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REVIEW

The Roles of Microbes in Stream Restorations

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Abstract

The goods and services provided by riverine systems are critical to humanity, and our reliance increases with our growing population and demands. As our activities expand, these systems continue to degrade throughout the world even as we try to restore them, and many efforts have not met expectations. One way to increase restoration effectiveness could be to explicitly design restorations to promote microbial communities, which are responsible for much of the organic matter breakdown, nutrient removal or transformation, pollutant removal, and biomass production in river ecosystems. In this paper, we discuss several design concepts that purposefully create conditions for these various microbial goods and services, and allow microbes to act as ecological restoration engineers. Focusing on microbial diversity and function could improve restoration effectiveness and overall ecosystem resilience to the stressors that caused the need for the restoration. Advances in next-generation sequencing now allow the use of microbial 'omics techniques (e.g., metagenomics, metatranscriptomics) to assess stream ecological conditions in similar fashion to fish and benthic macroinvertebrates. Using representative microbial communities from stream sediments, biofilms, and the water column may greatly advance assessment capabilities. Microbes can assess restorations and ecosystem function where animals may not currently be present, and thus may serve as diagnostics for the suitability of animal reintroductions. Emerging applications such as ecological metatranscriptomics may further advance our understanding of the roles of specific restoration designs towards ecological services as well as assess restoration effectiveness.

Keywords Stream restoration · Restoration ecology · Microbial ecology · Bioassessment · Biological indicators

Introduction

Stream restoration activities are increasingly critical in humanity's ongoing attempts to mitigate or reverse aquatic degradation. However, our interactions with freshwater ecosystems are a paradox. We rely on their many services from clean water for drinking, irrigation, and recreation to flood control and biodiversity maintenance and protection. Yet, we intentionally, and often unwittingly, use them for waste disposal, and we divert their flows for many purposes. These actions alter physical and chemical properties to the detriment of the ecosystem's inhabitants and have made freshwater systems some of the most sensitive and imperiled ecosystems on Earth [1–3]. Streams are particularly vulnerable to human activities and landscape change because they interact with uplands in a linear configuration. Actions at even the highest elevations of watersheds eventually reach and influence streams given enough precipitation. Persistent stressors can continue to influence streams with each runoff event, and the legacy of past insults may negatively impact stream ecosystems for decades [4, 5]. A few examples of such stressors in urbanized watersheds include eutrophication caused by increased inputs of nitrogen from agriculture and wastewater [6], acute toxicity from storm runoff for some fish species [7], chronically elevated stream conductivity from road salts [8], and more frequent flood

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events and unstable habitats [9] cumulatively leading to reduced biodiversity and ecosystem function [10, 11]. Consequently, in-stream biota are forced to endure, flee, or perish when confronted with multiple spatiotemporal dimensions of stressors compressed into what is functionally a one-dimensional environment for higher organisms [12].

Despite billions of US dollars spent on stream restorations [13], little evidence exists for ecological improvements in urbanized watersheds with our current approaches [14–17]. Even well-executed restorations that provide seemingly good biotic habitats and physical conditions may not return the desired ecological communities because of larger constraints, such as high levels of impervious surface cover, imposed by human activities in the surrounding watershed [10, 11, 18]. Nonetheless, restoration activities will continue in the USA and elsewhere because of government anti-degradation and water quality mandates and a long history of using in-channel construction in our efforts [19]. Therefore, new approaches and perspectives are needed to increase the chances of positive outcomes. One potentially beneficial approach is to design stream restorations to better harness the powers of stream microbiomes to enhance ecosystem functions. Microbes, including bacteria, archaea, protists, and microalgae, are dominant players in these ecosystem functions [20]. Microbial biomass is a primary food supply for stream food webs, and microbial metabolism drives cycling of biogeochemically active elements and transforms terrestrial organic matter, nutrients, and pollutants. Through these activities, microbes are intimately involved in limiting eutrophication, degrading toxins, and providing food to sustain stream biodiversity. Thus, facilitation of high functioning stream microbiomes, by focusing on details in restoration design to be more microbe-aware, has the potential to generate ecological benefits even in situations where enhancement or reintroduction of traditional aquatic indicator taxa is unsuccessful. In this paper, we describe ways in which microbes and their desired ecological functions may be more effectively incorporated into stream restorations and ways in which they can be used as diagnostics for tracking restoration success.

Microbiome enhancement has a long tradition in agriculture [21–24], and microbes are increasingly viewed as the primary agents of restoration in soil ecosystems [25, 26]. Microbial fertilizers or biofertilizers containing beneficial microbes have been shown to enhance soil restoration efforts [27, 28] and bioremediation [29, 30]. Many restoration strategies use microbes to encourage growth of specific plant or animal taxa that anchor restorations or serve as ecosystem engineers for jump-starting restoration. For example, inoculating soils with beneficial microbes via native soil enhancement or arbuscular mycorrhizae additions enhanced recovery of dune grasses [31–33] and prairie grasses [34–37]. Another approach is to use soil amendments to enhance growth of beneficial microbes, such as using biochar to improve wetland restorations [38].

Similar strategies may enhance stream restoration projects, but there are several challenges to overcome. Microbial communities are more transient in streams than soils (e.g., [39]) and are potentially less controllable for restoration purposes than higher organisms. The best approach in streams is probably to establish conditions that harness the continuous rain of microbes dispersed from landscapes and encourage them to colonize and perform desired functions in suitably created habitat zones for that desired function. Thus, the linear nature of streams makes them acutely vulnerable to disturbances, but the unidirectional, consistent flowpath has advantages for microbial dispersal and recolonization where it is difficult to create and maintain consistent geochemical gradients.

Microbes as Ecological Restoration Engineers

Microbes provide many different goods and services to target during restoration design, including organic matter breakdown, nutrient removal or transformation, pollutant removal, and biomass production (Table 1). But since microbial communities are immensely diverse and turn over rapidly, it is impractical to use restoration designs targeting specific microbial taxa in the same way that restoration design targets larger organisms. Instead, restoration designs that use a “build it and they will come” strategy are more likely to succeed at promoting microbial goods and services in contrast to the known limitations with higher organisms [40]. However, we know that various microbial goods and services require differing sets of environmental conditions to function and that streams are dynamic environments. For example, biomass production by microbial biofilms is favored under oxic conditions, but nutrient removal (via denitrification) requires anoxic conditions. Thus, a balanced approach is necessary in creating or manipulating suites of conditions that favor each of the desired microbial functions. At the same time, this balanced approach to restoration design may help the overall ecological system to better withstand the stressors that caused the degradation in the first place. To promote microbial goods and services, we propose that the keys are to retain organic matter within streams by creating storage within gravels, depositional areas, and debris jams, and to provide stable, hard substrates for microbial colonization.

An extreme example of site manipulation to maximize a microbial ecosystem service is the Regenerative Stormwater Conveyance (RSC) approach designed to use bacterial communities for denitrification [41] to achieve water quality requirements of the Clean Water Act [42]. A series of step pools are created within a stream, and each pool has a deep organic layer up to several meters thick to serve as an anoxic microbial bioreactor. Stormwater from the surrounding uplands is detained in the pools to reduce peak flows downstream, and nitrogen is removed as the stormwater infiltrates the organic layer. When designed correctly and properly sized for stormwater, RSC installations can return stream discharges to pre-development hydrograph shape and also reduce nutrients and suspended sediments [43].

RSC installations often underperform expectations of nitrogen removal for various reasons including position in the watershed, high seasonal water table, and under-sized pool and underlying media volumes [44, 45]. Seasonal performance differences may also be related to the quantity and quality of DOC and temperature-associated patterns of microbial activity [46]. But perhaps more concerning for stream restoration efforts is that, by maximizing design for nitrogen removal, RSC construction results in tradeoffs between nitrogen reductions and degraded communities of higher organisms due to low dissolved oxygen and sub-optimal habitats in the step pools [16] as well as the potential release of phosphorus under anoxic conditions [47]. A more integrated approach with restoration designs that use multiple microbial pathways could be more effective than trying to make a stream perform a single function exceeding its natural abilities [18].

Stream restorations creating a greater diversity of surface and subsurface physical conditions (Fig. 1) may help to achieve water quality goals while also providing redundancy and resilience to the overall system. Hyporheic zones are "biogeochemical hotspots" [48, 49] where the mixing of groundwater and surface water under a range of oxygen conditions promotes substantial microbial respiration to break down organic substrates and remove nutrients and pollutants [50–52]. Most of the overall ecosystem respiration in streams occurs via microbial activity in hyporheic zones [53, 54], and their potential importance in stream restoration is well known [55, 56]. Here and throughout stream ecosystems, microbes take up dissolved organic matter and convert this largely unavailable component into bacterial biomass that can be consumed by higher trophic levels [57, 58].

Restorations creating a balance of substrates with areas of hypoxic conditions juxtaposed with areas promoting aerobic hyporheic exchange will increase ecosystem benefits from microbial communities by harnessing both aerobic and anaerobic potential. Such conditions are often coincidentally created in restorations, but more microbe-aware designs specific to each project and structure can further enhance the functionality. Water surface height differences like those that form around in-stream structures (Fig. 1) create head gradients that generate rapid fluxes through hyporheic zones within the channel bed that feature anoxic sections upstream

Table 1 Stream restoration attributes that can promote microbial activity

Restoration attribute/activity	Goal	Ecological service provided by microbes	Other ecological services
Deep hyporheos	Deep anoxic and oxic zones	Denitrification (hypoxic); carbon processing (oxic) and storage; biomass	Habitat/refugia for fish and benthic macroinvertebrates
Vertical roughness elements (logs, boulders, debris jams)	Surface area for biofilms; stream flow energy dissipation and redirection	Microbial diversity; overall processing and uptake; biomass	Fish and benthic macroinvertebrate habitats; organic debris entrapment
Stable depositional areas	Increased longer-term storage of coarse organic materials	Carbon processing via leaf breakdown by fungi; some denitrification in hypoxic sediments	Fish and benthic macroinvertebrate habitats
Abrupt stream elevation changes	Create stable hypoxic zones (upstream) and oxic zones (downstream) via hydraulic head gradients	Denitrification (hypoxic) and carbon processing (oxic)	Fish and benthic macroinvertebrate habitats
Diverse channel bedforms and substrate particle sizes	Hydrodynamic diversity	Microbial uptake and processing; increased microbial diversity	Fish and benthic macroinvertebrate habitats
Connections to the floodplain	Energy dissipation from channel onto floodplain during high discharges; temporary pool creation	Nutrient capture via deposition and biogeochemical activity in temporary pools and sediments	Temporary waterfowl and amphibian habitat, biomass/food production by phyto- and zooplankton

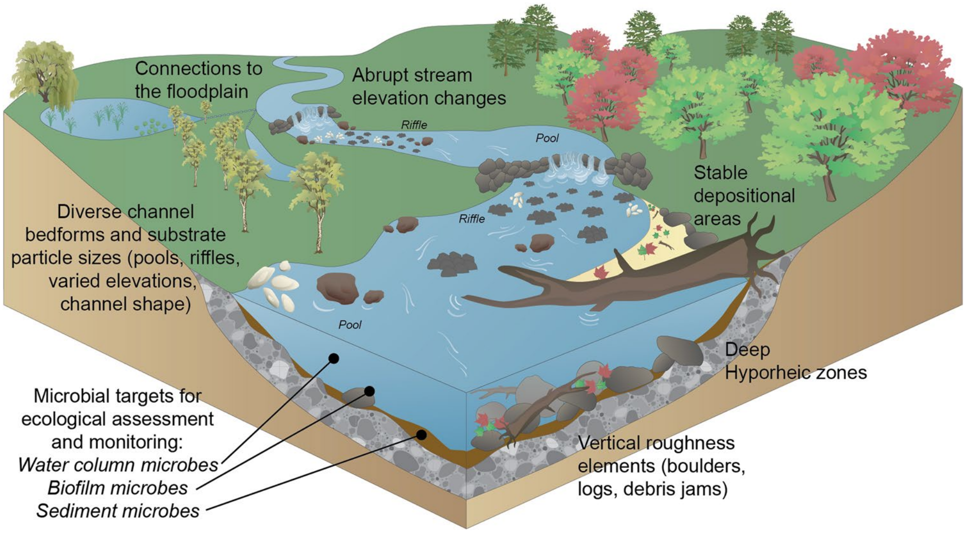

Fig. 1 Conceptual diagram of areas where stream restoration elements can enhance ecosystem services and assessments provided by microbes

in low-velocity pools and oxic conditions downstream in turbulent riffles [59]. The changes in surface height and composition of channel substrates substantially influence the water flux [60, 61], and these features can be controlled by the restoration design. Oxic zones around a structure can become net sources of nitrate (via microbial decomposition of organic N and nitrification of ammonium; [62]), while others become sinks for nitrate and dissolved oxygen (via denitrification and aerobic respiration; [63]). Designs that create consistent, reliable hyporheic mixing and exchange between oxic and anoxic environments through hydraulic head gradients can shift a system from one dominated by stochastic processes to a more deterministic system with elevated rates of biogeochemical processing [51].

Restoration designs for microbial processing need not be focused on restoration structures. Gravel bars and other coarse substrates that increase hyporheic depth or exchange can become enhanced sites for organic matter retention with increased compositional and functional diversity [64]. The stored organic matter in gravels increases nutrient retention [65] and can be the main carbon source for many microbial communities [66]. Microbial contribution to leaf breakdown can be higher in gravel than in debris jams where fungal and benthic macroinvertebrate activities are highest [67, 68]. Thus, intentionally created deep gravel bars may substantially increase ecological functions as well as provide additional habitats for benthic macroinvertebrates and fishes [69].

Careful thought to overall channel structure and streambed morphology in restorations can leverage microbial contributions (Fig. 1). Microbial diversity and differentiation in pool habitats are associated with stream substrate stability, with soft or more mobile sediments dominated by heterotrophic bacteria and more stable substrates encouraging photosynthetic organisms in rock biofilms [70]. Pool habitats and debris jams also promote substantial fungal activity on coarser organic substrates and can substantially exceed bacterial number and biomass in these depositional areas [67]. Increased physical heterogeneity, such as a diversity of hard substrates (rocks, logs) with complex shapes within the channel and areas for organic matter deposition and storage, will lead to greater surface areas available for microbial biofilm colonization. Biofilms can contain diverse members of the bacteria, archaea, and eukaryotes and are significant contributors to stream metabolism and material transfer [20, 71]. Diverse hydrodynamic conditions lead to variations in biofilm biomass and physical structure as well as increased resource use [72]. Thus, stream restorations containing high amounts of heterogeneity in both channel substrates and flow velocities should promote increased microbial activity for material transformations and increased microbial biomass available for direct consumption by benthic macroinvertebrates. Greater biofilm diversity and production will support more biomass of higher organisms with an overall effect of greater total biomass binding more nutrients that might otherwise contribute to eutrophic conditions and dead zones in downstream receiving waters. Integrated across entire stream reaches, the cumulative results could be substantial for restored streams.

Not all stream restoration activities are confined to the stream channel, and many of the long-term gains can be made by altering off-channel and upland conditions to protect the receiving stream. Reconnecting a stream to its floodplain by lower bank height or instream debris jams that mimic beaver dams (Fig. 1) not only dissipates the kinetic energy of flood events from the channel and onto the floodplain, but can also remove substantial amounts of nutrients and sediments through deposition and microbial processing [73–75]. Riparian forests and uplands also provide a regional and temporally variable source of microbial colonizers to allow the stream to change with varying environmental conditions [39].

In summary, we propose that more microbe-aware stream restorations can enhance the functioning of restored urban streams. Stream restorations in such highly modified watersheds usually fail to return the desired animal communities, and instead continue to reflect degraded conditions. Shifting to a microbe-aware focus leverages existing restoration approaches, and purposefully creates conditions that will enhance microbial function in both local reaches and impacted areas downstream (e.g., reduction of dead zones in large rivers and estuaries). We see this as an important research area in which future work should seek to determine if and how much increased functioning can be achieved with this strategy, and whether microbially enhanced restorations allow the return of desired higher organisms. To implement this strategy, restorations should focus on a balance of habitats and functions rather than maximizing one or two functions or services. Overly controlling environmental variation often results in additional problems or disappointments [76], whereas more balance should provide increased resilience to frequent disturbances and stressors in these highly modified systems.

Microbes as Ecological Indicators

The wide distribution and diversity of microbes make their use as environmental and ecological indicators an exciting new assessment and monitoring tool. We see this application as particularly promising for stream restorations, where traditional bioindicators (i.e., fish and benthic macroinvertebrates) are often lost to degradation, and absences may lead assessments to conclude a restoration was not ecologically successful. However, post-restoration conditions creating robust microbial communities and associated biogeochemical processes may allow higher organisms to re-establish.

Stream microbes are thus particularly promising for both ecological assessment and monitoring given their many useful attributes including broad distributions and dispersal abilities [77]; large taxonomic richness [78]; and wide functional diversity and redundancy across different environments and levels of degradation [79]. Microbiomes thus have potential use as hydrological predictors [80, 81] and ecologically relevant indicator taxa [82].

Microbes as ecological indicators have transitioned from light microscopy of benthic algae [83, 84] to genomic-based approaches using various gene sequence targets and next-generation sequencing. Periphyton- or diatom-based assessments such as the U.S. Geological Survey's National Water Quality Assessment Program [85–87] are currently being supplemented or replaced by DNA (meta-) barcoding using the *rbc*L chloroplast gene [88–90]. Genomic-based approaches using both 16S and 18S amplicon sequencing to include bacteria, archaea, and eukaryotes are increasingly being adopted by agencies such as the U.S. E.P.A. [91, 92]. Their use is supported by work to optimize taxonomic resolution [93], correlate microbial community structure with existing indices [82, 94], and develop watershed-specific metrics [95–97].

Specific riverine habitats harbor distinct microbial communities that reflect specific properties of the system (Fig. 1). Water column communities differ from stream sediments [98] and tend to reflect microbes in the surrounding uplands [99]. Water column microbes also correlate with watershed urbanization, impervious surfaces, and other landscape characteristics [39, 100, 101], which themselves strongly relate to ecological condition [102] and fish and benthic macroinvertebrate biodiversity [10, 11, 103]. Sediment microbes better reflect environmental conditions within a stream reach [100] and reproduce ecological assessments using traditional benthic macroinvertebrate monitoring [94]. Biofilms from hard surfaces also have their own distinct community structures and were the first microbial communities showing potential in assessments with genomic-based methods [95, 104, 105]. Biofilm communities on stable surfaces such as rocks may be very useful for assessing ecological conditions because they reflect time-integrated conditions where communities develop through ecological sorting.

Developing applications incorporating all aspects of river microbiomes may advance assessments beyond current abilities. We suggest exploring sampling regimes that include separate water column, sediment, and biofilm collections. A single water column collection may suffice in most systems due to homogenization via river flow, but multiple sediment samples should be collected in stable depositional areas and pooled. Biofilms should also be sampled across different substrates with hydrodynamic variability and can be pooled. Treating each habitat/community separately for library preparation and sequencing will facilitate comparisons among different habitats, yet allow the data from all environments to be combined to produce ecological assessments.

Applications using microbial indicators may face tensions between specificity and generality. The thousands of microbial species ("operational taxonomic units" or OTUs) in streams could be highly specific to environmental conditions or even symbiotic or parasitic to specific metazoan taxa. Such cases would make these OTUs very specific and useful as indicators for some types of monitoring. However, their over-specificity limits their use as general indicators and misses the bigger picture for ecological assessments. Hilderbrand et al. [94] found that grouping bacteria and archaea at the Order level assessed stream ecological condition with similar accuracy as finer levels of taxonomic resolution and avoided the issues with over-specificity. The approach was similar to Index of Biotic Integrity methods developed for fish [106] and benthic macroinvertebrates [107], which use community composition as diagnostic indicators. Similar to blood panels for human health, the proportions of different taxonomic or functional groups can be diagnostic of overall ecological condition. Although a taxonomic order may contain diverse ecological functions, there may be something ecologically diagnostic about the group. For example, the EPT (Ephemeroptera, Trichoptera, Plecoptera) triad of benthic macroinvertebrate orders is a diagnostic indicator of ecological condition because of general pollution intolerance despite this broad group containing numerous trophic groups and habitat uses [108]. Thus, we believe it is unlikely that individual OTUs can be used as general indicators because of the diversity of environmental conditions found even in undisturbed streams. As the numbers of classified microbial OTU sequences (and their functional roles) in databases increase, we will better understand the biogeography of microbes and taxonomic specificity required for ecological assessment at larger spatial scales.

An exciting emerging application to assess stream restoration effectiveness is through ecological metatranscriptomics [109, 110]. Expression of genes associated with ecosystem services such as denitrification and polyaromatic carbon degradation can be measured by sequencing the messenger RNA transcripts of bacteria, viruses, eukaryotes, and other groups individually or in total [111, 112]. Although this technology is in the early stages, ecological metatranscriptomes could be used to identify various functions and their relative levels of activity in streams. Restored streams could be compared against degraded and undisturbed streams to identify the degree to which restoration designs produce intended effects. Changes in ecosystem services could even be measured for individual restoration structures. If watershed conditions such as high amounts of impervious surfaces or intermittent or ephemeral streams preclude establishment of desired fish or benthic invertebrate species, such an ecosystem functional perspective could determine if restorations are accomplishing water quality goals even when they fail on biodiversity measures for eukaryotes.

Despite the immense number of projects, stream restoration is still a young science trying to inform applications to systems vital to humanity's well-being. With our current understanding, it may not be possible to restore many desired species to some systems because of watershed modifications. It becomes all the more critical for restorations to fully harness the power of microbes to enhance ecosystem services, and to evaluate ecological condition. We propose that microbial assessment will be a powerful tool to evaluate the effectiveness of restorations and different design approaches, and will help move this young science forward. Applications to restorations across all levels of degradation can benefit from a more resilient and functional system.

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Author Contribution Robert H. Hilderbrand conceived of the manuscript. Robert H. Hilderbrand, Ted Bambakidis, and Byron C. Crump conducted the literature review and wrote the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing Interests The authors declare no competing interests.

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