

## Microbial conservation in the Anthropocene

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.14124

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### *Ecosystem health in the Anthropocene*

Ecosystems are facing unprecedented challenges and decline as we enter the Anthropocene (Steffen et al., 2011). For instance, the earth's climate is already estimated to be 0.85 °C warmer than it was in 1880, affecting both terrestrial and marine ecosystems (IPCC, 2014). Much of this thermal energy (~60%) has been absorbed by the oceans, resulting in melting sea ice, rising sea levels, and record temperatures that have caused global mass coral bleaching events (Hughes et al., 2017). The simultaneous intensification of land use is causing extraordinary levels of erosion with substantial implications for nutrient and carbon cycling, land productivity and in turn, global socio-economic conditions (Borrelli et al., 2017). These mounting environmental pressures are forcing organisms to acclimate, migrate, or suffer reduced fitness and, potentially risk local extinction. Defining tolerance thresholds for individual species and entire communities is therefore a priority for environmental scientists, regulators and managers as they attempt to preserve ecosystems in the face of climate change and escalating human development; but this task is particularly challenging for microbial community conservation.

### *Quantifying microbial responses to environmental stress*

Microorganisms underpin ecosystem health, so establishing how, and to what extent environmental change alters microbial community structure and function is critical for informing ecosystem protection efforts. Globally, we have invested considerable resources into establishing spatial and temporal baselines for key microbial communities (Morris et al., 2005; Karl and Church, 2014; Bissett et al., 2016). This has led to substantive advances in our understanding of microbial community composition, however we still lack a mechanistic framework to reliably quantify how these microbial assemblages and their ecologically important functions respond to specific environmental stressors. As the first responders to environmental perturbation, microorganisms have the capacity to buffer or mitigate ecosystem changes. Yet, despite the critical importance of microorganisms for ecosystem function, most conservation and management endeavours still focus on iconic species (e.g. polar bears and whales) and macro-ecological communities (e.g. rainforests and coral reefs), with no major environmental initiatives investing in the health and function of the microbial ecosystems that underpin all life.

The impacts of environmental stress on microbial communities are generally described qualitatively due to the difficulty in applying valid stress-response data in a form that has ecological relevance. For instance, numerous studies assessed the impact of hydrocarbons and dispersants on native microbial populations during the massive Deepwater Horizon oil spill in the Gulf of Mexico, yet quantitative dose-response relationships to inform the development of regulatory guidelines have not been established (Hazen et al., 2010; Kleindienst et al., 2015). In addition, methods currently accepted by regulators to identify the toxic thresholds of pollutants on prokaryotes are generally restricted to quantifying changes in microbial growth, biomass or luminescence, and this is often applied to a narrow range of cultivated species (Shahsavari et al., 2017). Clearly this approach does not adequately assess impacts on the complex spectrum of microbial diversity and physiologies in most ecosystems. Microbial ecotoxicology is emerging as a new discipline that addresses this issue for the first time by applying a variety of analytical, enzymatic, toxicity and culture-independent techniques to define negative effects on microbial communities (Shahsavari et al., 2017).

### *Novel approaches to assessing microbial community responses to environmental stress*

There is an enormous opportunity (and challenge) to extend the microbial ecotoxicology approach to assess pollution, climate and cumulative environmental stresses for a broad range of microbial communities including free-living and host-associated microbiomes. More specifically, endeavours are needed to lever recent advances in omics technologies and imaging approaches to develop robust frameworks that reveal dose-response relationships and cause-effect pathways such that quantitative microbial data can be incorporated into regulatory, management and conservation guidelines. To

achieve this, the microbial ecology community must develop innovative yet standardised and ecologically meaningful approaches to quantify how entire communities of microorganisms respond to a broad suite of environmental perturbations based on changes to their (i) community composition, (ii) genomically encoded potential functions and (iii) actual metabolic activities. The development and uptake of such an approach would then provide a critical link between our fundamental understanding of microbial ecology and applied environmental and conservation science.

Ecological risk assessments are often poorly informed by response thresholds for a few species, whereas effective management strategies aim to target relevant populations, communities, and ecosystems (Anthony et al., 2015). A well-established approach to quantify risk thresholds posed to eukaryotic communities by pollutants is to model the variability in species sensitivities to various exposures (Posthuma et al., 2002). Here single-species stress threshold data for multiple taxa are combined as species sensitivity distributions (SSD) by fitting a statistical or empirical distribution model to the proportion of species affected as a function of stressor concentration, dose or level (Figure 1). Key strengths of the SSD probability models are that they can be tailored to global or local populations and can be used to identify the proportion of species affected within a community (Belanger et al., 2017). The SSD method has been applied as a decision support tool in environmental protection and management since the 1980s, being formally adopted for the derivation of environmental guidelines in 1985 in the United States and 1989 in Europe (Stephan et al., 1985; van Straalen and Denneman, 1989). In the last 30 years SSDs have been widely applied to develop risk thresholds and guidelines for eukaryotic communities to a variety of different local (e.g. nutrients, petroleum hydrocarbons) and global (e.g. temperature) stressors for compliance and spatial risk assessments (Del Signore et al., 2016).

Conceptually, such an approach could be translated to microbial communities by simply using taxon abundance data to generate stress-response relationships for each microbial OTU in a community. Automated curve fitting of each OTU could then be performed, enabling effect concentrations (EC) to be calculated from the fitted models (Knezevic et al., 2007). Stressor levels that affect 10% or 50% of each OTU ( $EC_{10}$  and  $EC_{50}$  respectively) would be interpolated from the model for all OTUs that remain stable in the control (no stressor treatments) over the duration of exposure. The multiple  $EC_x$  values (usually  $EC_{10}$ ) for each OTU would then be translated into SSDs to quantify the proportional impact on the overall microbial community, as well as establishing stressor levels (guidelines) that are protective of the desired percentage of microbial species / OTUs in any given community (Figure 1). Such a standardised molecular stress-response framework for microorganisms would have broad utility in terrestrial, marine or freshwater systems as well as for both free-living and host associated communities. Regulatory outcomes that could be realised using such a platform include: i) the derivation of protective thresholds for microbial communities across global scales, ii) the derivation of scenario-specific protective thresholds that more closely reflect local conditions (e.g. (Doolette et al., 2016)) and iii) identifying the causes of biological impact or expected impact to inform the need and focus for any remedial or management action. The first two applications would be protective of microbial communities and the 3rd would underpin restoration initiatives.

The ecological relevance of species protection values for microorganisms should also be validated by testing for loss of microbial function and/or activity in the same samples under the same conditions (Fig. 1). This is particularly important if one considers that niche partitioning, complex interaction networks and functional redundancy are key characteristics of most microbial ecosystems (Allison and Martiny, 2008). This could be achieved using a combination of metagenomic / metatranscriptomic sequencing (Birrer et al., 2017), and by incorporating recent developments in stable isotope analysis (for instance  $H_2^{18}O$  and or  $D_2O$  assays, (Aanderud and Lennon, 2011; Berry et al., 2015; Kopf et al., 2015)) or biorthogonal non-canonical amino acid tagging (Hatzenpichler et al.,

2016) that facilitate differentiation of active from non-active cells prior to meta-omic analyses (Singer et al., 2017). Stress-response curves could then be generated for each microbial function or pathway (instead of taxa), and the derived  $EC_{10}$ s or  $EC_{50}$ s used to generate functional sensitivity distributions (FSD). Regulatory guidelines that ensure protection of sensitive but ecologically important microbial functions could then be derived directly from FSDs (Figure 1). This approach would have enormous value if early identification of disruption to specific pathways could be used to avoid ecosystem tipping points. For instance, microbial functional changes in response to eutrophication or climate change can induce oxic-anoxic regime shifts, with cascading detrimental ecosystem effects (Bush et al., 2017). However, it is also important to note that high levels of functional redundancy in a microbial ecosystem may produce FSDs that have lower relative sensitivity than the corresponding SSDs. In some ecosystems it can also be challenging to establish the true extent of functional redundancy. For instance, ammonia oxidizers can have very different substrate affinities and loss of high affinity members could still have dramatic ecosystem impacts, even if ammonia oxidation as a generic function has a high  $EC_x$  value. This approach also misses yet to be discovered microbial functions which would be impossible to predict based on meta-omic or general activity data. Shifts in community structure may also have neutral effects on ecosystem function (Cravo-Laureau et al., 2017), hence a combined SSD/FSD approach should be employed for establishing guidelines. Adding to the challenge of adapting ecotoxicology techniques to complex microbial communities is the difficulty in differentiating the response due to the direct effect of the environmental pressure from the effect on biological interactions amongst the microorganisms (Cravo-Laureau et al., 2017). Impacts on microbial function may also act indirectly through altering community resistance or resilience (Cravo-Laureau et al., 2017), and differentiating between single and cumulative environmental stressors can be particularly problematic (Belanger et al., 2017). However despite these mechanistic uncertainties, we should also consider that regulatory thresholds would have the same outcome for ecosystem state, regardless of whether the effect is direct or indirect or we are assessing single or multiple stressors. Resolving these methodological and knowledge gaps that currently prevent accurate quantification of the impact of environmental pressures on microbial communities would transform our capacity to establish robust regulatory frameworks and facilitate early management interventions aimed at preserving the microbial communities underpinning ecosystem health.

#### Figure Legend

Figure 1: Framework for quantifying microbial sensitivity to environmental stress. Individual stress response curves (left panel) can be used to derive effect concentrations for each microbial OTU or function. A cumulative species sensitivity distribution (SSD, middle panel) can then be constructed to quantify the proportional impact on the overall microbial community (based on threshold effects for all OTUs in the left panel) and used to establish protective guidelines for community composition. The stress thresholds for individual microbial functions (left panel) can be used to construct functional sensitivity distributions (FSD, right panel), enabling establishment of protective guidelines for community function.

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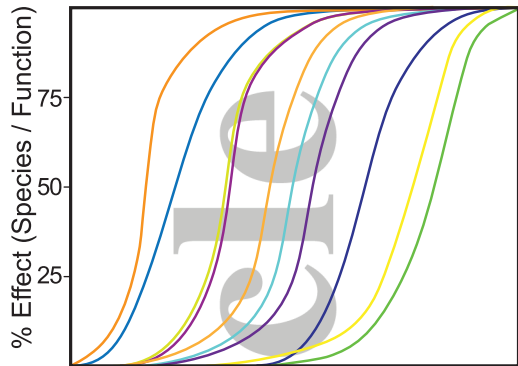
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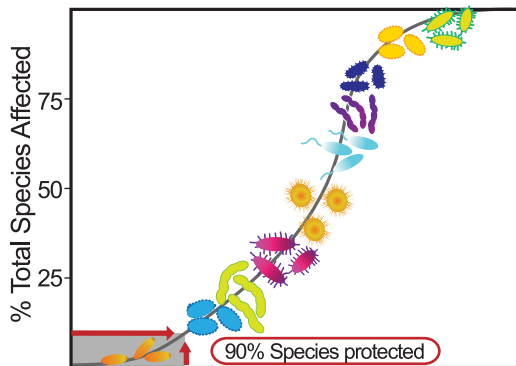
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Individual Stress Response Curves



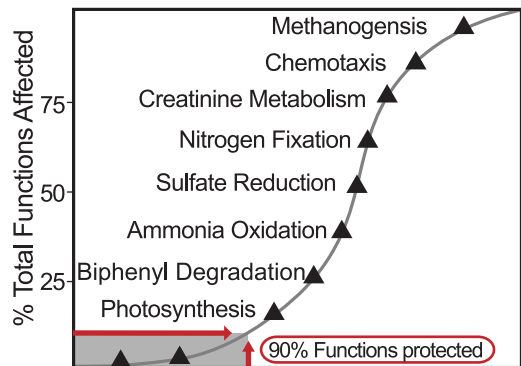
Increasing Stressor Level / Conc

Species Sensitivity Distribution (SSD)



Increasing Stressor Level / Conc

Functional Sensitivity Distribution (FSD)



Increasing Stressor Level / Conc

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