HYPOTHESIS

Chemodiversity in freshwater health

Dissolved organic matter may offer a way to track and restore the health of fresh waters

By **Andrew J. Tanentzap**[1] *and* **Jérémy A. Fonvielle**[2]

Freshwater ecosystems, such as lakes and rivers, have been deteriorating in many places worldwide (*1*). Growing research suggests that many aspects of freshwater health—that is, the maintenance of chemical, physical, and biological integrity—may depend on the composition of dissolved organic matter (DOM) (*2*). DOM consists of thousands of distinct organic compounds that mainly originate from different plant and animal remains. The resulting variety of compounds in DOM has been called chemodiversity to mirror the term biodiversity. Whereas past research tested how the environment shapes the chemodiversity of DOM (*3*, *4*), a new research frontier is understanding how healthy ecosystems may depend on chemodiversity so as to monitor and reverse declines in freshwater health.

The health of fresh waters, as in this small river outside Kuujjuaq, Canada, can be tracked by measuring chemodiversity.

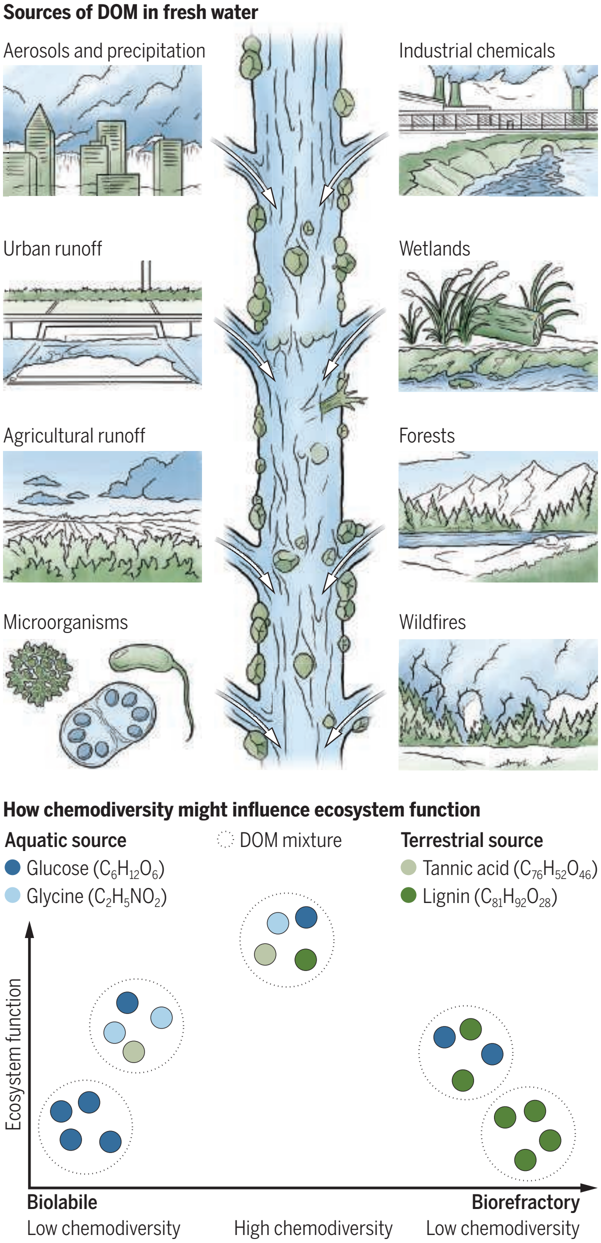
Four main lines of evidence indicate that different types of DOM influence the functioning of ecosystems. DOM from the land around fresh waters can supply nutrients to subsidize food web productivity (*5*). The elements bound to DOM, such as nitrogen and phosphorus, may result in undesirable effects, such as promoting concentrations of harmful bloom-causing algae species that impair water quality. Additionally, DOM can either reduce or enhance the toxicity of contaminants. For example, the highly toxic organometallic pollutant methylmercury, which is derived from natural and anthropogenic sources, can vary in bioavailability and accumulation through aquatic food webs owing to the composition of DOM. Thiols (sulfur-containing analogs of alcohols) can bind to methylmercury and reduce its bioavailability, and the concentration of thiols varies by many fold in the environment (*6*). Moreover, DOM can change the metabolism of microorganisms in ways that influence wider biogeochemical cycles. Heterotrophic microbes are the main consumers of DOM and annually respire 1.2 to 3.3 Pg carbon (1 Pg = 10^{15} g) of terrestrially derived DOM as carbon dioxide to acquire energy [adenosine triphosphate (ATP)] from organic compounds (*2*). However, compounds vary widely in the energy they release during degradation. Some compounds, such as those with greater ratios of oxygen to carbon atoms, require more energy to degrade, and so result in more respiration (*7*). Lastly, some types of DOM can modify the environment by reducing heat penetration into deep waters. Cooler deepwater temperatures can offset impacts of climate warming that reduce habitat for cold-water species and promote temperature-dependent heterotrophic respiration and release of greenhouse gases to the atmosphere.

DOM is thought to affect the functioning of fresh waters through the diverse characteristics of individual compounds, such as proteins, lipids, and lignin (which adds structure and strength to plants). Compounds vary in characteristics that include their elemental composition, chemical structure, and biological and photochemical reactivity. For example, some compounds, such as many carbohydrates produced by algae, are "biolabile" because they are more thermodynamically favorable for microbial degradation or meet microbial resource needs versus more "biorefractory" compounds, such as lignins produced by higher land plants (*8*). Therefore, DOM that is dominated by more biolabile compounds can be more quickly transferred into microbial biomass or provide energy to break down biorefractory compounds, thereby changing water clarity and carbon fluxes from fresh water (*9*). Any compounds chelated to DOM, including contaminants, may also be more quickly transferred to higher trophic levels in the food web. By contrast, DOM that is dominated by more biorefractory compounds may result in carbon accumulating in waters at higher concentrations (*10*). Because different sources of DOM (e.g., runoff and algal blooms) and modifiers of DOM (e.g., temperature, photoirradiation, and microorganisms) vary from subdaily to seasonal timescales, chemodiversity may also be very sensitive to change and thus an ideal measure of ecosystem health (see the figure).

Measuring chemodiversity can help predict the role of DOM in the environment. Traditional approaches to study DOM, such as those based on optical qualities of fresh water, measure the bulk properties of DOM. These approaches provide useful, albeit general, information about the composition of DOM, but are unable to resolve the origin and fate of individual compounds. Such approaches can therefore mask the influence of distinct compounds if most of the other compounds in a mixture are different. For example, optical properties such as fluorescence and absorbance may identify a large fraction of DOM as biolabile. However, the biolabile fraction may be associated with a few compounds that can undergo only highly specialized biochemical transformations by microbes, which optical properties cannot detect. This DOM mixture would have very different consequences for freshwater health (e.g., water clarity and food web productivity) compared with a mixture that had the same fraction of biolabile compounds, but which could undergo more numerous biochemical transformations.

The influence of dissolved organic matter on freshwater health

Dissolved organic matter (DOM) is a complex mixture of compounds from diverse sources in the catchment area of fresh waters. DOM undergoes biochemical modifications that influence physical, chemical, and biological processes such as nutrient cycling, carbon storage, light absorption, and food web interactions, which together determine ecosystem function. The chemical characteristics of DOM mixtures, called chemodiversity, can be determined by their sources and how labile the compounds are, which together influence ecosystem function and health.



High-resolution mass spectrometry is the state-of-the-art for measuring chemodiversity. By resolving the molecular composition of DOM, mass spectrometry can identify the characteristics of individual compounds, such as their thermodynamic traits and stoichiometry. This information can be used to summarize the variety and abundance of ecological roles (e.g., biochemical transformations, toxicity, and biolability) in chemodiversity and can be derived from measuring DOM rather than separately measuring many indicators of freshwater health, including organic nutrients, pollutants, and biological condition.

Measuring chemodiversity with high-resolution mass spectrometry also provides insight into the determinants of freshwater health. Common measures of ecosystem state (e.g., temperature, pH, and concentrations of nitrogen, phosphorus, and carbon) indicate the condition of a water body but not why a given state has arisen. Because different DOM sources have distinct composition, identifying the compounds associated with a given state can allow them to be linked to their sources. For example, a major tributary of Lake Erie shared nearly 30% more organic phosphorus compounds with cropland drainage than with wastewater effluent, suggesting that inorganic fertilizers were the main source of phosphorus pollution in this region (*11*). Although phosphorus concentrations can be detected with simple colorimetric reactions, it cannot be determined whether the phosphorus is derived from detergents in wastewater (characterized by phosphonic acids or linear alkyl benzene sulfonates) or agricultural runoff (enriched in tannins and lignins) (*11*). This information can only be revealed by measuring chemodiversity with high-resolution mass spectrometry, thus offering a more integrated way to assess the state and drivers of freshwater health.

Knowledge of chemodiversity measured with high-resolution mass spectrometry can add new information to improve freshwater health. For example, trace metals such as iron limit the growth of algae, including many toxic bloom-forming species. Trace metals are generally more available for uptake by algae when bound to organic compounds and form ligands. Measurements of iron concentrations or the optical properties of DOM cannot identify iron-binding compounds. By contrast, measuring chemodiversity can help determine whether iron is available for biological uptake and trigger interventions to reduce iron inputs, especially where these come from runoff.

Another example of the benefit of molecular-scale resolution comes from the discovery that a toxic compound from an anthropogenic source caused decades of unexplained mass die-offs in a socioeconomically important fish species (*Oncorhynchus kisutch*) in the Northwest US (*12*). The toxicant was a rubber antioxidant used in tires that washed into waterways from particles wearing off on roadways. The source of this toxicant could only be identified by comparing the chemodiversity of DOM of affected waterways with that of surrounding urban stormwater runoff.

There are other important benefits of resolving DOM at the molecular scale. Some organic compounds in DOM, such as polyphenols and highly unsaturated hydrocarbons, may be more likely to react with chlorine during drinking-water treatment to form by-products that are harmful to human health (*13*). However, the molecular identities of most of these by-products have not been described before. Only measurements of chemodiversity can start to identify these compounds, which is necessary to monitor their presence, identify their sources, and tailor treatments to reduce their presence.

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Monitoring of chemodiversity can be adapted for different aspects of freshwater health. For example, chemodiversity can be measured at subdaily or daily timescales to protect drinking-water quality, whereas seasonal measurements may be adequate to assess the state of food webs. By monitoring all compounds at once, it will also be possible to realize the benefits of both targeted approaches that track specific markers or contaminants and untargeted approaches that track the properties of DOM as a whole. Although there may be financial barriers to uptake, the per-sample cost of measuring chemodiversity is comparable to that of DNA sequencing, which is widely used for biodiversity monitoring.

Forecasting how the chemodiversity of DOM will affect freshwater health under global environmental change is an important future challenge. Climate warming has generally been associated with more biolabile compounds in DOM because heat directly promotes their production and decomposition by microbes, and indirectly promotes their production by changing land cover, such as favoring more nitrogen-rich vegetation (*8*). In cold climates, warming temperatures could also release previously preserved, highly biolabile compounds, such as carbohydrates, from thawing permafrost and melting glaciers (*14*). Linking freshwater health to changes in chemodiversity, such as from the release of biolabile compounds, would improve predictions of ecosystem impacts from environmental change. Similarly, the consequences of changes to precipitation regimes for chemodiversity and freshwater health remain poorly understood. Drought can extend water residence times that subject DOM to greater microbial processing and photochemical degradation (*2*, *14*), thereby leading to greater chemodiversity (*3*). However, drought can simultaneously reduce chemodiversity by suppressing runoff of terrestrial-derived DOM. Wetter climates with more flooding events may also have mixed outcomes by diluting DOM and increasing input from terrestrial sources.

Chemodiversity and thus the health of fresh waters may be further modified by other aspects of anthropogenic change. Examples include disturbances (such as wildfire and insect defoliation), eutrophication, acidification, land-use and land-cover change (including deforestation and urbanization), mining, and organic pollution (including synthetic polymers, per- and polyfluoroalkyl substances, chlorinated hydrocarbons, and improper wastewater discharge) (*14*). There are also longer-term questions about how heterotrophs might adapt to use new mixtures of DOM generated by environmental change. As microbes transform and release compounds into the environment, selection pressures on their metabolic pathways from changes in chemodiversity may feed back to alter the composition of DOM even further.

If chemodiversity is to be a useful measure of freshwater health, approaches are ultimately required to monitor and manipulate DOM at the scale of entire catchments. Although chemodiversity is measured at the molecular level, it reflects the contribution of different biogeochemical sources that supply a waterbody (*8*). Therefore, by characterizing different sources with high-resolution mass spectrometry and predicting their downstream mixing with hydrological and source-tracking models, chemodiversity and its implications for ecosystem health can be mapped across large spatial and temporal scales (*11*). DOM can also be manipulated at the catchment scale by cultivating different mixtures of vegetation around and within fresh water that contribute to downstream chemodiversity (*8*). For example, different plant lineages contain different metabolites in their leaves (*15*), which will leach into water from litterfall and soils, with different outcomes for ecosystem function and services. Ultimately, effective protection of fresh waters and their surrounding catchments can only emerge if the determinants of ecosystem health are understood. ■

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CELL SIGNALING

A mitotic stopwatch determines cell fate

Surveillance of mitotic timing prevents amplification of damaged cells

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Mitosis is a challenging time for cells owing to the potential for structural and numerical chromosomal abnormalities, termed chromosomal instability, which is a hallmark of cancer (*1*). In M phase, chromosomal instability is prevented by the spindle assembly checkpoint, which delays cell cycle progression until chromosomes are properly attached to spindle microtubules, ensuring proper separation into daughter cells (*2*). Recently, another M phase checkpoint was described: If a threshold mitotic duration is surpassed, proliferation of daughter cells is arrested in a manner that requires the tumor suppressor p53, the cell cycle inhibitor p21, ubiquitin-specific protease 28 (USP28), and p53-binding protein 1 (53BP1) (*3*–*6*). On page 1441 of this issue, Meitinger *et al.* (*7*) clarify how this process works, with extended mitosis leading to the assembly of a USP28-53BP1-p53 complex—the "mitotic stopwatch"—which mediates daughter cell arrest and may prevent cancer development.

Using human untransformed cell lines, Meitinger *et al.* show that increasing mitotic durations past a time threshold in the mother cell leads to progressively higher amounts of stopwatch complexes, resulting in p53 stabilization and high expression of p21 (a transcriptional target of p53) in the subsequent G_1 phase. Furthermore, the mitotic stopwatch complexes are stable enough to accumulate over multiple generations, enabling the arrest of cells that have experienced successive subthreshold extended mitoses. The outcomes experienced in G_1 because of prolonged mitoses (which vary between transient arrest, permanent arrest, or cell death) depend on the cell type being analyzed. In the case of transient arrest, however, the benefits of the "extra" time in G_1 and the