Chemical and microbial diversity covary in fresh water to influence ecosystem functioning

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Invisible to the naked eye lies a tremendous diversity of organic molecules and organisms that make major contributions to important biogeochemical cycles. However, how the diversity and composition of these two communities are interlinked remains poorly characterized in fresh waters, despite the potential for chemical and microbial diversity to promote one another. Here we exploited gradients in chemodiversity within a common microbial pool to test how chemical and biological diversity covary and characterized the implications for ecosystem functioning. We found that both chemodiversity and genes associated with organic matter decomposition increased as more plant litterfall accumulated in experimental lake sediments, consistent with scenarios of future environmental change. Chemical and microbial diversity were also positively correlated, with dissolved organic matter having stronger effects on microbes than vice versa. Under our experimental scenarios that increased sediment organic matter from 5 to 25% or darkened overlying waters by 2.5 times, the resulting increases in chemodiversity could increase greenhouse gas concentrations in lake sediments by an average of 1.5 to 2.7 times, when all of the other effects of litterfall and water color were considered. Our results open a major new avenue for research in aquatic ecosystems by exposing connections between chemical and microbial diversity and their implications for the global carbon cycle in greater detail than ever before.

carbon cycling | chemical diversity | microbial diversity | fresh waters

A handful of fresh water contains thousands of dissolved organic molecules of varying origin and composition (1, 2), but the biological significance of this chemodiversity remains largely unknown. Chemodiversity should have broad consequences for biogeochemical cycles (1), because variation in the composition of dissolved organic molecules influences the diversity, structure, and functioning of microbial communities (3–6). Microbial consortia also produce chemodiversity by degrading larger molecules into smaller structures and releasing new molecules into the environment, such as for communication and defense (7). These compounds can then feed back onto the composition of microbial communities (3). Recent advances in ultrahigh-resolution mass spectrometry (UHR-MS) coupled with next-generation sequencing now provide an exciting opportunity to test how chemodiversity and biodiversity are interlinked. However, this question has only been addressed in two estuarine systems (8, 9), and no study, to our knowledge, has integrated UHR-MS data with measures of both microbial and ecosystem functioning.

A central tenet in ecology—that more species coexist where there are more unique niches to be utilized (10)—can be extended to explain how chemodiversity and biodiversity may be associated. Broadly, the niche is defined by the requirements for a population to persist (11), and so one dimension of it should include the presence of other populations that facilitate these conditions. More microbial species can therefore be considered analogous to more niches that enable more chemical molecules to arise and coexist because the existence of many molecules depends on the presence of specific microbes that modify, uptake, and release organic matter (12, 13). Relatedly, for heterotrophic microbes, niches may take the form of molecular structures that offer different opportunities for decomposition and assimilation (14, 15). For example, dissolved organic molecules range from labile aliphatics to less reactive terrigenous phenolics (16). Different microbes will be associated with these different molecules (8), and so biodiversity should increase in more heterogeneous environments (9, 17).

Chemodiversity should ultimately relate to microbial and ecosystem functioning because greater biodiversity often promotes ecosystem functioning (6, 18–20). Biodiversity can explain increasingly more variation in ecosystem functioning where microbial functions are phylogenetically dispersed and facultative, as it will more closely reflect the diversity of traits present in communities (21). For example, microbial respiration from organic matter decomposition involves many pathways and potential species

Significance

Every drop of fresh water contains hundreds of different organic compounds, yet the biological role of this vast chemical diversity is largely a mystery. One hypothesis is that greater diversity may provide more opportunities for microbes to coexist, namely "diversity begets diversity." Here we find a close association between mixtures of chemicals and both the diversity of microorganisms in lake sediments and their potential to decompose plant litterfall. Increases in chemical diversity also elevated greenhouse gas concentrations by an average of 1.5 to 2.7 times under scenarios that simulated future environmental change. Overall, our findings advance our understanding of how life is connected to the chemical environment in ways that can influence important processes, such as carbon cycling.

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interactions, for example, breakdown of larger compounds by some taxa into smaller monomers for other taxa. Any change in biodiversity may therefore result in large changes in the processes that are represented in communities (7). This importance of biodiversity will diminish where taxa overlap functionally and show phenotypic plasticity (6, 22). Chemodiversity may also be a relatively stronger predictor of ecosystem functioning if it reflects abiotic conditions that govern biochemical processes [e.g., enzymatic decomposition (23)] and unmeasured biological variables (e.g., biomass) that explain additional variation to the effects of biodiversity (20, 21).

Here we tested whether chemodiversity was correlated with microbial diversity and which of these diversity measures explained more variation in greenhouse gas (GHG) concentrations in fresh water. Within small shallow lakes that dominate the world's freshwater area, littoral sediments can produce at least 1/4 of all CO_2, assuming similar surface areas of littoral and profundal zones (24–26). They can also produce more than 2/3 of all CH_4 (27). Much of this sedimentary CO_2 and CH_4 comes from terrestrial organic matter (tOM) that preferentially accumulates nearshore and contributes toward microbial decomposition and respiration (25, 27, 28). Sedimentary CO_2 production can also be stimulated by the release of organic matter and O_2 from macrophytes and periphyton in littoral zones (25), photochemical mineralization (29), and methanotrophy (30), and can also be consumed in clear waters with relatively high benthic primary production (26). For these reasons, we focused on measuring concentrations of dissolved gases at the sediment–water interface (i.e., the net outcome of production minus biological and photochemical oxidation), as opposed to microbial respiration alone. Because of the short distances (<0.75-m water depth), prevalent wave action, and relatively warm temperatures in the littoral zone that we study, much of this standing concentration is transferred from sediments to the atmosphere through diffusion, advection (e.g., resuspension), and ebullition (31–34).

We specifically tested the hypotheses that 1) greater chemodiversity in dissolved organic matter (DOM) should elevate microbial diversity because it provides more substrates that act as niches for decomposers, and 2) chemodiversity should be more strongly associated with GHG concentrations than microbial diversity. In addition to indirectly enhancing GHG concentrations by promoting microbial diversity, greater chemodiversity can provide more substrates for photooxidation that increases concentrations of CO_2 (35, 36) and other 1-carbon compounds (37). Both CO_2 and 1-carbon compounds such as formate, methanol, and methylamines ultimately act as terminal electron acceptors in the principal pathways of CH_4 production (38).

Our approach was to create gradients of chemodiversity within a common microbial species pool by adding tOM to artificial lake sediments in the littoral zone. Previous work has shown that chemodiversity correlates with the concentration and type of tOM in lake water (1), and both are expected to change in the near future. More fresh, particulate tOM accumulates in littoral zones as vegetation cover increases in surrounding catchments (39), which is occurring across most northern latitudes (40, 41). Future shifts in plant composition (42, 43) may also increase particulate tOM exported to littoral zones in addition to changing tOM quality (44). The accumulation of fresh particulate material in littoral zones contrasts with most other studies that focus on profundal sediments, where deposition primarily arises from flocculation of dissolved tOM (45). We also replicated the gradients in two lakes with contrasting light penetration to test how the associations between chemodiversity and biodiversity might change as northern waters darken because of increased tOM inputs (46). The experimental mesocosms mirrored the biogeochemistry of comparable sediment from the natural lakes in which they were deployed (47). Throughout, we defined chemodiversity from both the number and relative abundance of individual molecular formulas (MFs) in DOM using the Shannon–Wiener index (48). The index provides an "effective" number of types, which is the diversity in a community when all species or formulas are equally common. Thus, it corrects for variation in the evenness of community members that can bias richness counts (48). We similarly used the Shannon–Wiener index to define biodiversity from the number and relative abundance of microbial operational taxonomic units (OTUs) found in sediment around the DOM using metagenomic shotgun sequencing. Finally, we used information on chemical properties and cellular processes to derive indices of functional diversity (FD) for MFs and microbial metagenomes, respectively (49).

Results

Diversity in Relation to tOM and Lake Water Light Penetration. We found considerable chemodiversity and biodiversity in our sediments. After assigning MFs and OTUs to nonsingleton peaks and 16S rRNA sequences specific to microbes (i.e., bacteria and archaea), we discovered a total of 12,900 and 3,613 unique MFs and OTUs, respectively. Chemodiversity and microbial biodiversity of individual mesocosms ranged from an effective number of formulas (F_E) and species (S_E) of 1,875 to 3,418 and 31 to 689, respectively. There was also large compositional variation in MFs and OTUs, with the former filling the entire range of chemical space (*SI Appendix*, Figs. S1–S3).

Both chemodiversity and functional aspects of biodiversity varied similarly among global change scenarios. As expected, chemodiversity increased with the amount of tOM added to sediment when we also accounted for variation in MFs simply due to pore water dissolved organic carbon (DOC) concentrations ($t_{20} = 2.75$, $P = 0.012$; *SI Appendix*, Table S1). Consequently, an increase from 5 to 25% of tOM buried into sediment—as might happen from anthropogenic activities—increased F_E by a mean (95% CI) of 160 (152 to 169). Chemodiversity did not vary with the composition of tOM, but there was more diversity in the bioavailability and energetic rewards (i.e., potential energy yield) associated with DOM as more coniferous litterfall was added to sediment (*SI Appendix*, Table S1). Although microbial biodiversity did not change with tOM, the diversity of functional categories associated with extracellular enzymes that modify DOM increased as more tOM was added to sediment ($t_{21} = 3.45$, $P = 0.002$; *SI Appendix*, Table S1). This result was consistent when we calculated functional diversity for all of the genes associated with these categories (*SI Appendix*, Table S1).

Chemodiversity also responded to light penetration in the overlying water column. We found more chemodiversity in the dark lake ($t_{20} = 4.34$, $P < 0.001$), along with more diversity in molecular mass and aromaticity (*SI Appendix*, Table S1), both of which were positively correlated with chemodiversity (Pearson's correlation coefficient $r = 0.43$ and 0.30, respectively). This result could have arisen because of greater in-lake contributions and less photooxidation, as evidenced by lower relative abundances of oxygen-rich saturated and oxygen-rich aliphatic molecules, lower aromaticity, and a greater number of double-bond equivalents (*SI Appendix*, Fig. S4). However, the bioavailability and energetic rewards of DOM were less diverse in the dark lake (*SI Appendix*, Table S1), and both FD indices were negatively correlated with chemodiversity ($r = -0.59$ and -0.77, respectively).

Associations between Chemodiversity and Biodiversity. We found that chemodiversity and biodiversity were positively associated in two main ways. First, chemodiversity had twice as large an effect on biodiversity than vice versa ($t_{22} = 2.83$, $P = 0.010$; *SI Appendix*, Table S2). Microbial S_E consequently increased by a mean (95% CI) of 4.5 times (1.5 to 13.3) over the range of chemodiversity observed within mesocosms, when accounting for lake-level differences (Fig. 1*A*). Second, we found more similar mixtures of MFs tended to associate with more similar communities of microbial

OTUs (Mantel test: $r = 0.25$, $P = 0.022$). From pairs of mesocosms that had 60% similarity in their MFs to ones with 90% similarity, the proportion of shared microbial OTUs nearly doubled (Fig. 1*B*). None of the FD indices for MFs were correlated with microbial FD (for all, $P > 0.05$). The one exception was that a greater diversity in energetic rewards available from oxidative degradation was positively associated with FD calculated across all molecular functions associated with organic matter decomposition ($t_{22} = 2.71$, $P = 0.013$).

Links between Chemodiversity and Ecosystem Function. We found that chemodiversity promoted the concentration of GHG in sediment pore water, likely in part by enhancing microbial activity. Both CO_2 and CH_4 concentration increased with increasing chemodiversity ($t_{19} = 3.47$, $P = 0.003$ and $t_{19} = 2.63$, $P = 0.016$; Fig. 2) when we also accounted for the positive effect on mineralization from higher pore water DOC concentrations and the tOM additions (Table 1). Over the observed range of chemodiversity, CO_2 and CH_4 concentrations were consequently estimated to increase by a mean (95% CI) of 16 times (3 to 85) from 0.58 (0.20 to 1.68) to 9.21 (5.04 to 16.9) mg $C{\cdot}L^{-1}$ and 55 times (2 to 1,338) from 0.09 (0.01 to 0.72) to 5.14 (1.62 to 16.3) mg $C{\cdot}L^{-1}$, respectively, at the mean of all of the other model predictors (i.e., when tOM quantity, tOM quality, lake identity, and DOC concentration were held constant). The tOM additions themselves had no direct effect on GHGs (Table 1). This finding suggested that our results were not simply an artifact of both chemodiversity and GHGs independently covarying with sediment conditions, such as anoxia, created by the experimental treatments. Rather, tOM was indirectly responsible for changing GHG concentrations by shifting chemodiversity (*SI Appendix*, Fig. S5). Sediments with higher GHG concentrations were also not more anoxic (*SI Appendix*, Fig. S6), further supporting our interpretation that changes in tOM influenced GHGs by changing chemodiversity rather than through other environmental gradients. Models with microbial rather than chemical diversity also fitted the data more poorly, suggesting that chemodiversity had more complex effects on microbial processes than just changing biodiversity (Table 1). A greater diversity in bioavailability and less diversity in aromaticity were also associated with more CO_2 and CH_4, whereas greater diversity in energetic rewards was associated with more CH_4 only (*SI Appendix*, Table S3). Overall, all of the models explained a relatively large amount of variation in GHG concentrations, especially for CO_2, suggesting other unmeasured variables were of less importance (Table 1 and *SI Appendix*, Table S3).

Discussion

Our results show that the idea of greater niche diversity promoting species diversity can be extended to the interactions between heterotrophic organisms and the chemical environment that surrounds them. These findings support the few reports of positive associations between chemodiversity and biodiversity from estuarine systems (8, 9). We now advance beyond this work by also showing that levels of chemodiversity in fresh waters can have consequences for important ecosystem functions that are potentially larger than those arising solely from changes in biodiversity. We specifically found increases in chemodiversity that accompanied tOM inputs were more strongly associated with GHG concentrations than the quantity or quality of the tOM itself. One explanation for the latter result is that organic carbon is actively mineralized across a range of sediment conditions created by differences in tOM, including reduction–oxidation potentials (50, 51). Our results therefore uncover part of the mechanism by which increases in tOM into northern waters associated with global change may elevate carbon emissions from the land surface. Namely, increased tOM inputs can promote a greater diversity of chemical "niches" or substrates that provide more opportunities for biological and photochemical mineralization of DOM. An outstanding question is whether the potential GHG fluxes are large enough to offset predicted increases in the burial of carbon into lake sediments arising from similar drivers of global change, for example, enhanced primary production of forested catchments (52).

Our results suggest that the close molecular-level association between DOM and microbes can result in chemodiversity influencing ecosystem functioning. Different microbial communities have affinities for different carbon compounds (15, 53), partly because they have different functional genes for degrading macromolecules (54). These preferences would explain how a greater diversity of organic molecules provides more niches for microbes to occupy (8, 9) and increases carbon mineralization. Chemodiversity may also partly be an outcome of photooxidation that produces CO_2 (29, 35, 36), which can explain its closer association with ecosystem functioning than biodiversity. Similarly, chemodiversity can increase the activity of microbial communities without increasing their diversity. For example, two different isolates of the archaea *Methermicoccus shengliensis* produce CH_4 from at least 34 different carbon compounds (55). In this case, a greater diversity of compounds would promote ecosystem functioning irrespective of whether it increased microbial diversity. Microbes also produce many different compounds through catabolic processes that respire carbon (56), providing an additional

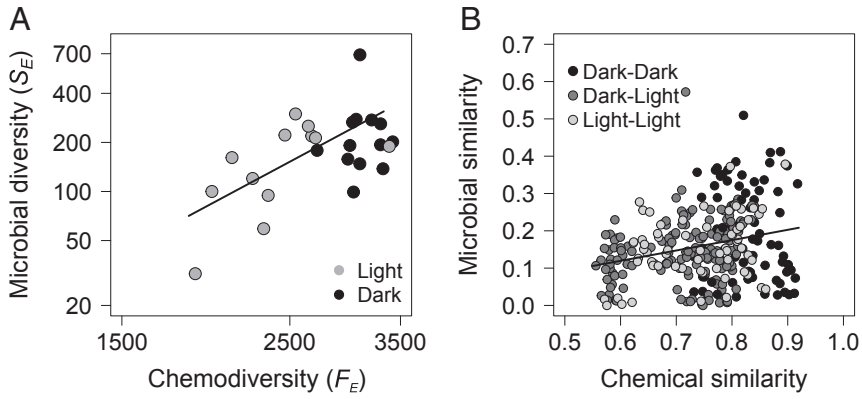

Fig. 1. Microbial and chemical diversity were positively correlated in sediments of a light- and dark-colored lake. (*A*) Effective number of microbial taxa (S_E) and molecular formulas (F_E) across 25 sediment mesocosms in a light and a dark lake. Marker gene sequences were clustered into operational taxonomic units (OTUs) based on their similarity to delineate microbial taxa. (*B*) All pairwise combinations across the 25 mesocosms in the Bray–Curtis similarity index for communities of OTUs and mixtures of molecular formulas. Values approaching 0 indicate two mesocosms do not share any species, whereas values of 1 indicate identical composition. Solid lines show line of best fit. $r = 0.60$ and 0.25 in *A* and *B*, respectively.

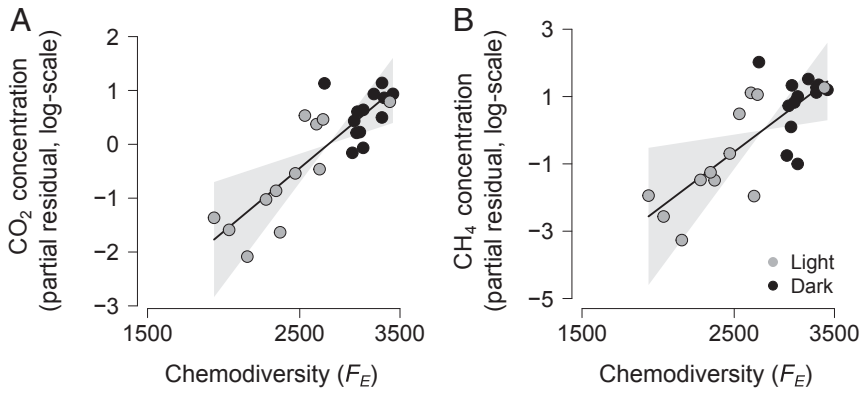

Fig. 2. Sediments had higher concentrations of greenhouse gases with increasing chemodiversity. Partial residuals from regressions predicting log-transformed (*A*) CO_2 and (*B*) CH_4 concentration across 25 sediment mesocosms in a light and a dark lake. Solid lines are mean model fit (±95% CI) at the mean of other variables. Model R^2 = 0.85 and 0.65 in *A* and *B*, respectively. Partial residuals visualize the relationship between chemodiversity and the greenhouse gases, when accounting for all of the other predictors in the multiple regression models. They were calculated in each model by adding the product of observed chemodiversity and its estimated effect to the difference between observed and predicted response values (91).

explanation for why chemodiversity can correlate more with GHG concentrations than biodiversity. The generation of time series can help future studies disentangle causality between chemodiversity and biodiversity, particularly by using dynamic modeling approaches (e.g., ref. 57), and may prove more fruitful than separately trying to control these variables under field conditions.

Our study has several limitations in addition to inferring causality. First, the UHR-MS approach was size-selective and excluded very small ionic compounds and larger colloidal aggregates. High–molecular-mass compounds (>1,000 Da) may be particularly bioavailable and drive much of microbial respiration (53). The analytical window of our UHR-MS approach spanned 93 to 2,000 Da and would have omitted some high–molecular-mass compounds, thereby decoupling chemodiversity from ecosystem functioning and potentially accounting for some unexplained variation in our statistical models. Nonetheless, we found that a considerable amount of ecosystem functioning was captured by our analytical window even if some of the DOM pool was excluded and we extracted dissolved carbon with relatively modest efficiency (*Methods*). This result is supported by studies showing that other features of ecosystem functioning are captured by the analytical approach used here (58, 59). Our UHR-MS approach is also among the most sensitive for detecting polar compounds. Detection limits in our samples likely range in femtomoles, given past work with model compounds in natural DOM (60), and thus are several orders of magnitude beneath presumed concentrations of the most limiting and biologically important organic compounds (61). We also did not measure particulate chemodiversity in the sedimentary phase, but current techniques can only identify about 100 compounds (62), which is <10% of that we observed in DOM. Microbial functioning has also been found to be much more dependent on this dissolved phase (63). Second, many structural isomers exist for each MF and these increase in number as DOM becomes more complex (64). Our analysis of chemical properties, however, generally complemented our chemodiversity findings. Third, we analyzed both living and nonliving fractions of DNA. Ecosystem functioning could have therefore been less correlated with biological diversity than chemodiversity if we sequenced taxa that were no longer metabolically active (65). Although evidence suggests that the resulting biases in measures of taxonomic diversity may be negligible, such as if nonactive cells are derived from those that were recently present (66), the future application

Table 1. Chemodiversity promoted ecosystem functioning

	Response			
	CO_2		CH_4	
Model term	+CD	+MD	+CD	+MD
Intercept	**3.16 (0.21)*** **	**2.81 (0.20)*** **	**1.11 (0.40)***	0.58 (0.36)
Dark lake	**−1.28 (0.34)** **	**−0.60 (0.29)***	−1.27 (0.65)	−0.26 (0.53)
DOC	**1.61 (0.19)*** **	**1.24 (0.17)*** **	**1.92 (0.36)*** **	**1.38 (0.30)*** **
tOM quantity	−0.29 (0.15)	−0.03 (0.15)	−0.25 (0.29)	0.12 (0.28)
tOM quality	−0.12 (0.16)	−0.13 (0.19)	−0.32 (0.31)	−0.33 (0.34)
Chemodiversity	**0.81 (0.23)** **	n/a	**1.17 (0.45)***	n/a
Microbial diversity	n/a	**0.30 (0.14)***	n/a	0.39 (0.26)
R^2	0.85	0.80	0.65	0.57
AICc	52.6	59.7	84.8	89.8

Cells are mean estimates (±SE) for effects in linear models separately predicting either log-transformed CO_2 or CH_4 concentration in sediment pore water. Model predictors included whether measurements were in the dark lake, DOC concentration in sediment pore water, tOM quantity (0, 5, 25, or 50% tOM), tOM quality (1/3, 1/2, or 2/3 deciduous tOM, estimated only where tOM quantity was >0%), and either chemodiversity (+CD) or microbial diversity (+MD). Models were refitted for each diversity metric. All predictors were scaled to a mean of 0 and SD of 1 so that effects were directly comparable. Bolded values were statistically significant at ***$P < 0.001$, **$P < 0.01$, *$P \leq 0.05$; degrees of freedom, 19. AICc, small-sample size-corrected version of the Akaike information criterion, for which models with smaller values indicate greater support. n/a, not available.

of metatranscriptomics could strengthen the results we report here. Finally, we may have underestimated the effects of biodiversity by not measuring eukaryotic diversity. For example, both fungi and benthic invertebrates contribute to carbon cycling in littoral zones, including under anoxic conditions that favor CH_4 production (67, 68). Importantly, none of these issues detract from the strong associations that we detected and the relatively large amounts of variation that we could explain in measured responses.

Northern waters are burying more carbon into sediment and darkening in color due to global change (46, 52), and our results suggest that these changes will increase chemodiversity with consequences for regional carbon cycles. For example, we substituted the increase in chemodiversity that we observed in our experiment with an increase in sediment tOM content from 5 to 25% in our statistical models of CO_2 and CH_4 concentrations. We found that the increase in chemodiversity alone (i.e., when all other variables were held constant, including the direct effects of litter input) was sufficient to raise GHG concentrations by a mean (95% CI) of 1.46 times (0.91 to 2.32) in CO_2 equivalents, assuming CH_4 had a global warming potential of 28. Similarly, our experimental design allowed us to isolate the effect of overlying water color on chemodiversity as we added identical tOM to each lake. In the light-colored lake, we found evidence of photooxidation that could have reduced chemodiversity and the pool of organic molecules available for microbial degradation (69, 70). Waters that were 2.5 times darker—well within the range predicted in the near future (71)—were subsequently sufficient to increase chemodiversity by a mean (95% CI) of 1.20 times (1.10 to 1.30). Adding this increase in chemodiversity to our models predicting GHGs again elevated their concentrations by a mean (95% CI) of 2.66 (1.04 to 7.05) when all other variables were held constant. As northern waters are increasingly recognized to be a major source in the global carbon cycle, such as one that can offset the terrestrial land sink (72), these findings are relevant for regional carbon budgets and climate models. More broadly, our work now provides strong evidence for how variation in chemodiversity widely reported in nature (1) influences biological communities and the functions that they perform.

Methods

Experimental Design. We submerged mesocosms on the bottom of the littoral zone (0.30- to 0.75-m water depth) of Lake Laurentian (46°27′9.74″N, 80°56′35.42″W) and Swan Lake (46°21′58.96″N, 81°3′48.58″W), Ontario, Canada during July 2015. Submergence exposed the experimental sediments to natural overlying water conditions with differing levels of light penetration. Swan Lake had nearly 2.5 times more light at the sediment surface (mean ± SE: 4,611 ± 70 lx; $n = 24$ loggers recording every 45 min for 38 d) than Lake Laurentian (1,847 ± 33 lx) over the course of our experiment. We hereafter refer to the two lakes as the "light" and "dark" lake, respectively.

We filled 17.5-L, 50.8 × 38.1 × 12.7 (height)-cm high-density polyethylene (HDPE) containers with 8 cm of sediment (total sediment volume ∼15 L) after ref. 47. Sediments consisted of 0, 5, 25, or 50% tOM (dry-weight basis) mixed with 9.5 kg of locally sourced inorganic material. The percentages were selected to represent the range of terrestrially derived OM observed in littoral sediments across the study region (73). For each nonzero tOM quantity, material was added in a 1:2, 1:1, or 2:1 dry-mass ratio of deciduous (primarily *Acer rubrum*, *Betula papyrifera*, *Populus tremuloides*, *Quercus* spp.) to coniferous (*Pinus* spp.) litterfall collected from nearby forests. Particle sizes and vertical structuring of all material were based on the average observed in littoral sediments beneath catchments that spanned the range of terrestrial influence in the study region (47, 73). Each mesocosm treatment was subsequently represented at least once across the two lakes (total $n = 25$ across 10 treatments). Although this meant losing replication, the treatments were continuous variables, namely the proportion of tOM and proportion of deciduous tOM. Analyzing them as such in a regression framework provided more statistical and predictive power, especially for upscaling estimates, than a traditional analysis-of-variance approach that maximized replicates per treatment at the expense of treatment variation (74).

Chemodiversity. We collected pore water from mesocosms between 7 and 9 September 2015. A 3-mL polypropylene syringe was secured horizontally immediately beneath the sediment surface along one side of the HDPE container prior to submergence in the lake. The wall of the syringe that faced the sediment was removed and covered in ca. 250-μm nylon mesh. Each sampling syringe was then connected to nylon tubing that was purged of water before any sample collection.

We measured chemodiversity using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). In the field, we passed 25 mL of pore water through a 0.5-μm glass fiber filter (Macherey-Nagel; MN 85/90) and into a preacidified (HCl, pH 2) 20-mL glass scintillation vial. After measuring DOC concentrations in the laboratory on a Shimadzu TOC-5000A, we extracted DOM from 5.5 mL of pore water using styrene divinyl benzene polymer solid-phase extraction cartridges (Agilent; Bond Elut PPL; 100 mg) after ref. 75. Extraction efficiencies were on average (±SE) 36 ± 3% on a carbon basis. The resulting methanol extracts were then diluted to yield a DOC concentration of 5 $mg{\cdot}L^{-1}$ in ultrapure water and MS-grade methanol and analyzed on a 15-T solariX (Bruker Daltonik) using electrospray ionization in negative mode. We accumulated 200 scans for each sample in a mass window of 93 to 2,000 Da. Masses occurring in ≥2 mesocosms that were above the method detection limit were assigned MFs allowing $C_{1-130}H_{1-200}O_{1-50}N_{0-4}S_{0-2}P_{0-1}$ (76). No peaks were retained that were >1,000 Da. Signal intensities of assigned peaks were normalized to the sum of all intensities within each sample.

We calculated two types of diversity measures. First, chemodiversity was calculated using the Shannon–Wiener index that accounts for the evenness of individual MFs in each sample (48). Second, we calculated four measures of FD based on the chemical properties of MFs. This approach assumes that compounds that react (i.e., "function") similarly have shared chemical properties (49). The metrics estimated diversity in 1) sizes of MFs, as inferred from the number of C atoms they contain; 2) bioavailability, whereby compounds with a higher H/C ratio are considered more easily biodegradable (77); 3) energetic rewards available from oxidative degradation (i.e., Gibbs energy per electron catalyzed), which should increase with the nominal oxidation state of carbon (78); and 4) aromaticity measured with the modified aromaticity index, which increases as molecules are less reactive (79). We used Rao's quadratic entropy as the FD statistic. Rao's quadratic entropy is one of the few metrics to meet all of the requirements of a functional diversity metric (80) and has been widely used (e.g., ref. 81), including in the context of DOM (49), allowing direct comparison with other studies. It also allows values to be readily interpreted as the expected difference between two molecules in a given functional property on the same scale as the associated property (49). For example, an FD for a molecular size of 100 means that two randomly chosen molecules in the dataset have an expected mass difference of 100 Da.

Biodiversity and Microbial Functioning. Microbial communities were characterized during pore water sampling from surface sediment grabs (ca. top 5 cm) that were freeze-dried immediately after collection in individual sterile bags. All environmental DNA was extracted in duplicate for each mesocosm using the Power Soil DNA Isolation Kit (Mo Bio Laboratories) according to the manufacturer's instructions and then pooled for downstream analyses. We included a water-only control in the extraction and all subsequent steps. Shotgun sequencing libraries were prepared with 1 ng of genomic DNA per sample using Nextera XT DNA Sample Preparation and dual barcoding with Nextera XT Indexes (Illumina). Libraries were quantified on a Qubit 3.0 fluorometer (Thermo Fisher Scientific) and Bioanalyzer HS DNA chip (Agilent) and pooled in equimolar concentrations into a single sample. Samples were then shotgun-sequenced on an Illumina NextSeq using 500/550 Mid Output Kit version 2 (300 cycles, paired-end).

Raw sequences were processed at a depth of ∼3.3 million reads per sample following a modified version of European Molecular Biology Laboratory-European Bioinformatics Institute (EBI) pipeline version 3.0 (82) and deposited in the EBI under project accession no. PRJEB18063. SeqPrep tool version 1.1 (https://github.com/jstjohn/SeqPrep) was used to merge paired-end overlapping reads and Trimmomatic version 0.35 was used to trim low-quality ends. Sequences with >10% undetermined nucleotides and <100 nt were removed using Biopython version 1.65. Noncoding RNAs were removed with HMMER version 3.1b1 (http://hmmer.org). We then taxonomically annotated sequences into OTUs with representative 16S sequences using QIIME version 1.9.1 with the SILVA reference database (release 128) at 97% sequence identity following the open-reference picking method with reverse-strand matching enabled. Between 1,141 and 10,036 16S sequence reads were obtained from each sample. All singletons were subsequently removed along with 4 OTUs out of the remaining 3,617 that were present in a negative water-only control with a relative abundance of >1%. Although OTUs can mask ecotypic diversity (83), broadscale diversity metrics derived from these classifications are highly correlated with those using exact sequence variants [mean Pearson's $r \geq 0.95$ (84)]. Moreover, taxonomic assignments irrespective of method can still mask

important functional differences among communities (e.g., ref. 83). For this reason, we also functionally annotated sequences with FragGeneScan version 1.31 using Gene Ontology (GO) terms, retrieving between 312,831 and 3,657,990 reads per sample. We retained the molecular-level activities performed by gene products involved in different types of DOM decomposition, selecting the highest-level, nonredundant terms in the GO hierarchy: "hydrolase activity," "oxidoreductase activity," "lyase activity," and "transferase activity."

We used both the OTU and subset GO data to estimate microbial biodiversity and FD, respectively, using the Shannon–Wiener index. The resulting estimates of microbial diversity were comparable to other shotgun sequencing studies (e.g., ref. 85) and a more deeply sequenced global analysis of amplicon data (86). Differences in sequencing depth may, however, limit such comparisons, including across samples in our study, but the Shannon–Wiener index is both less biased to sequencing depth than other metrics, such as Chao1 diversity (87, 88), and was adequately captured by most of our samples (*SI Appendix*, Fig. S3). Approaches that adjust for differences in sequencing depth prior to comparing diversity metrics have also been termed "inadmissible" because they discard data and reduce statistical power in downstream analyses (89). Nonetheless, rarefaction did not change any of our conclusions. We calculated the average Shannon–Wiener index after randomly resampling communities 100 times in each mesocosm to the lowest number of OTUs recovered in a single sample. Sample-specific diversity estimated with and without rarefaction was highly correlated ($r = 0.92$, $P < 0.001$) and no interpretations changed from the linear models except for the effect of microbial diversity on CO_2 concentrations ($t_{19} = 1.33$, $P = 0.199$). For FD, calculations were performed on a matrix of mesocosms by relative abundances for each of the four higher-level GO terms. Larger values indicated a wider and more even range of the four gene categories present in each community. This approach avoided the problem that some terms had more descendent functions than others, and so would have been weighted more heavily if we calculated FD on the disaggregated data. We nonetheless repeated the analysis for all descendent functions from the higher-level GO terms. The resulting matrix included 726 columns derived from 236, 155, 84, and 251 molecular functions associated with hydrolase activity, oxidoreductase activity, lyase activity, and transferase activity, respectively.

Ecosystem Functioning. During pore water sampling, we also analyzed dissolved CO_2 and CH_4 concentrations. In the field, we collected a 43-mL water sample into a 60-mL syringe that was preacidified with 2 mL of 0.5 M HCl before drawing in 15 mL of ambient air, closing the stopcock, and shaking for 2 min to equilibrate the air and acidified sample. We then collected 10 mL of headspace air in a syringe and concentrations of both CO_2 and CH_4 were measured on an SRI 8610C gas chromatograph fitted with a flame ionization detector and in-line methanizer within 24 h of collection (SRI Instruments). Concentrations were calibrated with a commercial gas standard of N_2 containing 1,008 ppm CO_2 and 10.2 ppm CH_4 (Praxair Canada). Final pore water concentrations of dissolved gases were calculated after ref. 90 by applying the Bunsen solubility coefficient and ideal gas law, accounting for pH and temperature measured in the field with a handheld meter (HI 9126; Hanna Instruments), and simultaneous measurements of the ambient air concentration of CO_2 and CH_4.

Statistical Analyses. We tested whether diversity varied with tOM additions using linear models. Both chemodiversity and all four molecular FD metrics were separately predicted given the quantity and quality (i.e., type) of tOM added to the sediment, while also allowing responses to vary simply because of lake identity and pore water DOC concentrations. We used the same model to predict biodiversity and microbial FD but without including DOC concentrations.

We also tested whether chemodiversity and biodiversity were associated in two ways. First, we modeled each metric as a linear function of the other and lake-specific differences. This approach does not explicitly assume the direction of causality, instead allowing the strength of directional associations to be compared because we scaled each response to a common scale with a mean of 0 and SD of 1. We used this same approach to compare each pairwise combination of molecular and microbial FD index. Second, we tested if more similar MFs were associated with more similar microbial OTUs, as expected if the two groups were responding to one another. We calculated the Bray–Curtis similarity index between all pairwise combinations of samples in each dataset of MFs and OTUs. We then correlated the resulting matrices using a Mantel test with Pearson's correlation coefficient and 999 permutations constrained within lake.

Finally, we tested whether chemodiversity promoted ecosystem functioning using linear models. We modeled log-transformed CO_2 and CH_4 concentrations in pore water given chemodiversity while also accounting for differences due to lake, pore water DOC concentrations, and experimental treatments. We refitted these models replacing chemodiversity with each molecular FD index and biodiversity. We could not include all metrics of MFs in a single model because of multicollinearity. Throughout, our goal was not to explain all variation in ecosystem functioning, as other unmeasured variables, such as inorganic nutrient concentrations, trace metals, and benthic photosynthesis, certainly influence GHG concentrations in lake sediments. We instead were interested in diversity-functioning relationships, widely tested elsewhere (18–21). In all linear models, continuous predictors were scaled to a mean of 0 and SD of 1 so that their effects were directly comparable. We used partial residual plots to visualize associations between chemodiversity and greenhouse gas concentrations given the other independent variables in the models. These plots are useful for visualizing associations between variables in higher-dimensional regression models (91). All statistical analyses were performed in R version 3.4.

Data Availability Statement. All data discussed in this paper have been made available to readers in external repositories.

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1. A. M. Kellerman, T. Dittmar, D. N. Kothawala, L. J. Tranvik, Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. *Nat. Commun.* **5**, 3804 (2014).
2. S. Wagner *et al*., Linking the molecular signature of heteroatomic dissolved organic matter to watershed characteristics in world rivers. *Environ. Sci. Technol.* **49**, 13798–13806 (2015).
3. K. E. Judd, B. C. Crump, G. W. Kling, Variation in dissolved organic matter controls bacterial production and community composition. *Ecology* **87**, 2068–2079 (2006).
4. V. Amaral, D. Graeber, D. Calliari, C. Alonso, Strong linkages between DOM optical properties and main clades of aquatic bacteria. *Limnol. Oceanogr.* **61**, 906–918 (2016).
5. J. B. Logue *et al*., Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *ISME J.* **10**, 533–545 (2016).
6. C. Orland *et al*., Microbiome functioning depends on individual and interactive effects of the environment and community structure. *ISME J.* **13**, 1–11 (2019).
7. E. B. Kujawinski, The impact of microbial metabolism on marine dissolved organic matter. *Annu. Rev. Mar. Sci.* **3**, 567–599 (2011).
8. H. Osterholz *et al*., Deciphering associations between dissolved organic molecules and bacterial communities in a pelagic marine system. *ISME J.* **10**, 1717–1730 (2016).
9. H. Osterholz, D. L. Kirchman, J. Niggemann, T. Dittmar, Diversity of bacterial communities and dissolved organic matter in a temperate estuary. *FEMS Microbiol. Ecol.* **94**, fiy119 (2018).
10. M. L. Rosenzweig, *Species Diversity in Space and Time* (Cambridge University Press, Cambridge, UK, 1995).
11. G. E. Hutchinson, Concluding remarks. *Cold Spring Harb. Symp. Quant. Biol.* **22**, 415–427 (1957).
12. H. Ogawa, Y. Amagai, I. Koike, K. Kaiser, R. Benner, Production of refractory dissolved organic matter by bacteria. *Science* **292**, 917–920 (2001).
13. L. Zou *et al*., Bacterial roles in the formation of high-molecular-weight dissolved organic matter in estuarine and coastal waters: Evidence from lipids and the compound-specific isotopic ratios. *Limnol. Oceanogr.* **49**, 297–302 (2004).
14. M. T. Cottrell, D. L. Kirchman, Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl. Environ. Microbiol.* **66**, 1692–1697 (2000).
15. L. Gómez-Consarnau, M. V. Lindh, J. M. Gasol, J. Pinhassi, Structuring of bacterioplankton communities by specific dissolved organic carbon compounds. *Environ. Microbiol.* **14**, 2361–2378 (2012).
16. A. Mostovaya, J. A. Hawkes, T. Dittmar, L. J. Tranvik, Molecular determinants of dissolved organic matter reactivity in lake water. *Front. Earth Sci.* **5**, 106 (2017).
17. E. E. Curd, J. B. H. Martiny, H. Li, T. B. Smith, Bacterial diversity is positively correlated with soil heterogeneity. *Ecosphere* **9**, e02079 (2018).
18. T. Bell, J. A. Newman, B. W. Silverman, S. L. Turner, A. K. Lilley, The contribution of species richness and composition to bacterial services. *Nature* **436**, 1157–1160 (2005).
19. M. Delgado-Baquerizo *et al*., Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. *J. Ecol.* **104**, 936–946 (2016).
20. M. Delgado-Baquerizo *et al*., Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nat. Commun.* **7**, 10541 (2016).
21. E. B. Graham *et al*., Microbes as engines of ecosystem function: When does community structure enhance predictions of ecosystem processes? *Front. Microbiol.* **7**, 214 (2016).

22. J. Comte, L. Fauteux, P. A. Del Giorgio, Links between metabolic plasticity and functional redundancy in freshwater bacterioplankton communities. *Front. Microbiol.* **4**, 112 (2013).
23. R. L. Bier *et al*., Linking microbial community structure and microbial processes: An empirical and conceptual overview. *FEMS Microbiol. Ecol.* **91**, fiv113 (2015).
24. A. Jonsson, M. Meili, A.-K. Bergström, M. Jansson, Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Örträsket, N. Sweden). *Limnol. Oceanogr.* **46**, 1691–1700 (2001).
25. M. L. Pace, Y. T. Prairie, "Respiration in lakes" in *Respiration in Aquatic Ecosystems*, P. del Giorgio, P. Williams, Eds. (Oxford University Press, Oxford, 2005), pp. 103–121.
26. J. Ask, J. Karlsson, M. Jansson, Net ecosystem production in clear-water and brown-water lakes. *Global Biogeochem. Cycles* **26**, GB10171 (2012).
27. S. Juutinen *et al*., Major implication of the littoral zone for methane release from boreal lakes. *Global Biogeochem. Cycles* **17**, 1117 (2003).
28. M. Schallenberg, J. Kalff, The ecology of sediment bacteria in lakes and comparisons with other aquatic ecosystems. *Ecology* **74**, 919–934 (1993).
29. B. Koehler, T. Landelius, G. A. Weyhenmeyer, N. Machida, L. J. Tranvik, Sunlight-induced carbon dioxide emissions from inland waters. *Global Biogeochem. Cycles* **28**, 696–711 (2014).
30. D. Bastviken, J. Ejlertsson, L. Tranvik, Measurement of methane oxidation in lakes: A comparison of methods. *Environ. Sci. Technol.* **36**, 3354–3361 (2002).
31. D. Bastviken, J. J. Cole, M. L. Pace, M. C. V. de Bogert, Fates of methane from different lake habitats: Connecting whole-lake budgets and CH_4 emissions. *J. Geophys. Res. Biogeosci.* **113**, G02024 (2008).
32. H. Hofmann, L. Federwisch, F. Peeters, Wave-induced release of methane: Littoral zones as source of methane in lakes. *Limnol. Oceanogr.* **55**, 1990–2000 (2010).
33. P. Kortelainen *et al*., Carbon evasion/accumulation ratio in boreal lakes is linked to nitrogen. *Global Biogeochem. Cycles* **27**, 363–374 (2013).
34. J. E. Fernández, F. Peeters, H. Hofmann, On the methane paradox: Transport from shallow water zones rather than in situ methanogenesis is the major source of CH_4 in the open surface water of lakes. *J. Geophys. Res. Biogeosci.* **121**, 2717–2726 (2016).
35. W. Granéli, M. Lindell, L. Tranvik, Photo-oxidative production of dissolved inorganic carbon in lakes of different humic content. *Limnol. Oceanogr.* **41**, 698–706 (1996).
36. D. Vachon, C. T. Solomon, P. A. del Giorgio, Reconstructing the seasonal dynamics and relative contribution of the major processes sustaining CO_2 emissions in northern lakes. *Limnol. Oceanogr.* **62**, 706–722 (2017).
37. K. Mopper *et al*., Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle. *Nature* **353**, 60–62 (1991).
38. R. K. Thauer, Biochemistry of methanogenesis: A tribute to Marjory Stephenson. 1998 Marjory Stephenson Prize Lecture. *Microbiology* **144**, 2377–2406 (1998).
39. A. J. Tanentzap *et al*., Forests fuel fish growth in freshwater deltas. *Nat. Commun.* **5**, 4077 (2014).
40. Z. Zhu *et al*., Greening of the Earth and its drivers. *Nat. Clim. Chang.* **6**, 791–795 (2016).
41. X.-P. Song *et al*., Global land change from 1982 to 2016. *Nature* **560**, 639–643 (2018).
42. L. Boisvert-Marsh, C. Périé, S. de Blois, Shifting with climate? Evidence for recent changes in tree species distribution at high latitudes. *Ecosphere* **5**, art83 (2014).
43. P. B. Reich *et al*., Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. *Nat. Clim. Chang.* **5**, 148–152 (2015).
44. J. S. Kominoski, L. B. Marczak, J. S. Richardson, Riparian forest composition affects stream litter decomposition despite similar microbial and invertebrate communities. *Ecology* **92**, 151–159 (2011).
45. E. von Wachenfeldt, L. J. Tranvik, Sedimentation in boreal lakes—The role of flocculation of allochthonous dissolved organic matter in the water column. *Ecosystems* **11**, 803–814 (2008).
46. I. F. Creed *et al*., Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. *Glob. Change Biol.* **24**, 3692–3714 (2018).
47. A. J. Tanentzap *et al*., Bridging between litterbags and whole-ecosystem experiments: A new approach for studying lake sediments. *J. Limnol.* **76**, 431–437 (2017).
48. L. Jost, Entropy and diversity. *Oikos* **113**, 363–375 (2006).
49. A. Mentges, C. Feenders, M. Seibt, B. Blasius, T. Dittmar, Functional molecular diversity of marine dissolved organic matter is reduced during degradation. *Front. Mar. Sci.* **4**, 194 (2017).
50. J. C. Angle *et al*., Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions. *Nat. Commun.* **8**, 1567 (2017).
51. T. DelSontro, P. A. del Giorgio, Y. T. Prairie, No longer a paradox: The interaction between physical transport and biological processes explains the spatial distribution of surface water methane within and across lakes. *Ecosystems* **21**, 1073–1087 (2018).
52. A. J. Heathcote, N. J. Anderson, Y. T. Prairie, D. R. Engstrom, P. A. del Giorgio, Large increases in carbon burial in northern lakes during the Anthropocene. *Nat. Commun.* **6**, 10016 (2015).
53. R. Benner, R. M. W. Amon, The size-reactivity continuum of major bioelements in the ocean. *Annu. Rev. Mar. Sci.* **7**, 185–205 (2015).
54. A. Fitch, C. Orland, D. Willer, E. J. S. Emilson, A. J. Tanentzap, Feasting on terrestrial organic matter: Dining in a dark lake changes microbial decomposition. *Glob. Change Biol.* **24**, 5110–5122 (2018).
55. D. Mayumi *et al*., Methane production from coal by a single methanogen. *Science* **354**, 222–225 (2016).
56. F. Guillemette, P. A. del Giorgio, Simultaneous consumption and production of fluorescent dissolved organic matter by lake bacterioplankton. *Environ. Microbiol.* **14**, 1432–1443 (2012).
57. G. Sugihara *et al*., Detecting causality in complex ecosystems. *Science* **338**, 496–500 (2012).
58. A. M. Kellerman, D. N. Kothawala, T. Dittmar, L. J. Tranvik, Persistence of dissolved organic matter in lakes related to its molecular characteristics. *Nat. Geosci.* **8**, 454–457 (2015).
59. M. Zark, U. Riebesell, T. Dittmar, Effects of ocean acidification on marine dissolved organic matter are not detectable over the succession of phytoplankton blooms. *Sci. Adv.* **1**, e1500531 (2015).
60. H. Waska, A. Koschinsky, M. J. Ruiz Chancho, T. Dittmar, Investigating the potential of solid-phase extraction and Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) for the isolation and identification of dissolved metal–organic complexes from natural waters. *Mar. Chem.* **173**, 78–92 (2015).
61. F. Azam, F. Malfatti, Microbial structuring of marine ecosystems. *Nat. Rev. Microbiol.* **5**, 782–791 (2007).
62. A. G. Bravo *et al*., Molecular composition of organic matter controls methylmercury formation in boreal lakes. *Nat. Commun.* **8**, 14255 (2017).
63. K. Attermeyer, K. Premke, T. Hornick, S. Hilt, H.-P. Grossart, Ecosystem-level studies of terrestrial carbon reveal contrasting bacterial metabolism in different aquatic habitats. *Ecology* **94**, 2754–2766 (2013).
64. M. Zark, J. Christoffers, T. Dittmar, Molecular properties of deep-sea dissolved organic matter are predictable by the central limit theorem: Evidence from tandem FT-ICR-MS. *Mar. Chem.* **191**, 9–15 (2017).
65. P. Carini *et al*., Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nat. Microbiol.* **2**, 16242 (2016).
66. J. T. Lennon, M. E. Muscarella, S. A. Placella, B. K. Lehmkuhl, How, when, and where relic DNA affects microbial diversity. *MBio* **9**, e00637-18 (2018).
67. C. M. Wurzbacher, F. Bärlocher, H.-P. Grossart, Fungi in lake ecosystems. *Aquat. Microb. Ecol.* **59**, 125–149 (2010).
68. R. I. Jones, J. Grey, Biogenic methane in freshwater food webs. *Freshw. Biol.* **56**, 213–229 (2011).
69. P. E. Rossel, A. V. Vähätalo, M. Witt, T. Dittmar, Molecular composition of dissolved organic matter from a wetland plant (*Juncus effusus*) after photochemical and microbial decomposition (1.25 yr): Common features with deep sea dissolved organic matter. *Org. Geochem.* **60**, 62–71 (2013).
70. E. B. Kujawinski *et al*., Microbial community structure affects marine dissolved organic matter composition. *Front. Mar. Sci.* **3**, 45 (2016).
71. G. A. Weyhenmeyer, R. A. Müller, M. Norman, L. J. Tranvik, Sensitivity of freshwaters to browning in response to future climate change. *Clim. Change* **134**, 225–239 (2016).
72. T. W. Drake, P. A. Raymond, R. G. M. Spencer, Terrestrial carbon inputs to inland waters: A current synthesis of estimates and uncertainty. *Limnol Oceanogr Lett* **3**, 132–142 (2018).
73. B. E. Wesolek, E. J. Szkokan-Emilson, J. M. Gunn, Assessment of littoral benthic invertebrate communities at the land–water interface in lakes recovering from severe acid- and metal-damage. *Hum. Ecol. Risk Assess.* **16**, 536–559 (2010).
74. K. L. Cottingham, J. T. Lennon, B. L. Brown, Knowing when to draw the line: Designing more informative ecological experiments. *Front. Ecol. Environ.* **3**, 145–152 (2005).
75. T. Dittmar, B. Koch, N. Hertkorn, G. Kattner, A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnol. Oceanogr. Methods* **6**, 230–235 (2008).
76. T. Riedel, T. Dittmar, A method detection limit for the analysis of natural organic matter via Fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* **86**, 8376–8382 (2014).
77. L. Sun, E. M. Perdue, J. L. Meyer, J. Weis, Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. *Limnol. Oceanogr.* **42**, 714–721 (1997).
78. D. E. LaRowe, P. Van Cappellen, Degradation of natural organic matter: A thermodynamic analysis. *Geochim. Cosmochim. Acta* **75**, 2030–2042 (2011).
79. B. P. Koch, T. Dittmar, From mass to structure: An aromaticity index for high-resolution mass data of natural organic matter. *Rapid Commun. Mass Spectrom.* **20**, 926–932 (2006).
80. Z. Botta-Dukát, Rao's quadratic entropy as a measure of functional diversity based on multiple traits. *J. Veg. Sci.* **16**, 533–540 (2005).
81. D. Schmera, J. Heino, J. Podani, T. Erős, S. Dolédec, Functional diversity: A review of methodology and current knowledge in freshwater macroinvertebrate research. *Hydrobiologia* **787**, 27–44 (2017).
82. A. Mitchell *et al*., EBI metagenomics in 2016—An expanding and evolving resource for the analysis and archiving of metagenomic data. *Nucleic Acids Res.* **44**, D595–D603 (2016).
83. A. B. Chase *et al*., Emergence of soil bacterial ecotypes along a climate gradient. *Environ. Microbiol.* **20**, 4112–4126 (2018).
84. S. I. Glassman, J. B. H. Martiny, Broadscale ecological patterns are robust to use of exact sequence variants versus operational taxonomic units. *mSphere* **3**, e00148-18 (2018).
85. C. Quince, A. W. Walker, J. T. Simpson, N. J. Loman, N. Segata, Shotgun metagenomics, from sampling to analysis. *Nat. Biotechnol.* **35**, 833–844 (2017).
86. L. R. Thompson *et al*.; Earth Microbiome Project Consortium, A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* **551**, 457–463 (2017).
87. D. Lundin *et al*., Which sequencing depth is sufficient to describe patterns in bacterial α- and β-diversity? *Environ. Microbiol. Rep.* **4**, 367–372 (2012).
88. A. T. Reese, R. R. Dunn, Drivers of microbiome biodiversity: A review of general rules, feces, and ignorance. *MBio* **9**, e01294-18 (2018).
89. P. J. McMurdie, S. Holmes, Waste not, want not: Why rarefying microbiome data is inadmissible. *PLoS Comput. Biol.* **10**, e1003531 (2014).
90. J. Åberg, M. Wallin, Evaluating a fast headspace method for measuring DIC and subsequent calculation of pCO_2 in freshwater systems. *Inland Waters* **4**, 157–166 (2014).
91. J. Fox, *Applied Regression Analysis and Generalized Linear Models* (Sage Publications, Thousand Oaks, CA, ed. 3, 2016).