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Identifying Early Ecological Disturbance Signals through Functional Shifts in Freshwater Microbial Communities

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Abstract: Freshwater systems face multiple coexisting stressors, but typical monitoring programs will not identify degradation until water chemistry falls outside set standards or changes have occurred in macroinvertebrate and fish communities. The microbes respond quickly (from hours to weeks) to environmental disturbances and facilitate key biogeochemical processes in freshwaters. This makes them promising indicators for early warning assessments. The current study investigates whether changes in functional attributes of freshwater microbial communities may act as an early signal of ecological disturbance before any structural and taxonomic change takes place. A systematic review of the literature on microbial resistance, resilience, functional redundancy, community assembly, and critical transition theories was coupled with a comparison of eight different functional indicators, from functional genes and enzymes to predicted metagenomic functionality. A simple simulation of indicator response to disturbance intensity was done afterwards. The synthesis shows that functional traits, especially the relative abundance of genes associated with nitrogen cycling and stress responses and the proportion of enzymes for acquisition of carbon, nitrogen, and phosphorus, respond more quickly and consistently to nutrient addition, pollution, warming, and changes in water flow regimes compared to taxonomic composition. In this experiment, functional gene indicators reached the level of detection 8.5 weeks prior to the macroinvertebrate indicator and four weeks prior to taxonomic community changes. Functional redundancy may obscure disturbances; therefore, increases in variation and delay in the recovery of functional indicators should be considered along with average values. A hierarchical approach to monitoring is suggested, which includes microbial functional assessment together with traditional biomonitoring.

Keywords: Freshwater Microbial Communities; Functional Shifts; Early-Warning Indicators; Ecological Disturbance; Functional Redundancy; Extracellular Enzymes; Biomonitoring; Ecosystem Resilience.

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I. Introduction

Freshwater ecosystems make up less than one percent of the Earth's surface but contain an outsized proportion of the world's biodiversity, as well as provide water for consumption, fishing, irrigation, nutrient retention, and recreational activities. They are also among the most endangered ecosystems in the world. Agricultural drainage, urban sewage, industrial pollution, flow control, climate change, and biological invasions interact in various ways that often deviate from the simple summation of their separate impacts. A continental-scale study of European rivers and lakes found that the presence of multiple stressors was the norm, and nutrient enrichment and hydromorphological modification were often accompanied by other stressors (Birk et al., 2020). In such circumstances, the detection of disturbances before they become expensive or irreparable is the key challenge of freshwater resource management.

But most standard monitoring programs tend to focus on measuring degradation rather than predicting it. Sampling using physico-chemical approaches happens at monthly or quarterly intervals, and it is based on fixed thresholds. In terms of biological monitoring, indicators like macroinvertebrates, diatoms, macrophytes, and fish, whose generation times range from a few weeks to several years, are used. And when changes are noted through these indicators, the process may have already taken place a few months back. According to theories regarding critical transitions, ecosystems, especially shallow lakes, are known to experience abrupt transitions from one state to another once they reach a certain threshold. And the amount of pressure needed to recover the ecosystem is often much greater than what led to the threshold being reached (Scheffer et al., 2009). Signals of generic early warnings include increased variance, increased autocorrelation, and decreased resistance from the system to disturbance (Scheffer et al., 2009).

Through food-web manipulation in the whole lake, the signals have been successfully detected about a year ahead of the actual transition taking place in the ecosystem Carpenter et al., (2011), while in laboratory populations, similar results have been found (Dai et al., 2012). Now comes the important question of what signals appear earlier in the ecosystem.

Microbial communities are also excellent candidates for such studies. Bacteria, archaea, microeukaryotes, and fungi provide the basis of the freshwater food web and are responsible for organic matter degradation, nitrogen transformations, phosphorus mineralization, methane cycling, and contaminant degradation. Bacterioplankton of freshwater represent distinct ecological groups with defined taxonomic groups, which include both oligotrophic and copiotrophic populations that vary depending on the supply of nutrients, quality of organic carbon, and oxygen availability (Newton et al., 2011). Because of the fast generation time, high abundance, and quick physiological adaptation, changes in the microbial community take place within hours or days, not months or years. There are three major attributes which describe the reactions of microbial community on perturbations – resistance (stability of composition and functionality), resilience (rate of recovery from perturbation), and functional redundancy (Allison & Martiny, 2008).

The presence of functional redundancy has a crucial effect on the interpretation of disturbance signals. The studies of the global ocean microbiome revealed that the distribution of metabolic functions is closely related to the environment regardless of significant differences in the taxonomic composition of the communities that perform those functions, which indicates that function may not be related to taxonomy (Louca et al., 2016). The trait-based and phylogenetic approaches show that a large number of microbial traits are conserved at certain phylogenetic levels, and their distribution at the community level is more indicative of the environment than species identity (Martiny et al., 2015). The community assembly framework shows that selection, dispersal, drift, and diversification together define the presence of particular taxa in a particular location, and thus, taxonomic changes may be non-stress-related (Nemergut et al., 2013). Therefore, the combination of all these ideas reveals that functional characteristics may be more indicative of ecosystem changes than taxonomic information.

Despite these advances, microbial functional indicators have not yet found their way into the statutory monitoring programs for freshwater. The existing techniques vary in their costs, sensitivities, mechanistic interpretability and processing time, but there is no objective criterion for selecting between them or for interpretation of their responses. Functional redundancies might provide additional resilience to mean rates of processes under moderate stress and thus mask changes at early stages – an issue that is often overlooked during the selection of indicators.

Objectives of this study include: (1) To assess the theoretical and empirical grounds for employing the microbial functional shifts as early signals of ecological perturbations; (2) To compare the major types of microbial functional indicators according to their represented functions, ability to respond to different types of stressors, response times and constraints; (3) To show, via a hypothetical response simulation, the expected lead time of functional indicators in comparison to taxonomic, chemical and macroinvertebrate indicators; and (4) To suggest the multi-tier scheme for monitoring based on microbial functional indicators.

Article structure: The first section introduces the problem of late detection in freshwater surveillance and importance of microbial community; Section II gives an overview on the materials and methods employed in synthesis, evaluation and simulation; Section III outlines the results obtained, which include comparative evaluation and simulation response time; Section IV discusses the mechanism, limitation and application of the result obtained; and Section V concludes the article.

II. Materials and Methods

Study Design

The project design included three parts: literature synthesis of existing knowledge about the response of microbes to disturbance in freshwater systems; comparative assessment of functional indicator types; and a heuristic approach for modeling the timing of the response of indicators. This approach was selected since the research question about whether microbial functions give an early warning compared to traditional indicators is interdisciplinary and involves microbial ecology, biogeochemistry, ecology, and monitoring and cannot be addressed with a single data set.

Literature Synthesis

Peer-reviewed literature was screened for the following four themes: (i) microbial resistance, resilience and functional redundancy; (ii) the connection between microbial taxonomy and function, covering both trait-based and assembly approaches; (iii) early warning signs and theories for critical transitions in aquatic ecosystems; and (iv) methodologies for characterizing microbial community function via molecular and biochemical means. The articles were selected when they provided information about (i) freshwater or other comparable aquatic systems, (ii) response of the system to some form of stress or environmental gradient, and (iii) either a functional feature of the community or some technique that could be used in order to measure this. Special emphasis was placed on research on high-resolution amplicon sequencing that provides resolution of exact sequence variants rather than clustering of operational taxonomic units (Callahan et al., 2016; Langille et al., 2013; Douglas et al., 2020).

Indicator Evaluation

Eight categories of functional microbial indicators were determined through synthesis: functional gene markers, gene transcripts, extracellular enzyme activity and stoichiometry, community-level physiological profiling, predicted functional profile, microbial respiration and carbon use efficiency, functional trait distribution, and statistical characteristics of functional time series (variance and autocorrelation). Each category was assessed according to the following five indicators: the ecosystem function represented by the indicator; analysis methodology; major stressors that can be detected by the method; characteristic response time after the occurrence of the stressor; and the major limitation in the interpretation of the indicator. The response times are presented in qualitative ranges according to the temporal scales described in the literature due to their high dependency on temperature and stressor strength.

Indicator-Response Simulation

As an example of how the indicators would be expected to respond to a disturbance, a numerical simulation was designed. A disturbance index S was devised for the 24-week monitoring period, taking zero values up to week 6 and rising to its maximum value of 1 at week 22 in a linear way. This could correspond to a gradually worsening disturbance factor, like, for instance, progressive nutrient enrichment. The response R of each indicator was calculated using a logistic equation with respect to with rescaling so that $R = 0$ when $S = 0$. All indicators had the same value for the steepness parameter k, which equaled 12. The half-response parameter S50 was chosen for each indicator based on the sensitivity rating obtained during the indicator evaluation, as follows: 0.22 for functional gene markers, 0.30 for extracellular enzyme ratios, 0.50 for taxonomic composition, 0.62 for water chemistry exceedance and 0.78 for a macroinvertebrate biotic index. A 20% detection threshold was chosen and the time when each indicator reached this threshold for the first time was registered.

Framework Development

The results from the synthesis, evaluation of indicators and simulation have been used to develop a hierarchical monitoring system. The system was intended to be able to operate along existing biological and physicochemical assessment systems, focus on indicators that would have favorable cost per information and use costly techniques for verification and diagnosis.

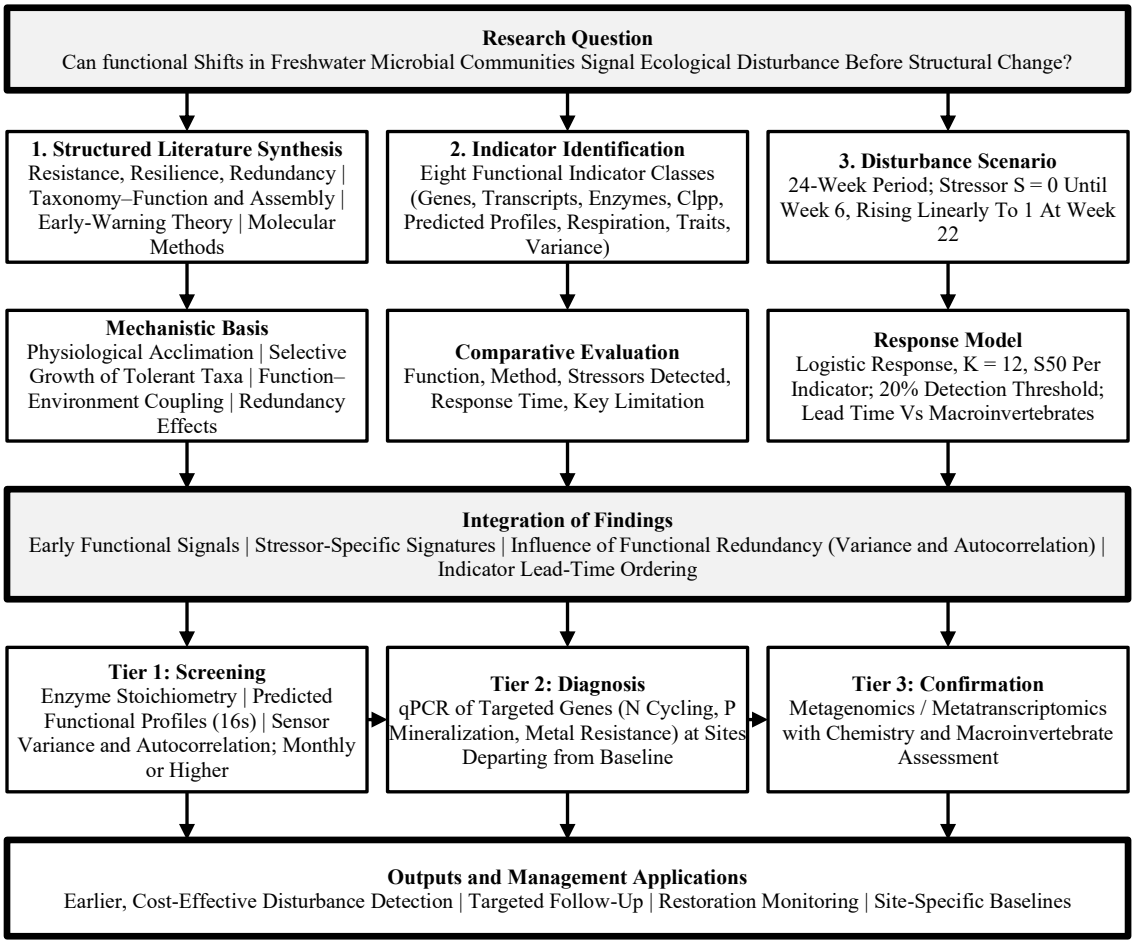


Figure 1: Proposed Multi-Tier Framework for Early Detection of Functional Shifts in Freshwater Microbial Communities

The multi-tier model shown in figure 1 has been developed to identify early functional shifts in microbial communities of freshwater ecosystems prior to the appearance of any ecological or structural changes. This model consists of literature synthesis, indicator development, disturbance modeling, mechanistic assessment, and response modeling of the early warning signals related to the environmental stress conditions. Additionally, it also includes the use of a three-tiered evaluation system comprising screening, diagnosis, and confirmation to differentiate between the functional change and the natural variation in the microbial community.

III. Results

Mechanistic Basis for Early Functional Signals

There were three main pathways for the alteration of functional traits in microorganisms prior to taxonomic changes or shifts in higher trophic levels. Firstly, the physiological acclimation takes place in hours to days due to the fact that microorganisms regulate their metabolism, gene expression and enzymes'

activity according to the change in nutrients, oxygen concentration, temperature and toxicity without changing the community composition. Secondly, the growth of some tolerant or opportunistic populations leads to the increase of the proportion of functional genes within days to weeks, even when the taxonomic structure of communities remains unchanged, because the functional genes may be carried only by small populations of communities (Allison & Martiny, 2008). Thirdly, the function is regulated by the environment rather than by taxonomic affiliation of the organism; hence, the environmental change can be detected faster via changes in functional profile (Louca et al., 2016; Martiny et al., 2015). The macroinvertebrate community integrates environmental stress for a longer time period and reacts to it via mortality, emigration and unsuccessful reproduction.

Comparative Evaluation of Functional Indicators

Table 1: Comparative Evaluation of Microbial Functional Indicator Classes for Early Detection of Ecological Disturbance in Freshwater Ecosystems

Indicator Class	Function Represented	Typical Method	Principal Stressors Detected	Response Time	Key Limitation
Functional gene markers (amoA, nirS/nirK, nosZ, phoD, metal-resistance genes)	Genetic potential for N and P cycling and stress tolerance	qPCR; shotgun metagenomics	Nutrient enrichment, metals, hypoxia	Days to 2 weeks	Reflects potential rather than realized activity
Gene transcripts	Realized expression of metabolic pathways	Metatranscriptomics (RNA sequencing)	Contaminants, oxygen depletion, warming	Hours to days	RNA instability; high cost
Extracellular enzyme activity and stoichiometry (BG, LAP, NAG, AP)	Relative C, N and P acquisition; nutrient limitation	Fluorometric microplate assays	Nutrient imbalance, organic loading, warming	Days to weeks	Strong diel and temperature dependence
Community-level physiological profiles	Breadth of carbon substrate use	Microplate substrate-utilization assays	Organic pollution, salinization	1 to 3 weeks	Culture-based bias toward fast-growing taxa
Predicted functional profiles	Inferred metagenomic content from 16S rRNA	Amplicon sequencing with PICRUSt2	Broad multi-stressor screening	1 to 3 weeks	Lower accuracy for poorly referenced freshwater taxa
Respiration and carbon-use efficiency	Organic matter mineralization and energy flow	O2 consumption; CO2 flux	Organic loading, warming, toxicants	Days to weeks	Confounded by temperature and substrate supply
Functional trait distributions (rRNA operon copy number, genome size)	Shift in life-history strategy toward copiotrophy	Metagenomics with trait databases	Nutrient pulses, flow disturbance	1 to 4 weeks	Incomplete trait data for freshwater lineages
Variance and autocorrelation of functional time series	Loss of resilience (critical slowing down)	High-frequency sensors; repeated assays	Approach to regime shifts	Precedes change in mean	Requires long, regularly spaced time series

Table 1 outlines the assessment of the eight groups of indicators. Functional gene markers and transcripts gave the most mechanistic information about the samples analyzed. Genes of ammonia monooxygenase (amoA), nitrite reductases (nirS and nirK), nitrous oxide reductase (nosZ) and alkaline phosphatase (phoD) were stimulated by nutrients and low oxygen; metal-resistance and oxidative-stress genes were stimulated by contaminants. Gene transcripts responded rapidly – within hours to days. Instability of RNA and the relatively high costs of analysis prevent regular use of transcripts for routine monitoring. Extracellular enzymes provided an optimal compromise in terms of sensitivity, price and interpretation. The ratio of β-glucosidase, leucine aminopeptidase, N-acetyl-glucosaminidase and alkaline phosphatase activities reflected the strategy of the community concerning acquisition of carbon, nitrogen and phosphorus.

Table 1 is a comparison of the eight classes of indicators according to the ecological function, analysis technique used, type of stressors identified, time of response post-disturbance onset, and main drawback.

Functional predictions based on the 16S rRNA amplicon sequencing enable the integration of functional screening at very little extra cost when a taxonomic study is done, particularly if the exact sequence variants are determined (Callahan et al., 2016; Langille et al., 2013; Douglas et al., 2020). The accuracy of the predictions, however, deteriorates for freshwater phyla poorly represented in the reference genome database (Newton et al., 2011). Community physiological profiling and respiration and carbon-use efficiency measures are relatively low-cost techniques that capture the activity of entire communities; however, they are subject to variation in both temperature and substrate availability, as well as providing relatively poor diagnostic discrimination. Changes in functional trait distributions, such as the trend towards greater ribosomal RNA operon copies found in fast-growing copiotrophs, suggest changes in life history strategies in response to nutrient influxes or hydrodynamic disturbances (Martiny et al., 2015). Lastly, the variability and autocorrelation in functional time series represent an entirely separate set of indicators, independent from shifts in mean values (Scheffer et al., 2009; Carpenter et al., 2011).

Simulated Indicator Response Timing

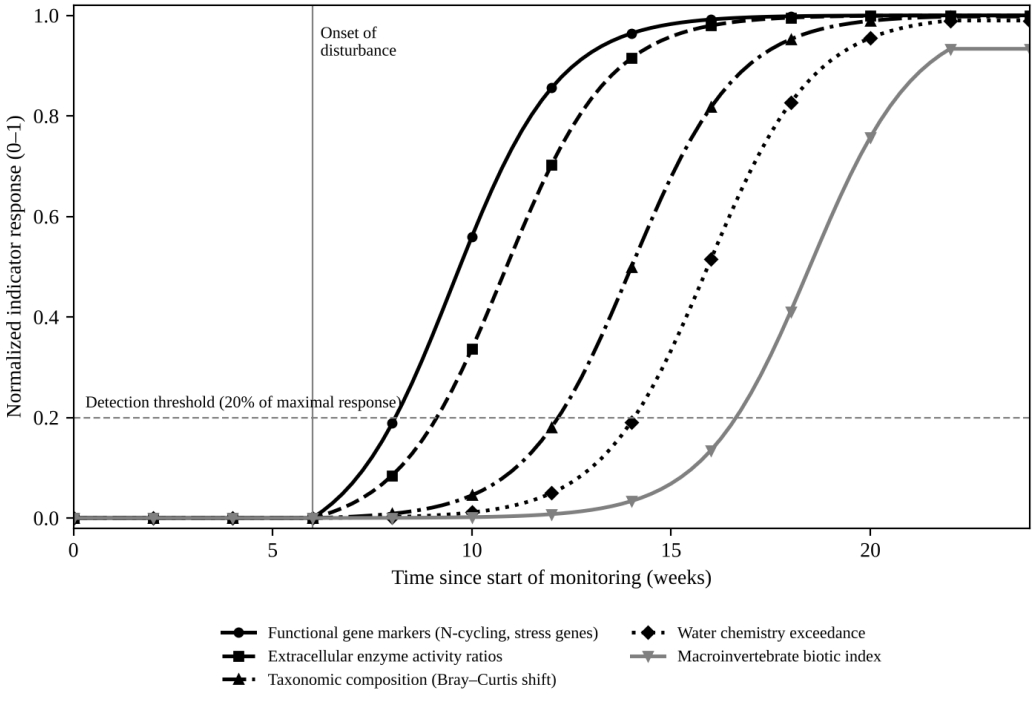


Figure 2: Simulated Response Timing of Microbial Functional, Taxonomic, Chemical and Macroinvertebrate Indicators Along a Gradually Intensifying Disturbance

The simulated response of the five indicator classes to the gradually increasing disturbance is shown in figure 2 below. The functional gene marker passed the 20 percent detection threshold in week 8.25, just 2.25 weeks after the start of disturbance. Extracellular enzyme activities passed the threshold in week 9.25. The taxonomic composition passed the threshold in week 12.25, water chemistry in week 14.25, and the macroinvertebrate biotic index in week 16.75, which was 10.75 weeks after the start of disturbance.

The response of five indicators is illustrated in figure 2 during a 24-week monitoring period during which the disturbance starts at 6 weeks and escalates linearly until 22 weeks. The dashed line represents the detection limit (20% of maximal response). The functional genes and the ratio of extracellular enzymes reach the threshold limit first followed by the taxonomic composition, water chemistry exceedance, and the macroinvertebrates biotic index.

In relation to the macroinvertebrate index, therefore, the functional gene markers offered a lead time of 8.5 weeks, the extracellular enzyme ratios of 7.5 weeks, the taxonomic composition of 4.5 weeks, and the water chemistry of 2.5 weeks. Functional gene markers were ahead of taxonomic turnover by 4.0 weeks. The order in which the indicators would have been simulated is indicative of the sensitivity hierarchy: Indicators based on physiological processes and functional gene abundance come first, followed by indicators that require any change in community membership, and finally, indicators that require the death and replacement of the multicellular organisms. The steep rise of each of the response curves further indicates that once the threshold was breached, the indicators based on functionality reached their maximum response very quickly, while the macroinvertebrate index did not.

Stressor-Specific Functional Signatures

The results of indicator assessment also indicated that various stresses produce different functional fingerprints, which can help diagnose the type of stressor. Nutrient enrichment resulted in higher abundance of genes responsible for ammonia oxidation and denitrification processes, a reduction in alkaline phosphatase activity compared to carbon acquiring enzymes in response to the alleviation of phosphorus limitation, and an adaptation to copiotrophy with higher copy numbers of ribosomal RNA operons (Newton et al., 2011; Martiny et al., 2015). Organic loading caused by wastewaters or agricultural effluents increased respiration and activity of carbon- and nitrogen-acquiring enzymes, often resulting in oxygen depletion and thus anaerobic processes. The presence of metal and organic contaminants resulted in increased abundance of resistance and detoxification genes and reduced substrate utilization diversity due to sensitive heterotrophs' extinction. Warming increased respiration and activity of enzymes in proportion to the temperature increase as well as the ratio of carbon- and nutrient-acquiring enzymes. Flow alteration resulted in changes to both sediment and water column connectivity, and this contributed to episodic events of nitrogen turnover and organic matter decomposition. Since some of the signatures above are similar, none of these functional attributes alone is enough for assigning cause; rather, the use of a combination of genetic markers and enzyme ratios allows a diagnosis to be made on likely causes.

Influence of Functional Redundancy

The analysis also revealed conditions when functional metrics would not deliver timely warning signals. If many species carry out the same function, the loss of sensitive species will be offset by tolerant species; hence, the mean rates of processes would remain constant despite the transformation of the community (Allison & Martiny, 2008). In such a case, the first sign of the disturbance would be the change in dynamics of the process rather than in the mean value of it – increased temporal variation, higher autocorrelation and slower recovery following natural disturbances like storms or seasonal mixing (Scheffer et al., 2009; Carpenter et al., 2011; Dai et al., 2012). Phylogenetically limited processes such as nitrification, methanotrophy or breakdown of specific pollutants, which require narrow specialization, were less redundant and more responsive to stress than general processes like heterotrophic respiration.

IV. Discussion

The results validate the hypothesis that functional changes in microbial communities of fresh water ecosystems are early indicators of ecological disturbance compared to the structural measures that form the basis of monitoring in existing systems. The mechanistic explanation behind this is that microbial communities undergo changes via physiological and selective responses in the early stages of their response, which affects functional changes before any changes to community composition or higher trophic levels are noticeable. This is consistent with the scientific finding that microbial functions are more dependent on the environment than the taxonomy Louca et al., (2016) and that a trait-based approach in describing communities is better able to indicate environmental filtering than taxonomically-oriented approaches (Martiny et al., 2015). It is also consistent with community assembly theory which suggests that some of the taxonomic changes are a result of dispersal and drift and not environmental selection (Nemergut et al., 2013).

This simulation underscores the practical importance of the differences described. A lead time of several weeks would be important to the manager since this would enable investigations into the sources of pollution, modification of treatment processes or agricultural practices, and timely action prior to toxic algal blooms, hypoxia, or fish kills. The numerical values in figure 2 are dependent on the assumption made and are not meant to be generalized; what is significant is the ranking of indicators, which stems from the difference in biological organization and generation time. Lead times empirically will differ based on temperature, residence time, type of stressor, and sampling interval. Fast-flowing River systems, for example, are constantly being resupplied with microbes from upstream, making local functional shifts difficult to detect, while lake and reservoir systems have longer residence times.

Functional redundancy is the foremost qualifying factor for the application of microbial functional indicators. In a community exhibiting high redundancy, stable mean process rates can be observed even as the capacity to withstand additional stress is lost, hence an observation of stable mean rate process is not an indicator of ecosystem health (Allison & Martiny, 2008). It is exactly in such a case where statistical indicators of critical slowing down come into play. An increase in variance and autocorrelation in functional time series before the transition point was seen in a whole lake study as well as in the collapse of laboratory populations, and theoretically it is expected that such indicators occur in all near-threshold systems (Scheffer et al., 2009) (Carpenter et al., 2011; Dai et al., 2012). Since high-frequency sensors measuring dissolved oxygen, pH and conductivity have the ability to measure the net effect of metabolism of the microbes, such time series data can easily be analyzed for such signals without much cost.

There are several methodological concerns that need to be addressed before the application of microbial functional indicators can become widespread. First, the lack of proper reference conditions since microbial functioning is seasonal and site-dependent, requiring baseline conditions determined for at least one annual cycle. Second, different methods used in molecular analysis in terms of sample preparation, primers, sequencing depth, bioinformatics, and the implementation of exact sequence variants could be standardized (Callahan et al., 2016). Third, while predicted functional composition may be a good tool for screening purposes, it should be confirmed through direct measurements such as quantitative PCR of target genes or enzyme assays, especially for lineages present in small numbers in reference databases (Newton et al., 2011; Langille et al., 2013; Douglas et al., 2020). Fourth, DNA-based methods show the potential rather than activity of microbial communities and thus should be considered along with the data on processes. Finally, the multi-stressor nature of most freshwater ecosystems necessitates the inclusion of land use, hydrological features, and point sources to understand which factor affects functioning (Birk et al., 2020).

Taking all of this into account, it is suggested to implement the monitoring approach using three tiers. The first tier involves regular screening based on the ratio of extracellular enzymes and functional predictions from amplicon data and sensor records, if available, analysis of variance and autocorrelation. Since these approaches are quite cheap, they may be performed at least once a month in various locations. In Tier 2, sites where screening parameters differ from baseline values will be investigated via qPCR for

functional genes like genes involved in nitrogen cycle, phosphorus mineralization and metal resistance to identify the cause of the stress. In the third tier, sites with confirmed disturbances will undergo analysis based on metagenomics or metatranscriptomics along with standard tests on chemicals and macroinvertebrates.

There are several limitations to this research effort which must be stated. Firstly, this is a qualitative synthesis and utilizes data from different studies with various experimental conditions, therefore, the values in the table only represent relative time scales and not absolute ones. Secondly, the simulated model takes into account the assumption that the stressor used is gradually and constantly increasing and all indicators have the same rate of response curve, which is often not the case since the stressor can have pulsed or seasonally varying effects and the indicators might have different shapes in addition to positions on the response curve. In addition to these two limitations, the simulation does not take into account the sampling error as well as natural variability which increases the threshold of detection and can reduce the lead time of noisy indicators.

This framework has implications even for ecosystem restoration. Since microbial activity is fast to react to the alleviation of stress, it may be employed to confirm the effectiveness of restoration activities, such as the decrease of nutrient load, creation of riparian buffers or restoration of flows, well before recovery of macroinvertebrates and fishes occurs. Trajectories of recovery of the ratios of enzymes and abundance of genes may thus become an intermediate step toward management actions.

V. Conclusion

The purpose of this investigation was to investigate if functional changes in microbial communities in fresh water could give advance notice of ecological disturbances relative to the traditional indicators of freshwater monitoring. Based on the synthesis of literature on resistance, resilience, redundancy, community assembly and critical transitions and on the results from both the evaluation of the different indicator groups and the response simulation, there is evidence for this hypothesis. Microbial functions react to nutrient loading, pollution, warming and hydrologic alterations within days to weeks, while changes in water quality and macroinvertebrate indices become apparent later on. Out of the eight different types of indicators assessed, functional genes and enzyme stoichiometry gave the best balance of sensitivity and interpretation and were easy to measure. Functional profile predictions and community physiological profiles served as cheap screening techniques. In the case study simulation, functional genes reacted 8.5 weeks prior to the macroinvertebrate index. The results also indicate that early functional signals need careful interpretation. Functional redundancy may help protect average rates of processes from fluctuations, and increased variance, higher autocorrelation and slower recovery after natural disturbances often reveal more information than single measures. Successful use of the approach requires the establishment of site-specific baseline data and repeated sampling, standardization of molecular and enzymatic techniques, and explicit connections between microbial signals and ecological functions of interest to managers. It is possible to design a tiered approach for monitoring that employs microbial functional screening for triggering targeted chemical and biological tests. This will enable a proactive, focused and less expensive management of freshwater resources. The next step in future research should include validation of thresholds of indicators based on observations and whole-ecosystem experiments.

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