

MINI-REVIEW

Can the black box be cracked? The augmentation of microbial ecology by high-resolution, automated sensing technologies

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Automated sensing technologies, ‘ASTs,’ are tools that can monitor environmental or microbial-related variables at increasingly high temporal resolution. Microbial ecologists are poised to use AST data to couple microbial structure, function and associated environmental observations on temporal scales pertinent to microbial processes. In the context of aquatic microbiology, we discuss three applications of ASTs: windows on the microbial world, adaptive sampling and adaptive management. We challenge microbial ecologists to push AST potential in helping to reveal relationships between microbial structure and function.

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Introduction

One challenge facing microbial ecology is the ability to link microbial composition and function (Waldrop *et al.*, 2000; Wagner *et al.*, 2002; Langenheder *et al.*, 2005; Ahlgren *et al.*, 2006). Microbial communities in ecosystems are basically analogous to a ‘black box’ where unknown specific microbial transformations underpinning ecosystem processes occur. Microorganisms function at different temporal and spatial scales than larger organisms (Prosser *et al.*, 2007), and observations of both the microbial variables and corresponding environmental parameters are often limited in resolution by manual analytical bottlenecks (Porter *et al.*, 2005). Automated sensing technologies (ASTs) enable environmental monitoring at temporal and spatial resolutions relevant to microbial community and process variation, allowing us to better decipher links between microbial composition and function.

A promising option is to use ASTs which ‘replace, augment, or surpass human observers of ecological

phenomena’ (Porter *et al.*, in press), and enable a more thorough understanding of ecological or environmental systems at fine temporal and spatial scales. Current ASTs allow physical, chemical or biological parameters to be monitored simultaneously with extremely fine-scale resolution. ASTs have been used to monitor diverse ecological environments ranging from engineered systems such as wastewater treatment facilities (Spanjers and van Lier, 2006) to pristine systems in as the high-arctic tundra (Edwards *et al.*, 2006). The technology exists to compile multiple variables into online accessible databases, allowing investigators to conveniently monitor their study sites and sensor performance from their office computer (Tilak *et al.*, 2007).

To maintain the momentum of these relatively young technologies, government funding initiatives have supported development and deployment of sensor technologies into the environment, as well as the expansion of cross-site sensor networks (Arzberger, 2004) (Supplementary Table S1). This has resulted in the development of large-scale initiatives to promote AST networks. Similarly, scientists eager to be at the forefront of AST-facilitated research have initiated grassroots networks of smaller, system-specific initiatives (Supplementary Table S1).

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AST observations will potentially transform microbial ecology by providing the ability to measure microbial responses that are more relevant to microbial scales. AST data will (1) provide a window into the black box to help correlate the complexity of microbial interactions with potential drivers within study systems, (2) enable the development of improved sampling regimes to capture events of interest and (3) provide knowledge to better manage systems. We will refer to these three applications as: windows on the microbial world, adaptive sampling and adaptive management.

Here, we highlight the use of ASTs in aquatic microbial ecology, though we recognize the application of AST in other ecosystems. We present an overview of the use of ASTs in microbial ecology, followed by some specific examples of subdisciplines within microbial ecology that may benefit from ASTs. Finally, we present some conclusions, recommendations and cautions.

Overview

Windows on the microbial world: using ASTs to couple environmental parameters and microbial dynamics

Microbial ecologists have a number of tools to monitor a range of environmental state variables that control cell growth and fate at high temporal and spatial resolution (Figure 1). However, many useful parameters cannot currently be measured

with off-the-shelf sensors and there is much variability in the maturity of available technologies.

Bottom-up control. Many microorganisms derive energy from solar radiation, and there are well-developed ASTs for assessing both the amount and quality of different wavelengths of solar radiation over depth and time (Palethorpe *et al.*, 2004). These data have been coupled to microbial response variables to understand phytoplankton photosynthesis and related physiology (Campanella *et al.*, 2001; Rodriguez *et al.*, 2002; Podola and Melkonian, 2005).

Microbial heterotrophs or mixotrophs use organic matter as an energy source. Established methods to autonomously measure chromophoric organic matter at high-frequency exist (Gallegos *et al.*, 2005), but are poor predictors of organic electron donors available to heterotrophs over biologically relevant timescales (Benner, 2003). The challenge to encompass the tremendous complexity of organic compounds presented to the microbial community, and the potential niches these diverse compounds present, has been partially addressed by the technological advances in electrochemical sensors (Rudnitskaya and Legin, 2008). One example of a versatile electrochemical sensor is the 'electronic tongue' which uses pulsed voltammetry to detect a wide range of redox-active organic and inorganic compounds (Gallardo *et al.*, 2003, 2004). Voltammetric microelectrodes can simultaneously measure

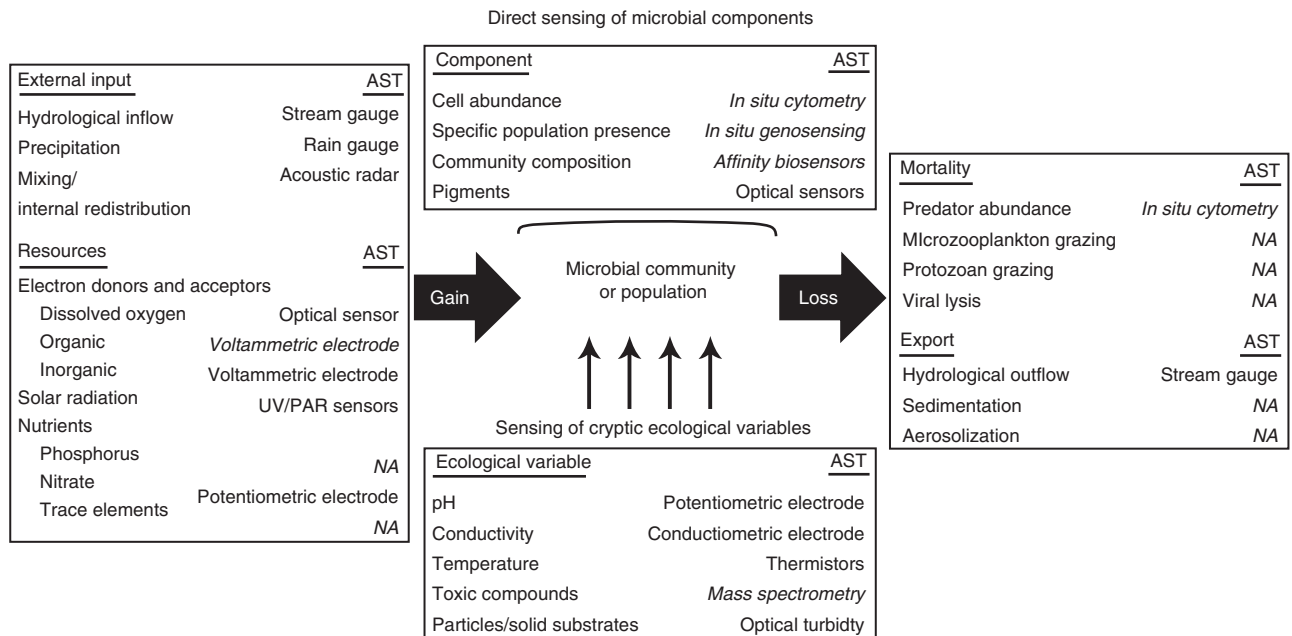


Figure 1 Conceptual model of variables influencing microbial communities in aquatic ecosystems, and general examples of automated sensing technologies (ASTs) available for observing these variables. In-development sensing technologies are given in italics. For high temporal resolution, reasonably priced, self-logging sondes can be equipped with sensors to measure temperature, conductivity, turbidity, dissolved oxygen, phytoplankton pigments, pH, light and oxidation reduction potential in bulk fluid. For high spatial resolution, microelectrodes can be precisely positioned to measure substrates such as oxygen, nitrogen species and sulfur species. NA indicates that the technology for automated sensing is not available.

temperature, oxygen and specific ions at the sub-millimeter scale. These ASTs have been used *in situ* for real-time data collection near thermal vents, salt marshes, microbial mats and in aquatic water columns (Luther *et al.*, 2008). Together with conductimetric (salinity) and potentiometric (pH, pCO₂) sensors, observations of aquatic and sediment pore-water chemistry can be gained relatively easily.

Nutrient and electron acceptor availability also control aquatic microbial community dynamics. Electrodes are commercially available and widely used to detect oxygen and nitrate for water quality monitoring, but often suffer from high detection limits and interference. New optical dissolved oxygen sensors offer improved accuracy and durability. Novel platforms such as the Spectrophotometric Elemental Analysis System show great promise for profiling of nitrate and phosphate (Adornato *et al.*, 2007). Microelectrodes have been developed for forms of nitrogen, sulfur and metals, though most require in-house high-skilled fabrication (Revsbech, 2005). Most have been used for short-term experiments rather than long-term automated monitoring.

Top-down control. There is a general lack of sensor technologies to probe and monitor mortality, or top-down, processes for microorganisms. Of the available options, image-based monitoring of potential bacterial predators is the most common. Currently no single AST or detection platform can effectively monitor organisms within the entire size-range of bacterivores. However, several available instruments use imaging and flow cytometry to quantify plankton cells, and offer improvements in taxonomic classification and field utility. Still, detecting and quantifying predator abundance are only indirect proxies for predation because current technology suffers from poor specificity (for example, singling out predators from organisms using other metabolic strategies; (Culverhouse *et al.*, 2006)). Predator abundance measurements are less valuable than predator feeding rates, however, feeding rate data will likely require normalization to predator abundance. We anticipate predator abundance data will inform activity as the technology improves.

Adaptive sampling: AST-observed environmental patterns initiating microbial observations

The technology to automatically collect microbial composition and process observations is constantly advancing, but has not yet achieved the same consistent utility as environmental sensors. Technologies for genetic sensing, or determining the proportion of photosynthetic pigments belonging to specific phytoplankton (Paul *et al.*, 2007), currently are not widely available or affordable. Therefore, the more immediate questions are when and where should microbial observations be collected to optimize correspondence with AST-derived

environmental measurements. This information is especially important as microbial ecology continues in this 'omics' age of massive, resource demanding data collection capabilities. ASTs will refine our adaptive sampling efforts to the most scientifically compelling sites and times to best collect metadata.

A key example of adaptive sampling is the work of Jones *et al.*, 2008. Overturn in a remote lake was observed following a typhoon disturbance using thermistor sensors, informing decisions to sample the lake for the microbial community response to the mixing (Jones *et al.*, 2008). Because of the typhoon and the isolated location of the study system, the physical (water column thermal stratification) and chemical (dissolved oxygen depletion) dynamics, hypothesized to drive microbial community dynamics following mixing, could not have been observed without ASTs. After observing the physical and chemical phenomena linked to water column re-stratification, the researchers decided to chase typhoon events with microbial community observations, and found that the bacterial communities 're-set' themselves deterministically following typhoon events and across years.

Adaptive management: ASTs informing microbial ecology in supervising natural resources and technical systems

Microbial ecology is increasingly linked to environmental systems management. Microbes influence and perturb aquatic ecosystem services, including drinking and wastewater treatment, recreation, irrigation, as well as commercial and recreational fisheries. Managers must evaluate the status of microbes and associated environmental parameters responding to microbe-mediated disturbance events. Improvements in ASTs for chemical and physical parameters and, to some extent, crude bulk biological measurements (for example, phytoplankton pigments) are becoming more frequently used to provide managers with high-resolution spatial and temporal data (Yentsch *et al.*, 2008). The coupling of molecular biological techniques to sensor systems allows increased 'species' or ecotype resolutions. Although this increased resolution is useful for informed management, sample collection and analysis remain separated in space and time. For this reason a number of groups are developing field amenable or autonomous sensors capable of performing molecular diagnostics *in situ*.

For example, harmful algal blooms negatively impact water quality, human health, fisheries, tourism and recreation, causing mass marine mortality events yearly (Paerl, 1988). Efforts are underway to facilitate *in situ* molecular detection of *Karenia brevis*, a dinoflagellate responsible for harmful algal blooms along the Florida Gulf Coast (Kirkpatrick *et al.*, 2004). Instrumentation has been developed to perform real-time Nucleic Acid Sequence-Based Amplification (RT-NASBA), to

detect *K. brevis* mRNA *in situ* (Casper *et al.*, 2004). The isothermal (41 °C) NASBA assay uses a trimeric enzyme mix coupled with molecular beacons to selectively amplify/detect RNA, thereby enabling the quantification of metabolically active cells and simplifying RNA extraction protocols. Similarly, the isothermal reaction diminishes instrument engineering constraints associated with operating software complexity, power requirements and instrument size and weight.

Hand-held, field portable instruments (Figure 2a) that use this in-field RNA extraction and NASBA technique have been developed (Casper *et al.*, 2007; Smith *et al.*, 2007; Smith and Fries, 2008). In addition, independent *in situ* sensors like the Autonomous Microbial Genosensor (AMG) (Figure 2b), designed to perform automated sample collection/filtration, RNA extraction/concentration and subsequent RT-NASBA detection, will enable around the clock sentinel capabilities. NASBA-based sensors may eventually provide the rapid and effective detection that is critical to the adaptive management of harmful algal blooms (Zingone and Enevoldsen, 2000).

Cost-effective automated molecular biosensors will likely advance to simultaneously monitor multiple organisms. Using current iterations of *in situ* molecular biosensors, this could potentially be achieved using multiplexed amplification assays, or through array-based approaches such as the Environmental Sample Processor (Greenfield *et al.*, 2006; Scholin *et al.*, 2008). These technologies are advancing rapidly, as demonstrated by improved and novel techniques incorporated into the Environmental Sample Processor, such as the recent assay sandwich hybridization-based molecular chip with probes for specific phylogenetic groups of aquatic bacteria (Preston *et al.*, 2009). For more information, microbial ecologists should refer to a recent survey of literature relating to the current state of optical ASTs and appropriate biosensor data handling (Rich *et al.*, 2008).

Subdisciplines of microbial ecology that could benefit from ASTs

Though not comprehensive, the following discussion targets subdisciplines within microbial ecology that are already using, or are poised to take advantage of, ASTs to advance their fields.

Global climate change microbial ecology. Global climate change microbial ecology will benefit from ASTs, as bacterial process, population or community changes could be linked to meteorological or climate observations (Levy *et al.*, 2007). The cascade of interactions between microbial respiration, carbon dioxide (CO₂), greenhouse gas production and terrestrial primary production can be tested in a large scale across ecosystem types using embedded sensor networks. As a start, the National Ecological

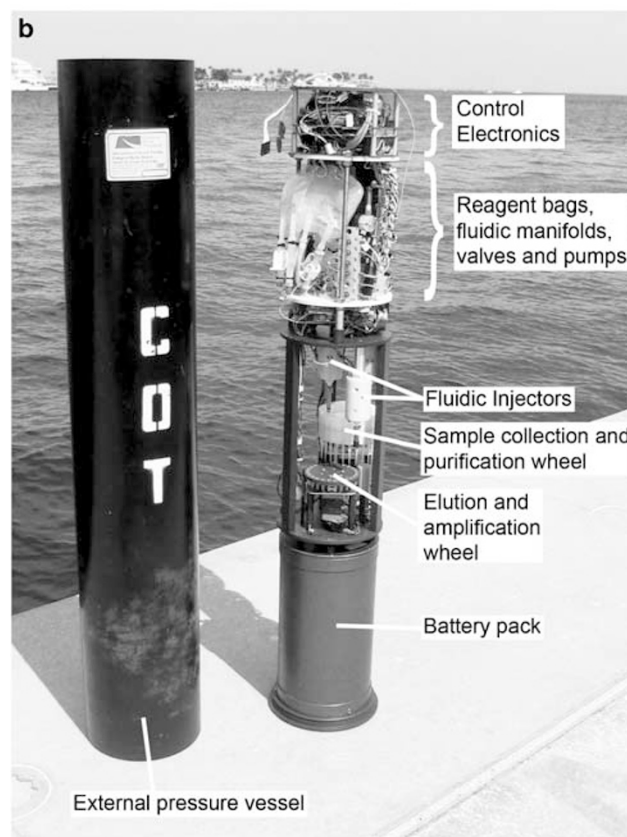


Figure 2 *In situ* Genetic Analysis (a) Example of a prototype hand-held RT-NASBA sensor (Casper *et al.*, 2007). The instrument is coupled with methods for in-field RNA extraction and allows an operator to perform molecular identification without access to extensive laboratory infrastructure (Casper *et al.*, 2007; Smith and Fries, 2008). (b) Image of the AMG highlighting associated control modules and components. The system is approximately 1.2m tall and 0.2m in diameter, and when deployed weighs approximately 40kg. When operating *in situ* the instrument is capable of reporting time stamped data from RT-NASBA reactions using a 802.11 WiFi connection as well as storing amplification data in non-volatile memory. To ensure quality control of the data the AMG is configured to perform four negative controls during deployment. Future inclusion of two-color fluorescence detection in the AMG and inclusion of an internal calibrator molecule (Casper *et al.*, 2005; Patterson *et al.*, 2005) will provide additional QA/QC of results, and allow for monitoring of amplification inhibitors or degradation of the stored NASBA chemistry.

Observatory Network plans to deploy and maintain intricate real-time sensor networks, and will potentially include ASTs that measure microbial composition or processes (King *et al.*, 2008). Within NEON or similar sensor networks, microbial ecologists, information technologists and ecosystem scientists will need to collaborate to understand microbial contributions to global change.

Carbon dioxide and methane are two potent greenhouse gases that are both produced and used by microbes. It was previously thought that the greenhouse gas flux from freshwater lakes was relatively small, but a recent analysis suggests that freshwater contributions to carbon (and other nutrient) cycling is underestimated because of the previously unrecognized influence of small lakes, impoundments and ponds (Downing *et al.*, 2006). During summer 2008, a prototype CO₂ sensor (Browne *et al.*, 2007), was fitted on an instrumented buoy during a whole-lake mixing manipulation (A Shade and KD McMahon, unpublished data). The researchers will be able to use these high-resolution data to decipher the coupling of CO₂ and bacterial community dynamics (measured using molecular fingerprinting techniques). We predict that ASTs similar to this CO₂ sensor will further enable coupling of global change relevant gas fluxes to microbial community dynamics.

Water quality. Advances in portable or autonomous mass spectrometers offer some solutions to monitor the threat of organic contaminants to surface and ground water quality (Richardson, 2001). These sensors are highly sensitive and can detect multiple chemical species simultaneously. The advent of membrane inlet technology has facilitated autonomous mass spectrometer operation for analysis of volatile halocarbons such as methane (Schluter and Gentz, 2008) and toluene (Short *et al.*, 2006). Engineering progress has made the platforms robust for extended field deployment, including continuous operation at fixed buoys or on autonomous underwater vehicles for spatial mapping (Hemond *et al.*, 2008). Though still in its infancy, autonomous mass spectrometry seems promising for ASTs directed at organic contaminants in aquatic systems.

A recent review of water quality ASTs can serve as a resource for aquatic microbial ecologists interested specifically in these applications (Glasgow *et al.*, 2004). This review also discusses satellite imagery and remote sensing for water quality applications.

Bioremediation. Bioremediation is controlled both by the composition and metabolic potential of the microbial community at hand and environmental constraints regulating microbial activity. This is another arena where ASTs may help scientists to understand and control microbial communities for optimal performance in contaminant removal.

Oxygen availability is a critical parameter that is tractable for high-frequency monitoring in bioreme-

diation situations (Koster *et al.*, 2006). Oxygen probes are available not only for homogenous aquatic systems, but also for heterogeneous matrices such as aquifers and other porous media where the electron acceptor limits biodegradation (Huang *et al.*, 2003). Redox potential also provides critical information about electron acceptor regimes that affect both microbial community composition and their ability to degrade organic pollutants (Alexander, 1999). For example, a recent study (Ruiz-Haas and Ingle, 2007) employed redox sensors to link oxygen to the onset and stability of reductive dechlorination; this work suggested that redox sensors are generally useful for monitoring multiple bioremediation processes.

Challenges and conclusions

Major challenges remain in broad scale integration of ASTs into existing or future environmental monitoring programs. Regulatory bodies must be enabled to adopt a shift from traditional laboratory-based analysis to point-of-use diagnostics. This will only occur when technologies evolve to the point where the hardware, consumables and personnel requirements (for deployment, retrieval and maintenance) are cost-effective, reliable and equal to, or surpass the sensitivity, specificity and throughput capacity of current analytical techniques. Also, AST data must be able to be readily interpreted within existing regulatory standards and historical framework.

An additional challenge is that of sensor drift and biofouling, which can go undetected unless appropriate quality assurance and control (QA/QC) is met. Sensor networks and organizations that share AST data with collaborators have particular interest in maintaining a standard of data quality, and there is some discussion to address these challenges (for example, Tilak *et al.*, (2007)). One suggestion for microbial ecologists to manage QA/QC is to maintain collaborations with AST networks, where resources for data management schemes are institutionalized to a common QA/QC standard and controlled vocabulary.

The sheer volume of data collected by ASTs can be overwhelming to manage and analyze, potentially causing us to lose sight of basic questions in our field. To prevent this, we need to optimize our sensor data management, data-sharing strategies, integration of sensor data with pre-existing data (for example, manual measurements from long-term ecological research databases) and knowledge of the computational protocols required to analyze these datasets correctly. Curation of time series data especially will be a daunting task, requiring consistent support, expertise and database management. For example, the United States Geological Survey (USGS, www.usgs.gov) has been collecting real-time stream gage data at thousands of sites since

1983, integrating these high-resolution data with point measurements and maintaining a publicly-accessible database (National Water Information Systems, NWIS, <http://waterdata.usgs.gov/nwis>). However, the USGS warns users that the real-time data are 'provisional' and not always quality-controlled. This reflects the lack of sufficient resources and personnel required to minimally curate these datasets.

The microbial ecology community has been actively discussing management, metadata collection and quality issues for genomic-related microbial research, which also generates massive amounts of data. Therefore, similar challenges face both AST data curation and genomic data (Gil *et al.*, 2007). These two topics inevitably will merge further as biosensor technology moves forward. The National Oceanic and Atmospheric Administration's National Data Buoy Center (<http://ndbc.noaa.gov/qc.shtml>) and similar cyberinfrastructure reports could serve as a starting point for microbial ecologists to make decisions about AST data management. The next generation of microbial ecologists should have formal training to develop a working balance of these skills (data management, statistics, information technology). We should start immediately to construct focused coursework and related research experiences for graduate students and professional development for young scientists.

Productive multidisciplinary collaboration, with engineers and information technology experts, is imperative to fully harness the potential of AST for microbial ecology. There are growing numbers of networks that aim to build these bridges among professionals interested in high-resolution environmental sensors (Hanson, 2007). We advocate participation in organizations or meetings that solicit science typical of automated sensing goals, but not necessarily specific to microbiology. For example, the International Society for Limnology 2007 meeting offered a series of automated sensor-centric sessions. Proposing AST-focused sessions at microbial ecology meetings would be productive for establishing microbial ecology centric networks that use AST data.

Finally, many AST datasets are publicly available in online accessible databases (Supplementary Table S1). These databases benefit researchers by allowing exploration and comparison of environmental parameters among systems, making it easier to link trends in ecosystem and microbial processes. A recent review emphasized the critical need for a general theory in microbial ecology: 'Theory is used to classify, interpret and predict the world around us. Without it, microbial ecology is merely the accumulation of situation-bound statements that are of limited predictive ability, providing microbiologists with few insights,' (Prosser *et al.*, 2007). The coupling of ASTs with microbial ecology may allow us to finally construct overarching theories for our field, and advance microbial ecology to the research forefront of our century.

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