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Wetland management using microbial indicators

Hidetoshi Urakawa [a,*], Anne E. Bernhard [b]

[a] Department of Marine and Ecological Sciences, Florida Gulf Coast University, Fort Myers, FL, USA
[b] Biology Department, Connecticut College, New London, CT, USA

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ABSTRACT

The use of biological indicators to monitor the health and functioning of wetlands has been an ongoing goal of wetland scientists and managers, and has focused primarily on monitoring the changes in macroorganisms, due to the relative ease of identifying and counting them. In recent years, the establishment of high-throughput sequencing techniques, development of assays for specific functional genes, and better quantitative measures are making it easier to get extensive diversity profiles and more robust abundance estimates of various microbial communities, and empowers us to explore wetland microbiomes and their role in ecosystem function. This heuristic search enables us to illuminate a spot light on minor populations of microbial communities, which were difficult to be scrutinized by more traditional molecular tools. Monitoring microbial indicators in response to nutrient loading, pollutants and redox potential is beneficial for wetland ecosystem management. Microbial populations can serve as the most sensitive and rapid bioindicator in response to various environmental changes. Evaluation of wetland condition and restoration cannot be met effectively by a single physical, chemical or biological parameter but a combination of multiple attributes is effective for robust wetland assessment and management. Various functional groups of microorganisms can be used as wetland assessment tools and provide a more profound understanding of microbial population dynamics and various direct microbial activity measurements. Understanding of the microbial communities controlling biogeochemical cycles in constructed wetlands could support optimizing performance of these promising treatment systems. This review focuses on a potential use of microorganisms as effective biological indicators for wetland management.

1. Introduction

Microorganisms play fundamental roles in wetland biogeochemistry through their versatile functions (Mitsch and Gosselink, 2015). Microbial communities respond to environmental alterations in a spatially and temporally highly dynamic fashion. In turn, microbial activity, biomass, and population dynamics may impact various chemical and physical conditions (e.g. pH, dissolved oxygen, nutrient availability and balance), and strongly shape wetland biomes and ecosystem functions (Fig. 1). It should be noted that wetlands can be an inorganic nutrient sink, a nutrient source, and a transformer of inorganic nutrients to organic nutrients (Mitsch and Gosselink, 2015). Therefore, an understanding of the impacts of nutrient inputs on biogeochemical processes, and the microbes that mediate the processes, is necessary to manage healthy wetland ecosystems.

Several lines of evidence have indicated that monitoring microorganisms as biological indicators in response to nutrient loading, pollutants, and redox potential is valuable for wetland management (Artman et al., 2008; Merkley et al., 2004; Paerl et al., 2003; Sims et al., 2013; Wright et al., 2009; Zhang et al., 2013). Due to their rapid growth rates and quick response to changes, microbial populations can serve as the most sensitive and rapid bioindicator in response to various pollutants (Parmar et al., 2016; Reddy and D'Angelo, 1997; Urakawa et al., 2012). Evaluation of wetland conditions and restoration efforts cannot be met effectively by a single physical, chemical or biological parameter, but a combination of multiple attributes is effective for robust wetland assessment and management (Mitsch and Gosselink, 2000; Sims et al., 2013). Various functional groups of microorganisms can be used as wetland assessment tools (Table 1), and a profound understanding of microbial population dynamics, functional redundancy and ecophysiology may support a strategic wetland assessment and management (Fig. 2).

Today 16S rRNA gene amplicon sequencing makes it easy to obtain comprehensive profiles of the phylogenetic diversity of microorganisms from various environmental samples, but 16S

* Corresponding author at: Florida Gulf Coast University, 10501 FGCU Blvd. S., Fort Myers, FL, 33965 USA.
E-mail address: hurakawa@fgcu.edu (H. Urakawa).

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0925-8574/

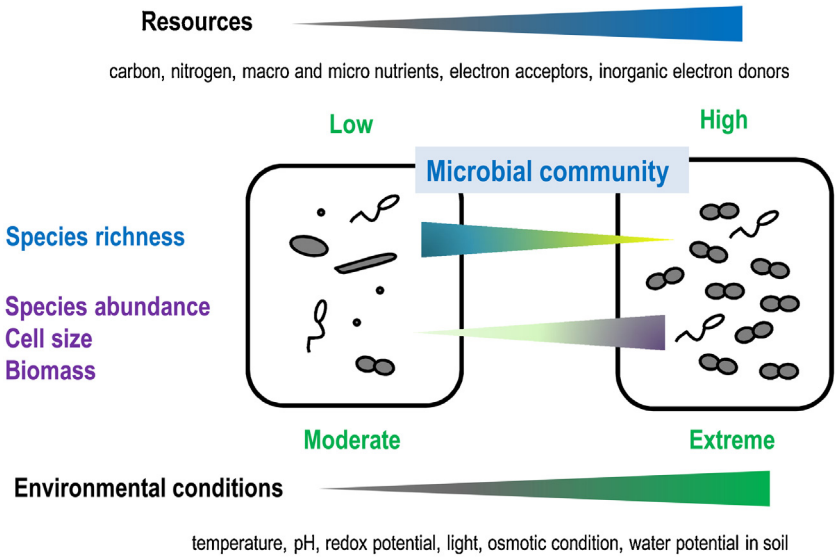

Fig. 1. Inputs of nutrient and other resources relevant to microbial communities in the wetland ecosystem. Species richness indicates the total number of different species present. Species abundance means the proportion of each species in the community. Moderate conditions may increase interactions among microbes, eukaryotic microbes and macro-organisms (e.g. plants, microbial predators). Extreme conditions (e.g. acid mine runoff, hypersaline lakes) decrease species richness but increase species abundance, cell size and biomass.

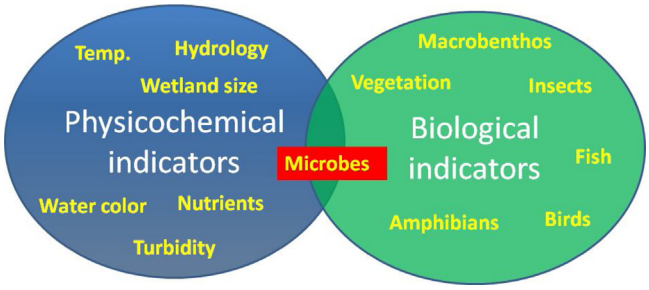

Fig. 2. Microbial indicators can be used for wetland management because they strongly couple with both physicochemical and biological indicators. Microbial indicators show rapid changes in response to the shift of environmental conditions and are extremely sensitive to environmental pollutants.

rRNA analyses do not necessarily relate to functional responses. To use microbes as bioindicators, genus-level ecotype identification (Fig. 3) and knowledge of ecophysiology of the microorganisms are necessary (Fig. 4). Since the vast majority of microorganisms have not yet been cultivated (Head et al., 1998), ecophysiology is often inferred from analysis of functional genes and transcripts. Phylogenetic and functional analyses using millions of sequences shall provide a detailed sketch of microbial diversity and functions in a wetland ecosystem. This review focuses on a potential use of microorganisms as an effective biological indicator for wetland management.

2. Microbial biomass and function

2.1. Bacterioplankton biomass and function

The biomass of bacterioplankton in wetlands may be regulated by available resources, habitats, and various environmental conditions in the ecosystem (Fig. 1). The abundance of bacterioplankton is also controlled by seasonal changes, and may be influenced by more long-term climate changes (Field et al., 1998). The total number of bacterioplankton is most accurately determined by the direct cell counting method using polycarbonate membrane filters, fluorochromes (e.g. 4′,6-diamidino-2-phenylindole [DAPI], SYBR Green I) and a fluorescence microscope (Faulwetter et al., 2009; Kepner and Pratt, 1994). With the combination of hybridization techniques (i.e. fluorescence *in situ* hybridization [FISH]) the number, morphology and spatial distribution of specific microbial populations can be selectively determined (Daims et al., 2015; Posch et al., 2009). The number of cells expected in wetland water columns is in the range of 10^5 to 10^6 cells/ml (Decamp and Warren, 2001; Hallberg and Johnson, 2005). The number of microbial cells may reach 10^7 cells/ml in nutrient-rich constructed wetlands or extreme conditions where microbial predation is limited (e.g. acid mine drainage) or there is very active decay of plants or animals (Fig. 1) (e.g. Decamp and Warren, 2001). Microbial biomass is also assessed by the compositional analyses of carbon, nitrogen and phosphorus. Wright et al. (2009) reported that microbial biomass P was somewhat more responsive to nutrient loading than biomass C and N, and can be a better marker to assess the level of eutrophication in the Everglades wetlands. It should be noted that bacteria in phosphate-limited freshwater environments can become a strong competitor of phytoplankton (Currie and Kalff, 1984), which may impact primary production. Under normal hydrological conditions, a large part of the water column is oxic, supporting high levels of aerobic heterotrophic activity, and constitutes one of the major microbial functions in the water column.

Bacterioplankton in some eutrophic surface waters may harbor a large fraction of Cyanobacteria. Because of their important role in primary production, their spatial and temporal abundance are of interest to researchers and freshwater monitoring programs (Paerl et al., 2016). The occurrence of massive cyanobacterial blooms in freshwater ecosystems is considered environmental pollution worldwide, and may be intensified by climate change (Paerl and Huisman, 2009; Paerl et al., 2016), so monitoring these populations is of critical importance (Fig. 5). Since commonly used chlorophyll *a* in vivo fluorescence cannot be used to accurately determine cyanobacterial abundance because their chlorophyll *a* locates in non-fluorescing photosystem I, analyzing phycobilin concentrations is preferred for detecting, quantifying, and monitoring cyanobacterial abundances (Seppälä et al., 2007). Generally, Cyanobacteria contain phycocyanin as an accessory pigment, and it can be used to fluorometrically differentiate Cyanobacteria from other eukaryotic algae by using narrow band interface filters that utilize excitation and emission wavelengths

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