organic carbon
OMP	organic micropollutant
PFAS	per- and polyfluoroalkyl substances
POC	particulate organic carbon
RNA	ribonucleic acid

SRP	soluble reactive phosphorus
TDN	total dissolved nitrogen
TMDL	total maximum daily load
TN	total nitrogen
TP	total phosphorus
UAV	uncrewed aerial vehicle
USGS	U.S. Geological Survey
WMA	USGS Water Resources Mission Area

Chemical Symbols

As	arsenic
C	carbon
Fe	iron
Hg	mercury
N	nitrogen
O	oxygen
P	phosphorus

Chapter B

Coupled Nutrient-Carbon Cycle Processes and Related Ecological-Flow Drivers of Aquatic Health

By Mark M. Dornblaser, Kimberly P. Wickland, Deborah A. Repert, Richard L. Smith, and Judson W. Harvey

Purpose and Scope

This chapter addresses knowledge gaps that, if filled, could improve predictions of aquatic ecosystem health as affected by coupled nutrient-carbon cycle processes and related ecological flow drivers. The gaps identified in this chapter are not intended to be comprehensive but are instead focused on key opportunities for the U.S. Geological Survey (USGS) Water Resources Mission Area (WMA, <https://www.usgs.gov/mission-areas/water-resources>). Nutrient effects on beneficial uses of water are not addressed in this chapter but are covered in Chapter E of Tesoriero and others (2024), “Improving Predictions of Nitrogen Effects on Beneficial Uses of Water,” the companion Open-File Report to this publication.

Statement of the Problem

The biogeochemical cycles of carbon (C), nitrogen (N), and phosphorus (P) are fundamental to life, and they are coupled to one another through basic stoichiometry requirements of organisms and oxidation-reduction reactions of metabolism (Sternier and Elser, 2002; Schlesinger and others, 2011). Human activities have radically changed the amount and distribution of these elements; for example, atmospheric carbon dioxide (CO₂) concentrations have increased by 40 percent or more since the beginning of the Industrial Revolution (Canadell and others, 2007), reactive N in the biosphere has doubled since 1950 (Galloway and others, 2008), and mining of P for fertilizers has redistributed it across the Earth (Gilbert, 2009). These individual and collective changes in C, N, and P are leading to the reorganizing of biological communities in terrestrial and aquatic systems, with important implications for ecosystem health (Finzi and others, 2011). This chapter summarizes gaps in our understanding of coupled nutrient-carbon cycle processes and related ecological flow regime (“eco-flow”) drivers (Suen and Eheart, 2006) and proposes a series of approaches across multiple scales (microbial-to-river reach) which will address these gaps, providing data and understanding that could aid ecosystem-health modeling efforts.

On an annual basis the U.S. Environmental Protection Agency (EPA) ranks by number the stakeholder-reported

impairments of aquatic health and beneficial use of surface waters across the Nation (EPA, 2017). Impairments associated with eutrophication of surface waters rank among the highest priorities, with excessive nutrients ranked number 2, low dissolved oxygen ranked number 4, high total suspended solids ranked number 6, and high temperatures ranked number 10 (EPA, 2017). Despite efforts to reduce nutrient inputs to waterways, land use and climate change have resulted in trends that are generally not improving. The EPA found 43 percent of national rivers and streams were of poor quality according to a rating based on total nitrogen (TN) with no change from 2008–2009 to 2013–2014; for total phosphorus (TP), they found that 58 percent of rivers and streams had poor quality in 2013–2014, up from 47 percent in 2008–2009 (EPA, 2017). U.S. Geological Survey (USGS) trend analysis for the years 1992–2012 showed that while nitrate (NO₃), TP, ammonium (NH₄), soluble reactive P (SRP), and TN were decreasing at a majority of urban sites, there was a lack of nutrient reduction at agricultural sites (Stets and others, 2020). An important question remains as to whether nutrient reduction efforts at agricultural sites have not been large enough, or whether legacy nutrients are causing a lag in river response to those efforts (Stackpole and others, 2019).

Eutrophication and other issues concerning surface-water quality are often assessed in the context of a single constituent; for example, TN or TP commonly is targeted for total maximum daily load (TMDL) regulation to address impaired waters, whereas other factors such as temperature, dissolved oxygen (O), fine sediment, dissolved and particulate organic carbon (DOC, POC), and antibiotics are not addressed. Also not addressed are the complex interactions among multiple biogeochemical processes and flow-related physical drivers (for example, water residence time, mixing and stratification, and oxygen reaeration) that influence the onset, severity, duration, and frequency of eutrophication events. A broad array of biologically mediated N cycle processes (including nitrification, denitrification, and microbial respiration), along with organic carbon (OC) reactions, strongly influence the ecological health of aquatic systems. These coupled or linked physical and biological reaction processes and their effects on the fate of constituents and on ecological health rarely are considered in regulatory measures to protect ecosystem health. Nevertheless, the coupled cycling of constituents and their interactions with physical mixing and gas exchange is foundational to water quality and ecosystem health (see fig. B1).

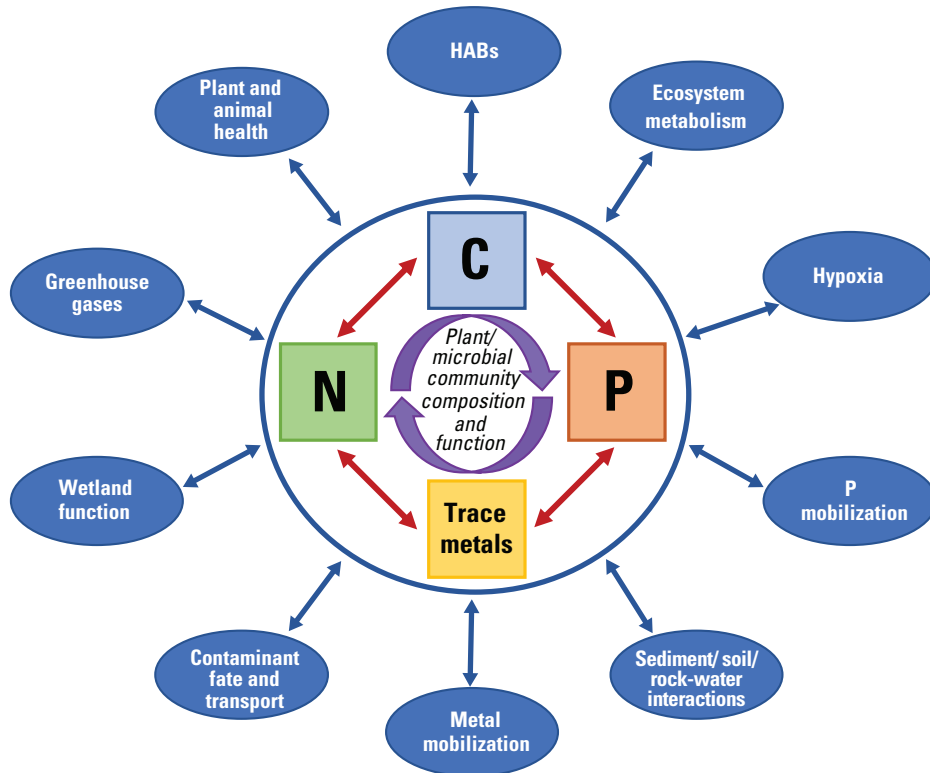


Figure B1. Diagram of coupled nutrient-carbon processes and their effect on aquatic eco-health. Inner circle coupled interactions (red arrows) have substantial consequences and feedback on key ecosystem functions and characteristics (blue arrows). Modified from Finzi and others (2011). C, carbon; N, nitrogen; P, phosphorus; HABs, harmful algal blooms.

An example of a coupled reaction is the linked mineralization reactions of an organic contaminant, which often result in the production of a related degradate compound, rather than complete disappearance of the parent compound. Coupled redox reactions can affect contaminant degradation, sequestration, and transport. For example, iron (Fe) oxidation coupled to nitrate reduction produces Fe oxides that sequester arsenic (As) and P. Sulfate reduction rates are a key consideration in modeling of mercury (Hg) cycling in streambeds (Marvin-DiPasquale and others, 2009). Another example is nutrient redox reactions that potentially affect reaction pathways and the form of gaseous end-products, such as nitrous oxide (N_2O), that affect greenhouse gas emissions, or the redox state of Fe that may lead to P sequestration by attachment to sediments under oxic conditions or release under anoxic conditions that can fuel harmful algal blooms (HABs). Process interactions can be either synergistic or antagonistic, resulting in positive or negative effects. For example, antibiotic inhibition of microbial processes in watersheds can inhibit or delay biogeochemical processes considered relevant to ecosystem health and can couple with hydraulic transport to downstream reaches, where subsequent processes may differ from upstream processes. Coupled constituents and processes are relevant to all stakeholders concerned with ecosystem health and can be considered

essential for modeling the overall effect of water quality and contaminants on ecosystems and for addressing impairment of aquatic ecological health (Finzi and others, 2011; Bruggeman and Bolding, 2014).

Status of Knowledge and Key Limitations

A summary of C-N-P constituents, their relevance to aquatic ecosystem health, and stakeholder level of concern is provided in table B1. The table provides information about primary constituents of concern, connections to potential aquatic and human impairments, and EPA rankings under nationwide Clean Water Act of 1972, in section 303(d) (CWA; 33 U.S.C. 1251 et seq.; <https://www.epa.gov/tmdl>) listings (EPA, 2017).

There has been substantial progress in characterizing sources and predicting the movement and attenuation of key constituents, particularly N, across the landscape and through surface water networks (National Research Council, 2002; Schmadel and others, 2019). Ecosystem syndromes such as excessive algal growth, high turbidity, hypoxia, and HABs often result from the combined effects of excess N, P, and OC coupled with high temperature and long residence times

Table B1. Summary of nutrient-carbon constituents, relevance to aquatic ecosystem health, and previous findings from the U.S. Environmental Protection Agency rankings (2017).

[DOC, dissolved organic carbon; TOC, total organic carbon; O, oxygen, Cl, chlorine; EPA, U.S. Environmental Protection Agency; HABs, harmful algal blooms; 303(d), Clean Water Act Section 303(d); TDN, total dissolved nitrogen; TN, total nitrogen; %, percent; NH₄, ammonium; N, nitrogen; NH₃, ammonia; mg/L, milligrams per liter; NO₃, nitrate; Fe, iron; P, phosphorus; TP, total phosphorus; TDP, total dissolved phosphorus or orthophosphate.]

Constituent	Relevance to aquatic ecosystem health	Previous findings ¹
DOC, TOC: including particulate organic carbon derived from in-stream and terrestrial sources strongly influenced by flow, agriculture, and urbanization; often stormflow generated from soils, wetlands, ponds, and reservoirs.	Fuels bacterial respiration which consumes O and may contribute to hypoxia; may affect nutrient availability for algae growth; binds with metals; reacts with Cl to form carcinogenic disinfection byproducts.	EPA does not specifically rank these constituents, but implicitly DOC and TOC are linked to nutrient, O, and eutrophication concerns.
Nutrients: fertilized agricultural and suburban areas, livestock pens and waste ponds, wastewater treatment plants, sewer, and septic.	Can lead to HABs, hypoxia/anoxia.	EPA-303(d): Ranks 2nd in national ranking of reported impairments; Ranks 6th as a “top 5” concern in 19% of large river basins.
TDN/TN: includes sources above as well as atmospheric deposition.	Can lead to HABs, hypoxia/anoxia.	EPA found 43% of national stream miles were of poor quality for TN in 2013–2014 and no change over 2008–2009.
NH ₄ : fertilizers, decomposition of organic matter, gas exchange, animal and human waste, N fixation, municipal effluent, atmospheric N deposition.	Toxic buildup in tissues and blood; pH and temperature can affect toxicity; can lead to HABs.	EPA: NH ₃ can be toxic to aquatic plants, invertebrates, and fish at concentrations less than 1 mg/L.
NO ₃ : fertilizers, animal and human waste, municipal effluent, atmospheric N deposition.	In drinking water—in the body, nitrate reduced to nitrite which reacts with Fe in hemoglobin causing methemoglobinemia (blue baby syndrome); can also lead to HABs.	EPA: NO ₃ , in conjunction with P, accelerates eutrophication; can lead to changes in aquatic species composition.
TP, TDP: Wastewater treatment; fertilizer; septic systems; animal manure runoff; also includes geogenic sources.	Can lead to HABs, increased biological O demand.	EPA found 58% of national stream miles were of poor quality for TP in 2013–2014, up from 47% in 2008–2009.

¹Information from EPA, 2017.

(Community Coordinating Group on Integrated Hydro-Terrestrial Modeling, 2020). Identifying and separating the role of contemporary and legacy sources of nutrients is a key concern. Distinguishing these sources requires understanding of complex hydrogeochemical interactions. For example, legacy P export depends on a soil’s mineral composition, organic matter content, and redox conditions (Stackpoole and others, 2019). Out-of-balance behaviors such as HABs suffocate aquatic communities, threaten water supplies and increase treatment costs, endanger recreation, lower property values, and pose long term challenges to biologically diverse and sustainable water resources. The USGS and other Federal agencies are only beginning to formulate an effective strategy for improving nationwide capabilities for predicting excess nutrients, hypoxia, and HABs (Community Coordinating Group on Integrated Hydro-Terrestrial Modeling, 2020).

A lack of understanding of coupled processes has resulted in substantial limitations for predicting and managing aquatic ecological health. For example, coupled nutrient-C reactions, ancillary physicochemical drivers such as temperature, redox, and pH, or coupling between physical processes such as residence time and reaeration of water bodies may have dominant outcomes for ecological health. “One of the most challenging steps in the development of coupled

hydrodynamic-biogeochemical models is the combination of multiple, often incompatible computer codes that describe individual physical, chemical, biological and geological processes” (Bruggeman and Bolding, 2004, p 249). Often there is a failure to recognize that N in the environment does not solely function as a nutrient as in the case where it is an electron acceptor in redox reactions. While individual N and P cycle processes are important, there are also abundant interactions with constituents that control their fate and transport throughout the hydrosphere by affecting N and P speciation and physical state. These processes often are coupled to the fate and transport of water quality constituents such as DOC and POC (Strauss and Lamberti, 2000). Stoichiometric relationships in biological uptake may drive proportional removal of N and P from flowing waters that lead to nutrient limitations for particular biological communities (Glibert, 2017). Furthermore, Fe and Mn precipitates on sediment that sequester or mobilize particular forms of N and P, subject to redox or pH conditions (Taguchi and others, 2020).

Microorganisms are coupled to numerous biogeochemical cycles in the environment (Falk and others, 2019) and microbial functional diversity is recognized among microbial ecologists as an essential link between biodiversity patterns and ecosystem functioning (Escalas and others, 2019). However, despite the fact

that biogeochemical cycles have coevolved and often directly affect the outcome of one another, these cycles and the associated microbial communities are often studied in isolation (Falkowski and others, 2008). Excessive nutrient and contaminant inputs to ground and surface waters from urban and agricultural land use activity can have dramatic effects on the microbial community composition and function and ultimately on water quality processes and ecosystem health. Microorganisms can serve as buffers against certain legacy pollutants or as bioindicators of stressed ecosystems. While the microbial community can adapt by using gene transfer mechanisms and functional redundancy, taxonomic and functional diversity indices can be used to assess increased or decreased stressors to a particular environment. “Investigations into active transcripts associated with nitrogen metabolism are key to understanding site-specific nutrient dynamics and ecosystem health” (Weisener and others, 2017, p. 702). By understanding how the microbial community responds to environmental stressors, researchers and managers can better predict how a particular environment will be able to respond to physical-chemical-biological drivers such as excess nutrients, chemical contaminants (for example, PFAS [per- and polyfluoroalkyl substances], antibiotics, nitrapyrin), and pathogens (Graham and others, 2016; Woodward and others, 2021).

Priority Knowledge Gaps in Coupled Nutrient-Carbon Processes Affecting Ecological Health

The USGS identified three main gap categories within this key topic. Detailed descriptions of each gap are presented in the following sections:

5. Multi-factor N, P, and labile C sources interacting with eco-flow drivers,
6. Redox processes controlling storage, processing, and release of N, P, Fe, As, and other contaminants,
7. Interaction between nutrient cycles and contaminants of emerging concern (CECs).

A summary of gaps, approaches, and expected outcomes is in table B2.

Gap 1. Multi-factor Nitrogen, Phosphorus, and Labile Carbon Sources Interacting with Eco-Flow Drivers

Key limitations in our understanding of coupled nutrient-carbon cycle processes include multi-factor N, P, and labile C sources and interactions with eco-flow drivers (includes river water residence time; reservoir storage; reaeration; water column light availability; associated thresholds controlling onset, severity, and duration of HABs and hypoxia/eutrophication and other adverse effects for ecosystems).

Spatial and Temporal Distributions of Labile Organic C Sources and Their Role in Coupled C-N-P Metabolic Reactions

Dissolved and particulate organic carbon (DOC, POC) are complex collections of thousands of different compounds with distinct chemical properties and turnover times that vary from minutes to millennia (Abbott and others, 2016). They provide a primary food source for food webs and can be highly reactive (“labile”). DOC and POC enter aquatic systems from the surrounding landscape with runoff and groundwater flows and are internally produced through in situ processes. Labile portions of OC are rapidly metabolized and assimilated as biomass or respired as CO₂ or methane (CH₄), while less labile OC persists and may accumulate or be transported to downstream environments. Metabolism of OC is tightly coupled with transformations in the N cycle, including denitrification (nitrate reduction to N₂) (Seitzinger, 1988; McClain and others, 2003), ammonification (Schlesinger, 1997), and nitrification (oxidation of ammonium [NH₄⁺] to nitrate [NO₃⁻]) (Strauss and Lamberti, 2000, 2002). The availability of labile (aka “biodegradable”) OC at any given place or time has important implications not only for C dynamics but also for N dynamics of aquatic ecosystems (for example, Wickland and others, 2012). For example, the presence of relatively high concentrations of labile or biodegradable DOC (BDOC) can lead to anoxia and nitrate reduction to ammonium (dissimilatory nitrate reduction, DNRA). Intermediate concentrations of BDOC often result in anoxia to suboxia and nitrate reduction to N gas (denitrification), while relatively low concentrations of BDOC will usually only couple to O consumption (aerobic respiration). The latter case will only consume nitrate in an assimilatory capacity. Conditions can exist in which mixed couples occur together in competition with each other, such as DNRA and denitrification. Simply quantifying DOC concentrations alone will not resolve the relative biodegradability of the DOC or the extent to which the DOC-nitrate reduction couple (that is, the potential for nitrate removal) could be occurring. Coupling between C and P cycling in freshwater systems is relatively less understood. However, there is evidence of close coupling of these cycles in freshwater aquatic microbial communities where labile soluble organic C enrichment can enhance microbial alkaline phosphatase production and thus increase P remineralization by organo-phosphate hydrolysis (Anderson, 2018). A comprehensive assessment of labile OC sources and degradation rates in aquatic systems, and spatial and temporal variabilities in systems of interest is necessary to accurately model coupled metabolic reactions and nutrient dynamics.

Relation Between Watershed Carbon, Nitrogen, and Phosphorus Biogeochemical Processes and Development of Harmful Algal Blooms

Nutrient concentrations and speciation are considered to be key components in the development of HABs (Gardner and others, 2017). The relations between N speciation, N:P ratios, and algal blooms are poorly understood, however. Nitrogen

cycle processes can control N speciation in sediments (Li and others, 2019), across the groundwater-surface water interface (Harvey and others, 2013; Smith and others, 2019), in suspended sediment (Xia and others, 2017), and alter diel N oxidation-reduction processes in lake water (Chen and others, 2021). These processes can also affect P transport, as denitrifying bacteria can sequester P intracellularly (Zaman and others, 2021) and potentially translocate P downstream to receiving lakes and reservoirs. Most water quality studies document total P, but much less is known regarding partitioning between aqueous and sorbed P and between organic and inorganic P. The dynamic nature of N:P ratios can alter the biodiversity of the microbial community, growth rates and trophic interactions. Yet, an understanding of which forms of N and P have the greater effect (as well as when and where) remains elusive. More research could incorporate nutrient inhibition, external stresses (light, temperature), and the resultant physiological response (Glibert, 2017). Furthermore, the development of hypoxia is related to high O₂ consumption during decomposition of OC from high algal production and leads to more acidic waters from the very high levels of CO₂ that are produced (Howarth and others, 2011). Perturbations to the carbonate cycle have been shown to affect the health of aquatic organisms particularly in estuarine systems (Green and others, 2009), but the full effects are not well known. Detailed understanding of N, P, and C sources, speciation, and processes in both surface and groundwater (Brookfield and others, 2021) could be developed in conjunction with studies monitoring HABs occurrences in lotic and lentic waters.

Beyond Streamflow for Prediction of Ecologically Relevant Flow and Physical Metrics

Streamflow is important for predicting water and constituent conveyance to downstream areas as well as quality of ecological habitat (Poff and others, 1997; Arthington and others, 2010; Eng and others, 2013). However, streamflow is far from complete as a predictor of the physical controls on water-quality and ecological functions of river corridors (Dunne and others, 1998; National Research Council, 2002; Jones and Stanley, 2016; Appling and others, 2018). For example, there is a gap in estimating river travel time (and average reservoir residence time) along with associated mixing and oxygen reaeration processes, and flood inundation processes (Scott and others, 2019) which have an important role in models of water-quality and ecological functions of the Nation's rivers and reservoirs (Harvey and Gooseff, 2015; Harvey and others, 2019; Harvey and Schmadel, 2021).

Gap 2. Redox Processes Controlling Storage, Processing, and Release of N, P, Fe, As, and Other Contaminants

This gap involves redox processes controlling storage, processing, and release of N, P, Fe, As, and other contaminants. This includes physical drivers (for example, reaeration), geochemical (for example, contact with geochemically reducing

sediments), and biological (for example, high labile carbon inputs) interactions in wastewater and septage ponds managed for water reuse, river-bank filtration zones, sediment retention ponds, reservoirs, and so forth.

Nitrogen, Phosphorus, Iron, and Arsenic Interactions in Wastewater and Septage Disposal

Nitrogen and P are common constituents in wastewater and septage disposal. The oxygen consuming capacity of those waters often results in anoxic zones in which P and geogenic As and Fe are solubilized and mobilized as a result of these disposal practices. If nitrate enters these zones, nitrate reduction can be coupled to Fe oxidation, leading to anoxic production of Fe oxides, which have the capacity to remove As, P, and other organic contaminants that can sorb onto the Fe oxides (Smith and others, 2017; Jamieson and others, 2018; Liu and others, 2019). Iron oxidation at oxic-anoxic boundaries can have the same effect, producing Fe oxides resulting in co-contaminant removal at the boundary interface. Thus, assessment of coupled processes is an important component for understanding co-contaminant transport and interaction in key water supply sources, wastewater disposal and reuse practices, wetlands, and groundwater-surface water interfaces. Studies could be done to determine rates of reaction, the sorption capacity, long-term fate of the sorbed constituents, and the extent to which Fe oxidation coupled to nitrate and (or) oxygen reduction occurs throughout the Nation's water supplies.

Gap 3. Interaction Between Nutrient Cycles and Contaminants of Emerging Concern

Interaction between nutrient cycles and contaminants of emerging concern (CECs), includes domestic and agricultural antibiotic effects on biogeochemistry, ecosystem metabolism, and co-contaminant fate and transport (such as microplastics effects on C and N processes).

Effects of Antibiotics (Domestic and Agricultural) on Water and Sediment Biogeochemistry and Co-Contaminant Fate and Transport

Natural and constructed wetlands are now used extensively across the United States as a means for mitigating nitrate losses to both surface and groundwater. While the use of wetlands as a treatment approach for nitrate-N is well known, the rates of nitrate removal and the ultimate fate of the nitrate-N are highly variable. Furthermore, N is not the sole contaminant that wetlands encounter. The presence of waterborne antibiotics, originating from both domestic and agricultural sources, is becoming increasingly common and can have an important effect on N cycle processes (Zou and others, 2019; Xu and others, 2020; Xu and others, 2021). The environmental consequences of these biologically active

compounds on the fate and transport of N can potentially be significant but are only beginning to be investigated. Controlled laboratory and field studies could be done to document the effect of antibiotics on the relative rates of N cycling processes in aquatic systems.

The effects of antibiotics on microbial communities are complex. Antibiotics can have a direct effect on the microbial community structure and also on the development and transfer of antibiotic resistance genes (ARGs), which have been considered pollutants of concern in recent years (Pruden and others, 2006). Chemical pollution (for example, antibiotics, heavy metals, biocides), physicochemical factors (for example, DO, salinity, TDN [total dissolved N]), and human activities (for example, wastewater treatment plants, agriculture, hospitals) can have dramatic influences on the propagation of ARGs (Yang and others, 2018). Studies focusing on antibiotics and nutrients could include approaches for determining the responses of microbial communities.

Interaction Between Nitrogen Cycle Processes and Contaminants of Emerging Concern

A wide variety of CECs (defined here as including pharmaceuticals, agricultural products, and personal care products) and organic micropollutants (OMPs) occur in the Nation's waters (Kolpin and others, 2002; Luo and others, 2014). Little is known about the effect of these chemicals on N cycle processes or, conversely, the potential for N redox reactions to degrade or assist in the degradation of CECs and OMPs in the environment. Those interactions that do occur could potentially be important for understanding the fate and transport of CECs and OMPs. Systematic studies including data reviews and lab experiments could be done to explore potential interactions. Nitropryrin is one CEC that is known to inhibit nitrification and has been detected recently in rivers and streams in Iowa (Woodward and others, 2016). The presence of nitropryrin and (or) its degradants in a stream could affect N speciation, depending on the nitropryrin concentration, with concomitant effects on N transport, microbial community structure and function, and downstream eutrophication. Nitrification has also been shown to biotransform OMPs containing alkyl, aliphatic hydroxyl, ether, and sulfide functional groups as well as substituted aromatic rings and aromatic primary amines (Su and others, 2021).

Effect of Microplastics on Carbon, Nitrogen, and Phosphorus Biogeochemical Processes

Evidence suggests that micro and nanoplastics are abundantly present in the environment (Adomat and Grischek, 2021; Lenaker and others, 2021) and that they can affect biogeochemical processes in sediment and wetland N cycling (Seeley and others, 2020; Zaman and others, 2021). The USGS has the in-house expertise to carry out experimental dosing studies to examine the effect of microplastics on N cycle processes. A wide variety of parameters could be

quantified using microcosms, mesocosms, or contained in situ chambers to assess N cycling, C cycling, greenhouse gas emissions, primary productivity (that is, relative to HABs), redox chemistry, macro fauna uptake, and trace element chemistry in the presence of microplastics. Added to that could be an examination of responses by different sediment or environment types or differences in other water quality parameters. These experimental studies are needed in the short-term to guide large-scale monitoring of plastic contaminants and to provide parameters and thresholds needed for modeling the effects on water quality.

Approaches to Address Gaps in Coupled Processes Affecting Water Availability

Below we provide four suggested approaches that potentially can be used to address the coupled-process gaps described in the previous section. The range of potential approaches are generally the same for each of the identified gaps; approaches are listed in order of scale, starting with microbial community-level assessment of the drivers, mechanistic studies of coupled processes, then working up to the ecosystem level using comprehensive measurements of coupled constituents at fixed stations, and finally to broader regional-scale assessments mapping N, P, C, and related eco-flow metrics across multiple scales. A final selection of approaches might involve one or more elements of each that will need to be tailored for any particular gap. A summary of gaps, approaches, and expected outcomes is provided in table B2.

Microbial Profiling

The use of metatranscriptomics (also known as “gene expression”) has become more common these days, going beyond determination of microbial community composition (metagenomics) and functional gene abundance (potential metabolism) to measuring gene expression to determine whether genes are being actively expressed or suppressed under specific environmental conditions. Whereas environmental deoxyribonucleic acid (DNA) sequencing can indicate the presence of a functional gene or a functional group of microbes, environmental ribonucleic acid (RNA) and gene expression (analysis of mRNA) indicates the active community and processes that are occurring within an environment, providing an assessment of the functional diversity of the community (Cristescu, 2019; Escalas and others, 2019; von Ammon and others, 2019; Cordier and others, 2021). These tools can be used to assess multiple biogeochemical processes and communities and link microbial functions to C, N, and P cycling processes. For example, coupling of chemical datasets with metatranscriptomics has been used to measure the response and coping mechanisms of the microbial community to xenobiotic stressors in sediments collected from the Detroit River (Falk and others, 2019).

Table B2. Summary of prioritized U.S. Geological Survey (USGS) knowledge gaps, approaches, and expected outcomes for water quality drivers of aquatic ecosystem health.

[C, carbon; N, nitrogen; P, phosphorus; HABs, harmful algal blooms; NO₃, nitrate; O, oxygen; Fe, iron; As, arsenic; CECs, contaminants of emerging concern; chl-a, chlorophyll a.]

Gap	Gap elements	Why	Approaches	Outcomes	References
Multi-factor N, P and labile C sources interacting with eco-flow drivers	Dominant sources of labile organic C. C, N, P biogeochemical processes that promote HABs and hypoxia. Need for ecologically relevant flow metrics beyond streamflow that predict eco-health (for example, river residence time, gas exchange, light attenuation).	C lability couples with N, P dynamics, affecting anoxia, HABs, and (or) NO ₃ removal. River residence time, mixing, and reaeration effects of dissolved O availability, redox status, contaminant concentrations, and balance between primary productivity, respiration, and nutrient reactions that influence outcomes for eco-health.	Coupled process and machine learning modeling studies to predict impact and recovery times of biological community structure and function following physical or water-quality disturbances. Microbial profiling to link microbial community function with processing of N, P, contaminants.	More accurate assessment and modeling of HABs, hypoxia, water quality, and ecological functions.	Wickland and others, 2012; Gardner and others, 2017; Harvey and Schmadel, 2021.
Redox processes controlling storage, processing, and release of N, P, Fe, As, and other contaminants	Wastewater/septage disposal controlling downstream redox conditions and N, P, Fe, As cycling. Redox controls on mobility and bioavailability of N and P.	P, As, Fe can be mobilized or removed from wastewater sources, depending on nutrient concentrations, oxic and anoxic status.	“Fast mapping” of streambed redox status and related eco-flow drivers (river residence time) to build eco-metrics for model inputs.	Improved modeling of contaminants relative to drinking water sources and wastewater reuse.	Smith and others, 2017; Jamieson and others, 2018; Li and others, 2019.
Interaction between nutrient cycles and CECs	Effect of antibiotics on water/sediment biogeochemistry. Interactions with N cycle controlling CECs. Effect of microplastics on C and N biogeochemical processes.	Antibiotics affecting pollutants of concern; N redox reactions may degrade CECs; microplastics affect nutrient biogeochemistry, potentially affecting HABs formation.	Integrated eco-health metrics (sensor-based metabolism and remote sensing of turbidity and chl-a) combined with eco-flow metrics (for example, river residence time, gas exchange, light attenuation) as proxy indicators of water quality threats to ecological health. Comprehensive measurement of water-quality and eco-health predictors (dissolved oxygen in addition to temperature, nutrients, and specific conductance) to better serve stakeholder needs.	Improved prediction of N loads; improved understanding of CEC degradation; guide large-scale monitoring of microplastics	Pruden and others, 2006; Luo and others, 2014; Adomat and Grischek, 2021; Xu and others, 2021.

8 Water-Quality Processes Affecting Aquatic Ecosystem Health

Metatranscriptomics in combination with metagenomics can be used to assess changes to microbial processing of nutrients and other contaminants resulting from agricultural runoff, fires, HABs, other chronic or extreme events, and provide information on linkages between physical, hydrologic, and chemical processes. It can be used to answer questions such as:

- Does repeated or seasonal exposure to certain chemicals, such as seasonal application of agricultural chemicals, eventually lead to adaptation by the microbial community?
- How does the function of the microbial community change in the presence of mixtures of contaminants?
- How quickly does the microbial community recover after extreme events, such as floods, wildfires, and so forth?

Mechanistic Studies of Coupled Processes

Quantitative information about kinetics of coupled processes and environmental factors that control process rates is essential information for predicting and understanding the function of the processes relative to water quality assessment and ecosystem health. Mechanistic studies of coupled processes can be conducted in controlled laboratory settings using freshly collected environmental materials (soil, sediment, water, biofilms, and so forth) to quantify potential rates of reactions, competition between various reaction couples, and responses to experimental manipulation (for example, temperature, substrate concentration, presence of other contaminants). These experiments control environmental variables that might occur across time and space within a watershed and quantify process response. Such results are extremely important for interpreting bulk changes in water and sediment geochemistry and for predicting effects of disturbances or other environmental changes. Field experiments, utilizing chemical and isotopic tracers, and carried out in conjunction with laboratory experiments, could provide invaluable information regarding in situ processes and rates. Reach-scale and hyporheic zone field experiments using conservative and reactive tracers have been used to measure denitrification rates (Böhlke and others, 2009; Harvey and others, 2013) as well as methane and nitrous oxide emissions (Smith and Böhlke, 2019) in streams heavily affected by agricultural inputs. Systematic experimental sampling across a watershed can incorporate process differences related to hydrology, landscape setting, groundwater inputs, sediment types, and contaminant exposures. Such an approach is also needed to integrate and scale up the effects of coupled processes from local to more regional settings, which is a major gap in our understanding of the role that coupled processes have in region-wide ecosystem health.

Comprehensive Measurements of Coupled Constituents at Fixed Stations

The USGS currently operates approximately 2,100 water quality stations around the country, with more to be added under

the Next Generation Water Observing Stations (NGWOS) program. These stations can be equipped with a wide array of instrumentation that encompasses the range of parameters of interest in multi-constituent process studies, including multi-parameter sondes measuring water temperature, pH, specific conductance, dissolved O, turbidity, fluorescent dissolved organic matter (FDOM), and chlorophyll coupled with dissolved nitrate, phosphate, and carbon dioxide sensors. While NGWOS stations often have the purpose to integrate signals over large river basins, focus could also be directed towards comparing small watersheds with contrasting characteristics. Comprehensive measurements at both NGWOS and smaller scale watersheds could improve understanding and parameterization of watershed responses to N-P-C drivers. High frequency continuous sensor output measurements can help resolve processes such as metabolism, gas emissions, and so forth, which vary over short time scales (diel, high-flow, seasonal) that may not be resolved with discrete sampling protocols. Increasing and improving discrete sampling and analysis is also needed, however, to improve integrated constituent studies. Discrete sampling is essential for measuring important and informative chemical parameters (for example, isotopes of various constituents) that cannot be analyzed continuously or remotely. Added to this will be demands for new analytical capabilities as new contaminants of concern arise. The wealth of information collected from NGWOS stations and small watersheds can be leveraged with mapping and lab/field process studies in the two approaches described above and in the next approach to provide modelers with information crucial to model development, and to provide stakeholders with the data and interpretation necessary to make informed decisions.

Mapping of Nitrogen, Phosphorus, Carbon, and Related Eco-Flow Metrics Across Multiple Scales

The USGS NGWOS fixed-location water quality stations do not represent the full continuum of stream reach conditions (Crawford and others, 2016). A multi-scale approach could adequately address nutrient and C processing in rivers from the small watersheds hosting a single fixed-station to large basins hosting a number of fixed-stations scale. This scaling approach could provide modelers and stakeholders with an integrated “picture” of nutrient-C processing to facilitate model development and decision making. Better information about the geographic setting of freshwater resources is also needed to enhance our understanding of the scale, rate, and consequences of coupled biogeochemical reactions. The ongoing expansion of data collection including standard techniques along with rapidly developing new laboratory capabilities, high-frequency continuous sensor output and high-spatial resolution tools, remote sensing, and unmanned vehicles offers an unprecedented opportunity to describe aquatic resources more fully across the United States.

The tools that are currently available for estimation of river travel time and reservoir residence time throughout the United States are outdated and based on limited data sources (Harvey and Schmadel, 2021). Travel time estimates are

essential for tracking how water-quality disturbances are propagated to downstream areas. Numerous stream tracer tests have been carried out in the past 60 years in the United States, but results have not been synthesized and analyzed to produce a model estimator for downstream propagation, dispersion and dilution, and for estimates of the time required for water-quality disturbances such as river spills to clear from the river corridor. Improved travel-time estimation can advance the prediction of water availability at any scale. Examples include (1) apportioning river contaminant loading and removal rates across watersheds, states, and regions; (2) incorporating contaminant legacy storage times and releases back to river corridors in water quality models; and (3) predicting downstream effects of water quality disturbance with early warning for downstream water users.

Remote sensing has been used to measure stream parameters such as width, depth, and streamflow, but recent advances in sensor technology and data processing are setting the stage for an expansion of remote sensing as a tool for mapping water quality from the scale of rivers down to a few meters (Tomsett and Leyland, 2019; Topp and others, 2020). Sensor data is becoming more available from platforms including satellites, manned aircraft, and uncrewed aerial vehicles (UAVs or drones). Published research has already shown the promise of remote sensing in modeling N and P (Lillesand and others, 1983; He and others, 2008; Torbick and others, 2013), dissolved O (Wang and others, 2004; Toming and others, 2016), and heavy metals (Choe and others, 2008; Fichot and others, 2016). Remote sensing has also been used to map chlorophyll a (Duan and others, 2007) and harmful cyanobacteria (Kudela and others, 2015; Oyama and others, 2015), total suspended sediment (Telmer and others, 2006; Riaza and others, 2012; Brando and others, 2015), colored dissolved organic matter (CDOM; a proxy for DOC and possibly methylmercury) (Griffin and others, 2011; Fichot and others, 2016), and water clarity (Olmanson and others, 2008; Sheela and others, 2011). While limitations with these technologies exist regarding repeatability, accuracy, and data processing, we are in a “golden age” of remote sensing as it applies to rivers (Tomsett and Leyland, 2019), and the USGS has the expertise to meet these challenges and be a leader in this field. Remote sensing of water quality needs continual improvement through ground-truthing measurements. This is another area where USGS has led the field, demonstrating the utility of high-speed high-resolution mapping of rivers for a number of water quality parameters (Crawford and others, 2015), including nitrate (Loken and others, 2018) and nitrous oxide (Turner and others, 2016). Boats can be equipped with water quality sensors that include temperature, conductance, O, pH, CDOM, turbidity, total algae, chlorophyll a, nitrate, ammonia, nitrous oxide, carbon dioxide, and methane. These boats equipped with multi-parameter sondes have the advantage of being able to map entire stretches of river such as the upper Mississippi (Crawford and others, 2016; Turner and others, 2016; Loken and others, 2018). The systems are mobile, allowing the mapping of river conditions across watersheds at

various scales. They can also be used seasonally and on short notice, such as during storm or flooding events, HABs, and contamination events, and they can be timed to coincide with airborne and satellite overflights. They not only provide ground-truthing for remote sensing but can locate hot spots of coupled nutrient-C cycling such as wastewater disposal sites, drinking water treatment facilities, and tributary confluences and provide a river-scale method for calculating greenhouse gas emissions. Combined with the nutrient-C process studies described in previous sections above, this multi-scale data collection/mapping effort aligns with USGS WMA program goals for aquatic eco-health and water quality management moving into the future.

Timelines

Proposed timelines of prioritized USGS approaches to closing knowledge gaps for water quality drivers of aquatic ecosystem health are summarized in table B3.

Near-Term (2-Year) Advancements

1. **Microbial Profiling:** Incorporate DNA and RNA sampling at USGS Integrated Water Science (IWS) basins, perhaps the Illinois River Basin where anthropogenically-affected waters and sediments are evident. These studies could be combined with the exploratory mechanistic studies of coupled processes to assess how the microbial community adapts to changing conditions by exploiting new pathways and (or) employing stress-response mechanisms. A few USGS labs currently have the tools and expertise to extract and analyze samples for DNA, RNA, and gene abundance (for example, Laboratory and Analytical Services Division [LASD], Geology Energy and Minerals [GEM], and Upper Midwest Water Science Center [WSC]). Microarrays or geochips can simultaneously measure the expression level of thousands of functional genes important to biogeochemical processes or can identify gene changes in response to contaminants or other organisms.
2. **Mechanistic Studies:** Focus on C-N-P coupling in detail in a selected WMA IWS basin with exploratory experiments to test the effects of target CECs and OMPs found in the basin. Emphasis could be placed on deriving rate constants, temperature and other seasonal effects, and key locations for biogeochemical processing in the watershed (headwater vs. large channel streams, bed sediment vs. suspended sediment, gaining vs. losing groundwater reaches). This would include collaboration with modelers to begin incorporating results into process/transport models.
3. **Comprehensive measurements of coupled constituents** at fixed stations, focusing on IWS basins: Conduct regular sampling and analyses of high-priority N, C, and

Table B3. Timeline summary of prioritized U.S. Geological Survey (USGS) approaches to closing knowledge gaps for coupled nutrient-carbon cycle processes and related ecological-flow drivers of aquatic health.

[yr, year; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; C, carbon, N, nitrogen; P, phosphorus; IWS, Integrated Water Science; CEC, contaminant of emerging concern; NGWOS, Next Generation Water Observing Stations.]

Advancements	Microbial profiling	Mechanistic studies of coupled processes	Coupled constituents at fixed stations	Mapping nutrients and carbon across multiple scales
Near-term (2-yr)	Include DNA/RNA sampling at an IWS basin site using microarrays to measure the microbial community response to environmental stressors.	Measure physicochemical parameters that affect C-N-P coupled rates at a select IWS basin site, incorporate results into process/transport models.	Identify reaches between fixed stations for intensive mechanistic studies, install “experimental sensors” at select stations, install wells to monitor groundwater inputs.	Analyze remote sensing-water quality data relationships to aid modeling, use new technological platforms to ground-truth models at select IWS basin site
Mid-term (2- to 5-yr)	Utilize predictive biology software to incorporate microbial physiology and community dynamics into reactive and process-based transport models.	Assess nutrient-CEC coupled processes, incorporate early results in models, select other IWS basins for study.	Carry out seasonal and diel studies using tracer tests to identify coupled biological and geochemical processes, permanently install experimental sensors at select stations.	Utilize the increased data and imagery collection by NGWOS stations to inform model predictions and stakeholders.
Long-term (10-yr)	Incorporate in situ automated molecular samplers into other IWS basin study sites	Expand lab quantification studies to other IWS basin sites, carry out field tracer tests to ground-truth process couple results and modeling efforts.	Expand capabilities to other NGWOS sites, incorporate measurement results into process-based models.	Use remote sensing and mapping platforms to map nutrients, C, and other parameters across multiple scales at all IWS basin sites

P constituents to complement and ground-truth sensor data at select fixed stations in NGWOS basins. Install “experimental sensors” that are not core NGWOS sensors at select fixed stations for testing and verification. Identify one or more reaches between fixed stations for intensive mechanistic studies to link sources and in-stream processes to observations at fixed stations across scales. Identify areas of regional groundwater inputs and design and install sampling wells and (or) multi-level samplers, along with streambed temperature arrays, at select input locations. This work could focus on IWS basins and can expand to other geographic areas of scientific interest.

4. Enhanced across-scale mapping of nutrients and carbon: Building on expanding USGS sensor networks, remote sensing offers low-hanging fruit including the AquaSat database (Ross and others, 2019), a set of 600,000 Landsat spectral reflectance measurements paired with field measurements of total suspended sediment, DOC, chlorophyll a, and secchi depth. This data set could be mined to examine remote sensing-water quality relationships, and aid in processing and modeling approaches. In addition, boats could be easily outfitted to run as high-speed measurement platforms to begin ground-truthing and creating heat maps for decision makers.

5. Assimilation and analysis of underutilized channel corridor data to estimate ecologically relevant flow and physical metrics beyond streamflow: Across-scale estimation of eco-flow metrics in all rivers and reservoirs of the Nation would be instrumental in coupled nutrient-carbon-and eco-flow modeling that makes use of the enhanced sensor networks and remote sensing described above in item 4 “Enhanced across-scale mapping of nutrients and carbon.” Priority eco-flow drivers beyond streamflow include:

- River and reservoir travel time (residence time) as well as river and reservoir depth and volume as they vary with streamflow;
- longitudinal mixing by dispersion as well as vertical water mixing which influence dilution of constituent concentrations and contact with reactive bed sediments;
- turbulence and vertical mixing and its effects on oxygen reaeration which lessens metabolic stress on organisms and decreases the probability of hypoxia;
- off-channel exchange flows that lengthen the travel time and activate water-quality and ecological functions of backwaters, riparian wetlands, and floodplains, including providing low-flow habitat and prolonging low-flow

season return flows from near-stream (riparian and floodplain) storages;

- contaminant releases from legacy storage areas near or within the river corridor that are currently unaccounted for in water quality models are needed to prioritize land conservation versus instream restoration practices; and
- light availability within water column influenced by turbidity and riparian and topographic shading, substantially affects algal growth and potential for HABs.

Mid-Term (2- to 5-Year) Advancements

1. Microbial profiling: Work with modelers to incorporate molecular results into reactive transport models. Predictive biology software such as KBase (Arkin and others, 2018) could be used for modeling microbial physiology and community dynamics.
 2. Mechanistic studies could include other process couples pertinent to the selected basin, such as exploring the effect of microplastics and antibiotics on N, P, and C cycling as well as targeted studies during HABs events. Initial modeling using the first 2-year results could be conducted and compared with basin water quality data to test the development of the modeling and provide feedback on laboratory measurements and approaches. Also, selection of additional IWS basin(s) for expanding the study could be conducted.
 3. Comprehensive measurements of coupled constituents at fixed stations: Analysis of reaches between fixed stations selected for detailed study, which would include comprehensive seasonal and diel investigations with tracer tests to identify coupled biological and geochemical processes in water and sediments, biological communities, and physical attributes of reaches. Permanent installation of “experimental sensors” at select fixed stations after verification and ground-truthing.
 4. Mapping of nutrients and carbon across multiple scales: There will likely be significant advances in remote sensing of water quality parameters in the next five years, hopefully with progress in technical capabilities and processing capacity. The potential exponential increase in available imagery over that time frame, combined with a new influx of data from NGWOS stations and fast limnology missions, could provide massive amounts of information with which to inform modelers and decision makers.
2. “For aquatic ecosystems especially, the next breakthrough of this revolution is now expected to be the development and deployment of low-cost, automated and miniaturized in-situ environmental nucleic acids (eDNA and RNA) samplers (Carr and others, 2017; Gan and others, 2017). These may be integrated into autonomous instruments for broadscale and continuous ecosystem monitoring programmes (Brandt and others, 2016; Bohan and others, 2017; Aguzzi and others, 2019; Benway and others, 2019; Levin and others, 2019).”
 3. Mechanistic studies could include expanding the laboratory quantification efforts to other IWS sites, conducting field tests with tracers to ground truth the combined laboratory/modeling results, more detailed examination of process couples that were deemed relevant in the earlier tests, and application of the model(s) to other data-rich sites where laboratory process assessments have not been conducted to test the suitability for extrapolation. Work with remote sensing and prediction models to coordinate experiments with HABs events.
 4. Comprehensive measurements of coupled constituents at fixed stations: Expand capabilities and measurements to other NGWOS sites for incorporation into statistical and process-based models.
 5. Mapping of nutrients and carbon across multiple scales: We anticipate that within 10 years the USGS could be in a position to take advantage of all remote sensing and high-speed mapping platforms.

Expected Outcomes

- Improved biogeochemical modelling from process studies that will lead to increased predictive capabilities;
- New tools and methodologies as a result of advances in remote sensing connectivity to aquatic eco-health metrics;
- Broad applicability and transferability across reach, basin, region, and national scales, beginning with a proof-of-concept approach in the Illinois River IWS basin;
- Serves WMA priorities through synergy with Integrated Watershed Availability Assessments (IWAA), IWP, and NGWOS program goals;
- Serves stakeholders and decision makers through improved mapping capabilities that help visualize annual, seasonal, and storm trends as well as identifying hot spots and other areas of concern.

Long-Term (10-Year) Advancements

1. Microbial profiling: Include the incorporation of in situ samplers into ecosystem monitoring programs. As pointed out in Cordier and others, (2021, p. 2940):

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