Integrating ecology into biotechnology
Katherine D McMahon¹, Hector Garcia Martin² and Philip Hugenholtz²

New high-throughput culture-independent molecular tools are allowing the scientific community to characterize and understand the microbial communities underpinning environmental biotechnology processes in unprecedented ways. By creatively leveraging these new data sources, microbial ecology has the potential to transition from a purely descriptive to a predictive framework, in which ecological principles are integrated and exploited to engineer systems that are biologically optimized for the desired goal. To achieve this goal, ecology, engineering and microbiology curricula need to be changed from the very root to better promote interdisciplinarity.

Addresses
¹ Department of Civil and Environmental Engineering, University of Wisconsin-Madison, 1415 Engineering Drive, Madison, WI, USA
² Microbial Ecology Program, DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA, USA

Corresponding author: McMahon, Katherine D (tmcmahon@engr.wisc.edu)

Introduction
Ecology is the study of the distribution and abundance of organisms and their biotic and abiotic interactions in an environmental setting. Biotechnological processes often rely on microbial organisms contained within an engineered environment designed to allow some level of operator control. All too often, however, such processes overlook or ignore the microbial communities that underpin the process. This has not necessarily been due to a lack of interest in the underlying communities, but primarily because of a lack of tools with which to dissect and monitor microbial organisms in a cost-effective and timely fashion. To date, efforts to describe microbial communities of microbial organisms in a cost-effective and timely fashion have been largely descriptive. Because of a lack of tools with which to dissect and monitor the process, this has not necessarily been due to a lack of interest in the underlying communities, but primarily because of a lack of tools with which to dissect and monitor microbial organisms in a cost-effective and timely fashion. To date, efforts to describe microbial communities of microbial organisms in an engineered environment designed to allow some level of operator control. All too often, however, such processes overlook or ignore the microbial communities that underpin the process. This has not necessarily been due to a lack of interest in the underlying communities, but primarily because of a lack of tools with which to dissect and monitor microbial organisms in a cost-effective and timely fashion. To date, efforts to describe microbial communities of microbial organisms in an engineered environment designed to allow some level of operator control. All too often, however, such processes overlook or ignore the microbial communities that underpin the process. This has not necessarily been due to a lack of interest in the underlying communities, but primarily because of a lack of tools with which to dissect and monitor microbial organisms in a cost-effective and timely fashion. To date, efforts to describe microbial communities of microbial organisms in an engineered environment designed to allow some level of operator control. All too often, however, such processes overlook or ignore the microbial communities that underpin the process. This has not necessarily been due to a lack of interest in the underlying communities, but primarily because of a lack of tools with which to dissect and monitor microbial organisms in a cost-effective and timely fashion.

From tools to principles
Many authors before us have remarked on the revolutionary impact that molecular tools have had on the disciplines of microbial ecology and environmental biotechnology over the past two decades [2,3,4,5]. As we continue to move beyond the cataloging of 16S rRNA genes and begin to catalog whole (meta)genomes, (meta)transcriptomes, (meta)proteomes, and ultimately (meta)metabolomes [1,6,7], the need to work within a framework designed to integrate data at a previously unimaginable scale is becoming ever greater. We will need to turn to the disciplines of ecology and ecosystem science for guidance.

Here we emphasize the need to go beyond descriptive science and apply theoretical ecology to understand, model, and manage environmental biotechnology systems. The basic principles of ecology developed during a century of study focused on how macroscale organisms interact with their environment and fellow community members should be mined by environmental biotechnologists for useful conceptual frameworks.

Environmental biotechnology systems tend to be engineered to select for a few high-performing functional groups that may or may not be represented by a single coherent phylogenetic group. Examples include nitrifiers in activated sludge [8], reductive dechlorinators in contaminated groundwater [9] or methanogens in anaerobic digesters [10]. Engineers and microbial ecologists alike have been preoccupied by the search for ‘super bugs’ capable of carrying out these kinds of crucial processes quickly, reliably, and predictably. At first glance this approach seems quite reasonable: enrich for the best organism available to get the job done for the least cost. However, when addressed within an ecological framework, the objective of designing and operating stable and resilient high-performing systems could be met in strikingly different ways.

A disturbing mind-set
Perhaps the most widely appreciated concept borrowed from ecologists by environmental biotechnologists is the proposed relationship between biodiversity and system...
stability [3°,5,11]. The latter can be characterized by measures of resistance, resilience, and scale of temporal variability. Although ecologists still debate whether positive correlations between diversity and stability are universal (and therefore useful for predictive purposes) [12–15], much theoretical and experimental work has been done to explore the processes that could be generating this relationship. The uncoupling of stability in ecosystem function and community composition is of particular interest as environmental biotechnologists seek to understand the significance of observed community dynamics in the face of stable process performance [5,16].

One of the most puzzling observations made by ecologists was elegantly articulated by Hutchinson in the classic ‘paradox of the plankton’ [17]. Simply put, why do we observe multiple species with over-lapping niches (i.e. functions) co-existing in an ecosystem, when the principles of competitive exclusion dictate that they should not? A popular answer to this question points to the influence of habitat heterogeneity in both time and space, which is the foundation of the Intermediate Disturbance Hypothesis [18]. This conceptual framework integrates observed patterns of community succession and spatial heterogeneity. Disturbances occurring at different scales and frequencies create patches (either in space or time) that harbor communities in varying stages of succession towards a climax. Most ecosystems we observe are not yet at equilibrium, which would be characterized by competitive exclusion by the fittest species. Thus, any community under the influence of periodic disturbances acting externally to the system would not be expected to be at equilibrium. Community succession was recently linked to the development of stable process performance in a circulation flush toilet [19]. Convergent succession appeared to occur in a fivecompartment anaerobic migrating blanket reactor perturbed by sulfate [20] and in activated sludge systems fed with non-ionic surfactants [21].

Indeed, environmental biotechnologists must consider the concept of disturbance from several perspectives. First, variability in abiotic system components is unavoidable: for example, wastewater treatment influents are extremely heterogeneous in time. If ecosystem function (process performance) is maintained by functional redundancy in the face of disturbance, then our engineering objective should be to maximize diversity. An activated sludge system operated to remove surfactants performed better and was more resistant to invasion by competitors when the communities were more diverse [22]. On the other hand, engineered disturbances can be used to control community assembly [5,16]. Methanogenic digesters were found to recover more quickly from organic overload conditions when their communities had been subjected to previous perturbations [10]. Competition between acetoclastic methanogen species with different kinetic characteristics could be manipulated using a defined digester feeding strategy [23]. Fluctuating performance and community dynamics were correlated in denitrifying bioreactors, and process instability was induced by altering nitrate loadings [24]. Pulsed nutrient dynamics provided to activated sludge organisms generated community functions that were different depending on the pulse regime [25]. Interestingly, community composition was more distinct across systems than could be explained by the different pulse regimes explored, suggesting that stochastic inter-specific interactions played a major role in structuring the bacterial assemblages. This observation hints at the need to consider principles of non-linear dynamics and complexity theory, particularly in the absence of external abiotic disturbances (see also below) [26°]. Future challenges include the need to determine what type of disturbance regimes will satisfy our engineering goal of process stability. These may be very process specific.

Community assembly: from pattern to process

If our design objectives become directed toward maintaining maximal diversity and functional redundancy, how does this influence our choice of process configuration and mode of operation? We could turn to neutral models such as the Unified Theory of Biodiversity and Biogeography [27] when considering ways to predict and control community assembly. This framework, based on the Island Theory of Biogeography [28], was developed mainly to explain patterns observed in communities of macroscale organisms such as plants and birds, although it is based on general principles applicable even to the microbial world. It proposes a stochastic model of community assembly that relies primarily on rates of immigration and extinction, with biodiversity being ultimately constrained by the area (or volume) sampled and dispersal limitations. Microbial ecologists have only recently attempted to search for and describe such spatial patterns of taxa distribution [29°,30]. However, methodological limitations continue to plague interpretation of such data [31], as does a lack of information about dispersal rates and mechanisms. Different microbial taxa appear to be influenced to varying degrees by dispersal limitation. For example, narrowly defined phylogenetic groups of *Candidatus* Accumulibacter phos- phatis, a model polyphosphate-accumulating organism in activated sludge, appear to be globally dispersed [32°,33] (V Kunin et al., unpublished), while populations of *Sulfu- lobus*, a thermophilic archaeon found in hot springs, showed evidence for geographic isolation [34]. Much effort will be required to understand and quantify dispersal characteristics for microbial taxa that are important in environmental biotechnology systems. Still, neutral community assembly models are appealing because of their simple architecture and potential for parameterization [35,36°].

Other more deterministic approaches to predicting the outcome of community assembly weigh more heavily the importance of interspecific competition and niche
partitioning. Although this framework often seems diametrically opposed to the neutral theories of Hubbell [27] and Bell [37], ecologists have recently argued that neutral patterns of distribution and abundance are merely an emergent property [38] of more deterministic processes that rely on local selection [39,40,41**,42–44]. Theoretical ecologists are themselves still working to develop conceptual models to capture the tension between stabilizing mechanisms (i.e. niches) and fitness equivalence (neutrality and habitat filtering) that is expected to create observed patterns of community assembly and species-coexistence [45–47]. The usefulness of this approach has already been demonstrated for human gut microbial communities [48]. Principles of non-linear dynamics have also been invoked to explain oscillatory behavior among populations of competitors in the absence of disturbance [26**,49]. These and other related concepts could easily be applied to studies of environmental biotechnology systems designed to observe the community dynamics resulting from non-equilibrium conditions [50–52], and to make predictions about community development. Microbial ecologists and environmental biotechnologists will only benefit from becoming and remaining engaged in this effort to further develop such models.

An additional layer of complexity: trophic food web interactions

Microbial communities and populations in engineered systems are also subject to ‘top-down’ control exerted by predators such as viruses (bacteriophage) and protozoan bacteriovores (e.g. ciliates and flagellates). However, few studies have addressed either type of predator–prey dynamic. It is noteworthy that models designed to predict process performance, such as the series of Activated Sludge Models [53], do not include food-web interactions of any kind, despite the wealth of theoretical information available from studies of aquatic microbial food webs. In particular, viral diversity and abundance in environmental biotechnology systems is expected to exceed that of prokaryotic and eukaryotic organisms. A survey of prokaryotic community composition in engineered systems. Again, the most promising approach for directly accessing viral communities is through the use of high-throughput culture-independent molecular methods [58].

Describe, explain, predict, control

Ecologists have long used models to try to quantitatively understand ecosystems. The goal of modeling is to integrate all available data to distill the main processes that drive the system. A good model should then be capable of predicting the behavior of the system to the required resolution. In fact, it is fair to say that if you cannot predict the behavior of the system you do not really understand it.

The challenge before us is to effectively integrate community-wide high-resolution molecular data into a quantitative predictive framework. One promising approach is flux balance analysis (FBA), a widely used method for metabolic modeling of microorganisms [59,60]. Although other approaches for modeling cellular processes have been developed [61–66], their use in genome-scale simulations is hampered by the requirement for a large number of parameters and associated computational complexity. FBA does not require kinetic parameters for the reactions involved and can be scaled to deal with complete genomes. Moreover, FBA has been shown to provide predictions of growth rates [67**], growth of gene deletion mutants [68,69*] and metabolic fluxes [70], compatible with experimental data in a high number of cases for Escherichia coli and other species [71,72].

FBA produces results by finding the set of metabolic fluxes that maximizes a given goal (typically growth rate [59]) within a set of constraints. The two fundamental constraints are the reaction stoichiometry for the metabolic network inferred from the metabolic reconstruction and empirical data on measurable fluxes (e.g. acetate uptake or growth rate); other constraints, such as gene regulation, refine this basic model [73]. Presently, genome-scale FBA has been restricted to single pure-culture model organisms, such as E. coli. Very recently, FBA has been successfully applied to a simple mutualistic co-culture system comprising a sulfate reducer and methanogen with available genome sequences [74**]. Several ecologically relevant characteristics including co-culture cell ratios and metabolic fluxes could be predicted using this approach. This heralds the beginning of whole genome-based community modeling at the molecular level. The next step will be to make use of metagenomic datasets in a similar way.
Environmental biotechnology

Bridging the gap between the disciplines

We have learned much about the links between community diversity, composition, and process performance in environmental biotechnology systems by applying the powerful molecular tools developed by microbiologists. However, there is still a great need to develop new curricula and concerted research efforts to better integrate the knowledge and tools of molecular microbiology and engineering [2]. We propose, as have others [3•,5,75], that students and practitioners of environmental biotechnology should also embrace the principles of Ecology, with a capital ‘E’. Even microbiologists need more exposure to ecology and ecosystem science in their undergraduate and graduate coursework, and as part of continuing professional development [76]. Many approaches to experimental design and data analysis (particularly the use of statistics) that are fundamental to ecology are completely foreign to microbiologists classically trained in a scientific culture born of the Delft School [77] and a continued fixation with microbial pathogens. Indeed, all three disciplines (microbiology, engineering and ecology) would benefit from more conversation and interaction. Ecologists should recognize engineered processes as valid model ecosystems that are simplified enough to be tractable, yet still ‘wild’ enough to be relevant [75].

Conclusions

These are exciting times to be working with biological systems. Microbial ecology as a discipline is on the verge of making major contributions to the understanding of natural environments and the beneficial use of natural processes in engineered systems. Researchers are applying complex microbial communities to develop truly innovative approaches to their trade.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


23. Conklin A, Stensel HD, Ferguson J: Growth kinetics and competition between Methanosarcina and Methanosaeta in
Integrating ecology into biotechnology

McMahon, Martin and Hugenholtz


www.sciencedirect.com

Please cite this article in press as: McMahon KD, et al., Integrating ecology into biotechnology, Curr Opin Biotechnol (2007), doi:10.1016/j.copbio.2007.04.007
6 Environmental biotechnology


