Implications of microbial community assembly for ecosystem restoration: patterns, process, and potential

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Abstract.

While it is now widely accepted that microorganisms provide essential functions in restoration ecology, the nature of relationships between microbial community assembly and ecosystem recovery remain unclear. There has been a longstanding challenge to decipher whether microorganisms facilitate or simply follow ecosystem recovery, and evidence for each is mixed at best. We propose that understanding microbial community assembly processes during ecosystem restoration is critical to optimizing management strategies. We examine how the connection between environment, community structure, and function is fundamentally underpinned by the processes governing community assembly of these microbial communities. We review important factors to consider in evaluating microbial community structure in the context of ecosystem recovery as revealed in studies of microbial succession: 1) variation in community assembly processes, 2) measurable microbial community attributes, and 3) linkages to ecosystem function. We seek to empower restoration ecology with microbial assembly and successional understandings that can generate actionable insights and vital contexts for ecosystem restoration efforts.

Keywords: microbiome, disturbance ecology, succession, restoration ecology, microbial community assembly, community ecology
Introduction.

Restoration of degraded ecosystems is essential to maintaining global biodiversity and ecosystem services [1-4]. Yet despite large economic investments [5], restored ecosystems are often strikingly different in species composition and edaphic factors than their more pristine counterparts [6-10]. While restoration efforts have long focused on aboveground communities [11-13], there is increasing interest in the role of belowground systems, including their interactions with aboveground processes, as restoration tools. A central challenge in this new line of inquiry, as succinctly stated by Harris [14], is whether microorganisms follow or facilitate restoration. That is, do microorganisms simply reflect broader ecosystem succession or can they drive the restoration of degraded ecosystems?

Much evidence has been gathered to support both sides of this argument [6, 15-24]. For instance, restored habitats may promote microbial pollutant degradation [25, 26] and increased diversity and function of soil microbiota [20, 27, 28]; conversely mycorrhizal fungi are widely considered as facilitators of plant establishment [6, 23, 24]. In reality, each side of the equation likely plays a role in ecosystem restoration, with the relative balance dependent on ecosystem and species characteristics. Indeed, Van der Bij et al. [24] suggest the simultaneous application of belowground and aboveground biomass as an optimal restoration strategy. Understanding the coupled changes of below- and aboveground ecosystems during ecosystem restoration may be key to improving its efficacy. Whether microorganisms follow or facilitate restoration, application of concepts in microbial community assembly and succession can better attainment of desired restoration outcomes.

The study of ecological succession has long provided testable theory to understand factors that control changes in community structure and ecosystem function over time. More
recently, the application and examination of such theory to microbial communities has built insights into when and how microbial community structure changes through ecosystem recovery. Increasingly, ecologists seek to understand how and why these changes will mediate impacts on ecosystem function, and related theory can thus inform belowground dimensions of restoration efforts. In particular, microbial community assembly processes have been noted as an important driver of microbial community structure and function during natural and managed secondary succession.

Here we seek to empower restoration ecologists with microbial assembly and successional understandings that can generate actionable insights and vital contexts for ecosystem restoration efforts. To that end, we examine how the connection between environment, community structure, and function is fundamentally underpinned by the processes governing community assembly of microbial communities. We review important factors to consider in evaluating microbial community structure in the context of ecosystem recovery as revealed in studies of microbial succession: 1) variation in community assembly processes, 2) measurable microbial community attributes, and 3) linkages to ecosystem function. Ultimately, we provide guidance on how assembly processes may help unify variable results of empirical studies assessing biodiversity-ecosystem function and environment-ecosystem function relationships to reveal opportunities for guiding microbial mediated biogeochemistry in restoration.

Successional Trends in Community Assembly.

There is much debate surrounding the roles of microbial community succession and assembly in ecosystem restoration. Some insist upon unpredictable patterns in microbial
communities through secondary succession and restoration [29-32]; more recently others highlight demonstrable patterns along these trajectories [21, 33, 34]. Underpinning these patterns are community assembly processes. Existing understandings from microbial succession on when and how community assembly is driven by more deterministic or stochastic processes, in turn informs strategies of when and how microbial communities may be influenced for restoration purposes.

Microbial community assembly is determined through the balance of deterministic and stochastic processes, each of which can operate across distinct spatial, temporal, and genetic variables [35-39]. Determinism is largely governed by the interplay of homogeneous vs. variable selective pressures; while stochasticity results from a combination of dispersal, drift, and dispersal limitation [37]. At the highest level, selection results from environmental conditions, or changes in those conditions through successional trajectories. Key factors influencing the selective pressures imposed on various microbial clades include temperature [40], moisture [41], salinity [42, 43], pH [44, 45], vegetation type [46-49], and resource availability [50-53].

Homogeneous selection through space or time results in specialized communities with low diversity, while variable selection can result in high diversity through niche-based processes [37, 38]. Similarly, variation in body size and dispersal traits (e.g., sporulation, flagella) can influence dispersal and dispersal limitation in taxon-specific ways [35, 36, 54-58]. In turn, the ratio of immigration/emigration influences community biodiversity. Differences in assembly between bacteria and fungi [59], heterotrophs and autotrophs [36], specialists and generalists [54], and abundant and rare taxa [60-63] influence community composition.

While there are multiple possible trajectories of assembly processes through succession [33], we present commonly observed patterns, as well as trajectories that may be beneficial for
restoration efforts in Figure 1 and Box 1. We note that while we generally discuss assembly at the community-level, variation in assembly can also be assessed across microbial clades as discussed above [36, 60, 64-66]. Generally, there is an early period of stochasticity during secondary succession and restoration that is succeeded by increasing determinism [33, 67], although Dini-Andreote et al. [33] note that secondary succession may begin under a deterministic environment depending on disturbance type. Other factors that can influence assembly trajectories include priority effects whereby initial colonizers set successional trajectories despite similar environments [68-70] and life history strategies, with fast growing r-strategists having an advantage in early succession [71].

In the case of secondary succession, the type and strength of disturbance can impact the initial degree of stochasticity. Strong disturbances lead to a “blank slate” environment that can be easily colonized via immigration. Extreme disturbances can also result in low resource availability, limit microbial biomass, and limit the survival of sensitive species [21, 72]. In contrast, resilient community members remain after weaker disturbances, supporting competition and niche differentiation. As environmental stress declines through restoration, these attributes tend to recover, increasing selective pressures and opening new ecological niches [72-75].

**Linkages between Microbial Assembly and Ecosystem Function.**

The connections between successional trajectories in species composition and ecosystem functions are essential to promoting the long-term success of restoration efforts. Vital to this line of inquiry is understanding how communities that assemble through time evoke changes in community diversity, species and trait abundances, and interactions between community members that ultimately impact gene expression.
The biodiversity-ecosystem function (BEF) is a central tenet of ecosystem ecology and is increasingly recognized as an important fundamental of microbial systems [76-81]. BEF research posits that biodiversity supports enhanced rates of ecosystem functions through ecological mechanisms such as niche or resource use complementarity, mass or sampling effects in which more diverse communities include members with a trait of interest, and functional redundancy or insurance in which organisms or communities contain multiple copies of a trait of interest [82-84]; all of which may point to a role for rare species in supporting ecosystem functions [85, 86]. Yet, the nature of the BEF relationships can vary with assembly processes, ecosystem attributes, and the amount of redundancy in a community [rev. in 82]. In some cases, increases in diversity may even decrease ecosystem function; for example, through the dilution of traits of interest from immigration [54].

Community assembly therefore provides a framework for understanding the nuances of the BEF relationship. Determinism and stochasticity both denote ecological processes acting on organisms, or traits contained therein, that impact both their activities and overall community composition. For instance, selection can optimize microbial metabolism through species sorting [87, 88]. Communities with a history of consistent selection may be particularly well-adapted and contain efficient pathways for catalyzing biogeochemical processes and cell maintenance [54]. Alternatively, selection may exclude taxa that metabolize more scarce resources, resulting in decreased community metabolism [82, 89, 90]; and Graham et al. [35] have suggested that multiple competing selective pressures can optimize community function. Various stochastic processes can also alternatively increase or decrease ecosystem functions. Under high dispersal rates, communities may be more likely to contain species with more traits that are beneficial to a function of interest [91, 92] and allow for more niche complementarity [93]. This may be
particularly important in early succession [33, 67]. Conversely, dispersal may add maladapted organisms to a community (i.e., mass effect or source-sink dynamics [94, 95]) resulting in decreased function, an effect that may vary with niche breadth [54]. Dispersal limitation can also inhibit the ability of organisms to reach their optional environment or decrease the diversity of immigrating organisms, lowering community function [54, 77, 96]. In total, it maybe be that a combination of diverse assembly processes acting on a varied suite of traits optimizes community functions [54].

Underlying the relationship between microbial diversity, community composition, and ecosystem function is the coupling of response and effect traits (Figure 2). Response traits determine species composition through interactions with the environment (e.g., spore formation, life history), while effect traits correspond to the functional roles of taxa (e.g., biogeochemical cycling, [97, 98]). The strength of the relationship between response and effect traits determines the impact of changes in community assembly on ecosystem functions. In turn, this is contingent on phylogenetic conservation of traits, or ecological coherence in which physiological diversity aligns with phylogenetic diversity [21, 99-101]. When determinism reigns, the coupling between effect and response traits varies depending on whether the environment selects for clades of organisms with optimal functional traits or acts on response traits that are unrelated to the ecosystem process of interest [82, 102-105]. When stochasticity reigns, community composition will largely depend on effect traits that facilitate or impede dispersal (e.g., spore formation), and thus response traits that dictate function may be decoupled from the environment. When a single response trait is dominant, community composition may be more easily manipulated by restorations efforts. For example, Knelman et al. [53] showed that nutrient addition to a nutrient limited glacial environment could dramatically advance succession.
Key Microbial Indicators for Assessing Assembly and Succession.

Because microorganisms respond quickly to changes in the environment, microbial communities are used in restoration ecology to assess the status of an ecosystem relative to a reference condition or to enhance ecosystem recovery through inoculations [14, 21, 106]. The return of soil microbial properties such as diversity and nutrient cycling are often considered to be indicative of restoration success [107]. More details on common metrics used to describe microbial communities, their relationships to community assembly and ecosystem function, and their implementation in restoration ecology are provided in Table 1. We provide a brief discussion of each below.

Diversity metrics are perhaps the most longstanding descriptors for microbial community composition. Indeed, many have pointed to microbial community diversity as a major driver of ecosystem health and soil fertility. Alpha- (within community), beta- (across local communities), and gamma- (across regional communities) diversity each serve as easy-to-measure assessments of ecosystem trajectories. At a finer level of resolution, trait-based approaches provide information on changes in response traits (e.g., changes in life history strategies) and effect traits (e.g., changes in carbon and nutrient cycling) through restoration [102, 104, 105]. For instance, fungal:bacterial ratios, ribosomal gene copy numbers, or shifts in bacterial and fungal composition are often leveraged to infer changes in r- vs. k- selection [14, 22, 71, 108-110]. Many studies also pair these metrics with changes in corresponding effect traits, particularly those related to biogeochemical cycles. Common observations include a shift from nitrogen cycling to C cycling as secondary succession proceeds [34, 65, 111, 112], greater C storage [113, 114] and aggregation [115, 116], and lower water stress [115, 116]. Finally, microbial community assembly is increasingly being studied by restoration ecologists. Most rely on neutral
or null modeling approaches to infer the strengths of determinism (e.g., variable and homogenous selection via $\beta$NTI as proposed by Stegen et al. [37]) and stochasticity (e.g. $R_{\text{bray}}$ [37]).

**Applications in Restoration Ecology.**

The multitude of drivers and organismal interactions are a major challenge to evaluating and enhancing successes in restoration ecology. Given the large economic cost of restoration, it is vital that we garner a better understanding surrounding the trajectories and timeframes of restoration efforts. Without active intervention, ecosystem recoveries can occur exceedingly slowly [117, 118]. Belowground microbial communities present an opportunity to dramatically enhance and assess restoration outcomes [6, 108, 119]. For instance, there are many examples of restoration efforts focused solely on aboveground ecosystems that are unable to recreate reference conditions [7-9, 120-122], and microbial communities may be particularly important to facilitating the establishment of late successional species [6, 24, 123, 124].

Plant-microbial interactions are central to understanding the roles microorganisms play in restoration, particularly given the strong attention to management of vegetation. With some notable exceptions (e.g., mycorrhizal associations), it remains unclear how plants and the soil microbiome interact to drive ecosystem assembly and succession [14, 24], yet vegetation is an important factor in compositional and functional changes in microbial communities [6, 71, 125]. Plants have direct mutualistic and antagonistic relationships with soil microorganisms [126, 127] as well as indirect impacts through the transfer of phosynthate to decomposers and nutrient cyclers [128, 129]. Invasive species in particular have been noted to changed soil microbial community structure and function [130-132]. Conversely, inoculation with various microbes or
whole soils has been shown to have bottom-up effects on plant establishment during restoration [24, 127, 133].

Given this context, there are interesting ways in which soil microbial communities may aid in restoration (Table 2). First, microorganisms may serve as an early indicator of recovery [14, 21]. With the relative affordability of amplicon sequencing, microbial community composition could provide value as part of rapid ecosystem function assessments [134]. A deeper understanding of microbial communities within the context of the ecological theory presented may also serve to help leverage microbes create “shortcuts” for restoration [14].

Targeted inoculations with microbiota have proven to override priority effects and aid in plant re-establishment under some circumstances [24, 64, 73, 135]. Inoculations that allow for faster plant re-establishment may inevitably provide shortcuts in which ecosystems bypass successional stages in route to a recovered state [14]. These strategies may provide the most value when coupled with habitat manipulations that support microbiota of interest [127, 133].

The discussion here is intended to provide an overview of the importance of microbial community structure and how it relates to changes that occur during ecosystem recovery. While it is clear that microorganisms provide essential functions in restoration ecology, new implementations of microbial ecology during restoration may help bolster management successes. For instance, management of belowground systems could be directed towards improving gamma diversity so as to maximize the sampling effect during early succession, towards facilitating niche diversity, or towards creating selective environments that maximize response-effect trait linkages. The vast potential of microorganisms for the benefit of ecosystem restoration remains largely untapped, and a greater understanding of the ecological context in which these organisms reside provides an avenue for more fully realizing their power.
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Figure 1. Example changes in community assembly through restoration. Placing management strategies in the context of community assembly can help to optimize restoration outcomes. The balance of assembly processes is denoted on the vertical axis, with the gray line denoting an equal balance of determinism and stochasticity. Scenario 1 (blue) represents a tightly regulated, low diversity system for targeted (but limited) functions, for example contaminant remediation. In scenario 2 (red), early colonizers in a deterministic set the stage for succession. Selection wanes as the ecosystem recovers and a more stochastic late-stage ecosystem supports diverse and multifunctional microorganisms. Scenario 3 hosts an initial community with a mix of diversity that progresses into a specialized community under increasing determinism. Likewise, scenario 4 begins with a similar community under high stochasticity but ends with an even balance of determinism and selection. Such a trajectory may optimize the balance between specialists and generalists for high rates of ecosystem functioning. Finally, scenario 5 represents the influence of priority effects in which early colonizers hold an advantage through succession. This may require targeted inoculations to change microbial communities.
Figure 2. Relationships between environmental change, microbial traits, community composition, and ecosystem function. Changes in the environment impact the interplay between response and effect traits, which in turn influence microbial community composition. In turn, the environment and microbiome collectively determine ecosystem functions.
Box 1. Assembly-driven Considerations for Restoration Ecology.

Factors Influencing The Balance of Assembly Processes During Restoration
•Severity and Duration of Initial Disturbance
•Successional Stage
•Natural vs. Artificial Disturbance
•Strength of Environmental Pressures

Considerations for Restoration under Deterministic Microbial Assembly
•Environmental controls (pH, temperature, salinity, resource availability)
•Functional redundancy
•Niche Complementarity
•Response-effect trait linkages for traits of interest

Considerations for Restoration under Stochastic Microbial Assembly
•Metacommunity Composition
•Niche Breadth
•Priority and Mass/Sampling Effects
•Dispersal rate and/or limitations
Table 1. Common metrics of microbial community composition, relations to assembly processes and ecosystem function, and implications for restoration.

<table>
<thead>
<tr>
<th>Genomics-enabled Variables</th>
<th>Common Metrics</th>
<th>Assembly Process Implications</th>
<th>Ecosystem Function Implications</th>
<th>Restoration Implications</th>
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</thead>
</table>
| Alpha Diversity            | Shannon, Simpson, Chao1, Species Richness | • Elevated stochasticity (homogenizing dispersal) can lead to increased alpha diversity  
• The strength of selection (determinism) also influences alpha diversity, with strong selection in one direction generally leading to low alpha diversity | • High alpha diversity can lead to multifunctionality and/or low rates of a specific function through a dilution effect  
• Low alpha diversity may indicate specialized function or low rate of biogeochemical cycling | • Indicator of ecosystem health and multifunctionality |
| Beta and Gamma Diversity   | Alpha Diversity Metrics plus Ordination Groupings, $\beta$-Dispersion | • Elevated stochasticity or determinism can lead to increased (dispersal limitation; variable selection) or decreased (homogenizing dispersal; homogeneous selection) beta- and gamma-diversity | • High beta and/or gamma diversity may bolster local biogeochemical functions through rescue effects  
• Comparative metrics can indicate changes in function across time or space | • Can be used to assess microbial trajectories through time and/or similarities to natural or restored ecosystems  
• Potential for enhancement of restoration through rescue effect |
| Community Composition      | Species/trait (Relative) Abundances, Differential Expression and Gene Abundance | • Homogenizing dispersal or homogeneous selection can lead to more even species/trait distributions across communities  
• The balance of assembly processes interacts with environmental conditions to determine the relative abundance of determine distributions of response traits | • Can be used as an assessment of genetic potential for a biogeochemical process of interest | • Can be used as early indicators of restorative success  
• Can be manipulated to support restoration outcomes |
| Response traits            | Gene ratios, 16S rRNA gene copy number, Bacterial:fungal ratio; Resource Acquisition Traits; Resource Use Traits; Stress Tolerance Traits; Life History Traits | • The balance of assembly processes interacts with environmental conditions to determine the relative abundance of determine distributions of response traits | • Invoke and/or interact with effect traits that impact biogeochemical function | • Can denote successional stage and trajectory of community composition  
• Can be manipulated to support restoration outcomes |
| Effect traits              | (Relative) Abundance of Specific Genes or Taxa related to Biogeochemical Processes | • The balance of assembly processes interacts with environmental conditions and response traits to determine the relative abundance of determine distributions of effect traits | • Under optimal environmental conditions, effect traits have a direct impact on function  
• Impact mediated by environment | • Can denote the trajectory of functional recovery through successional stages  
• Can be manipulated to support restoration outcomes |
| Stochasticity vs. Determinism | Neutral and Null Models, $\beta$NTI, RCbray | • Direct measurements indicating the balance of assembly processes | • The balance of assembly processes can reveal the nature of expected biodiversity-ecosystem function relationships | • Can denote successional stage and trajectories in composition and function  
• Can be manipulated to support restoration outcomes |
Table 2. Examples of community assembly and management interventions.

<table>
<thead>
<tr>
<th>Balance of Assembly Processes</th>
<th>Example Management Intervention</th>
<th>Reference</th>
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</thead>
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<tr>
<td>Deterministic Assembly; Strong response-effect trait linkage</td>
<td>Altering soil environment (homogeneous or variable selection)</td>
<td>Addition of a single nutrient drives poorly developed soil bacterial communities to resemble late succession community state (Knelman et. al, 2014)</td>
</tr>
<tr>
<td>Stochastic Assembly; Dispersal/Immigration limitation</td>
<td>Altering microbial immigration rates</td>
<td>Increases in immigration (soil addition) improves abundance of key microbes and removal of estrogens in wastewater treatment (Pholchan et al., 2013)</td>
</tr>
<tr>
<td>Stochastic x Deterministic Assembly: Priority Effects</td>
<td>Manipulating microbial colonization order</td>
<td>Inoculation order of phyllosphere with bacterial community members results in distinct end communities (Carlström et al. 2019)</td>
</tr>
<tr>
<td>Stochastic x Deterministic Assembly: Plant Microbe Interactions</td>
<td>Controlling presence/absence of specific microbes</td>
<td>Availability of ectomycorrhizal fungi (dispersal limitation) hinders success of exotic Pinaceae (Nuñez et al. 2009)</td>
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