

Microbial Community Responses to Natural and Anthropogenic

Disturbances in Aquatic Systems

by

Christopher Spencer Farinholt Ward

Marine Science & Conservation
Duke University

Date: _____

Approved:

Dana Hunt, Supervisor

Richard Di Giulio

P. Lee Ferguson

Hans Paerl

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy
in Marine Science & Conservation in the
Graduate School of Duke University

2015

ABSTRACT

Microbial Community Responses to Natural and Anthropogenic
Disturbances in Aquatic Systems

by

Christopher Spencer Farinholt Ward

Marine Science & Conservation
Duke University

Date: _____

Approved:

Dana Hunt, Supervisor

Richard Di Giulio

P. Lee Ferguson

Hans Paerl

An abstract of a dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy
in Marine Science & Conservation in the
Graduate School of Duke University

2015

Copyright by
Christopher Spencer Farinholt Ward
2015

Abstract

Aquatic ecosystems are highly dynamic environments, suggesting that resident microbial communities to respond and adapt to these environmental changes. However, despite the frequency of disturbances in aquatic ecosystems such as annual cycles in environmental parameters and episodic storm events, few studies have examined the impacts of disturbances on aquatic microbial communities. In this dissertation, I investigate community- and taxon-level responses to natural and anthropogenic disturbances in the coastal ocean and wetland mesocosms using ribosomal RNA gene library sequencing. In my first chapter, I present an overview on disturbances in microbial communities and describe microbial interaction network-based approaches for predicting disturbance effects. In my second chapter, I use three years of weekly coastal ocean samples to identify transitions between distinct summer- and winter-associated taxa that occur across the microbial community over relatively short time intervals. Using the same time series, I find that episodic disturbances involve in rapid turnover of both abundant and conditionally rare taxa depending on environmental conditions and initial community composition. Finally, I investigate the microbial responses to acute and chronic environmental loading of an emerging contaminant in replicated wetland mesocosms. Despite the antimicrobial properties of silver nanoparticles, community changes in both treatments appear to be dominated by indirect effects through aquatic plant die-off, though the timing, duration, and magnitude of responses vary. Together, my dissertation demonstrates that associations between microbial taxa, environmental factors, and other components of the ecosystem all contribute to community response to disturbance. By exploring community responses to disturbance, new insights can be

gained into the resistance and resilience of microbial communities in response to environmental drivers of community change.

Contents

Abstract	iv
List of Tables.....	ix
List of Figures.....	x
Acknowledgements.....	xi
1. Introduction.....	1
1.1 Microbes as important responders to ecosystem changes.....	2
1.2 Microbial interactions in community assembly.....	4
1.3 Using association networks to explore disturbance.....	6
1.4 Using storms as models to explore disturbance propagation	11
1.5 Conclusions	14
2. Seasonal threshold effects on microbial community composition in the temperate coastal ocean.....	16
2.1 Introduction	16
2.2 Results and Discussion.....	19
2.3 Conclusions.....	29
2.4 Experimental approaches.....	30
2.4.1 Environmental sampling.....	30
2.4.2 DNA extraction	30
2.4.3 Sequence processing.....	31
2.4.4 Characterization of microbial community	32
2.4.5 Temperature shift experiments	32
2.5 Acknowledgments	33
3. Differential responses to disturbances by variable taxa in the coastal bacterioplankton	34

3.1 Introduction	34
3.2 Results	36
3.2.1 Environmental variation in the coastal ocean.....	36
3.2.2 Community variation over time	37
3.2.3 Identification of highly variable and conditionally rare taxa	39
3.2.4 Contributions of highly variable and conditionally rare taxa during disturbances	41
3.3 Discussion	43
3.4 Conclusions	45
3.5 Experimental approaches.....	45
3.5.1 Microbial community sequence and environmental data	45
3.5.2 Assessment of community composition change	46
3.5.3 Detection of highly variable, conditionally rare, and highly abundant taxa	47
3.5 Acknowledgments	48
4. Indirect effects of silver nanoparticle environmental loading overwhelm direct microbial toxicity in wetland mesocosms	49
4.1 Introduction	49
4.2 Results and Discussion.....	53
4.2.1 Complicating factors of environmentally realistic studies	54
4.2.2 Acute treatment.....	55
4.2.3 Chronic treatments.....	59
4.3 Conclusions	62
4.4 Experimental approaches.....	63
4.4.1 Field site and experimental treatments.....	63
4.4.2 Sample collection.....	64
4.4.3 DNA extraction and sequencing.....	64

4.4.4 Sequence processing	65
4.4.5 Flow cytometry	66
4.5 Acknowledgments	66
5. Conclusions	67
Biography	86

List of Tables

Table 1: Comparisons of community compositions between treatments over time.....	62
--	----

List of Figures

Figure 1: Schematic for tracking disturbance transmission through a microbial community.....	13
Figure 2: Composition of partial 16S rRNA gene sequences for weekly samples from the Pivers Island Coastal Observatory (PICO) over three years (Jan 2011 – Dec 2013).	21
Figure 3: Biplot of canonical correspondence analysis (CCA), based on microbial community composition and environmental variables for each sample from the PICO time series.	23
Figure 4: Seasonal patterns of the 100 most abundant OTUs for weekly samples over the entire three-year PICO time series.	25
Figure 5: Spring and autumn transitions of seasonally-associated OTU clusters versus temperature.	27
Figure 6: Average fold change in the relative abundances of OTUs belonging to winter- and summer-associated clusters.	29
Figure 7: Location and environmental conditions of a highly dynamic, temperate coastal system.	37
Figure 8: Community dissimilarity over short time intervals across three years (Jan 2011 – Dec 2013).	39
Figure 9: Characterization of highly variable, conditionally rare, and abundant taxa.	40
Figure 10: Temporal patterns of OTUs belonging to highly variable and conditionally rare taxa groups over the three-year PICO time series	42
Figure 11: Prokaryotic cell abundances over time in aquatic zones of mesocosms.	56
Figure 12: Phylum-level composition of 16S rRNA gene libraries of mesocosm samples over one year.	58
Figure 13: Treatment effects on community diversity.	59
Figure 14: Characteristics of study location.	71
Figure 15: Increased community diversity during temperature transitions.	72
Figure 16: Relative abundance patterns of clusters identified for the 100 most abundant OTUs.	73

Acknowledgements

I need to begin by thanking my advisor, Dr. Dana Hunt, for her patience and encouragement throughout my time in her lab. Her high expectations and dedication to the pursuit of good science has been inspiring. I would also like to thank the rest of my committee – Dr. Rich Di Giulio, Dr. Lee Ferguson, Dr. Diana Nemergut, and Dr. Hans Paerl – for their support and their many helpful discussions.

I am indebted to the members of the Hunt and Johnson labs past and present for their camaraderie and support throughout the years. In particular, I am grateful to Charmaine Yung, Katy Davis, Yajuan Lin, Sara Blinebry, and Alyse Larkin for countless discussions that directly or indirectly helped to develop many of the ideas in this dissertation. A big thanks to the PICO time series team and the CEINT chronic mesocosm crew for their field and lab expertise and assistance.

I would like to thank the great staff and faculty both on Main Campus and at the Marine Lab. A special thanks to Rachel Lo Piccolo and Eve Marion for their endless help throughout the process.

This research was made possible with financial support from the National Science Foundation (GRF to CSW, OCE 1322950 & OCE1416665 to DEH), the Environmental Protection Agency (NSF/EPA EF-0830093 to DEH), and the Gordon and Betty Moore Foundation (GBMF3768 to DEH).

1. Introduction

This chapter has been published as:

Hunt D. E., and Ward C. S. (2015). A network-based approach to disturbance transmission through microbial interactions. *Frontiers in Microbiology*, doi: 10.3389/fmicb.2015.01182.

Microbes numerically dominate aquatic ecosystems and play key roles in the biogeochemistry and the health of these environments. Due to their short generations times and high diversity, microbial communities are among the first responders to environmental changes, including natural and anthropogenic disturbances such as storms, pollutant releases, and upwelling. These disturbances affect members of the microbial communities both directly or indirectly through interactions with impacted community members. Thus, interactions can influence disturbance propagation through the microbial community by either expanding the range of organisms affected or buffering the influence of disturbance. For example, interactions may expand the number of disturbance-affected taxa by favoring a competitor or buffer the impacts of disturbance when a potential disturbance-responsive clade's growth is limited by an essential microbial partner. Here, we discuss the potential to use inferred ecological association networks to examine how disturbances propagate through microbial communities focusing on a case study of a coastal community's response to a storm. This approach will offer greater insight into how disturbances can produce community-wide impacts on aquatic environments following transient changes in environmental parameters.

1.1 Microbes as important responders to ecosystem changes

Most people and development reside near water bodies, so human activities profoundly affect both freshwater and marine ecosystems (Vitousek et al., 1997). In these aquatic environments, microbes are the numerically- and often the biomass-dominant organisms, thus how they respond to anthropogenic impacts determines both ecosystem health and biogeochemical rates. Although a large body of research explores microbial responses to long-term human alteration of the environment (e.g. climate change, ocean acidification), here we focus on pulse disturbance events that disrupt “ecosystem, community, or population structure and [change] resources, substrate availability or the physical environment” (White and Pickett, 1985). High levels of diversity and short generation times make aquatic microbes a sensitive model system to explore disturbance, but also complicate tracking the impacts and progression of disturbance. The wide range of pulse disturbances affecting aquatic environments including storms, snowmelt, mixing/upwelling, and chemical or sewage spills allows microbial ecologists to probe community responses to environmental changes.

In general, microbial communities are not resistant, which is defined by Allison and Martiny (2008) as the degree to which microbial composition remains unchanged in the face of disturbance. This low resistance is likely due to the wide range of genetic and physiological targets present in diverse microbial communities as well as microbes’ short generation times, which allow observation of both positive (increased growth) and negative (death, impaired growth) responses. Following a disturbance, community resistance and resilience are generally determined by comparing community composition at specific time points (Shade, et al., 2012). A metric of community recovery,

microbial resilience is generally defined as a return to the initial community composition (Allison and Martiny, 2008; Shade, et al., 2012). However, aquatic microbial communities are highly dynamic and continually change in response to seasonal environmental variables (e.g. light and temperature) or subsequent disturbances (Chow et al., 2013; Needham et al., 2013; Yung et al., 2015). Thus, we define the resilience of an aquatic microbial community as the rate at which the community composition returns to a non-disturbed state following a disturbance. This definition of resilience requires understanding the disturbance-independent temporal dynamics of microbial communities. Although marine microbial communities exhibit regular seasonal patterns at monthly time scales (Fuhrman et al., 2006; Gilbert et al., 2012; Giovannoni and Vergin, 2012), high resolution and repeated annual sampling reveals shorter-term and inter-annual variability at the days to weeks time scale of disturbance responses (El-Swais et al., 2014), complicating differentiation of disturbance responses from annual patterns and stochasticity. However, even if the seasonal community trajectory is known, challenges to measuring microbial responses to disturbance include confounding factors such as unrelated changes in environmental variables, stochasticity in response and recovery, dispersal limitation, genomic evolution to become resistant to disturbances, and microbial interactions with other organisms (Shade, et al., 2012; Nemergut et al., 2013). We propose to begin addressing the importance of microbial interactions to gain new insights into the mechanisms underlying the resistance and resilience of microbial communities.

1.2 Microbial interactions in community assembly

As identifying the drivers of microbial community composition is complex, most investigators first consider environmental selection, and generally secondarily address other aspects of community assembly: dispersal, drift (stochasticity), and diversification (mutation) (Vellend, 2010; Hanson et al., 2012; Nemergut et al., 2013). However, dispersal may limit the viable population present even when conditions favor growth (Caporaso et al., 2012; Hanson et al., 2012) or alternatively, environmental changes may not persist long enough for viable cells to respond (Hutchinson, 1961). An emphasis on deterministic processes also ignores the role of stochasticity in community assembly and the potential for communities with different compositions to carry out the same processes at the same rates (e.g. functional redundancy) (Werner et al., 2011; Bissett et al., 2013; Hellweger et al., 2014; Zhou et al., 2014). Further, microbial genomes evolve in response to disturbance; they can develop resistance to stressors, such as antibiotics or heavy metals, alter metabolic capabilities, and change physiological niche width (Riehle et al., 2003; Davies and Davies, 2010). Although microbial communities are shaped by a combination of selection, drift, dispersal and evolution, there is value in addressing subsets of these factors – here we focus on selection via biological interactions following disturbance.

Microbial ecology research currently emphasizes the role of interactions in the community response to environmental changes and disturbances (Faust et al., 2012; Bissett et al., 2013; Fuhrman et al., 2015). Although some examples of relationships between specific taxa and environmental variables exist (Field et al., 1997; Johnson et al., 2006; Yung et al., 2015), interactions between aquatic microbes have not been well explored. Even for predation by viruses and grazers, one of the best-studied microbial

interactions, much still remains to be discovered about the interaction specificity (Sullivan et al., 2003; Apple et al., 2011). However, the nature of biological interactions may be dictated by characteristics of dominant aquatic bacteria; the most abundant marine populations (e.g. *Pelagibacter*, *Prochlorococcus*) are known for their streamlined genomes, small cell sizes, and efficient use of resources (Giovannoni et al., 2014). Some of the evolutionary success of these organisms may be due to their conservation of limited resources by shedding genes encoding critical functions and outsourcing these functions to other members of the community (Black Queen Hypothesis; Morris et al., 2012). For example, both *Pelagibacter* and *Prochlorococcus* have lost the gene for catalase, which protects cells from hydrogen peroxide; as hydrogen peroxide diffuses through cell membranes, other members of the microbial community can protect catalase non-producers (Morris et al., 2011, 2012). Yet aquatic organisms with complex genomes have also evolved required interactions with other organisms; many eukaryotic algae have a B₁₂-dependent methionine synthase rather than the B₁₂-independent version, despite the fact that B₁₂ is only synthesized by prokaryotes. This suggests that interactions with other organisms evolve due to net ecological advantage rather than purely genome streamlining. Although outsourcing key requirements may be ecologically advantageous, long distances between cells, on average ~100 μm (Hunt et al., 2010), may exclude specific types of biological interactions for planktonic organisms such as syntrophy where physical coupling allows efficient transfer between cells (Boetius et al., 2000; Malfatti and Azam, 2010). For truly free-living organisms, interactions likely involve diffusible compounds, suggesting that interaction partners may not be highly specific or involve complex regulation. Experimental evidence supports complementation of lost capabilities by nonspecific interaction partners: a range of

reduced sulfur sources can be used by SAR11 (Tripp et al., 2008) and many bacteria can provide B₁₂ for auxotrophs (Croft et al., 2005). Additionally, some obligate relationships, at least in artificial laboratory conditions, do not involve regulation or signaling (Durham et al., 2015), while others are regulated (Kazamia et al., 2012), suggesting a number of potential strategies for interactions. Although outsourcing key functions is thought to be evolutionarily adaptive, interactions also incur costs: B₁₂ additions have been shown to stimulate phytoplankton, implying that an interaction limits algal growth (Sañudo-Wilhelmy et al., 2006; Bertrand et al., 2007). While experimentally verified interactions between microbes remain rare, the success of aquatic organisms may stem at least partially from outsourcing key functions. Thus, increasingly, microbial ecologists are incorporating interactions into our understanding of microbial communities, including interaction-mediated transmission of disturbance, resistance, and resilience.

1.3 Using association networks to explore disturbance

In general, microbial interactions cannot be directly observed, thus ecological relationships are instead inferred based on environmental observations of co-occurrence patterns and synchronous population dynamics (Ruan et al., 2006; Steele et al., 2011; Faust et al., 2012). Patterns of microbial relative abundance obtained from communities sampled over spatial or temporal gradients are used to generate correlation-based association networks of potential interactions between operational taxonomic units (OTUs) and between OTUs and environmental variables (Barberan et al., 2012; Faust et al., 2012; Fuhrman et al., 2015). These correlations are interpreted to capture biological mutualisms such as cross-feeding and exchange of metabolites (Kazamia et al., 2012; Morris et al., 2012), functional redundancy (Eiler et al., 2012; Needham et al., 2013), or

antagonism through competition or predation (Pernthaler and Amann, 2005). In addition to the well-known biases of DNA extraction, PCR amplification and in inferring patterns of organismal abundance from library relative abundance data (Polz and Cavanaugh, 1998; Acinas et al., 2004; Friedman and Alm, 2012), association networks also suffer from a number of network-specific limitations. First, association networks assume that 16S rRNA-based OTUs are ecologically coherent in spite of known microdiversity (Hunt et al., 2008) and physiologically identical under all environmental conditions, e.g. does not account for phenotypic plasticity based on environmental conditions (Nemergut et al., 2013; Worden et al., 2015). Second, associations may serve as proxies for specific environmental conditions or niches rather than indicating true interactions (Fuhrman et al., 2015). Finally, metrics of association strength are not standard and depend on the metric chosen, number of samples, taxa relative abundance, beta diversity, and data normalization (Ruan et al., 2006; Faust et al., 2012; Friedman and Alm, 2012; Berry and Widder, 2014). Currently, this field also lacks methods to add additional support for interactions such as observed physical associations to networks (Malfatti and Azam, 2010; de Vargas et al., 2015). While acknowledging the limitations of correlation-based association networks, we believe this technique has the potential to inform our understanding of aquatic microbial community dynamics

Recently, association networks were employed to predict the bacterial response to disturbance (Bissett et al., 2013); expanding on this work, we propose to use network approaches to quantitatively examine the importance of interactions in altering the taxa affected by disturbance. Of particular promise are techniques developed in information technology and social learning, where interactions transmit signals between nodes, much in the same way that initial disturbance-induced changes in an OTU's abundance

may in turn affect the abundance of its interaction partners at later time points. One technique to look at disturbance transmission, information flow analysis can model the transmission of disturbance through the interaction network using the interaction strength and considering all possible paths in a network (Missiuro et al., 2009). Information flow analysis accounts for the strength of inferred interactions, enabling prediction of how changes in the relative abundance of a specific organism or value of an environmental variable will affect the microbial community, and thus provides a metric of predicted community resistance. Additionally, network-based diffusion analysis could be used to determine quantitatively whether association networks help to explain the propagation of disturbance through the community (Franz and Nunn, 2009). Operationally, association networks would be used to predict the temporal dynamics of microbial community composition following disturbance. The effects of disturbance on rest of the community (changes in OTU relative abundances) can be predicted using information flow analysis. This predicted community composition would be compared to the actual community composition following a disturbance and community changes predicted from a randomized network generated by preserving the association network topology but repeatedly, randomly assigning OTUs to network nodes. Thus if the association network's inferred interactions are truly important in the community's disturbance response, the true association network should more closely match the observed community responses compared to a set of randomized networks. These methods will quantify the importance of interactions and predict community responses to specific environmental conditions, enhancing our understanding of the role interactions play in disturbance.

Although these techniques are potentially powerful methods to track community responses to disturbance, there are a number of logistical considerations in using association networks to follow the propagation of disturbance through microbial communities. First, network-based analyses require large datasets both pre- and post-disturbance synoptic with community changes to develop an association network and track the disturbance response, respectively. As disturbance-responsive taxa are often rare, they may not be well-represented in association networks which generally require taxa to be present in most samples (Shade et al., 2014). Moreover, taxa which can respond quickly to environmental changes may exhibit fewer, or different types of biological interactions than the streamlined genome oligotrophs which dominate many aquatic environments (Polz et al., 2006). Additionally, microbial community composition, generally measured using small subunit ribosomal RNA genes, may not be sufficiently sensitive to detect a disturbance response due to the time for cells to reproduce or predation of responsive taxa, necessitating the use of alternative metrics such as activity measurements (Berga et al., 2012; Hunt et al., 2013). Finally, dispersal may limit the response of taxa even under conditions which favor growth. With the relatively short time scales of pulse disturbances, it may be necessary to include prior relative abundance in predicting an OTU's potential responsiveness to disturbance. With all of these caveats in place, we suggest first studying time periods when disturbances are predicted to produce large changes in the microbial community.

Theoretically, anthropogenic disturbances should have the greatest impact when highly connected taxa change their abundance or activity. Research on networks has shown that disturbances that target central "keystone" nodes dramatically alter the rest of the network (Albert et al., 2000; Montoya and Sol, 2002). Ecological theory posits the

existence of keystone taxa – which may impact multiple members of the community through either positive interactions (production of substrates or co-factors utilized by other microbes) or competitive exclusion, predation, disease or habitat modification (Power and Tilman, 1996). Keystone organisms are often defined as those with disproportionate ecological roles given their relative abundance (Power and Tilman, 1996); however, as microbial ecology lacks techniques to remove specific OTUs and quantify the ecosystem effect, here we operationally define keystones as taxa located at the hubs of association networks with an increased number of network connections relative to abundance (high mean degree); however, other metrics take into account the betweenness and closeness centralities of the node as well as strength of interactions (Missiuro et al., 2009; Bissett et al., 2013; Berry and Widder, 2014; Peura et al., 2015). Yet many network hubs may be artifacts of network construction rather than true keystone taxa (Berry and Widder, 2014). Although the concept of keystone taxa has not been thoroughly explored in microbial ecology, previous studies have suggested that microbial community activity and succession is driven by interactions with phytoplankton (Azam et al., 1983; Kent et al., 2007). The factors that promote phytoplankton growth are generally well known: light, inorganic nutrients, specific temperature ranges; and phytoplankton are the dominant primary producers in most aquatic systems. These photosynthetic organisms shape the microbial community through primary production, but at the same time outsource the production of essential functions (e.g. hydrogen peroxide detoxification) to the broader community (Cole, 1982; Kazamia et al., 2012; Morris et al., 2012). Other taxa interact with phytoplankton through photosynthate consumption, degradation of detrital material, symbiosis, and predation (Croft et al., 2005; Stocker et al., 2008; Morris et al., 2011; Teeling et al., 2012; Durham et

al., 2015). Finally, phytoplankton serve as hubs in association networks (Steele et al., 2011) and could function as keystone organisms in aquatic ecosystems. While the ecological roles of some potential keystone taxa have been identified, e.g. nitrogen-fixing bacteria (Tyson et al., 2005), for most network hubs there is no known keystone function (Steele et al., 2011; Bissett et al., 2013). Thus the phytoplankton, where growth-promoting factors and relationships with other microbes are relatively well-characterized, represent an ideal model system in which to explore the biological interactions that underlie association networks during pulse disturbances.

1.4 Using storms as models to explore disturbance propagation

Storms represent complex, pulse disturbances that integrate both natural and human impacts. Storm-driven rain and wind events increase turbidity and introduce nutrients, organic material, and microbes from both the benthos and land into aquatic systems; while anthropogenic activity increases nutrient fluxes, impacts the timing of freshwater inputs, and contributes other chemical pollutants. Thus storms are multifaceted disturbances; yet, unlike some discrete disturbances (e.g. Deepwater Horizon oil spill), they occur frequently enough to allow comparison across different storms, environments, and microbial communities (Berga et al., 2012; Yeo et al., 2013). Here we use storms as a model disturbance to explore using association networks to track the propagation of disturbance through the microbial community.

To investigate this concept further, we will follow the progression of storm-mediated impacts on a simplified microbial community association network where an alga serves as a keystone microbe and a network hub. In our model system (Fig. 1) the major storm impact is an increase in nutrients (Iluz et al., 2009; Johnson et al., 2013); and

the first microbial community responder is the keystone algal OTU, which is positively correlated with nutrient levels. Using association networks prepared from non-disturbance data (Fig. 1 A&B), we can infer which other OTUs are likely to respond to a change in algal abundance. With high resolution post-storm sampling, we can observe changes in OTUs correlated with the early responders, as shown by lines (edges) connecting these taxa to the alga, which should exhibit changes in activity or relative abundance at intermediate time points if that OTU is dependent on the alga e.g. through metabolism of photosynthate (Fig. 1 C&D: yellow circles). At still later time points, the disturbance may propagate to taxa which interact with the yellow OTUs (Fig. 1D; green circles). Alternately, at this same time point, OTUs with inferred relationships with the alga, but utilizing detritus associated with bloom termination rather than photosynthate from active algal cells may exhibit increases in relative abundance (Fig. 1 C&D; yellow circles)(Teeling et al., 2012). Thus network-based approaches can offer biological insights into phytoplankton-bacterial interactions, the propagation and persistence of disturbance (Fig. 1), and community stability (Carpenter et al., 2011; Veraart et al., 2012). Even anecdotal observations of how OTUs respond to disturbance can generate hypotheses that can be verified using more controlled laboratory or manipulation experiments.

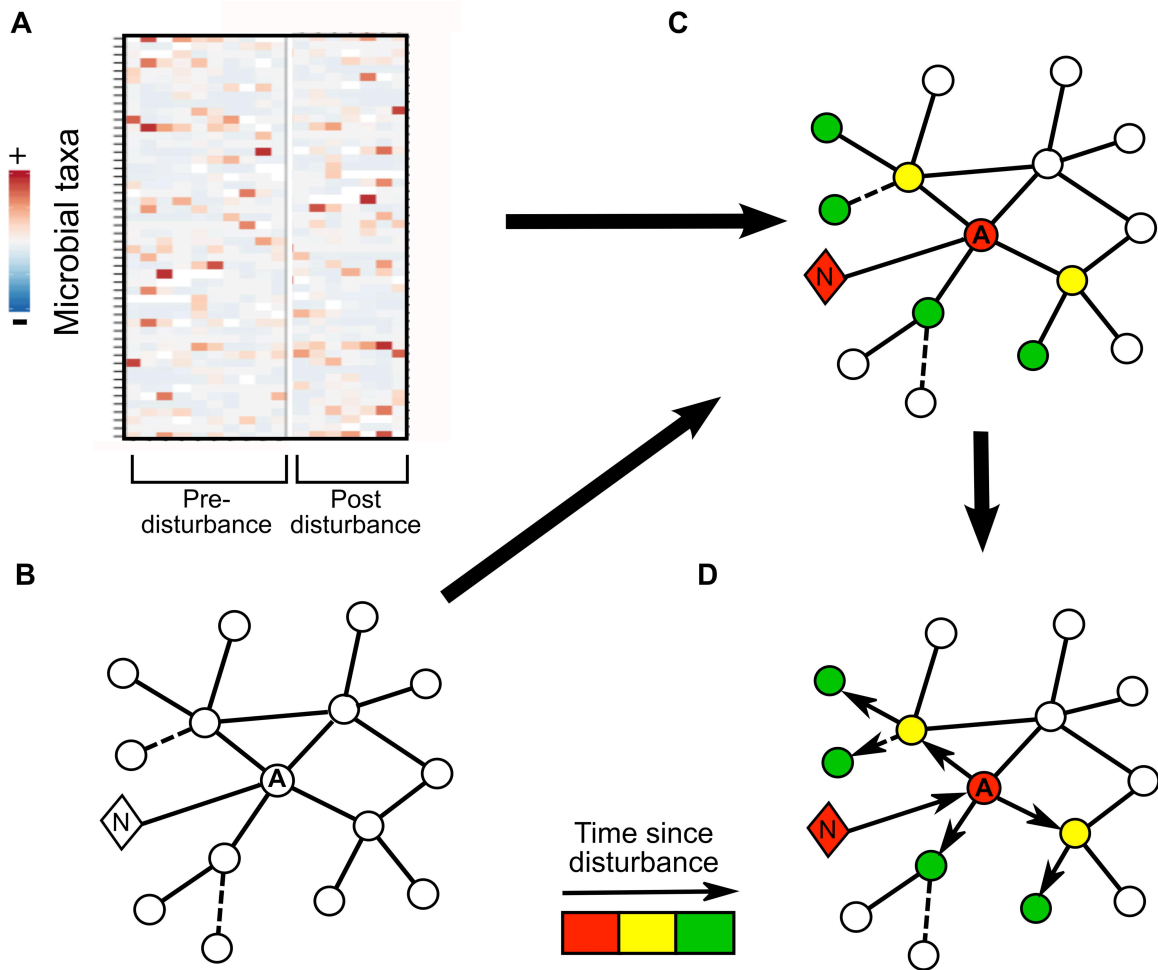


Figure 1: Schematic for tracking disturbance transmission through a microbial community. (A) Repeated community observations pre-disturbance are used to develop (B) a correlation-based association network for the microbial community. The circles represent operational taxonomic units (OTUs), with the keystone algal OTU denoted with an A, the diamond represents nutrients and is labeled with an N, solid lines connecting shapes indicate statistically significant positive correlations and dashed lines negative correlations between the connected taxa or environmental parameters. The same environment is intensively sampled following a storm to track short-term alterations in environmental variables and community composition. (C) The post-disturbance community composition from three time points is overlaid onto the interaction network to track the propagation of disturbance through the community: the red coloring indicates the changes directly following the storm: an increase in nutrients and shortly thereafter increased algal abundance. Yellow coloring indicates OTUs which display relative abundance changes at the second time point following disturbance and green those OTUs which change in relative abundance in the final period. (D) Arrows indicate the direction of inferred disturbance propagation based on the timing of observed changes in OTU relative abundance.

Here, we have presented a cartoon storm as a pulse of nutrients, in reality storms and other ecological disturbances are complex. In addition to nutrients, storms introduce

human pollutants into aquatic ecosystems, including pesticides, oil, untreated human waste, etc. that will have direct and interaction-mediated effects on the microbial community. Unlike our simple example in Figure 1, there may be multiple, competing impacts on our keystone algal OTU. For example, chemical herbicides such as atrazine impact phytoplankton due to the conservation of photosystem II between cyanobacteria, algae, and plants (Huber, 1993). While, the specific impacts of most chemicals are correlated with concentration; another herbicide class of synthetic auxins (e.g. 2,4-dichlorophenoxyacetic acid) is toxic to cyanobacteria at high concentrations but stimulates growth at lower levels (Mishra and Pandey, 1989), a subtlety which is not readily incorporated into association networks. Among other anthropogenic pollutants, fungicides are generally less specific than herbicides, targeting highly conserved cellular processes such as respiration and thus directly affect a range of microbes (DeLorenzo et al., 2001; Casida, 2010; Yang et al., 2011). Thus, along with nutrients, storms introduce a cocktail of chemicals to aquatic environments, complicating evaluation of direct and indirect community effects on the microbial community.

1.5 Conclusions

Here, we discuss the potential for association networks to track the propagation and persistence of disturbance in a microbial community. We have identified two major opportunities afforded by this approach: (1) to quantify the importance of interactions a microbial community's response to disturbance and (2) to generate biological hypotheses about the network's inferred interactions. However, a major challenge of this approach is that to characterize a microbial community's resistance and resilience we first need to understand disturbance-independent microbial community dynamics

(Shade, et al., 2012), suggesting the need for long-term monitoring of key study sites. Although the vast amounts of data required can appear daunting, specific taxa have been shown to repeatedly respond to storms (Jones et al., 2008) and the field is beginning to identify general characteristics of disturbance-responsive organisms (Shade et al., 2014), suggesting that there are conserved rules that govern microbial communities' disturbance responses. However, to tease apart the effects of factors that tend to co-vary in the environment, for example, separating the stimulatory effects of increasing nitrogen versus organic carbon, there is an additional role for controlled, replicated manipulations of natural aquatic communities. Beyond community changes, these experiments will also provide predictions about the alteration and restoration of ecosystem function following a disturbance, either by linking specific taxa to functions or by identifying the types of disturbance which may be most likely to disrupt specific processes (Amend et al., 2015). An association network-based approach to analyzing microbial community disturbances and experimental manipulations will provide a basis to mechanistically predict community response to both pulse and press environmental changes.

2. Seasonal threshold effects on microbial community composition in the temperate coastal ocean

2.1 Introduction

Microbial communities exhibit pronounced changes over an annual cycle, yet the mechanics by which microbial taxa respond to environmental changes are poorly characterized. In order to gain greater insight into seasonal microbial community changes, we tracked weekly changes in the microbial community over a three-year period at a temperate coastal time series. Remarkably, temporal changes in microbial community composition are not continuous, rather the community transitions rapidly between winter and summer assemblages over relatively short time intervals. Although a number of variables influence microbial communities, temperature is most strongly linked to these community- and taxa-level seasonal changes. Thus we hypothesized that temperature alone could induce transitions between seasonally-associated microbial communities. In order to test the potential for temperature (rather than other highly correlated environmental variables) to alter the community composition, we incubated seawater poised at this transition point at ambient and experimentally warmed (+5 °C) conditions. In both the warmed and ambient mesocosms, the summer taxa increased and the winter taxa remained at the same relative abundance or declined, suggesting that the temperature threshold may be more narrow than previously assumed or alternatively that summer-associated taxa are responsive to bottle effects. In spite of mixed results from mesocosm incubations, the observation of a rapid response to environmental changes suggests that rather than microbes responding linearly to environmental variables that threshold responses may drive microbial community dynamics. Thus this data suggest that climate change-induced warming of the surface

oceans may induce earlier and potentially geographically wider switches in seasonally-associated microbial communities. If communities differ in their functional capacities or rates, even small temperature shifts could exert profound differences in the biogeochemical functioning of marine ecosystems.

Planktonic microbes dominate biomass and biogeochemistry in the world's oceans. There is still not a consensus of how these communities are assembled (Nemergut et al., 2013), yet understanding the relative importance of environmental selection, stochasticity, dispersal, and evolution in microbial community assembly is essential to predicting ecosystem responses to environmental alterations including climate change (Doney et al., 2012). The processes determining microbial community and population composition are often identified using observational sampling, experimental community manipulations or cultures of model organisms (Gilbert et al., 2012; Johnson et al., 2006; Eren et al., 2013). Although observational sampling accurately captures complex associations between microbial community composition and environmental conditions, attributing responses to specific factors can be difficult due to covariation between variables such as with seasonal factors, e.g. temperature, light, and day length (Gilbert et al., 2012; Yung et al., 2015). Experimental manipulations incorporate the complexity of microbial communities, and enable us to test the impacts of individual environmental factors in isolation. The community response, whether at the compositional, metagenomic, or metatranscriptomic level, must be robust relative to the influence of bottle and stochastic effects (Baltar et al., 2015). Finally, culturable model organisms can be used to link field observations with microbial physiology; however laboratory conditions cannot fully replicate a complex environment and many environmentally relevant organisms cannot be cultured (Johnson et al., 2006; Yung et al.,

2015). Moreover, it may not be possible or desirable to extrapolate observations from single bacterial strains or clades to the entire microbial community. Despite these challenges, researchers generally agree that bacterioplankton respond to both physical conditions (e.g. temperature) and resource availability (e.g. nutrients, organic matter)(Fuhrman et al., 2006; Steele et al., 2011; Gilbert et al., 2012; Salter et al., 2015; Yung et al., 2015). In addition to identifying important environmental factors, these studies have also suggested that interactions between microbes and other organisms may be important in determining the community composition (Kent et al., 2007; Fuhrman et al., 2015).

In order to overcome some of the limitations of individual observational, manipulative or culture-based experiments, interest has grown in using trait-based approaches to examine fundamental drivers and trade-offs in marine microbes to identify unifying principles. Consistent biological responses across taxa and geographic locations can be used to identify key environmental variables that influence microbial community composition (Kent et al., 2007; Thomas et al., 2012). Again these approaches identify important drivers of bacterioplankton community composition that change with both season and latitude (temperature, light, day length, mixed layer depth)(Gilbert et al., 2012; El-Swais et al., 2014). However, many of these studies suggest that microbial communities respond in a linear manner to environmental changes, implying geographic changes in response to climate change factors, such as sea surface temperature warming, and perhaps localized extinctions in the tropics (Thomas et al., 2012). However high resolution long-term studies at a single location may be well suited to studying the nature of bacterioplankton responses to environmental changes, using seasonal patterns of environmental variability as a proxy for long-term environmental changes.

The temperate North Atlantic Ocean represents an ideal system to study the responses of bacterioplankton communities to changes in environmental conditions with strong seasonal patterns in variables such as temperature and pH (Johnson et al., 2013; Yung et al., 2015). Common environmental sampling schemes of monthly oceanographic time series or single research cruises generally cannot resolve within-season dynamics or resolve the progression of rare events (Gilbert et al., 2012; Hunt and Ward, 2015). Sampling at higher temporal or spatial resolution (Needham et al., 2013; El-Swais et al., 2014; Lindh et al., 2015) can resolve short-term dynamics such as phytoplankton blooms but often this sampling is not sufficiently resolved to place responses to these events in the context of inter-annual or ocean basin-wide changes.

Here, we examine weekly changes in the bacterioplankton community over a three-year period (January 2011-December 2013) in relation to a suite of environmental variables to identify key drivers of microbial community changes over annual cycles.

2.2 Results and Discussion

In order to identify environmental drivers of bacterioplankton community changes, bacterial community composition and a suite of environmental variables were measured weekly for three years as part of the Pivers Island Coastal Observatory (PICO: Fig. 14). The PICO time series, located at the Beaufort Inlet, Beaufort NC USA, captures the dynamics of the temperate coastal ocean, including large seasonal changes in environmental variables such as light, temperature, and pH (Johnson et al., 2013). Although the sampling site is located at the mouth of an estuary and in relatively shallow water (depth ~4.5m), environmental conditions including salinity are similar to the coastal ocean. However, unlike other coastal systems with strong seasonal upwelling (Chow et al., 2013), nutrients are low throughout the year, with infrequent pulses

following wind and rain events (Johnson et al., 2013). To explore microbial community changes in this time series, we sequenced the V3-V4 region of the 16S rRNA gene using primers for both bacteria and archaea (Kozich et al., 2013) and clustered sequences into operational taxonomic units (OTUs) of at least 97% similarity (Edgar, 2013). As observed in other coastal locations, the bacterioplankton are dominated by common marine bacteria including SAR11, OCS155 and *Synechococcus* (Fig. 2)(Gilbert et al., 2012; Chow et al., 2013).

With both high-resolution (weekly) sampling and several years of observations, this dataset is well-positioned to examine annual cycles of bacterial community composition. Like other time series, this dataset exhibits strong, repeated annual patterns in both the composition of the microbial community at the Family level (Fig. 2). In winter, the community is characterized by a high relative abundance of SAR11 and other Proteobacteria, while the summer community is enriched in photosynthetic taxa such as *Synechococcus* and eukaryotic chloroplast sequences, *Actinobacter OCS155*, and *Roseobacter*. Although we observed relatively high abundance of the cyanobacterium *Prochlorococcus* in the summer using flow cytometry, in these libraries they are not differentiated from the numerically more abundant *Synechococcus* due to high sequence similarity in this portion of the 16S rRNA gene. Despite gross similarities across seasons at the family level, the bacterioplankton community is fairly different between winter and summer. But as we have previously observed at this site, at fine phylogenetic scales the bacterioplankton community further changes over the annual cycle, potentially driven by the seasonal cycling of environmental variables such as temperature (Yung et al., 2015).

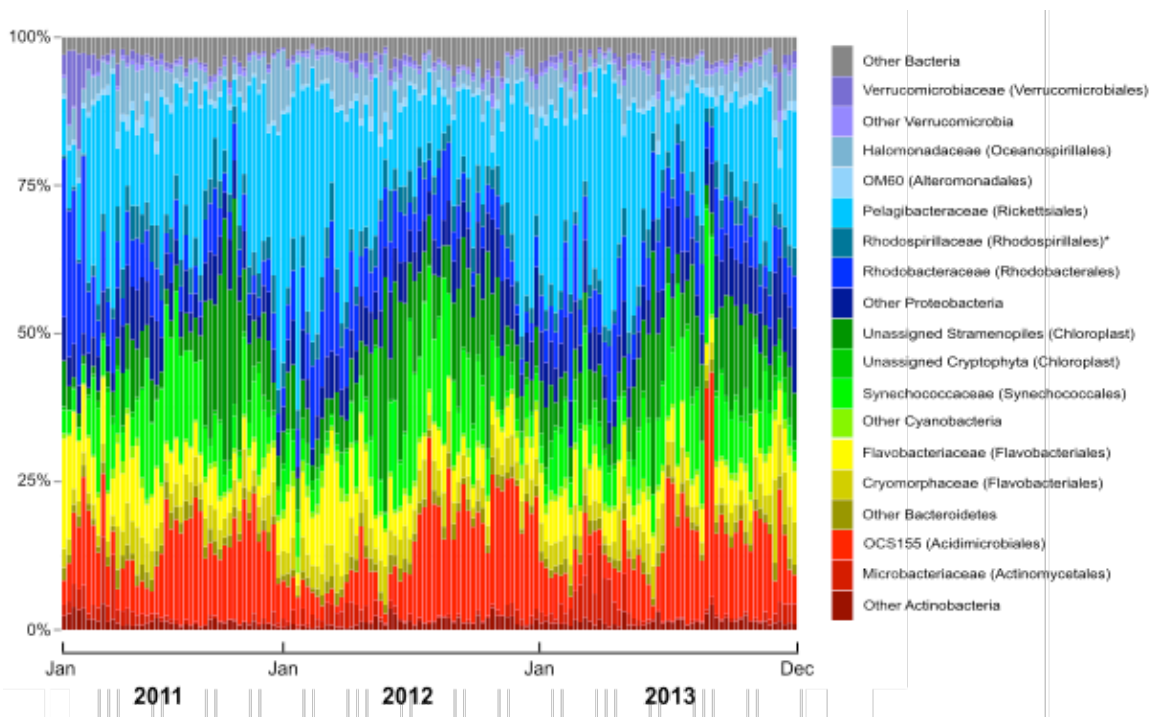


Figure 2: Composition of partial 16S rRNA gene sequences for weekly samples from the Pivers Island Coastal Observatory (PICO) over three years (Jan 2011 – Dec 2013). Taxonomic classification was made using RDP Classifier following OTU clustering (97% sequence identity). Taxonomy was assigned at the level of Family, with Order indicated in parentheses. OTUs not identified at the family level were grouped at the lowest level of taxonomy identified. Taxa comprising less than 1% of total abundance over the entire dataset are grouped as “Other”.

For example, looking at the Genus level we observe that two genera within the Rhodobacter – Octadecabacter and an unclassified Roseobacter genus – appear to alternate in relative abundance, with Octadecabacter peaking in the winter. In contrast, the unclassified Roseobacter genus exhibits its highest relative abundance in late summer. If family-level phylogeny is indicative of shared metabolic capabilities (A. Martiny, personal communication), this result suggests that distinct OTUs might play similar ecological roles in temporal microenvironments (Hunt et al., 2008; Yung et al., 2015).

To identify which variables might be responsible for community and taxa switching in this environment, we examined microbial community changes along with a

constrained set of environmental variables using canonical correspondence analysis (CCA: Fig. 3). The CCA plot, colored by year day, shows that the microbial community follows a repeated annual trajectory. However, it also becomes apparent that the community forms winter and summer clusters with relatively short transitions between these distinct community compositions (Fig. 3). These distinct winter and summer clusters are in contrast to other time series which observe distinct spring and fall microbial communities presumably linked to spring and fall phytoplankton blooms (Chow et al., 2013; El-Swais et al., 2014). This time series does not exhibit a regular annual cycle in chlorophyll *a* (Johnson et al., 2013), thus this site lacks characteristic bloom periods that might alter microbial community composition or obscure other seasonal signals. To identify the environmental drivers of these changes, the CCA includes a constrained set of environmental variables determined using forward stepwise selection ($P < 0.05$). Among the variables tested (temperature, no-sky projected daily insolation, salinity, chlorophyll *a*, and ammonium), the environmental drivers that explained the most community variability are temperature and insolation, although all variables included were statistically significant ($P < 0.05$). This result mirrors that of previous marine time series which found a large “seasonal driver” (a combination of light (day length) and water temperature metrics) of either OTU richness or community diversity (Gilbert et al., 2012; Chow et al., 2013). Due to high correlation between multiple seasonal environmental variables, it can be difficult to deconvolve the contributions of each. However, the high resolution and repeated annual cycles captured in the PICO time series, this dataset can offer unique insight into both the patterns and environmental drivers of bacterioplankton diversity.

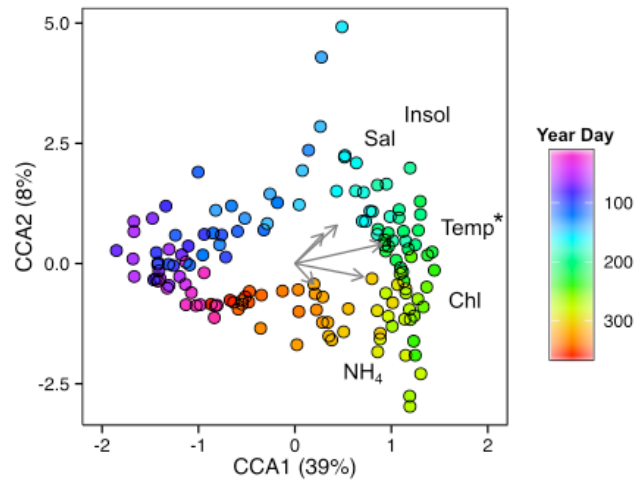


Figure 3: Biplot of canonical correspondence analysis (CCA), based on microbial community composition and environmental variables for each sample from the PICO time series. The percents of variation in the microbial community explained by the first two axes indicated in parentheses on the axis label. The constrained set of environmental variables used in the CCA is represented as vectors: temperature (Temp), projected no-sky daily insolation (Insol), salinity (Sal), chlorophyll (Chl), and ammonium (NH₄). The microbial community compositions from each time point are represented as circles; the color gradient indicates the day of the year. All included environmental variables are statistically significant ($P < 0.05$), as assessed by the marginal effects of terms; temperature (asterisk) had the highest explanatory value.

However, individual taxa may have distinct responses to environmental variables which may be obscured when looking at the whole community level; thus we propose to examine the temporal dynamics of individual OTUs. Although at the Family level most taxa are present throughout the year (Fig. 2), at the OTU-scale there is a much stronger seasonal pattern. The heatmap of the 100 most abundant OTUs, which account for 68% of the total sequence reads, across weekly samples visually shows concordant transitions occurring at seasonal boundaries (Fig. 4). Thus, to better understand the drivers of the observed community patterns, we used soft clustering to identify shared patterns in the relative abundance of these OTUs across the three years of environmental sampling (Fig. 4). We visually combined these clusters into four groups based on similarities in their relative abundance patterns: ubiquitous, sporadic, summer-associated, and winter-associated (Fig. 4). Both the patterns of OTU relative abundance and the centroids of each cluster (Fig. 4, Fig. 16) highlight the shared seasonality of the

groups and as well as between-cluster differences in both relative abundance and initiation time (lag) with a group (Fig. 4). The ubiquitous group consists solely of SAR11 and this cluster remains relatively highly and evenly abundant throughout the time series (Fig. 16). This ubiquity of SAR11 OTUs (in contrast with other groups which are more seasonal) has been previously explained by the observation of sub-OTU level ecological specialization {Eren *et al.*, 2013}, suggesting that finer-scale phylogenetic resolution will be necessary to resolve ecologically distinct SAR11 types. Two OTUs identified as diatom chloroplast sequences were assigned to the sporadic group which appears to increase following storms (Fig. 4, Fig. 16). However the majority (84 %) of the top 100 OTUs were assigned to either summer- or winter-peaking clusters, suggesting this is the dominant pattern in microbial community variability at this site. Moreover, transitions between summer and winter communities are not dominated by new taxa but rather reflect co-occurrence of both winter and summer groups when alpha diversity peaks (Fig. 15). This finding is in contrast to observed winter diversity maxima in the English Channel (Gilbert *et al.*, 2012) which may be an artifact of a more even bacterioplankton community in the winter combined with limited sampling depth. The predominance of summer- and winter-associated groups suggests that certain ecological niches are occupied sequentially by distinct OTUs during the course of the year. However, despite these strong seasonal associations, taxa can be present in the non-preferred season, in fact, some clusters (e.g. Clusters 3 and 9) are present throughout the year but change in relative abundance (Fig. 16). It is unclear whether these seasonally-associated but ubiquitous OTUs are more generalist taxa, if subOTU-level ecological specialization occurs, or if additional seasonal environmental variables influence

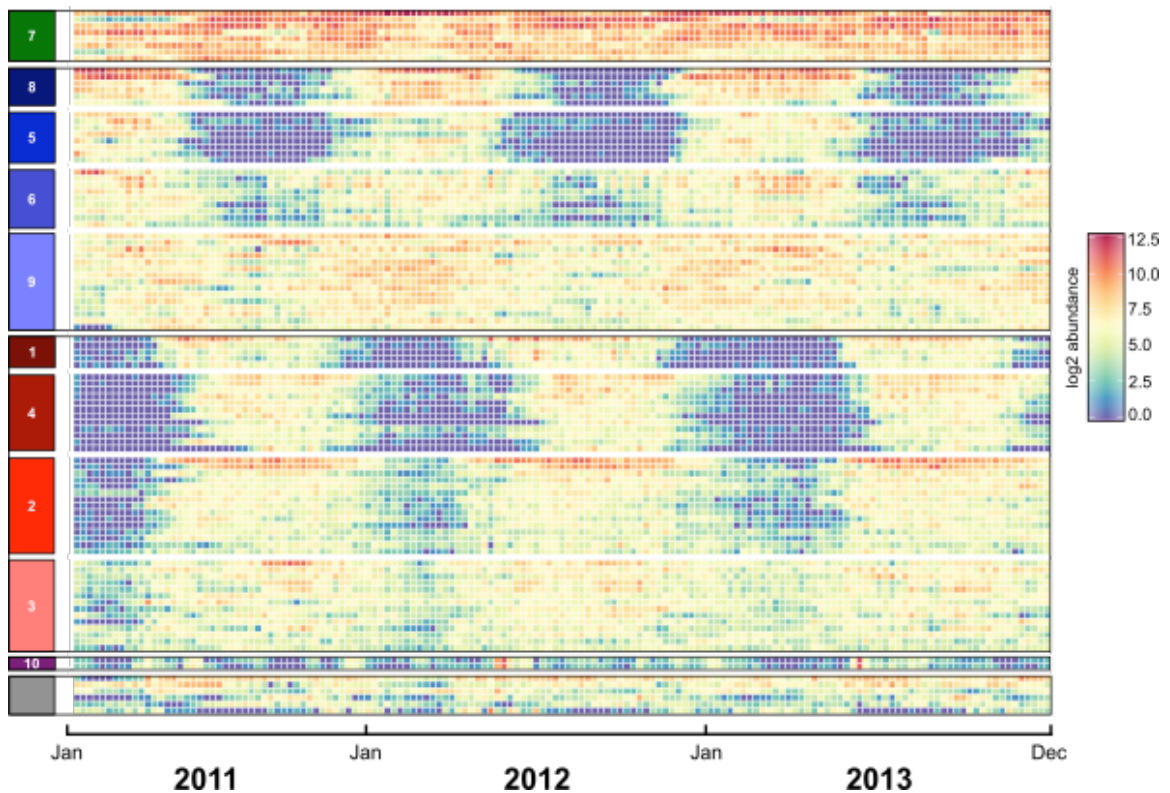


Figure 4: Seasonal patterns of the 100 most abundant OTUs for weekly samples over the entire three-year PICO time series. The heatmap represents the log₂ relative abundance for each 97% identity OTU post-processing. OTUs are ordered based on patterns observed in soft clustering (Fig. 16), shown as numbered boxes on the left hand side of the figure. These clusters were further categorized by seasonal patterns into ubiquitous (green), winter-associated (blue), summer-associated (red), sporadic (purple) groups; the remaining OTUs (gray) were not statistically assigned to clusters.

microbial abundance patterns (Yung et al., 2015). In spite of some variability in the lag and seasonal fidelity of specific OTUs, the strongly entrained seasonal signal suggests the potential for an annually repeated driver of transitions between summer and winter microbial communities.

We posit two possible drivers for these repeated seasonal environmental transitions: (1) there is a community-wide biological tradeoff that occurs at specific environmental thresholds which cause the community to transition (Yung et al., 2015), or (2) that a keystone organism changes in abundance or activity likely due to an environmental factor, and triggers a community-wide change mediated by interactions

with the keystone (Hunt and Ward, 2015). Given the difficulties in establishing the impact of keystone taxa, we focus on potential environmental drivers of community change, although observational data will not be able to separate the environmental driver versus interaction-driven transition hypotheses. However, it should be noted that even an interaction-driven transition required a seasonally-sensitive change in the keystone organism. Although it can be difficult to separate seasonally-associated variables, given the significance of temperature for community composition (Fig. 3), we predict that temperature may drive the seasonal distributions of OTUs. Closer examination of seasonally-associated clusters reveals dramatic nonlinear responses of cluster relative abundances over a temperature range of 19°C-24°C (Fig. 5). One problem with purely observational data is the difficulty in separating the impacts of environmental variables which are correlated with one another, in this case seasonally-changing variables: temperature and light. However, while insolation follows regular annual patterns, temperature is more variable across years due to weather changes. Supporting the importance of temperature driver over light, the timing of cluster transitions varies on a yearly basis, for example, a cold spell in early 2013 delayed seasonal warming and the appearance of summer-associated OTUs. Additionally, although light is important for phytoplankton which potentially serve as keystone microbes and affect the microbial community through production of labile organic material (Hunt and Ward, 2015), temperature has the potential to directly impact a broad range of microbes including photosynthetic taxa through metabolic kinetics (Brown et al., 2004; Allen et al., 2005). Moreover, recent work has suggested that growth at low temperatures comes with a fitness cost (as measured by growth rate) at higher temperatures, highlighting the improbability of thermal generalists (Yung et al., 2015). Thus, a number of lines of

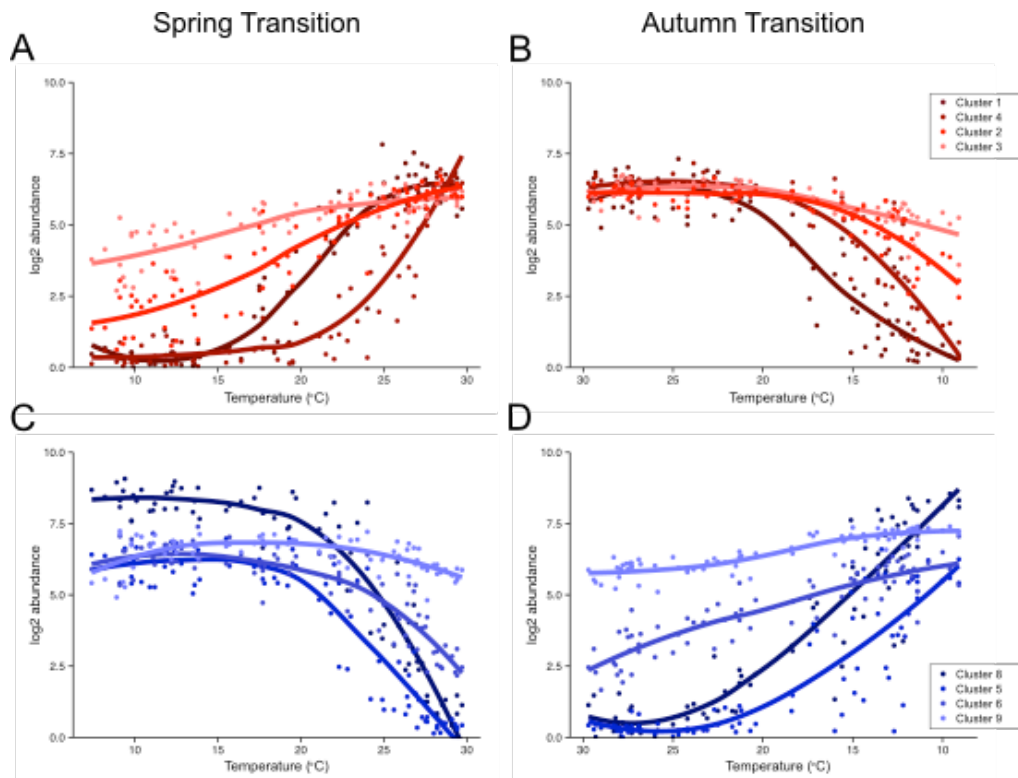


Figure 5: Spring and autumn transitions of seasonally-associated OTU clusters versus temperature. The 3-year time series has been separated into seasonal transitions, the spring transition (A and C) is shown in panels for observations from the yearly observed minimum to maximum temperatures. The autumn transition (B and D) is depicted using data from observed over the period from the yearly maximal to minimal temperatures. Centroids representing the relative abundance of each cluster for all three years of data are plotted as both observed (points) and fitted (lines) for summer- (A and B) and winter- (C and D) associated clusters.

evidence point to temperature as the key driver of these community transitions and suggest that temperature-mediated community transitions likely occur in temperate regions of the world's oceans which experience large annual variability in temperature.

Although temperature is consistently a key driver of microbial composition (Fuhrman et al., 2006, 2008; Johnson et al., 2006; Yung et al., 2015), these repeated annual transitions at the OTU level have not been observed previously in time series data. OTU transitions may be less pronounced for time series which do not have such large annual changes in temperature (~20 °C at the PICO study site). Alternatively, transitions may occur at specific biological threshold (19-24 °C) not crossed at all locations or these

transitions are more readily apparent with higher-resolution sampling and the absence of seasonally-associated phytoplankton blooms. In addition to strong correlations with temperature, we wanted to experimentally test the importance of a temperature threshold on the bacterioplankton community, we compared microbial community composition in replicate ambient (19°C) and experimentally warmed (24 °C) mesocosms (10 L) incubated for five days under otherwise uniform conditions in the lab. While we acknowledge the limitations of bottle effects (Pernthaler and Amann, 2005; Hammes et al., 2010), mesocosm experiments enable the isolation of temperature from other environmental variables. Additionally, this experimental approach is unable to differentiate between our competing temperature and keystone organism-mediated community change hypotheses. However, for summer-associated microbes to bloom in the warmed mesocosm under a keystone-microbe hypothesis, the keystone organism itself would have to be temperature-sensitive, arguing for an indirect temperature effect. For the 100 most abundant taxa from the three-year time series, the summer-associated OTUs on average increased in both the warmed and ambient mesocosms (Fig. 6), suggesting that the microbial community was not yet at equilibrium with the rapidly warming, ambient environmental conditions. However, we noted a marked decrease in winter-associated clusters in the 24°C mesocosms, suggesting that their transition temperature was reached. Thus although we cannot rule out alternatives such as interactions facilitating the observed microbial community changes, temperature alone has a significant impact on the microbial community at this temperate coastal site.

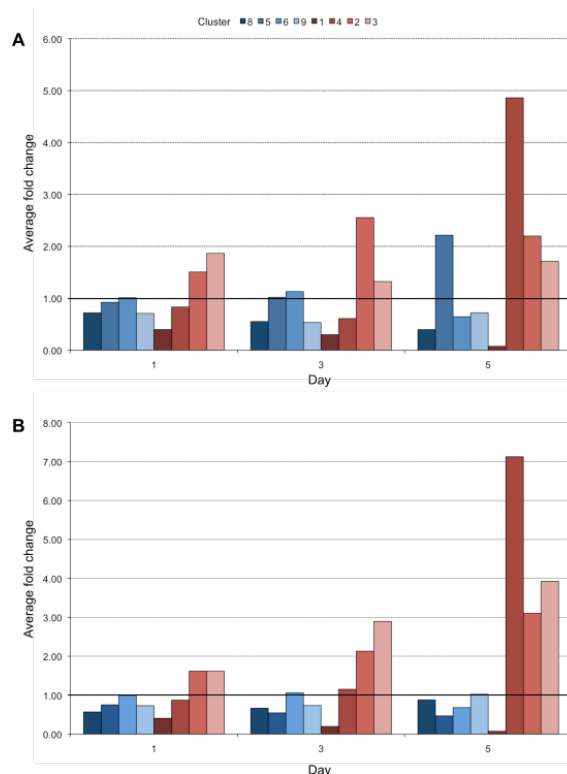


Figure 6: Average fold change in the relative abundances of OTUs belonging to winter- and summer-associated clusters. OTUs recovered from library sequencing of ambient (A) and experimentally warmed (B) mesocosms were assigned to previously determined clusters. Winter-associated clusters are represented in blue shades while summer-associated clusters are represented in red shades. Black line indicates fold change of 1 (no change) relative to Day 0.

2.3 Conclusions

Although observational measurements are one of the primary means that environmental scientists use to predict the ecologies of the largely unculturable microbial inhabitants of the world's oceans, it can be difficult to link these observations to specific environmental drivers. Here we show that a dynamic, microbial community inhabiting a temperate coastal site exhibits distinct winter and summer assemblages that are apparently mediated by temperature changes. That this state transition occurs over a relatively narrow temperature threshold has key implications for climate change, suggesting all temperature increases are not created equal, and that community and

potentially rates of biogeochemical cycling change over narrow environmental thresholds. Thus climate change-mediated increases in sea surface temperatures may not result in small changes in community composition or the poleward expansion of temperate taxa but the wholesale transition to a new microbial community.

2.4 Experimental approaches

2.4.1 Environmental sampling

Samples were collected at the Pivers Island Coastal Observatory (PICO) site (34.7181°N 76.6707°W) at Beaufort Inlet weekly from January 2011 to December 2013. Seawater was collected at 10:30 AM local time using a 5-L Niskin bottle centered at 1 m or a peristaltic pump with the tubing open at 1 m, and processed within one hour. Methods for determination of surface water temperature, pH, salinity, dissolved inorganic nutrients concentrations, chlorophyll (Chl) *a* concentration, and bacterioplankton and phytoplankton numbers were described previously (Johnson et al., 2013; Hunt et al., 2013).

2.4.2 DNA extraction

Microbial biomass was collected by filtering ~1 L of seawater through a 0.22-micron Sterivex filter (Millipore), and filters were stored at -80°C until extraction. Nucleic acids were extracted as described previously (Massana et al., 1997), with some modifications. In brief, cells were lysed by bead-beating on ice three times for 30 secs in lysis solution (0.75 M sucrose, 40 mM EDTA, 50 mM Tris pH 8.0), followed by consecutive incubations with lysozyme (60 mg/mL; 37°C) and SDS (1%; 55°C). DNA was purified by phenol-chloroform extraction, RNase treatment, isopropanol precipitation, and PCR inhibitor removal (Zymo). DNA concentration was measured using a NanoDrop ND-1000.

Microbial communities were characterized using a dual index primer approach (Kozich et al., 2013) with the following universal bacterial and archaeal primer region to target the 16S rRNA gene: S-D-Bact-0341-b-S-17 (16S F V3; CCTACGGGNGGCWSCAG) and Arch806 (Hugoni et al., 2013; 16S R V4; GGACTACNVGGGTWTCTAAT). PCR reactions contained 20 ng of template gDNA and 0.4 U of Q5 DNA polymerase (NEB) as well as a final concentration of 200 μ M dNTPs, 2 mM MgCl₂, and 0.5 μ M primers. PCR reactions were thermocycled using the following protocol: 98°C for 30 sec, and 28 cycles at 98°C for 10 sec, 55°C for 30 sec and 72°C for 30 sec, with a final extension at 72°C for 2 min. Triplicate reactions per sample were pooled and gel purified. In total, 151 libraries were paired end (2x 250bp) sequenced on the MiSeq (Illumina) at Duke's Genome Sequencing and Analysis Core Facility.

2.4.3 Sequence processing

Sequences were demultiplexed and assigned to corresponding samples using CASAVA (Illumina). Sequences were processed using USEARCH v7 (Edgar, 2010, Edgar, et al., 2011, Edgar, 2013). Briefly, low quality sequence ends were trimmed at Phred quality (Q) of 30 using a 10-bp running window. Paired end reads were merged when reads had a ≥ 10 bp overlap with no mismatches; the resulting joined sequences were then filtered to remove reads with expected errors > 1 or a length < 400 nt. At this point, singleton sequences were excluded and the remaining sequences were assigned to OTUs of 98.5% pairwise identity using the centroid-based clustering UPARSE-OTU algorithm (Edgar, 2013), resulting in OTUs of at least 97% similarity. Chimeras were removed at the OTU clustering step and using ChimeraSlayer in UCHIME (Edgar, et al., 2011). OTUs occurring less than five times in the entire dataset were removed, yielding a total of 10,357 OTUs. Libraries were subsampled to 20,082 reads per library. The most common sequence in each OTU was automatically aligned using PyNAST; the

alignment was filtered to remove gaps and hypervariable regions using a lane mask, and used to construct a maximum-likelihood tree using FastTree. The taxonomies of representative sequences were classified using the RDP naïve Bayesian classifier using the Greengenes version 13.5 database.

2.4.4 Characterization of microbial community

Canonical correspondence analysis (CCA)(Terbraak and Verdonschot, 1995) was used to visualize the temporal dynamics of microbial community composition and to identify the environmental and biological factors that were most closely associated with compositional changes. Microbial communities were related to a constrained set of environmental and biological variables using CCA implemented in the Vegan R package (Oksanen et al., 2015). The constrained parameter set was determined prior by performing a stepwise selection (Akaike information criterion, 999 permutations per step) using the 'step' function in the Vegan R package. To reveal synchronous dynamics in the 100 most abundant OTUs, they were clustered using fuzzy c-means clustering (Mfuzz; Kumar & Futschik, 2007). The number of clusters was set to 10 and the fuzzifier coefficient set to 1.25. OTUs with a membership value of at least 0.7 were assigned to corresponding clusters (94 OTUs assigned, 6 unassigned).

2.4.5 Temperature shift experiments

Acid-washed 10-L glass bottles were filled using a peristaltic pump with PICO seawater from 1 meter pre-filtered through a 63- μ m plankton net to exclude large cells and particles. Seawater samples were collected on April 14, 2015 at 10:30AM local time when the water temperature at 1 m was 19.0°C. Duplicate mesocosms were incubated at ambient and elevated temperatures (19°C and 24°C, respectively) on a 14h/10 h light/dark cycle with a light intensity of 80 microEin/m²•s. The mesocosms were manually inverted twice daily. Mesocosms were sampled for DNA, flow cytometry, and

extracted chlorophyll on Days 0, 1, 3, and 5, and samples were processed as for the weekly PICO sampling. 16S rRNA gene library sequences were added to the existing OTU database and subsampled to a depth of 5,700 sequences and processed as described above for other samples.

2.5 Acknowledgments

The authors would like to acknowledge the entire PICO team for help with environmental sampling. This work was supported by grants from the Gordon and Betty Moore Foundation (GBMF3768 to DEH) and the National Science Foundation (OCE 1322950 to DEH and OCE1416665 to DEH and ZIJ), as well as a NSF Graduate Research Fellowship to CSW. This work will be submitted for publication, with Charmaine Yung, Katherine Davis, Sara Blinebry, Tiffany Williams, Zackary Johnson, and Dana Hunt as co-authors.

3. Differential responses to disturbances by variable taxa in the coastal bacterioplankton

3.1 Introduction

Marine microbial communities have well-characterized annual patterns, yet how they change in response to episodic disturbances is not well known. Here we examine short-term microbial community variability and explore the contributions of rare and abundant taxa to the compositional changes in the whole community. The V3-V4 regions of 16S rRNA gene were analyzed by library sequencing over three years at a highly dynamic coastal site. Disturbances involving turnover of nearly half of the community over one or two weeks occurred numerous times throughout the study. Some disturbances occurred following small storms while larger rain events did not always result in community disturbances. Conditionally rare taxa contributed to the community changes in some, but not all of the disturbances. Our findings suggest that abundant and rare microbial taxa can drive community changes during disturbances and that conditions of the microbial community may set the stage for disturbance responses.

Most microorganisms live in variable environments, yet this is especially true of coastal bacterioplankton. The temperate coastal ocean is a highly dynamic ecosystem that continuously undergoes changes in environmental factors such as light, temperature, acidity, salinity, and nutrients (Johnson et al., 2013). Bacterioplankton rapidly turn over, suggesting that microbial community composition should exhibit changes in response to environmental fluctuations (Lindh et al., 2015). However, detecting episodic disturbances requires characterizing the dynamics of microbial communities on relevant temporal scales (Gilbert et al., 2012; Needham et al., 2013; El-

Swais et al., 2014). Consequently, the frequency and extent of biological response among diverse microbial taxa experiencing environmental fluctuations *in situ* remains poorly understood (Ottesen et al., 2013).

In the coastal ocean, physical and biological processes including seasonal, diel and tidal cycles and episodic storms contribute to temporal variability of environmental conditions on multiple time scales (Johnson et al., 2013). How such variation influences the resident microbial community remains an active question in microbial ecology. Seasonal shifts in microbial community composition have been demonstrated in marine environments around the globe (Fuhrman et al., 2006; Gilbert et al., 2009; Andersson et al., 2010; Salter et al., 2015), indicating that over longer time periods (i.e., ≥ 1 year) seasonal environmental patterns typically overwhelm variability from other processes. Yet over shorter time periods, microbial communities can be exposed to dramatic changes in other abiotic variables, imposing strong environmental forcing (Iluz et al., 2009; Johnson et al., 2013). Given that microbial communities typically exhibit low resistance to disturbances (Allison and Martiny, 2008; Shade et al., 2012), community composition would be expected to change in response to short-term environmental changes (Needham et al., 2013). In coastal areas, storms represent repeated episodic disturbances that introduce wind-driven mixing, freshwater runoff, and pulses of allochthonous nutrients and organic material to marine ecosystems (Paerl et al., 2001, 2010; Yeo et al., 2013). Previous microbial community studies of coastal storm disturbances have focused on a single event or perturbation type in high temporal and/or spatial resolution (Yannarell et al., 2007; Jones et al., 2008; Yeo et al., 2013); this may also overlook other less well-characterized causes of short-term variability. Here, we present an approach to studying disturbances in microbial communities that focuses

on identifying extreme compositional changes in order to avoid making *a priori* assumptions of what events may constitute disturbances.

In the previous chapter, a three-year time series of 16S rRNA gene amplicon libraries was used to characterize the seasonal patterns of bacterioplankton communities at a temperate coastal site (Beaufort, NC). The highly dynamic nature of this marine system, in combination with the temporal resolution of our time series, presents an ideal opportunity to comprehensively investigate patterns and drivers of short-term variability in bacterioplankton and phytoplankton communities. Here, we examine microbial responses during pulse disturbances in the coastal ocean on the community and OTU levels.

3.2 Results

3.2.1 Environmental variation in the coastal ocean

Environmental variables that may influence microbial communities were examined in 151 water samples taken from the coast of North Carolina weekly between January 2011 and December 2013, as part of the time series at Pivers Island Coastal Observatory (PICO; Fig. 7A). Two major features contribute to the environmental variation at PICO: (1) large annual cycles in water temperature, and (2) frequent, often large rainfall events. In contrast to water temperatures which vary on a seasonal basis, rainfall occurred sporadically throughout the time series with no clear temporal pattern. Rainfall positively affected inorganic nutrient concentrations, which were otherwise low throughout the time series (Fig. 7B). The concentrations of inorganic nutrients peaked following Hurricane Irene (August 27, 2011) and a large coastal storm that followed four weeks later; however, overall the magnitude of nutrient increase was not highly correlated to the amount of rainfall (Fig. 7B).

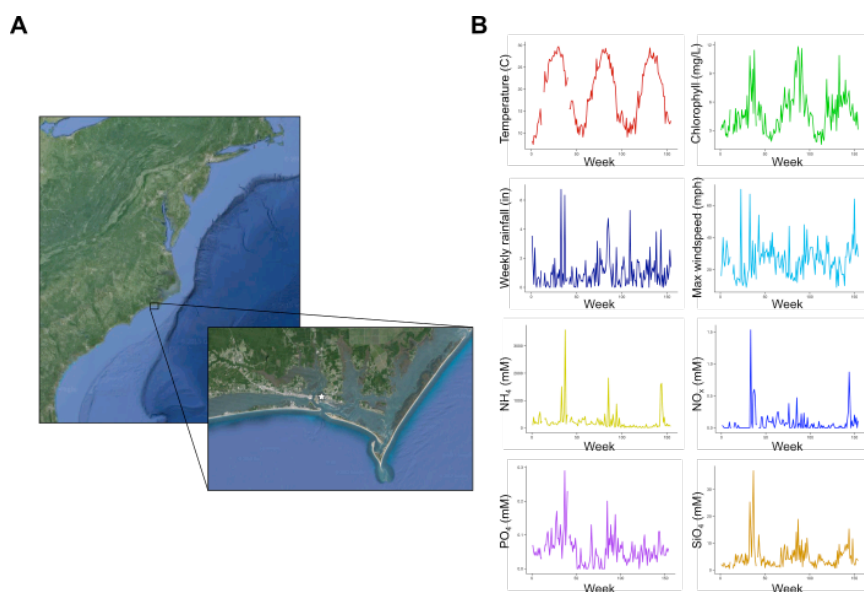


Figure 7: Location and environmental conditions of a highly dynamic, temperate coastal system. (A) Map of western North Atlantic Ocean coast, with an inset of the Newport River Estuarine System. The study site is indicated by the white star on Pivers Island Coastal Observatory (PICO), approximately 1.4 miles to Beaufort Inlet. (B) Plots of environmental variables (temperature, chlorophyll, weekly rainfall, maximum wind speed, NH_4^+ , $\text{NO}_2+\text{NO}_3^-$, PO_4^- , SiO_4^-) over the 3-year study period.

3.2.2 Community variation over time

Community composition of bacterioplankton and phytoplankton communities was assessed by sequencing partial 16S rRNA genes from the PICO time series and clustering gene sequences into operational taxonomic units (OTUs) of at least 97% similarity. We calculated temporal changes in microbial community composition over one- and two-week intervals to comprehensively quantify the extent of short-term variability. High community dissimilarity indicates large microbial community changes over that time period; notably this study was agnostic to measured changes in environmental variables over these time intervals.

Over the three-year time series, community dissimilarities over both one- and two-week intervals tend to fluctuate between 0.20 and 0.40 with intermittent spikes of elevated values (Fig. 8). Replicate samples filtered within one hour had community

dissimilarity values of 0.24 ± 0.01 ($n=3$), indicating the lower sensitivity threshold of the approach. Detected disturbances were defined as time periods in which the community dissimilarity exceeds the 95th percentile of observed values (Bray-Curtis dissimilarities of 0.46 and 0.48 for one- and two-week intervals respectively). Because several disturbances occurred consecutively or in quick succession, five distinct disturbance periods were detected using each time interval, with overlapping disturbances in spring 2012 (Disturbance 3), spring 2013 (Disturbance 5), and summer 2013 (Disturbance 6)(Fig. 8). There was general agreement between disturbances detected using one and two-week intervals. Disturbances were distributed fairly evenly across season, and no consistent pattern was discernable in relation to environmental variables, particularly rainfall and inorganic nutrients. For instance, Disturbance 1 occurred following large weekly rainfall and spike in inorganic nutrients; however, other large rainfalls and/or nutrient spikes did not coincide with other disturbances (Fig. 7). However, the disturbance with the largest community dissimilarity (that is to say, the most extreme disturbance) happened in spring 2012 during a period of repeated small rainstorms (weekly rainfall ≈ 2.5 cm) when nutrient levels remained low. Interestingly, Disturbance 5 occurred during a similar period in spring 2013.

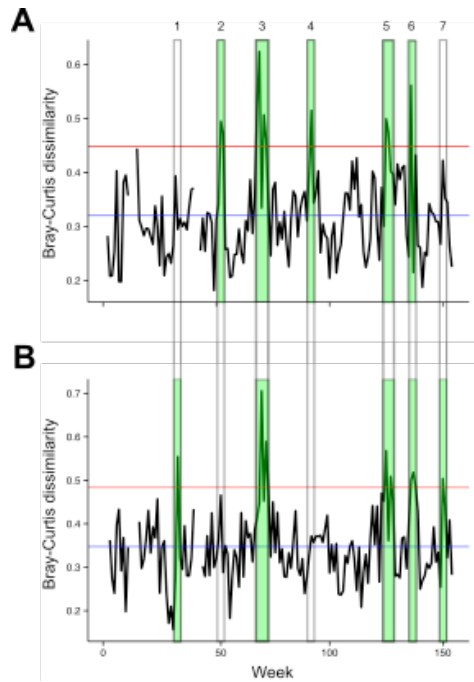


Figure 8: Community dissimilarity over short time scales across three years (Jan 2011 – Dec 2013). Bray-Curtis dissimilarity (black line) was calculated over one- (A) or two- (B) week intervals. Mean value (blue line) and 95th percentile (red line) are shown. Green boxes indicate periods when community dissimilarity exceed 95th percentile (disturbance). Disturbance periods are chronologically numbered above the boxes.

3.2.3 Identification of highly variable and conditionally rare taxa

To investigate how different taxa types may contribute to pulse disturbances in the coastal microbial community, we followed the dynamics of individual OTU abundances over time. If rare taxa become transiently abundant, we can identify them based on characteristics of their abundance patterns. Two classifications were used: highly variable, which is based on relative changes in abundances, and conditionally rare, which is based on distribution of abundances (see Experimental Approaches for details); for contrast, the dynamics of the 50 most abundant OTUs were provided as well. To reduce the likelihood of selecting taxa whose abundance patterns do not exhibit typical ‘boom-and-bust’ dynamics (i.e., false positives), conservative thresholds of variability (variability=1.25) and conditional rarity (bimodality=0.9) were selected. This

resulted in relatively few highly variable taxa (41 OTUs) and conditionally rare taxa (34 OTUs) being selected. 17 OTUs overlap between the two groups (Fig. 9); there was no overlap between the 50 most abundant OTUs and the conditionally rare or highly variable taxa (Fig. 9A).

The OTUs in these three potentially disturbance-responsive groups were identified to the taxonomic level of Class (Fig. 9B). The makeup of the fifty most abundant taxa reflected the taxonomic composition of the whole microbial community, consisting primarily of Alphaproteobacteria, Acidimicrobia, Flavobacteria, Synechococcophycidae, and Stramenopiles (chloroplast)(Fig. 9B). In comparison, the highly variable and conditionally rare taxa represented a broad range of phylogenetic groups, many of which are found in very low relative abundance in the whole microbial community. Stramenopiles comprised a large percentage of both groups (>30%), and were also enriched for Planctomycetes, Flavobacteria, and Chloroflexi classes (Fig. 9B).

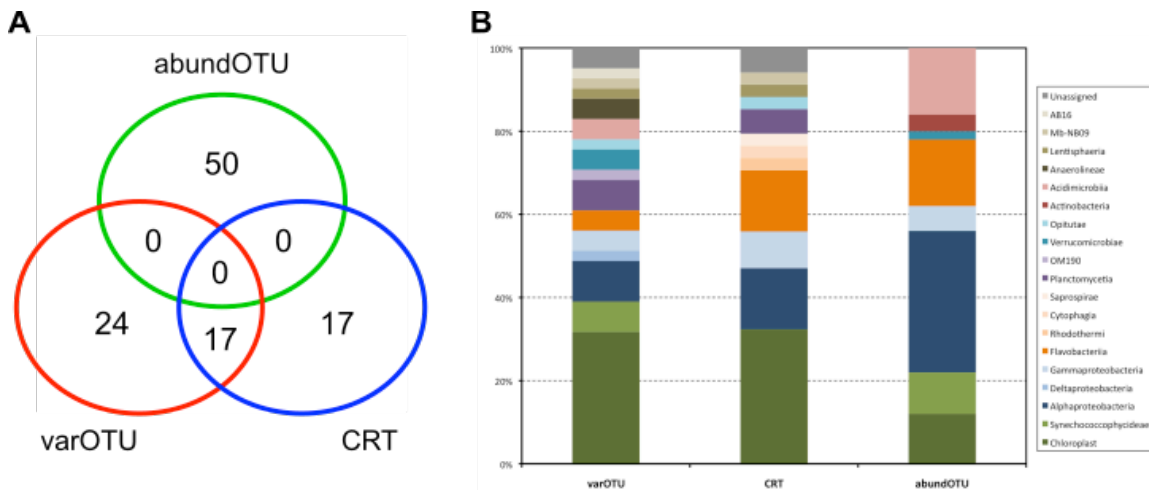


Figure 9: Characterization of highly variable, conditionally rare, and abundant taxa. (A) Distribution of taxa identified as belonging to one or more of the three groups. There are 24 and 17 unique OTUs identified between the highly variable (varOTU) and conditionally rare taxa (CRT) with 17 OTUs shared between the two groups; none of the 50 abundant OTUs (abundOTU) were shared. (B) Taxonomic composition of OTU groups. Taxonomic classification was made using RDP Classifier following OTU clustering (97% sequence identity). Taxonomy was assigned at the Class level.

3.2.4 Contributions of highly variable and conditionally rare taxa during disturbances

The contributions of highly variable and conditionally rare taxa to the community changes were examined (Fig. 10A). To determine the contribution of OTU groups to the community dissimilarity of the entire community (whole community dissimilarity), we calculated the proportion of Bray-Curtis dissimilarity attributable to the highly variable, conditionally rare, and 50 most abundant taxa (see Experimental Approaches). Over the time series, the highly variable and conditionally rare taxa normally contributed little to the whole community dissimilarity (average 1% contribution), but on few occasions contributed up to 31% (Fig. 10A), disproportionate to both the number of OTUs and their relative abundances. Increased contributions to whole community dissimilarity of highly variable or conditionally rare taxa roughly follow their increased relative abundances (Fig. 10B). This is particularly true of the Stramenopile OTUs belonging to both the highly variable and conditionally rare taxa that reach high relative abundance. In comparison, the 50 most abundant taxa consistently contributed between 30% and 65% of the whole community dissimilarity, due to their far greater abundances.

To examine the potential roles that highly variable and conditionally rare taxa may play in responding to coastal pulse disturbances, we focused on the contributions of whole community dissimilarity during the detected disturbances. Since the two-week interval provides a time period more in line with microbial *de novo* growth and proliferation, disturbances detected over two-week intervals were used. Highly variable and conditionally rare taxa contributed a combined 25% and 31% of the whole community dissimilarity during Disturbances 3 and 5, respectively. In contrast, during the other disturbances the contributions of these groups did not exceed 2% of the whole

community dissimilarity, and instead the most abundant taxa increased in their contribution of community dissimilarity.

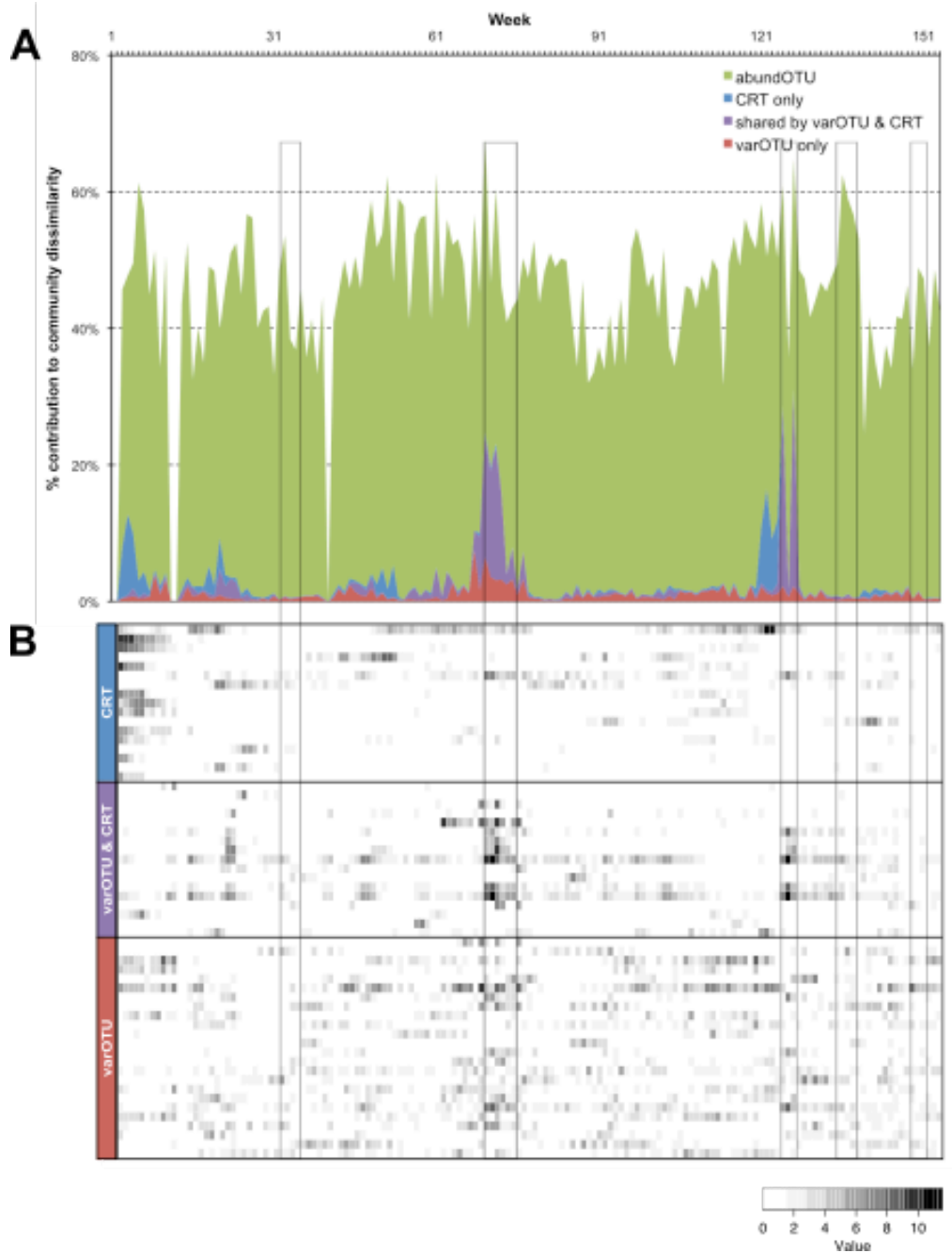


Figure 10: Temporal patterns of OTUs belonging to highly variable and conditionally rare taxa groups over the three-year PICO time series. (A) Contributions of varOTU only (red), shared by varOTU&CRT (purple), CRT (blue), abundant (green) to community dissimilarity over a two-week interval. (B) The heatmap represents the log₂ relative abundances of OTUs belonging to varOTU only (red), shared between varOTU and CRT (purple), and CRT only (blue). Black boxes indicate the periods of detected disturbances.

3.3 Discussion

It is critical to assess the contributions by abundant and rare marine microorganisms to disturbance in light of the high environmental and community variability in coastal ecosystems (Johnson et al., 2013; Needham et al., 2013). Although recent studies have indicated that marine microbial communities have the capacity to rapidly respond to changes in environmental conditions (Iluz et al., 2009; Gilbert et al., 2012; Lindh et al., 2015), and pulse disturbances can impact microbial communities in the coastal ocean (Yeo et al., 2013), little data are available on the frequency and range of causes of pulse disturbances. To explore this issue, we aimed to comprehensively examine pulse disturbances by identifying disturbances by their community response rather than deciding on their most likely cause(s) *a priori*. The results from our weekly time series at a coastal site indicate that the microbial community turns over on weekly and biweekly time scales. Disturbances in which nearly half of the community changes (Bray-Curtis dissimilarity ≈ 0.5) were observed numerous times through the time series, and up to three times in a single year (2013). These disturbances were associated with environmental changes of varying apparent strengths. In fact, most of the detected disturbances occurred during periods which, based on their meteorological and environmental conditions, would not have been expected to elicit dramatic responses from the microbial community. This finding suggests that by only focusing on community changes following extreme weather events, such as hurricanes, typhoons, and other large storms (Yannarell et al., 2007; Jones et al., 2008; Yeo et al., 2013), researchers may be overlooking other significant disturbances to the coastal microbial community.

Our findings also demonstrate that a small number of normally rare taxa can increase at various times during the time series and contribute to the changes in the whole community. Taxonomic classification of the highly variable and conditional rare taxa indicate that the many belong to microbial clades that include fast-growing, opportunistic bacterioplankton and phytoplankton (del Giorgio and Gasol, 2008; Lauro et al., 2009). These findings expand on a recent study that also found that conditionally rare taxa disproportionately contribute to community dissimilarity in a marine microbial community, as well as a range of other ecosystems (Shade et al., 2014). In our study, the highly variable and conditionally rare taxa contributed substantially to the community changes accompanying two disturbances (Disturbances 3 and 5). It has previously been suggested that increased abundances of normally rare taxa may be triggered by favorable environmental conditions (Pedrós-Alió, 2006; Sogin et al., 2006; Jones and Lennon, 2010). Given the similarities between the two disturbances in regards to contributions to community dissimilarity and abundance profiles of the taxa groups, closer examination of the environmental conditions is warranted. Both disturbances occurred during spring following repeated storms over 3 and 4 weeks. At least two non-exclusive mechanisms could explain the results. In this study site, the community undergoes state shifts within a temperature window during spring (when the two disturbances occurred) and autumn (Chapter 2); during these transition periods when fewer taxa have established dominance, the community may be more invasible by rare microorganisms than during summer or winter (Davis et al., 2005; Litchman, 2010). At these times, the dominant taxa may respond to disturbance conditions (e.g. increased nutrients) by increasing in abundance. A second explanation involves the varying rates of nutrient loading that would occur during continual versus acute rainfall. Terrestrial runoff that brings a sustained nutrient supply may support the exponential growth of

rare taxa over multiple generations that would be necessary to reach abundance (Newton & McMahon, 2011; Pinckney, Paerl, & Harrington, 1999). Storm events also alter the hydrologic processes (i.e., flushing and residence times) of receiving waters. This too would differentially affect microbial communities, to favor microbes whose growth rates are in line with flushing rates (Hall et al., 2013; Paerl et al., 2014). Disturbances that do not include significant responses of abundant taxa may reflect release from top-down controls; predation pressure can be escaped temporarily due to differential timing of responses to enhanced nutrient conditions by bacteria and their predators (Pernthaler, 2005).

3.4 Conclusions

This study highlights the value of examining microbial responses to environmental changes on both the community and OTU levels. Though our data provide better understanding of microbial community variation in relation to environmental changes in the coastal ocean, further *in situ* observations and/or controlled experimental manipulations are necessary to assess whether microbial communities and individual taxa respond consistently to given environmental stimuli. The observed relationship between environmental changes and extent of community turnover indicates that we need to reassess our notions of how microbial communities are influenced by their environment.

3.5 Experimental approaches

3.5.1 Microbial community sequence and environmental data

The microbial time series used in this study is described in Chapter 2 of this dissertation, with descriptions, quality control, and normalization of the microbial

community sequencing and environmental data sets. In brief, samples were collected at the Pivers Island Coastal Observatory (PICO) site (34.7181°N 76.6707°W) at the mouth of the Newport River Estuary weekly over a three-year period from January 2011 to December 2013. Nucleic acids were extracted from 0.22-micron Sterivex filters (Millipore), and libraries of prokaryotic 16S rRNA genes were generated as described by Kozich et al., 2013. In total, 151 libraries covering 2011-2013 were sequenced on the MiSeq (Illumina) using the 2x 250 bp protocol at Duke's Genome Sequencing and Analysis Core Facility. Sequences were processed using USEARCH v7 (Edgar, 2010, Edgar, *et al.*, 2011, Edgar, 2013) with stringent quality filtering parameters detailed in Chapter 2. Merged paired end sequences were clustered into OTUs in which all assigned sequences are at least 97% similarity. This was done using centroid-based clustering UPARSE-OTU algorithm (Edgar, 2013) with a pairwise identity of 98.5% to the centroid. Reads were assigned to OTUs to generate an OTU table. OTUs with fewer than 5 total sequences were removed from the OTU table, and sequence read counts were rarefied to a common depth of 20,082 reads per library.

3.5.2 Assessment of community composition change

To examine short-term variability in microbial community, we quantified differences in community composition over one- and two-week intervals using Bray-Curtis dissimilarity. Extreme changes in community composition (Bray-Curtis dissimilarity values exceeding 95th percentile) were considered to be community disturbances. Analysis of community dissimilarity was performed using the Vegan R package.

3.5.3 Detection of highly variable, conditionally rare, and highly abundant taxa

Two approaches were used to select taxa with dynamic abundance patterns that may potentially make considerable contributions to community change. Highly variable taxa exhibit population dynamics with large changes in abundance, relative to their overall abundance. Variability is calculated as follows:

$$\text{var} = \frac{\sum |x_t - x_{t-1}|}{\sum x_t}$$

and highly variable taxa were identified as having variability values greater than 1.25 and relative abundance exceeding 0.0025% of the total community at least once.

Conditionally rare taxa have low abundances at most times but occasionally increase to high abundance. Accordingly, conditionally rare taxa would exhibit bimodal distributions of abundance (Shade et al., 2014). The coefficient of bimodality is computed as:

$$b = \frac{(1 + \text{skewness}^2)}{(\text{kurtosis} + 3)}$$

where $\text{skewness} = \frac{\sum (x_t - \bar{x})^3 / n}{[\sum (x_t - \bar{x})^2 / n]^{3/2}}$ and $\text{kurtosis} = \frac{\sum (x_t - \bar{x})^4 / n}{[\sum (x_t - \bar{x})^2 / n]^2}$, and was

implemented using custom R scripts (Shade et al., 2014). Conditionally rare taxa were identified as having bimodality values greater than 0.90 and relative abundance exceeding 0.0025% of the total community at least once. Highly abundant taxa were 50 taxa with the highest total sequence counts.

For each subset, we calculated the community dissimilarity attributed to the taxa identified as highly variable, conditionally rare, and highly abundant. To do so, Bray-Curtis dissimilarity was calculated using just the identified taxa to determine the

numerator, while the denominator was determined for all taxa of the community (Shade et al., 2014). In this manner, we partitioned the Bray-Curtis dissimilarity to the identified OTU groups and determined the fractions of contribution by dividing Bray-Curtis dissimilarities of highly variable, conditionally rare, and highly abundant taxa by the total community Bray-Curtis dissimilarity.

3.5 Acknowledgments

This work was supported by grants from the Gordon and Betty Moore Foundation (GBMF3768 to DEH) and the National Science Foundation (OCE 1322950 to DEH and OCE1416665 to DEH and ZIJ), as well as a NSF Graduate Research Fellowship to CSW. The authors would like to acknowledge the entire PICO team for help with environmental sampling. This work will be submitted for publication, with Charmaine Yung, Zackary Johnson, and Dana Hunt as co-authors.

4. Indirect effects of silver nanoparticle environmental loading overwhelm direct microbial toxicity in wetland mesocosms

4.1 Introduction

Microbial communities are central to the productivity and biogeochemistry of wetland ecosystems. Silver nanoparticles (AgNP) are emerging contaminants of aquatic ecosystems, including wetlands, with known antimicrobial properties. Nevertheless, the direct and indirect effects of AgNP on aquatic microbial communities remain poorly understood. Here, we use high-throughput sequencing to examine compositional changes in aquatic microbial communities in response to acute and chronic AgNP environmental loading scenarios over one year. Using a set of replicated wetland microcosms, we observed that the both acute and chronic AgNP treatments alter community composition, though the effects differ in timing, duration, and magnitude of disturbance response. In acute mesocosms, observed disturbance responses occurred immediately with *Flectobacillus sp.* dominating the community but differences dissipated after one week. In chronic mesocosms, a less substantial community response was observed initially but prolonged changes in chronic mesocosm communities were observed at later time points. The disturbance responses of both acute and chronic mesocosm communities included decreased alpha diversity and enrichments in Bacteroidetes, a phylum whose members often degrade complex organic matter. These results suggest that community changes do not primarily reflect direct responses to AgNP toxicity, but rather indirect effects of AgNP through plant die-off and subsequent release of dissolved organic carbon. This highlights the need to investigate the effects of environmental loading within environmentally realistic systems that incorporate

environmental media, multiple levels of biological interactions, and field conditions. Contamination of wetlands with emerging antimicrobial compounds has the potential to influence microbial system-level processes.

Silver nanoparticles (AgNP) are the most commonly used nanomaterial in consumer products (Scientific Committee on Emerging and Newly Identified Health Risks, 2010; The Project on Emerging Nanotechnologies, 2015). As of September 2015, there were over 400 consumer products known to contain AgNPs (The Project on Emerging Nanotechnologies, 2015). Silver nanoparticles are primarily included in consumer products for their antimicrobial properties (Scientific Committee on Emerging and Newly Identified Health Risks, 2010). Given widespread use, AgNP inevitably make their ways into residential and industrial waste streams (Benn and Westerhoff, 2008; Blaser et al., 2008; Hagendorfer et al., 2010), with up to 10% of the AgNP entering wastewater treatment plants (WWTP) predicted to be released in wastewater effluent into aquatic ecosystems (Blaser et al., 2008; Kaegi et al., 2011, 2013; Keller et al., 2013). Given their antimicrobial properties, AgNP could potentially alter rates of important biogeochemical processes, such as primary production (Navarro et al., 2008; Yin et al., 2012), secondary production (Pradhan et al., 2011), and nitrogen cycling (Throbäck et al., 2007; Choi and Hu, 2009).

In the past decade, growing attention has been paid to the environmental implications of nanomaterials in consumer products. However, most toxicological studies of silver nanoparticles have used pure cultures or simplified experimental systems under controlled laboratory conditions (Navarro et al., 2008; Fabrega et al., 2009; Bernhardt et al., 2010; Alito and Gunsch, 2014). These studies have been instrumental in

revealing mechanisms of toxicity, yet have done little for understanding toxicological effects on diverse microbial communities in natural ecosystems (Bernhardt et al., 2010).

In a previous study, the biological responses to acute AgNP additions were investigated in a replicated wetland mesocosm experiment (Colman et al., 2014). Due to their biocidal properties, AgNP addition was expected to reduce microbial and plant biomass. Indeed, AgNP addition reduced the abundance of submersed and floating macrophytes as well as phytoplankton. Plant death contributed to increased dissolved organic carbon (DOC) into the water compartment, which in turn led to a rapid increase in prokaryote abundance in the week following AgNP addition. These important interactions between organisms highlight the necessity of maintaining as much environmental complexity as possible in ecotoxicological studies. This follow-up experiment considers the more environmentally realistic scenario of chronic environmental loading of AgNP (Collin et al., 2014).

Due to the differing dosing schedules, the acute and chronic AgNP treatments are expected to elicit different responses from the microbial communities. While both acute and chronic AgNP treatments are considered disturbances, or causal events that alter the abiotic or biotic environment with potential influence on the resident community (Rykiel, 1985; Glasby and Underwood, 1996; Shade et al., 2012), the acute AgNP treatment is intended to constitute a pulse disturbance, or a discrete, short-term event, while chronic AgNP treatment is intended to constitute a press disturbance, or a long-term or continuous process (Bender et al., 1984). Pulse disturbances frequently enrich the resident microbial community with resistant and/or opportunistic populations (Shade et al., 2010; Lamendella et al., 2014), which favor or are tolerant of the disturbance conditions, and can capitalize on resource availability or reduced competition (Allison and Martiny, 2008; Shade et al., 2012). Thus pulse disturbances

frequently result in blooms and reduced community diversity (Shade, et al., 2012; Jones et al., 2013; Lamendella et al., 2014). As communities recover, both deterministic and stochastic processes contribute to community assembly, and the extent of each contribution may vary at different stages of disturbance recovery (Nemergut et al., 2013). In a study of a wildfire pulse disturbance, stochastic processes dominated initially (4 weeks post), but gave way to deterministic processes during recolonization, presumably before returning to equilibrium (Ferrenberg et al., 2013). However, it is possible that community re-assembly following different disturbance types in different ecosystems may follow different sequences, or that additional re-assembly stages may exist. While pulse disturbances may temporarily change the environmental conditions of the ecosystem, they otherwise allow ecosystems to remain within its normal bounds and to recover to the non-disturbed state (Niemi et al., 1990). In contrast, press disturbances force an ecosystem to a different set of conditions by immediately or incrementally intensifying over time. Under these conditions, the forcing selects for taxa that are inherently tolerant or become so through adaptation (Rainey and Travisano, 1998; Rainey, 1998; Cohan, 2002; Cohan, 2005). Adaptation within semi-closed systems can set replicated communities on differing trajectories, resulting in high heterogeneity within replicates (Widenfalk et al., 2008). While there have been numerous studies on pulse and press disturbances, few have addressed both disturbance types in the same experimental setup. This study provides us with a unique opportunity to examine the differential ecological outcomes that result from environmental realistic scenarios of AgNP loading.

In conjunction with a larger study conducted through the Center of Environmental Implications of Nanotechnology (CEINT) to characterize ecosystem effects of nanoparticle exposure, the aquatic zones of wetland mesocosms were sampled monthly for one year (August 2013-August 2014). Through sequencing partial 16S rRNA

gene libraries, we profiled the microbial community composition over time in order to compare the longitudinal effects of acute and chronic environmental loading of AgNP. Dramatic effects on microbial community composition were observed in both AgNP treatments, yet the magnitude, timing, and duration of impacts varied between acute and chronic AgNP treatments.

4.2 Results and Discussion

This study was designed to examine differences in the microbial community responses to acute and chronic environmental loading scenarios. For acute AgNP treatment, 450 mg of AgNP (6 nm diameter, gum arabic coating) were added on Day 0 to the aquatic portion of triplicate wetland mesocosms to result in an expected concentration of 1.25 mg/L. This was half the quantity added previously (Colman et al., 2014); as dramatic changes were observed previously, biological responses would still be expected with lower dosing. This treatment was intended to simulate a spill or sudden release of large quantities of AgNP upstream of a wetland environment. However, it is worth noting that no AgNP spills have been documented (Bernhardt et al., 2010). The chronic AgNP treatment, in which the AgNP were added incrementally every week throughout the yearlong study, was intended to simulate slow, consistent introduction of AgNP leading to an eventual accumulation in the system. In addition to no-nanoparticle addition controls, sulfidized AgNP were added to another set of triplicate mesocosms to simulate the product of nanoparticle aging, which frequently occurs in WWTP. Given the differences in the duration and intensity of the disturbances, it is expected that the direct microbial response to the acute disturbance should be of larger magnitude initially but would dampen over time compared to chronic effects that might not be immediately observed but intensify over time (Grimm and Wissel, 1997).

However, as observed in the previous study, indirect effects of AgNP loading can result from interactions between components of a complex ecological system.

4.2.1 Complicating factors of environmentally realistic studies

Mesocosm studies enable us to examine community responses within realistic environments in replication. However, due to the complexity of ecological systems, mesocosms can frequently have high variability of both physicochemical and biological components (Caquet et al., 2000; Clements and Rohr, 2009). In the initial setup phase of this experiment, substantial variation in environmental variables of the mesocosms was observed. In order to minimize the potential confounding effect of different environmental conditions within a treatment, mesocosms were divided into treatment groups prior to the start of the experiment to distribute variation in water chemistry, dissolved gasses, plant cover, and sediment organic matter content (B. Colman, personal communication).

High heterogeneity between communities within a treatment was a general feature of this experiment. Initial community compositions varied greatly (36%-38% beta dispersal) and high levels of within-treatment variation continued through the duration of the experiment. Rarefaction analysis (data not shown) suggests that this level of heterogeneity is unlikely to be an artifact of insufficient sequencing depth. Rather, community heterogeneity was likely contributed by a combination of several ecological factors, including environmental filtering due to abiotic differences in replicate mesocosms, stochastic assembly processes, and/or functional redundancy (Nemergut et al., 2013b). Coarse ecological coherence at high bacterial taxonomic levels may enable related taxa with functional similarities to fill the same ecological niche in replicate mesocosms (Philippot et al., 2010; Koeppeel and Wu, 2012), offering a potential

explanation for why within-treatment community differences decrease at high taxonomic levels (i.e., phylum). Nevertheless, despite high community and environmental heterogeneity, pronounced treatment effects were observed. Although a larger sample size would be desirable for a more robust conclusion, this result suggests that effects of AgNP additions overwhelm other influences on microbial community composition at periods of greatest impact.

4.2.2 Acute treatment

Acute AgNP additions had a brief negative effect on prokaryotic cell abundances (Fig. 11). From Day 0 to Day 3 of the experiment, there was no detectable difference in prokaryotic cell abundances between acute and control mesocosms ($P=0.9$, t test). However on Day 7, the aquatic compartments of acute AgNP mesocosms contained significantly fewer prokaryotic cells compared to the control mesocosms ($P=0.03$, t test). This is in contrast to the previous experiment where a large increase ($\approx 70\%$) in bacteria was observed over the first week (Colman et al., 2014). There are several potential factors that may have contributed to this difference. In this experiment, half the quantity of AgNP was added to the acute mesocosms and there was less duckweed biomass on the surface of the aquatic zone surface on Day 0 (B. Colman, personal communication). The combined effect of a smaller AgNP dose, which may have elicited less of a response from the aquatic plants, and less aquatic plants may have resulted in a smaller increase in DOC, as was observed during the first week (B. Colman, personal communication). DOC can stimulate bacterial growth, as well as bind AgNP to reduce bioavailability and toxicity (Lowry et al., 2012; Unrine et al., 2012). Thus, one or more of these factors may have shifted the net effects of AgNP addition on bacterial abundances from positive to negative.

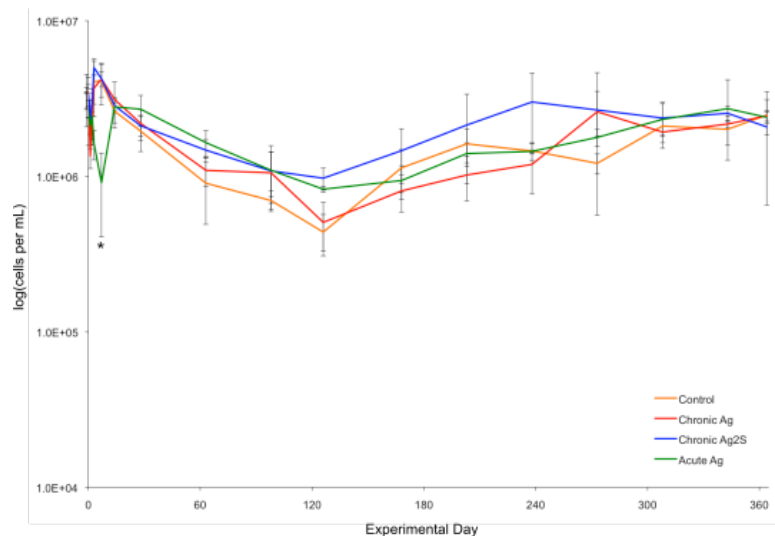


Figure 11: Prokaryotic cell abundances over time in aquatic zones of mesocosms. Control (orange), chronic AgNP (red), chronic Ag.SNP (blue), acute AgNP (green). Values are mean \pm standard deviation for n=3 for all treatments.

The effects of acute AgNP treatment on microbial community composition were dramatic but transient. Acute mesocosm communities changed rapidly during the initial time period (Days 1-7; Fig. 12). Within 24 h of dosing (Day 1), community composition in acute treatment changed significantly from Day 0 as well as in comparison to controls, with enrichment of bacterial phylum Bacteroidetes and reduced representation of the phyla Verrucomicrobia, Cyanobacteria, and Actinobacteria (Fig. 12). By Day 3, 85-95% of library sequences from acute mesocosms consisted of a single operational taxonomic unit (OTU) identified as *Flectobacillus sp.* (Bacteroidetes). Differences between acute and control communities, including enrichment of Bacteroidetes, continued to Day 7, but by Day 14 the compositions of acute mesocosms were no longer statistically different from controls, and remained so for the remainder of the study (Fig. 12, Table 1).

In spite of their high relative abundance in this experiment, Bacteroidetes are not known to be particularly resistant to silver or other heavy metals (Sheng and Liu, 2011). However, Bacteroidetes play an important role in the degradation of highly complex

organic matter (Kirchman, 2002; Thomas et al., 2011; Gómez-Pereira et al., 2012), and frequently occur at sites characterized by high DOC (Eiler and Bertilsson, 2004, 2007; Kolmonen et al., 2004). Bacteroidetes also are presumed to be fast-growing *r* strategists (Gómez-Pereira et al., 2012), which fits with their rapid rise to dominance following acute dosing.

Due to the Bacteroidetes bloom in the first week of the experiment, and particularly the dominance of a single *Flectobacillus* OTU during this time period, the diversity of the acute mesocosm communities were greatly reduced. At the time of greatest impact (Day 3), alpha diversity, as measured by Simpson's index, was dramatically reduced in acute mesocosms (0.20 ± 0.08) compared to control mesocosms (0.92 ± 0.01 ; $P < 0.0001$, ANOSIM)(Fig. 13). However, this severe reduction in diversity did not persist for long, as by Day 7 alpha diversity in acute mesocosms had returned to that of controls ($P = 0.25$, ANOSIM). In addition, acute treatments had short-term effects on community heterogeneity within replicate mesocosms. On Day 3, the community heterogeneity in acute mesocosms was much lower than in controls (5.8% vs. 35%; $P < 0.001$, permutation test for homogeneity of multivariate dispersions). Convergence on similar community compositions likely indicates a strong role for selection. However, on Day 7, heterogeneity of acute mesocosm communities increased relative to controls (45% vs. 37%; $P = 0.2$, permutation test for homogeneity of multivariate dispersions), and by Day 14, heterogeneity of acute and control mesocosms were nearly identical (39% vs. 38%; $P = 0.9$, permutation test for homogeneity of multivariate dispersions). Although contributions of niche vs. neutral processes should be tested explicitly, the observed patterns in community heterogeneity in acute mesocosm communities suggest that the disturbance elicits a reproducible community response, while the recovery was far more variable between replicate communities.

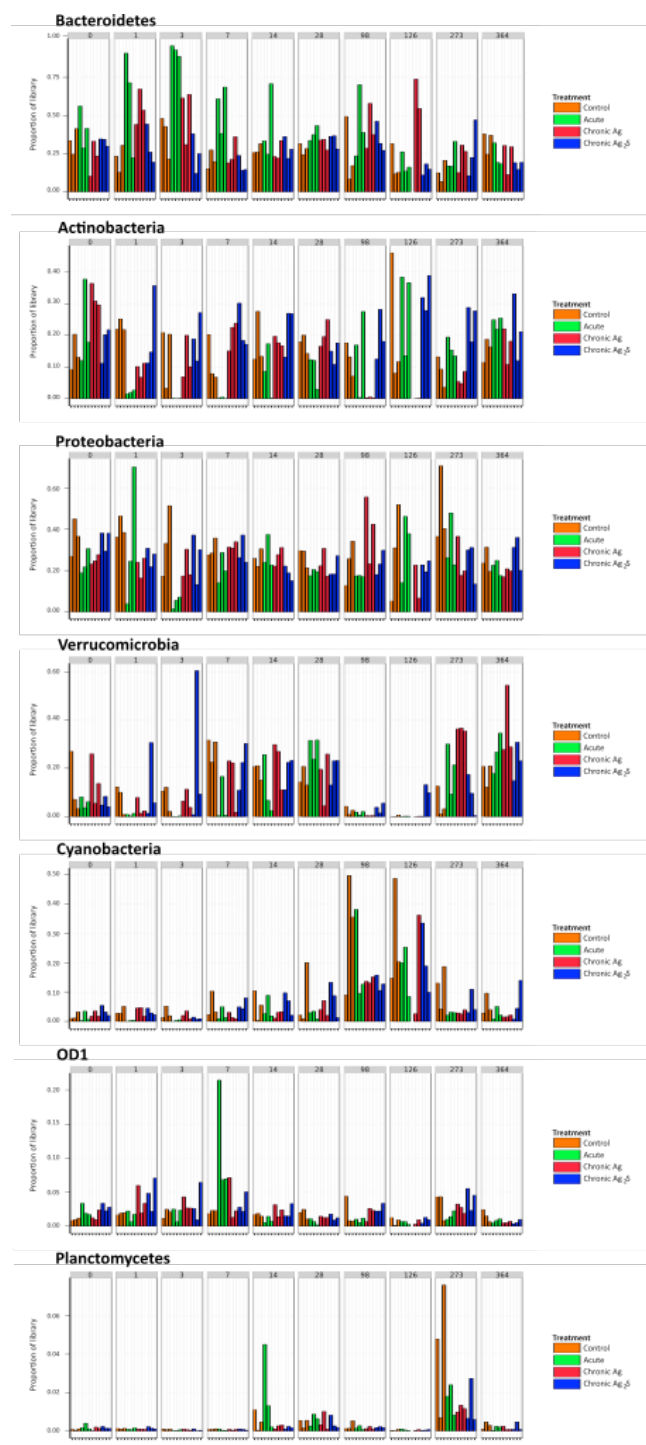


Figure 12: Phylum-level composition of 16S rRNA gene libraries of mesocosm samples over one year. Taxonomic classification was made using RDP Classifier following OTU clustering. Experimental day is relative to the start of dosing on August 13, 2013 (Day 0). Control (orange), acute AgNP (green), chronic AgNP (red), chronic Ag.SNP (blue).

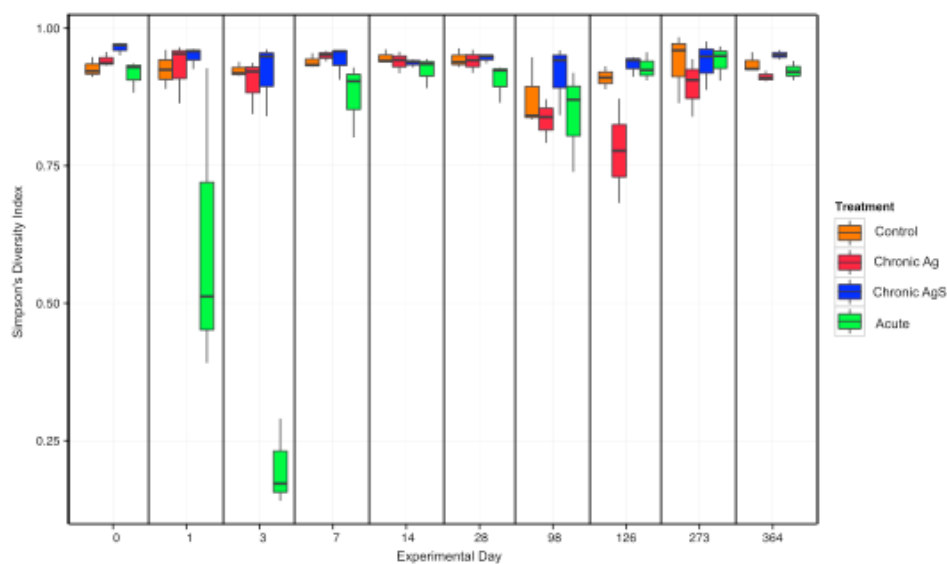


Figure 13: Treatment effects on community diversity. Box and whiskers plot showing the distribution of Simpson's diversity values, with the center line within boxes indicating the mean value and whiskers extending to minimum and maximum values. Control (orange), chronic AgNP (red), chronic Ag.SNP (blue), acute AgNP (green).

4.2.3 Chronic treatments

While chronic mesocosms never exhibited dramatic differences in total bacterial abundances relative to control mesocosms (Fig. 11), community composition in chronic treatments also displayed increases in the same *Flectobacillus* OTU on Day 1 in a similar but less extreme change as in the acute mesocosms (Fig. 12), even though mesocosms received a small fraction ($1/52^{th}$) of the dosing. For the rest of the initial period (Days 3-28), bacterial community composition did not show significant differences between chronic AgNP and control mesocosms (Table 1). As expected, the major compositional changes in microbial communities exposed to chronic AgNP treatment were delayed, but persisted for longer duration relative to the acute exposure. On Days 98 and 126 following dosing, the chronic AgNP mesocosm communities differed from both the

control and the other treatments (Fig. 12). For these time points, community composition in chronic treatments exhibited an enrichment of Bacteroidetes and an absence of Actinobacteria. On Day 126, a Flavobacteria bloom dominated chronic mesocosms, making up 30-64% of the library sequences, and decreased community diversity (0.78 ± 0.13 vs. 0.91 ± 0.02 , ANOSIM; Fig. 13). Despite continued weekly dosing of AgNP, community differences with the control and acute treatment were no longer statistically significant at 9 and 12 months following dosing (Days 273 and 364)(Table 1).

The transience of the community response varies from what would be expected, as press disturbances typically cause unidirectional community change (Widenfalk et al., 2008; Shade, et al., 2012). However, an interactive effect between treatment and year day was detected (ADONIS, Treatment:Day $P=0.013$) with several possible explanations. First, microbial community changes observed on Days 98 and 126 may have been the direct effect of AgNP as silver levels built up in the system; later time points may show less of an effect as silver resistance spread through the community (Silver, 2003). Second, microbial responses may be due to indirect effects mediated through plants: temperature stress could make plants more susceptible to AgNP toxicity (Koch and Erskine, 2001; Koch et al., 2007; Ramegowda and Senthil-Kumar, 2014). Elevated DOC, as was observed (B. Colman, personal communication), could in turn explain the increase in Bacteroidetes at the winter time points, reminiscent of the community changes in the acute treatment. Warming spring temperatures would ease plant stress and changes in the microbial communities would dissipate.

Surprisingly, there were no observable differences in community heterogeneity between chronic mesocosms and control (32-40% vs. 26-42%; $P>0.2$, permutation test for homogeneity of multivariate dispersions). We had predicted that chronic additions

would increase community heterogeneity between replicates. However, changes in community heterogeneity in response to chronic additions may not have been detected due to inadequate temporal resolution or high variation in initial communities that overshadowed more subtle changes. Alternatively, it is possible that chronic additions during winter functioned more like pulse disturbances, by eliciting DOC release from cold-stressed plants leading to Bacteroidetes enrichments.

Unlike chronic AgNP additions, chronic Ag₂SNP additions, which are meant to simulate aged AgNP (Lowry et al., 2012), did not result in significant differences in microbial communities compared to controls. This is in line with previous studies that have shown diminished toxicity of sulfidized AgNP (Choi and Hu, 2009; Levard et al., 2012; Reinsch et al., 2012). Others have suggested that AgNP would be rapidly sulfidized in natural and artificial systems, such as WWTP (Lowry et al., 2012).

Table 1: Comparisons of community compositions between treatments over time. Statistically significant treatments effects ($P < 0.05$) as determined by ADONIS are indicated in bold.

Factor	df	F	P		F	P
<i>Acute</i>						
Day 0	1,4	0.96624	0.6014			
Day 1	1,4	5.1852	0.1			
Day 3	1,4	13.251	0.001389	**	Week 1	9.9717 0.001 ***
Day 7	1,4	2.7378	0.1			
Day 14	1,4	2.2349	0.1			
Day 28	1,4	1.9446	0.001389	**		
Day 98	1,4	0.98525	0.4014			
Day 126	1,4	1.1613	0.3			
Day 273	1,4	1.5503	0.1			
Day 364	1,4	2.075	0.1			
<i>Chronic</i>						
Day 0	1,4	1.2265	0.3			
Day 1	1,4	2.9324	0.001389	**		
Day 3	1,4	1.196	0.3			
Day 7	1,4	1.1505	0.3			
Day 14	1,4	1.0017	0.5			
Day 28	1,4	1.2649	0.1014			
Day 98	1,4	2.389	0.001389	**	Winter	2.6534 0.003 **
Day 126	1,3	1.4194	0.1			
Day 273	1,4	1.8819	0.1			
Day 364	1,4	2.075	0.1			
<i>Chronic Aged</i>						
Day 0	1,4	0.98215	0.7			
Day 1	1,4	1.2534	0.2			
Day 3	1,4	1.3145	0.2			
Day 7	1,4	1.1863	0.3			
Day 14	1,4	0.83378	0.5			
Day 28	1,4	0.80397	0.6014			
Day 98	1,4	0.94496	0.4014			
Day 126	1,4	0.94496	0.6			
Day 273	1,3	1.4549	0.1083			
Day 364	1,3	1.6522	0.1			

4.3 Conclusions

The effects of disturbance on a diverse community in a complex natural ecosystem are dependent on the disturbance regime (the type and magnitude of stress),

the stability of the community, and the natural fluctuations of the environment (Allison and Martiny, 2008; Shade et al., 2012; Nemergut et al., 2013b). Despite the differences in timing, duration, and magnitude of impacts between acute and chronic AgNP treatments, we observed similar responses in the microbial communities – enrichment of Bacteroidetes, loss of Actinobacteria, and decreased diversity. AgNP-mediated DOC release from aquatic plants were likely responsible for the observed changes in acute and chronic AgNP mesocosms. Direct effects of AgNP additions on microbial community composition should not be ruled out, but were overwhelmed by indirect AgNP effects and high variation in mesocosm community compositions. AgNP additions led to surprising and dramatic systemic impacts on microbial community through a cascade of biotic interactions. Accordingly, studying environmental realistic dosing scenarios within experimental setups that retain the complexity of natural ecosystems is essential to determining potential outcomes of environmental contamination.

4.4 Experimental approaches

4.4.1 Field site and experimental treatments

Our study took place at the Center of Environmental Implications of Nanotechnology (CEINT) mesocosm site in Duke University Forest in Durham, North Carolina. The design and construction of wetland mesocosms was described previously (Lowry et al., 2012; Colman et al., 2014). In brief, slantboard mesocosms measured 3.66 x 1.22 x 0.81 m, and consisted of three components: an aquatic zone, a transition zone, and an upland zone. In the aquatic compartment, *Egeria densa* and duckweed *Landoltia punctata* were added (Carolina Biological). Mesocosm water columns were initially inoculated with 250 mL of unfiltered water from a local wetland; other organisms were

added or allowed to colonize the mesocosms, as described in Colman *et al.*, 2014. To simulate dispersal and connectivity with a larger wetland system, mesocosms were re-inoculated every two weeks with 250 mL water from local wetlands, which were pre-filtered through a 200- μ m filter.

A total of 12 mesocosms were subjected to four treatments (three replicates per treatment). Prior to treatment assignment, individual mesocosms were randomized such that environmental variation was distributed across treatments. In the controls, deionized water was added on Day 0 (August 13, 2013). For the acute AgNP mesocosms, 450 mg gum arabic-coated AgNPs (diameter 6 nm) suspended in deionized water and diluted in mesocosm water were added on Day 0. There were two sets of chronic mesocosms which were dosed weekly with 8.7 mg of gum arabic-coated AgNPs and sulfidized AgNP (Ag_sSNPs). Despite differences in dosing regimen, over the course of the study all NP treatments received 450 mg NPs.

4.4.2 Sample collection

We focused our sampling on the water column of the aquatic zone. Samples were taken at least monthly from August 2013 to August 2014, with higher frequency during the first month. From each mesocosm, 300 mL of water was collected from near-surface (0.25-m depth) by submerging sterile polypropylene bottles. Microbial biomass was collected by filtering 250 mL of water through 0.22-micron Supor filters. Filters were stored at -80°C until analysis.

4.4.3 DNA extraction and sequencing

DNA extraction was performed according to manufacturer's instructions (DNeasy Tissue kit; Qiagen), with the addition of bead beating (0.25 g zirconium, 0.1-mm; BioSpec) for 3x 30 sec at 4,800 rpm. DNA concentration was measured with NanoDrop ND-1000.

Prokaryotic 16S rRNA genes were amplified with primers containing unique combinations of 8-nucleotide index sequences to produce dual-indexed PCR products, as outlined in Kozich *et al.*, 2013. The general degenerate primers were S-D-Bact-0341-b-S-17 (Klindworth *et al.*, 2013; 16S F V3; 5'- CCTACGGGNGGCWSCAG -3') and Arch806 (Hugoni *et al.*, 2013; 16S R V4; 5'- GGACTACNVGGGTWTCTAAT -3'). PCR amplification was carried out in a total volume of 20 μ L containing 20 ng of template DNA, 200 μ M dNTPs, 2 mM MgCl₂, 0.5 μ M primers, 0.4 U of Q5 DNA polymerase (NEB). The amplification conditions comprised of steps at 98°C for 30 sec, and 28 cycles at 98°C for 10 sec, 55°C for 30 sec and 72°C for 30 sec, with a final extension at 72°C for 2 min. Triplicate reactions per sample were pooled and gel purified. In total, 119 libraries were sequenced on the Illumina MiSeq using the 2x 250 bp paired-end protocol at Duke's Genome Sequencing and Analysis Core Facility.

4.4.4 Sequence processing

Sequences were demultiplexed and assigned to corresponding samples using CASAVA (Illumina). Sequences were processed in USEARCH (Edgar, 2010, Edgar, *et al.*, 2011, Edgar, 2013). Briefly, sequence reads were trimmed using a 10-nt running window with a minimum mean Phred quality (Q) score of 30. Paired-end reads were merged and the resulting contigs were quality-filtered to remove reads with expected errors > 0.5 and shorter than 400 nt. Sequence contigs were then dereplicated and singletons were discarded. Contigs were clustered into OTUs at least 97% similarity using a centroid-based clustering UPARSE-OTU algorithm (Edgar, 2013) with a pairwise identity of 98.5% to the centroid. The OTU clustering step includes the removal of reads containing chimeric models; an additional reference-based chimera filtering step was performed using UCHIME (Edgar, *et al.*, 2011) and the ChimeraSlayer reference database version microbiomeutil-r20110519.

4.4.5 Flow cytometry

Microbial abundances were enumerated using a BD FACSCalibur Flow Cytometer. Samples for quantification of phytoplankton used natural pigments, while samples for quantification of non-photosynthetic prokaryotes were first stained with SYBR Green-I, as previously described (Marie et al., 1997; Johnson et al., 2010).

4.5 Acknowledgments

The authors would like to acknowledge the entire CEINT chronic mesocosm team for study design and logistics. In particular, thanks to Ben Colman, Anna Fedders, and Carley Gwin for thoughtful discussions and help with sampling. This work was supported by grants from the National Science Foundation and the Environmental Protection Agency (NSF/EPA EF-0830093), as well as a NSF Graduate Research Fellowship to CSW. This work will be submitted for publication, with Carley Gwin, Tiffany Williams, Amy Ardis, Benjamin Colman, Claudia Gunsch, Emily Bernhardt, and Dana Hunt as co-authors.

5. Conclusions

Throughout my dissertation research, I have sought to understand microbial communities, the processes which govern their structure, and the environmental drivers which influence their change. I focused on disturbances, as a means to study how the microbial community changes in response to discrete environmental changes. In this work, I used high-throughput 16S rRNA gene sequencing to examine community- and taxon-level changes in planktonic microbial communities in response to natural seasonal and episodic disturbances as well as anthropogenic pulse and press disturbances in aquatic systems in order to gain insights into their ecology.

My second chapter showed that seasonal environmental changes are major drivers of microbial community composition in a temperate coastal time series. Using ribosomal RNA gene libraries from a three-year weekly time series of a temperate coastal ocean site, I found that annual repeating patterns in the microbial community composition result from distinct summer- and winter-associated taxa which switch in dominance. During spring and autumn transitions, seasonally-associated taxa overlapped, and communities peak in alpha diversities. Analysis of environmental variables indicated that temperature was strongly linked to these community- and taxon-level seasonal changes.

Despite strong seasonally-entrained patterns in the microbial community, a number of episodic disturbances were evident in this time series. A correlation between community change and nutrient or salinity values was hypothesized, however we found no clear relationship. In fact, the largest changes in environmental variables did not produce the most dramatic microbial community changes. Further, I found that disturbance-responsive taxa apparently depend on the stability of the initial microbial community, with rare taxa contributing a larger percentage of the community change

during the spring community transition period. This data suggested that further research should involve identifying the conditions which prime distinct sets of disturbance-responsive taxa.

Finally, I examined how differing disturbance scenarios affect the community responses. I used a replicated mesocosm experiment to examine how pulse versus press applications of an emerging contaminant alter microbial community responses. The microbial community responses to silver nanoparticle application in both acute and chronic treatments had surprising similarities despite the differences in magnitude and duration of the treatments and appeared to reflect indirect silver nanoparticle effects through pulses of aquatic plant die-off, rather than direct toxic responses. This finding demonstrated that within complex ecosystems, disturbance effects can be mediated by interactions between microbial taxa and components of the ecosystems, highlighting the importance of studying disturbance and ecotoxicological responses in environmentally realistic systems.

In this work, I considered the microbial community responses to a variety of natural and anthropogenic disturbances in aquatic systems, through an observational time series of a coastal ocean microbial community and an experimental study in wetland mesocosms. Each chapter revealed new insights into the ecology of microbial communities, as I have described above. Yet across all, I observed that seasonal dynamics was the dominant underlying pattern in microbial community composition on a longer time scale (Chapter 2). Episodic disturbances introduced second-order variability to this baseline community trajectory. However rather than being related to the magnitude of the environmental perturbation, the response of the microbial community to a given disturbance appeared to depend on the composition and stability of the community at that time. This was most evident in the comparison of spring versus

summer disturbances (Chapter 3), where large community changes followed repeated rainstorms with no appreciable environmental changes while moderate community changes accompanied a hurricane with dramatic and long-lasting environmental changes. The importance of community seasonality for influencing stability may not be constrained to just the microbial community, but also extend to the larger ecosystem. Microbial community responses to chronic silver nanoparticle treatment were most severe during winter months, possibly a result of decreased stability of the aquatic plant community (Chapter 4).

This work has advanced our understanding of how microorganisms operate in dynamic environments by demonstrating both unique features as well as commonalities in microbial community responses to changing conditions. My research generated experimentally testable hypotheses concerning how microbial communities and individual taxa respond to environmental drivers. First, microbial keystone taxa may play central roles in mediating and synchronizing microbial responses to key environmental drivers, namely temperature and nutrients. Second, microbial communities are responsive to environmental changes, but their responses are more dependent on their initial community composition than type or magnitude of disturbance. This research has laid the groundwork for future studies into how microbial taxa interact within their community and with their environment.

While this dissertation examined the changes in the microbial community on the community and taxon levels, I see incorporating microbial interactions as a particularly promising approach to pursue in studying disturbances to microbial communities. Microbial ecology has long understood the importance of interactions in influencing microbial community structure, yet only recently have researchers had tools and techniques available to them to investigate them. As I outline in Chapter 1, network-

based approaches can be used to reveal the propagation of disturbance effects. This may reveal mechanistic underpinnings of disturbance responses in microbial communities and in turn improve our ability to predictively model community changes to disturbances.

Appendix A: Supplementary Figures for Chapter Two

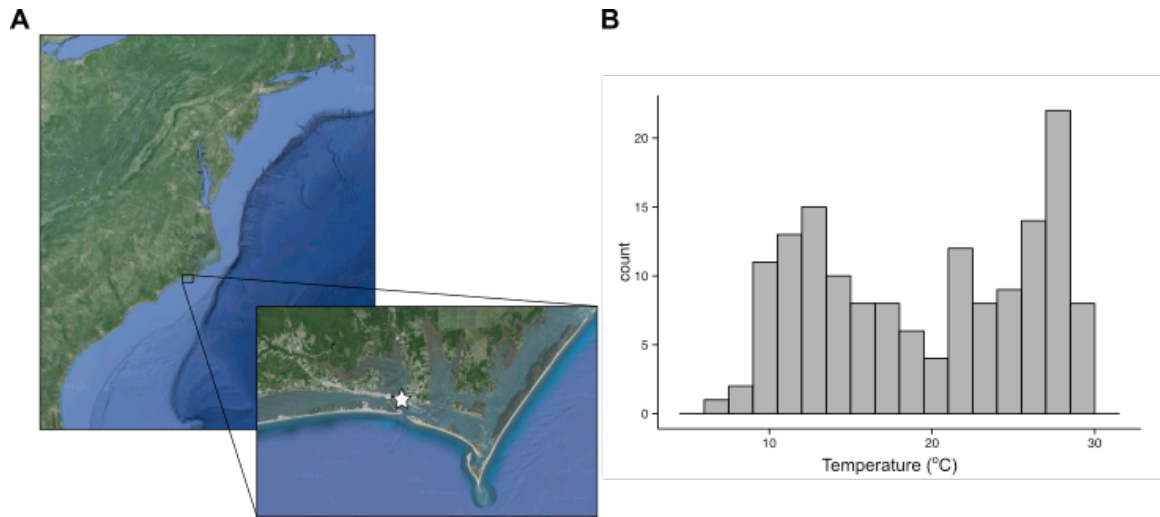


Figure 14: Characteristics of study location. (A) Map of US East Coast with inset showing the Pivers Island Coastal Observatory sampling location (star). (B) Distribution of observed water temperatures ($^{\circ}\text{C}$) in weekly environmental samples collected over three years.

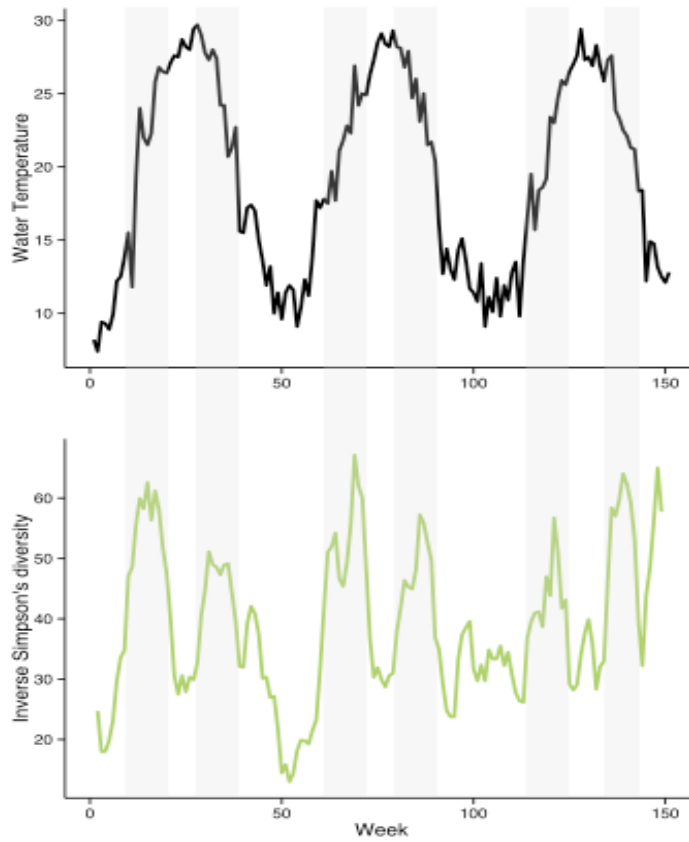


Figure 15: Increased community diversity during temperature transitions. (A) Water temperatures versus time (weeks elapsed since initial sampling). (B) Shannon's diversity of microbial communities in weekly water samples versus time (weeks since start of sampling). Gray highlighted sections correspond to elevated diversity values.

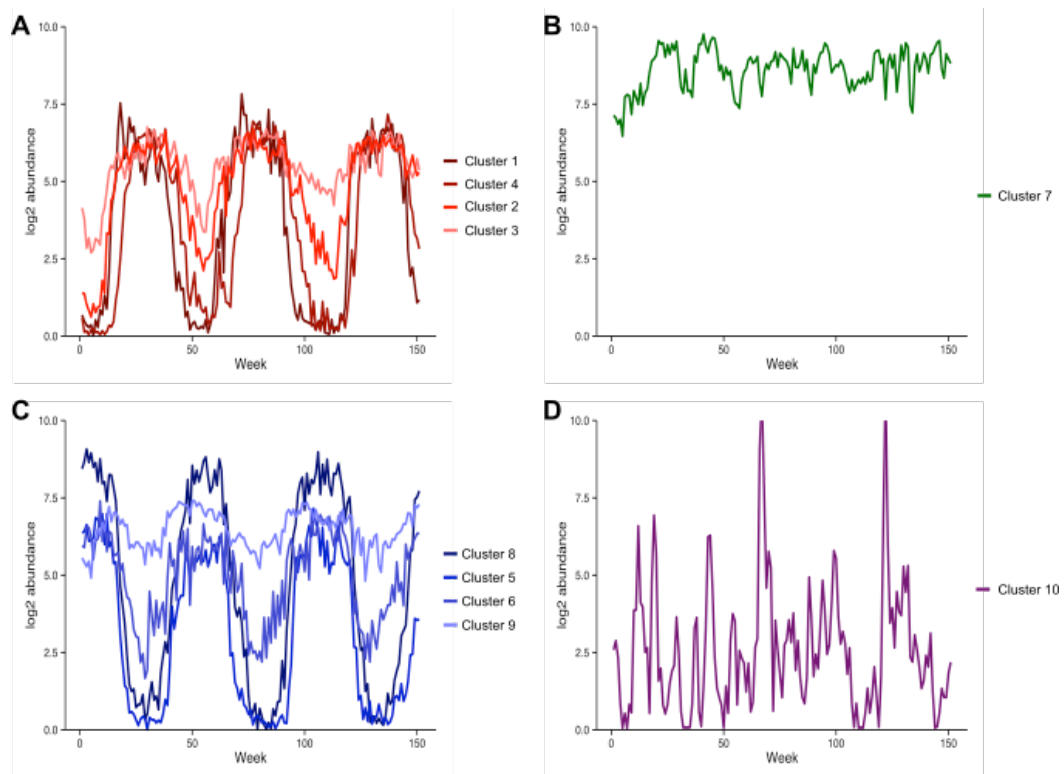


Figure 16: Relative abundance patterns of clusters identified for the 100 most abundant OTUs. Plots show the temporal dynamics of clusters within summer-associated (A), ubiquitous (B), winter-associated (C), and sporadic (D) groups. Lines shown are the cluster centroids for all three years of weekly PICO samples (2011-2013).

References

- Acinas, S.G., Klepac-Ceraj, V., Hunt, D.E., Pharino, C., Ceraj, I., Distel, D.L., and Polz, M.F. (2004) Fine-scale phylogenetic architecture of a complex bacterial community. *Nature* **430**: 551–554.
- Albert, R., Jeong, H., and Barabasi, A.L. (2000) Error and attack tolerance of complex networks. *Nature* **406**: 378–382.
- Alito, C.L. and Gunsch, C.K. (2014) Assessing the effects of silver nanoparticles on biological nutrient removal in bench-scale activated sludge sequencing batch reactors. *Environ. Sci. Technol.* **48**: 970–6.
- Allen, A., Gillooly, J., and Brown, J. (2005) Linking the global carbon cycle to individual metabolism. *Funct. Ecol.* **19**: 202–13.
- Allison, S.D. and Martiny, J.B. (2008) Resistance, resilience, and redundancy in microbial communities. *Proc Natl Acad Sci U S A* **105 Suppl**: 11512–11519.
- Amend, A.S., Martiny, A.C., Allison, S.D., Berlemont, R., Goulden, M.L., Lu, Y., et al. (2015) Microbial response to simulated global change is phylogenetically conserved and linked with functional potential. *ISME J.* 1–10.
- Andersson, A.F., Riemann, L., and Bertilsson, S. (2010) Pyrosequencing reveals contrasting seasonal dynamics of taxa within Baltic Sea bacterioplankton communities. *ISME J.* **4**: 171–81.
- Apple, J.K., Strom, S.L., Palenik, B., and Brahamsha, B. (2011) Variability in protist grazing and growth on different marine *Synechococcus* isolates. *Appl. Environ. Microbiol.* **77**: 3074–84.
- Azam, F., Fenchel, T., Field, J., Gray, J., Meyer-Reil, L., and Thingstad, F. (1983) The Ecological Role of Water-Column Microbes in the Sea. *Mar. Ecol. Prog. Ser.* **10**: 257–263.
- Baltar, F., Palovaara, J., Unrein, F., Catala, P., Hornák, K., Šimek, K., et al. (2015) Marine bacterial community structure resilience to changes in protist predation under phytoplankton bloom conditions. *ISME J* **11 August** :
- Barberan, A., Bates, S.T., Casamayor, E.O., and Fierer, N. (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J* **6**: 343–351.
- Bender, E. a, Case, T.J., Gilpin, M.E., and Feb, N. (1984) Perturbation experiments in community ecology: Theory and practice. *Ecology* **65**: 1–13.
- Benn, T.M. and Westerhoff, P. (2008) Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol.* **42**: 4133–9.
- Berga, M., Szekely, A.J., and Langenheder, S. (2012) Effects of Disturbance Intensity and Frequency on Bacterial Community Composition and Function. *PLoS One* **7**:
- Berga, M., Székely, A.J., and Langenheder, S. (2012) Effects of disturbance intensity and frequency on bacterial community composition and function. *PLoS One* **7**: e36959.

- Bernhardt, E.S., Colman, B.P., Hochella, M.F., Cardinale, B.J., Nisbet, R.M., Richardson, C.J., and Yin, L. (2010) An Ecological Perspective on Nanomaterial Impacts in the Environment. *J. Environ. Qual.* **39**: 1954.
- Bernhardt, E.S., Colman, B.P., Hochella, M.F., Cardinale, B.J., Nisbet, R.M., Richardson, C.J., and Yin, L. (2010) An ecological perspective on nanomaterial impacts in the environment. *J. Environ. Qual.* **39**: 1954–1965.
- Berry, D. and Widder, S. (2014) Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Front. Microbiol.* **5**: 1–14.
- Bertrand, E.M., Saito, M. a., Rose, J.M., Riesselman, C.R., Lohan, M.C., Noble, A.E., et al. (2007) Vitamin B12 and iron colimitation of phytoplankton growth in the Ross Sea. *Limnol. Oceanogr.* **52**: 1079–1093.
- Bissett, A., Brown, M. V., Siciliano, S.D., and Thrall, P.H. (2013) Microbial community responses to anthropogenically induced environmental change: Towards a systems approach. *Ecol. Lett.* **16**: 128–139.
- Blaser, S.A., Scheringer, M., Macleod, M., and Hungerbühler, K. (2008) Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles. *Sci. Total Environ.* **390**: 396–409.
- Boetius, A., Ravensschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., et al. (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* **407**: 623–626.
- Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., and West, G.B. (2004) Toward a metabolic theory of ecology. *Ecology* **85**: 1771–1789.
- Caporaso, J.G., Paszkiewicz, K., Field, D., Knight, R., and Gilbert, J. a (2012) The Western English Channel contains a persistent microbial seed bank. *ISME J.* **6**: 1089–93.
- Caquet, T., Lagadic, L., and Sheffield, S.R. (2000) Mesocosms in ecotoxicology (1): Outdoor aquatic systems. *Rev. Environ. Contam. Toxicol.* **165**: 1–38.
- Carpenter, S.R., Cole, J.J., Pace, M.L., Batt, R., Brock, W. a, Cline, T., et al. (2011) Early warnings of regime shifts: a whole-ecosystem experiment. *Science* **332**: 1079–1082.
- Casida, J.E. (2010) Pest Toxicology: The Primary Mechanisms of Pesticide Action. *Chem. Res. Toxicol.* **22**: 609–619.
- Choi, O.K. and Hu, Z.Q. (2009) Nitrification inhibition by silver nanoparticles. *Water Sci. Technol.* **59**: 1699–702.
- Chow, C.-E.T., Sachdeva, R., Cram, J. a, Steele, J. a, Needham, D.M., Patel, A., et al. (2013) Temporal variability and coherence of euphotic zone bacterial communities over a decade in the Southern California Bight. *ISME J.* **7**: 2259–73.
- Chow, C.E., Sachdeva, R., Cram, J.A., Steele, J.A., Needham, D.M., Patel, A., et al. (2013) Temporal variability and coherence of euphotic zone bacterial communities over a decade in the Southern California Bight. *ISME J.*

- Clements, W.H. and Rohr, J.R. (2009) Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. *Environ. Toxicol. Chem.* **28**: 1789–1800.
- Cohan, F.M. (2005) Periodic selection and ecological diversity in bacteria. In, *Selective sweep.*, pp. 78–93.
- Cohan, F.M. (2002) What are bacterial species? *Annu. Rev. Microbiol.* **56**: 457–87.
- Cole, J.J. (1982) Interactions Between Bacteria and Algae in Aquatic Ecosystems. *Annu. Rev. Ecol. Syst.* **13**: 291–314.
- Collin, B., Auffan, M., Johnson, A.C., Kaur, I., Keller, A. a., Lazareva, A., et al. (2014) Environmental release, fate and ecotoxicological effects of manufactured ceria nanomaterials. *Environ. Sci. Nano* **1**: 533–548.
- Colman, B.P., Espinasse, B., Richardson, C.J., Matson, C.W., Lowry, G. V, Hunt, D.E., et al. (2014) Emerging Contaminant or an Old Toxin in Disguise? Silver Nanoparticle Impacts on Ecosystems. *Environ. Sci. Technol.*
- Croft, M.T., Lawrence, A.D., Raux-Deery, E., Warren, M.J., and Smith, A.G. (2005) Algae acquire vitamin B12 through a symbiotic relationship with bacteria. *Nature* **438**: 90–3.
- Davies, J. and Davies, D. (2010) Origins and Evolution of Antibiotic Resistance. *Microbiol. Mol. Biol. Rev.* **74**: 417–433.
- Davis, M.A., Thompson, K., and Grime, J.P. (2005) Invasibility: the local mechanism driving community assembly and species diversity. *Ecography (Cop.)*. **5**: 696–704.
- DeLorenzo, M.E., Scott, G.I., and Ross, P.E. (2001) Toxicity of pesticides to aquatic microorganisms: A review. *Environ. Toxicol. Chem.* **20**: 84–98.
- Doney, S.C., Ruckelshaus, M., Emmett Duffy, J., Barry, J.P., Chan, F., English, C. a., et al. (2012) Climate Change Impacts on Marine Ecosystems. *Ann. Rev. Mar. Sci.* **4**: 11–37.
- Durham, B.P., Sharma, S., Luo, H., Smith, C.B., Amin, S.A., Bender, S.J., et al. (2015) Cryptic carbon and sulfur cycling between surface ocean plankton. *Proc. Natl. Acad. Sci.* **112**: 453–457.
- Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **10**: 996–8.
- Eiler, A. and Bertilsson, S. (2004) Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. *Environ. Microbiol.* **6**: 1228–43.
- Eiler, A. and Bertilsson, S. (2007) Flavobacteria blooms in four eutrophic lakes: linking population dynamics of freshwater bacterioplankton to resource availability. *Appl. Environ. Microbiol.* **73**: 3511–8.
- Eiler, A., Heinrich, F., and Bertilsson, S. (2012) Coherent dynamics and association networks among lake bacterioplankton taxa. *ISME J.* **6**: 330–42.
- El-Swais, H., Dunn, K.A., Bielawski, J.P., Li, W.K.W., and Walsh, D.A. (2014) Seasonal assemblages and short-lived blooms in coastal north-west Atlantic Ocean bacterioplankton. *Environ. Microbiol.* **2424**: 1–20.

- Eren, a. M., Maignien, L., Sul, W.J., Murphy, L.G., Grim, S.L., Morrison, H.G., and Sogin, M.L. (2013) Oligotyping: differentiating between closely related microbial taxa using 16S rRNA gene data. *Methods Ecol. Evol.* **4**: 1111–1119.
- Fabrega, J., Fawcett, S.R., Renshaw, J.C., and Lead, J.R. (2009) Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environ. Sci. Technol.* **43**: 7285–7290.
- Faust, K., Sathirapongsasuti, J.F., Izard, J., Segata, N., Gevers, D., Raes, J., and Huttenhower, C. (2012) Microbial Co-occurrence Relationships in the Human Microbiome. *Plos Comput. Biol.* **8**:
- Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D., et al. (2013) Changes in assembly processes in soil bacterial communities following a wildfire disturbance. *ISME J.* **7**: 1102–11.
- Field, K.G., Gordon, D., Wright, T., Rappé, M., Urbach, E., Vergin, K., and Giovannoni, S.J. (1997) Diversity and depth-specific distribution of SAR11 cluster rRNA genes from marine planktonic bacteria. *Appl. Environ. Microbiol.* **63**: 63–70.
- Franz, M. and Nunn, C.L. (2009) Network-based diffusion analysis: a new method for detecting social learning. *Proc. R. Soc. Biol.* **276**: 1829–36.
- Friedman, J. and Alm, E.J. (2012) Inferring correlation networks from genomic survey data. *PLoS Comput. Biol.* **8**: e1002687.
- Fuhrman, J. a, Steele, J. a, Hewson, I., Schwalbach, M.S., Brown, M. V, Green, J.L., and Brown, J.H. (2008) A latitudinal diversity gradient in planktonic marine bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **105**: 7774–8.
- Fuhrman, J.A., Cram, J.A., and Needham, D.M. (2015) Marine microbial community dynamics and their ecological interpretation. *Nat. Rev. Microbiol.*
- Fuhrman, J.A., Hewson, I., Schwalbach, M.S., Steele, J.A., Brown, M. V, and Naeem, S. (2006) Annually reoccurring bacterial communities are predictable from ocean conditions. *Proc Natl Acad Sci U S A* **103**: 13104–13109.
- Gilbert, J.A., Field, D., Swift, P., Newbold, L., Oliver, A., Smyth, T., et al. (2009) The seasonal structure of microbial communities in the Western English Channel. *Env. Microbiol* **11**: 3132–3139.
- Gilbert, J.A., Steele, J.A., Caporaso, J.G., Steinbruck, L., Reeder, J., Temperton, B., et al. (2012) Defining seasonal marine microbial community dynamics. *ISME J* **6**: 298–308.
- Del Giorgio, P.A. and Gasol, J.M. (2008) Physiological Structure and Single-Cell Activity in Marine Bacterioplankton. In, *Microbial Ecology of the Oceans.*, pp. 243–298.
- Giovannoni, S.J., Cameron Thrash, J., and Temperton, B. (2014) Implications of streamlining theory for microbial ecology. *ISME J.* **8**: 1–13.
- Giovannoni, S.J. and Vergin, K.L. (2012) Seasonality in ocean microbial communities. *Science* **335**: 671–6.
- Glasby, T.M. and Underwood, a. J. (1996) Sampling to differentiate between pulse and press perturbations. *Environ. Monit. Assess.* **42**: 241–252.

- Gómez-Pereira, P.R., Schüler, M., Fuchs, B.M., Bennke, C., Teeling, H., Waldmann, J., et al. (2012) Genomic content of uncultured Bacteroidetes from contrasting oceanic provinces in the North Atlantic Ocean. *Environ. Microbiol.* **14**: 52–66.
- Grimm, V. and Wissel, C. (1997) Babel, or the ecological stability discussions: An inventory and analysis of terminology and a guide for avoiding confusion. *Oecologia* **109**: 323–334.
- Hagendorfer, H., Lorenz, C., Kaegi, R., Sinnet, B., Gehrig, R., Goetz, N. V., et al. (2010) Size-fractionated characterization and quantification of nanoparticle release rates from a consumer spray product containing engineered nanoparticles. *J. Nanoparticle Res.* **12**: 2481–2494.
- Hall, N.S., Paerl, H.W., Peierls, B.L., Whipple, A.C., and Rossignol, K.L. (2013) Effects of climatic variability on phytoplankton community structure and bloom development in the eutrophic, microtidal, New River Estuary, North Carolina, USA. *Estuar. Coast. Shelf Sci.* **117**: 70–82.
- Hammes, F., Vital, M., and Egli, T. (2010) Critical evaluation of the volumetric “bottle effect” on microbial batch growth. *Appl. Environ. Microbiol.* **76**: 1278–81.
- Hanson, C. a, Fuhrman, J. a, Horner-Devine, M.C., and Martiny, J.B.H. (2012) Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.* **10**: 497–506.
- Hellweger, F.L., van Sebille, E., Fredrick, N.D., and Sebille, E. Van (2014) Biogeographic patterns in ocean microbes emerge in a neutral agent-based model. *Science (80-.)*. **345**: 1346–1349.
- Huber, W. (1993) Ecotoxicological Relevance of Atrazine in Aquatic Systems. *Environ. Toxicol. Chem.* **12**: 1865–1881.
- Hugoni, M., Taib, N., Debroas, D., Domaizon, I., and Jouan, I. (2013) Structure of the rare archaeal biosphere and seasonal dynamics of active ecotypes in surface coastal waters. *Proc Natl Acad Sci U S A* **110**: 6004–6009.
- Hunt, D.E., David, L. a, Gevers, D., Preheim, S.P., Alm, E.J., and Polz, M.F. (2008) Resource partitioning and sympatric differentiation among closely related bacterioplankton. *Science* **320**: 1081–5.
- Hunt, D.E., Lin, Y.J., Church, M.J., Karl, D.M., Tringe, S.G., Izzo, L.K., and Johnson, Z.I. (2013) Relationship between Abundance and Specific Activity of Bacterioplankton in Open Ocean Surface Waters. *Appl Env. Microbiol* **79**: 177–184.
- Hunt, D.E., Ortega-retuerta, E., and Nelson, C.E. (2010) Connections between bacteria and organic matter in aquatic ecosystems: Linking microscale ecology to global carbon cycling. In, *Eco-DAS VIII Symposium Proceedings, ASLO.*, pp. 110–128.
- Hunt, D.E. and Ward, C.S. (2015) A network-based approach to disturbance transmission through microbial interactions. *Front Microbiol* **6**:
- Hutchinson, G.E. (1961) The Paradox of the Plankton. *Am. Nat.* **95**: 137–145.
- Iluz, D., Dishon, G., Capuzzo, E., Meeder, E., Astoreca, R., Montecino, V., et al. (2009) Short-term variability in primary productivity during a wind-driven diatom bloom in the Gulf of Eilat (Aqaba). *Aquat. Microb. Ecol.* **56**: 205–215.

- Johnson, Z.I., Shyam, R., Ritchie, A.E., Mioni, C., Lance, V.P., Murray, J.W., and Zinser, E.R. (2010) The effect of iron- and light-limitation on phytoplankton communities of deep chlorophyll maxima of the western Pacific Ocean. *J. Mar. Res.* **68**: 283–308.
- Johnson, Z.I., Wheeler, B.J., Blinby, S.K., Carlson, C.M., Ward, C.S., and Hunt, D.E. (2013) Dramatic variability of the carbonate system at a temperate coastal ocean site (beaufort, north Carolina, USA) is regulated by physical and biogeochemical processes on multiple timescales. *PLoS One* **8**: e85117.
- Johnson, Z.I., Zinser, E.R., Coe, A., McNulty, N.P., Woodward, E.M.S., and Chisholm, S.W. (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science* (80-.). **311**: 1737–1740.
- Jones, A.C., Liao, T.S.V., Najar, F.Z., Roe, B. a, Hambright, K.D., and Caron, D. a (2013) Seasonality and disturbance: annual pattern and response of the bacterial and microbial eukaryotic assemblages in a freshwater ecosystem. *Environ. Microbiol.* **15**: 2557–72.
- Jones, S.E., Chiu, C.-Y., Kratz, T.K., Wu, J.-T., Shade, A., and McMahon, K.D. (2008) Typhoons initiate predictable change in aquatic bacterial communities. *Limnol. Oceanogr.* **53**: 1319–1326.
- Jones, S.E., Chiu, C.Y., Kratz, T.K., Wu, J.T., Shade, A., and McMahon, K.D. (2008) Typhoons initiate predictable change in aquatic bacterial communities. *Limnol. Oceanogr.* **53**: 1319–1326.
- Jones, S.E. and Lennon, J.T. (2010) Dormancy contributes to the maintenance of microbial diversity. *Proc Natl Acad Sci U S A* **107**: 5881–5886.
- Kaegi, R., Voegelin, A., Ort, C., Sinnet, B., Thalmann, B., Krismer, J., et al. (2013) Fate and transformation of silver nanoparticles in urban wastewater systems. *Water Res.* **47**: 3866–3877.
- Kaegi, R., Voegelin, A., Sinnet, B., Zuleeg, S., Hagendorfer, H., Burkhardt, M., and Siegrist, H. (2011) Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. *Environ. Sci. Technol.* **45**: 3902–3908.
- Kazamia, E., Czesnick, H., Nguyen, T.T., Croft, M.T., Sherwood, E., Sasso, S., et al. (2012) Mutualistic interactions between vitamin B(12) -dependent algae and heterotrophic bacteria exhibit regulation. *Env. Microbiol* **14**: 1466–1476.
- Keller, A. a., McFerran, S., Lazareva, A., and Suh, S. (2013) Global life cycle releases of engineered nanomaterials. *J. Nanoparticle Res.* **15**: 1692.
- Kent, A.D., Yannarell, A.C., Rusak, J.A., Triplett, E.W., and McMahon, K.D. (2007) Synchrony in aquatic microbial community dynamics. *ISME J* **1**: 38–47.
- Kirchman, D.L. (2002) The ecology of *Cytophaga-Flavobacteria* in aquatic environments. *FEMS Microbiol. Ecol.* **39**: 91–100.
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., and Glockner, F.O. (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41**: 1–11.
- Koch, M.S. and Erskine, J.M. (2001) Sulfide as a phytotoxin to the tropical seagrass *Thalassia testudinum*: interactions with light, salinity and temperature. *J. Exp. Mar. Bio. Ecol.* **266**: 81–95.

- Koch, M.S., Schopmeyer, S., Kyhn-Hansen, C., and Madden, C.J. (2007) Synergistic effects of high temperature and sulfide on tropical seagrass. *J. Exp. Mar. Bio. Ecol.* **341**: 91–101.
- Koepfel, A.F. and Wu, M. (2012) Lineage-dependent ecological coherence in bacteria. *FEMS Microbiol. Ecol.* **81**: 574–582.
- Kolmonen, E., Sivonen, K., Rapala, J., and Haukka, K. (2004) Diversity of cyanobacteria and heterotrophic bacteria in cyanobacterial blooms in Lake Joutikas, Finland. *Aquat. Microb. Ecol.* **36**: 201–211.
- Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Patrick, D., and Arbor, A. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.*
- Kumar, L. and Futschik, M. (2007) Bioinformatics Mfuzz : A software package for soft clustering of microarray data Bioinformatics. 5–7.
- Lamendella, R., Strutt, S., Borglin, S., Chakraborty, R., Tas, N., Mason, O.U., et al. (2014) Assessment of the Deepwater Horizon oil spill impact on Gulf coast microbial communities. *Front. Microbiol.* **5**: 130.
- Lauro, F.M., McDougald, D., Thomas, T., Williams, T.J., Egan, S., Rice, S., et al. (2009) The genomic basis of trophic strategy in marine bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **106**: 15527–15533.
- Levard, C., Hotze, E.M., Lowry, G. V., and Brown, G.E. (2012) Environmental transformations of silver nanoparticles: Impact on stability and toxicity. *Environ. Sci. Technol.* **46**: 6900–6914.
- Lindh, M. V., Sjöstedt, J., Andersson, A.F., Baltar, F., Hugerth, L.W., Lundin, D., et al. (2015) Disentangling seasonal bacterioplankton population dynamics by high-frequency sampling. *Environ. Microbiol.* **17**: 2459–2476.
- Litchman, E. (2010) Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. *Ecol. Lett.* **13**: 1560–72.
- Lowry, G. V., Espinasse, B.P., Badireddy, A.R., Richardson, C.J., Reinsch, B.C., Bryant, L.D., et al. (2012) Long-term transformation and fate of manufactured Ag nanoparticles in a simulated large scale freshwater emergent wetland. *Environ. Sci. Technol.* **46**: 7027–36.
- Malfatti, F. and Azam, F. (2010) Atomic force microscopy reveals microscale networks and possible symbioses among pelagic Marine Bacteria. *Aquat. Microb. Ecol.* **58**: 1–14.
- Marie, D., Partensky, F., Jacquet, S., and Vaulot, D. (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Appl. Environ. Microbiol.* **63**: 186–193.
- Massana, R., Murray, A.E., Preston, C.M., and DeLong, E.F. (1997) Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. *Appl. Environ. Microbiol.* **63**: 50–56.
- Mishra, A. and Pandey, A. (1989) Toxicity of three herbicides to some nitrogen-fixing cyanobacteria. *Ecotoxicol. Environ. Saf.* **17**: 236–46.

- Missiuro, P. V., Liu, K.S., Zou, L.H., Ross, B.C., Zhao, G.Y., Liu, J.S., and Ge, H. (2009) Information Flow Analysis of Interactome Networks. *Plos Comput. Biol.* **5**:
- Montoya, J.M. and Sol, R. V (2002) Small world patterns in food webs. *J. Theor. Biol.* **214**: 405–412.
- Morris, J.J., Johnson, Z.I., Szul, M.J., Keller, M., and Zinser, E.R. (2011) Dependence of the cyanobacterium *Prochlorococcus* on hydrogen peroxide scavenging microbes for growth at the ocean's surface. *PLoS One* **6**: e16805.
- Morris, J.J., Lenski, R.E., and Zinser, E.R. (2012) The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. *MBio* **3**: e00036–12–.
- Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., et al. (2008) Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. *Environ. Sci. Technol.* **42**: 8959–8964.
- Needham, D.M., Chow, C.E., Cram, J.A., Sachdeva, R., Parada, A., and Fuhrman, J.A. (2013) Short-term observations of marine bacterial and viral communities: patterns, connections and resilience. *ISME J* **7**: 1274–1285.
- Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., et al. (2013a) Patterns and processes of microbial community assembly. *Microbiol. Mol. Biol. Rev.* **77**: 342–56.
- Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., et al. (2013b) Patterns and processes of microbial community assembly. *Microbiol. Mol. Biol. Rev.* **77**: 342–56.
- Newton, R.J. and McMahon, K.D. (2011) Seasonal differences in bacterial community composition following nutrient additions in a eutrophic lake. *Environ. Microbiol.* **13**: 887–899.
- Niemi, G.J., DeVore, P., Detenbeck, N., Taylor, D., Lima, A., Pastor, J., et al. (1990) Overview of case studies on recovery of aquatic systems from disturbance. *Environ. Manage.* **14**: 571–587.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., et al. (2015) vegan: Community Ecology Package. R package version 2.2-1. <http://CRAN.R-project.org/package=vegan>.
- Ottesen, E. a, Young, C.R., Eppley, J.M., Ryan, J.P., Chavez, F.P., Scholin, C. a, and DeLong, E.F. (2013) Pattern and synchrony of gene expression among sympatric marine microbial populations. *Proc. Natl. Acad. Sci. U. S. A.* **110**: E488–97.
- Paerl, H.W., Bales, J.D., Ausley, L.W., Buzzelli, C.P., Crowder, L.B., Eby, L. a, et al. (2001) Ecosystem impacts of three sequential hurricanes (Dennis, Floyd, and Irene) on the United States' largest lagoonal estuary, Pamlico Sound, NC. *Proc. Natl. Acad. Sci. U. S. A.* **98**: 5655–60.
- Paerl, H.W., Hall, N.S., Peierls, B.L., Rossignol, K.L., and Joyner, A.R. (2014) Hydrologic Variability and Its Control of Phytoplankton Community Structure and Function in Two Shallow, Coastal, Lagoonal Ecosystems: The Neuse and New River Estuaries, North Carolina, USA. *Estuaries and Coasts* **37**: 31–45.
- Paerl, H.W., Rossignol, K.L., Hall, S.N., Peierls, B.L., and Wetz, M.S. (2010) Phytoplankton community indicators of short- and long-term ecological change in the anthropogenically and climatically impacted neuse river estuary, North Carolina, USA. *Estuaries and Coasts* **33**: 485–497.
- Pedrós-Alió, C. (2006) Marine microbial diversity: can it be determined? *Trends Microbiol.* **14**: 257–263.

- Pernthaler, J. (2005) Predation on prokaryotes in the water column and its ecological implications. *Nat. Rev. Microbiol.* **3**: 537–546.
- Pernthaler, J. and Amann, R.I. (2005) Fate of heterotrophic microbes in pelagic habitats: focus on populations. *Microbiol. Mol. Biol. Rev.* **69**: 440–461.
- Peura, S., Bertilsson, S., Jones, R.I., and Eiler, A. (2015) Resistant Microbial Cooccurrence Patterns Inferred by Network Topology. *Appl. Environ. Microbiol.* **81**: 2090–2097.
- Philippot, L., Andersson, S.G.E., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., and Hallin, S. (2010) The ecological coherence of high bacterial taxonomic ranks. *Nat. Rev. Microbiol.* **8**: 523–529.
- Pinckney, J.L., Paerl, H.W., and Harrington, M.B. (1999) Responses of the Phytoplankton Community Growth Rate to Nutrient Pulses in Variable Estuarine Environments. *J. Phycol.* **35**: 1455–1463.
- Polz, M.F. and Cavanaugh, C.M. (1998) Bias in template-to-product ratios in multitemplate PCR. *Appl. Environ. Microbiol.* **64**: 3724–3730.
- Polz, M.F., Hunt, D.E., Preheim, S.P., and Weinreich, D.M. (2006) Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **361**: 2009–2021.
- Power, M.E. and Tilman, D. (1996) Challenges in the quest for keystones. *Bioscience* **46**: 609.
- Pradhan, A., Seena, S., Pascoal, C., and Cássio, F. (2011) Can Metal Nanoparticles Be a Threat to Microbial Decomposers of Plant Litter in Streams? *Microb. Ecol.* **62**: 58–68.
- Rainey, P.B. and Travisano, M. (1998) Adaptive radiation in a heterogeneous environment. *Nature* **394**: 69–72.
- Rainey PB, T.M. (1998) Adaptive radiation in a heterogeneous environment. *Nature*.
- Ramegowda, V. and Senthil-Kumar, M. (2014) The interactive effects of simultaneous biotic and abiotic stresses on plants: Mechanistic understanding from drought and pathogen combination. *J. Plant Physiol.* **176C**: 47–54.
- Reinsch, B.C., Levard, C., Li, Z., Ma, R., Wise, a., Gregory, K.B., et al. (2012) Sulfidation of silver nanoparticles decreases Escherichia coli growth inhibition. *Environ. Sci. Technol.* **46**: 6992–7000.
- Riehle, M.M., Bennett, A.F., Lenski, R.E., and Long, A.D. (2003) Evolutionary changes in heat-inducible gene expression in lines of Escherichia coli adapted to high temperature. *Physiol. Genomics* **14**: 47–58.
- Ruan, Q., Dutta, D., Schwalbach, M.S., Steele, J.A., Fuhrman, J.A., and Sun, F. (2006) Local similarity analysis reveals unique associations among marine bacterioplankton species and environmental factors. *Bioinformatics* **22**: 2532–2538.
- Rykiel, E.J. (1985) Towards a definition of ecological disturbance. *Aust. J. Ecol.* **10**: 361–365.
- Salter, I., Galand, P.E., Fagervold, S.K., Lebaron, P., Obermosterer, I., Oliver, M.J., et al. (2015) Seasonal dynamics of active SAR11 ecotypes in the oligotrophic Northwest Mediterranean Sea. *ISME J* **9**: 347–360.

- Sañudo-Wilhelmy, S. a., Gobler, C.J., Okbamichael, M., and Taylor, G.T. (2006) Regulation of phytoplankton dynamics by vitamin B12. *Geophys. Res. Lett.* **33**: 10–13.
- Scientific Committee on Emerging and Newly Identified Health Risks (2010) Scientific Basis for the Definition of the Term “ Nanomaterial .”
- Shade, A., Chiu, C.Y., and McMahon, K.D. (2010) Differential bacterial dynamics promote emergent community robustness to lake mixing: an epilimnion to hypolimnion transplant experiment. *Env. Microbiol* **12**: 455–466.
- Shade, A., Jones, S.E., Caporaso, J.G., Handelsman, J., Knight, R., Fierer, N., and Gilbert, J. a (2014) Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. *MBio* **5**: e01371–14.
- Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Burgmann, H., et al. (2012) Fundamentals of microbial community resistance and resilience. *Front Microbiol* **3**: 417.
- Shade, A., Read, J.S., Youngblut, N.D., Fierer, N., Knight, R., Kratz, T.K., et al. (2012) Lake microbial communities are resilient after a whole-ecosystem disturbance. *ISME J* **6**: 2153–2167.
- Sheng, Z. and Liu, Y. (2011) Effects of silver nanoparticles on wastewater biofilms. *Water Res.* **45**: 6039–6050.
- Silver, S. (2003) Bacterial silver resistance: Molecular biology and uses and misuses of silver compounds. *FEMS Microbiol. Rev.* **27**: 341–353.
- Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R., et al. (2006) Microbial diversity in the deep sea and the underexplored “rare biosphere.” *Proc Natl Acad Sci U S A* **103**: 12115–12120.
- Steele, J.A., Countway, P.D., Xia, L., Vigil, P.D., Beman, J.M., Kim, D.Y., et al. (2011) Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *ISME J* **5**: 1414–1425.
- Stocker, R., Seymour, J.R., Samadani, A., Hunt, D.E., and Polz, M.F. (2008) Rapid chemotactic response enables marine bacteria to exploit ephemeral microscale nutrient patches. *Proc. Natl. Acad. Sci. U. S. A.* **105**: 4209–4214.
- Sullivan, M.B., Waterbury, J.B., and Chisholm, S.W. (2003) Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*. *Nature* **424**: 1047–1051.
- Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, a., Bennke, C.M., et al. (2012) Substrate-Controlled Succession of Marine Bacterioplankton Populations Induced by a Phytoplankton Bloom. *Science (80-.).* **336**: 608–611.
- Terbraak, C.J.F. and Verdonschot, P.F.M. (1995) Canonical Correspondence-Analysis and Related Multivariate Methods in Aquatic Ecology. *Aquat. Sci.* **57**: 255–289.
- The Project on Emerging Nanotechnologies (2015) Nanotechnology Consumer Products Inventory. <http://www.nanotechproject.org/inventories/consume>.
- Thomas, F., Hehemann, J.-H., Rebuffet, E., Czjzek, M., and Michel, G. (2011) Environmental and gut bacteroidetes: the food connection. *Front. Microbiol.* **2**: 93.

- Thomas, M.K., Kremer, C.T., Klausmeier, C. a., and Litchman, E. (2012) A Global Pattern of Thermal Adaptation in Marine Phytoplankton. *Science (80-.)*. **338**: 1085–1089.
- Throbäck, I.N., Johansson, M., Rosenquist, M., Pell, M., Hansson, M., and Hallin, S. (2007) Silver (Ag⁺) reduces denitrification and induces enrichment of novel nirK genotypes in soil. *FEMS Microbiol. Lett.* **270**: 189–94.
- Tripp, H.J., Kitner, J.B., Schwalbach, M.S., Dacey, J.W.H., Wilhelm, L.J., and Giovannoni, S.J. (2008) SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature* **452**: 741–744.
- Tyson, G.W., Lo, I., Baker, B.J., Allen, E.E., Hugenholtz, P., and Banfield, J.F. (2005) Genome-directed isolation of the key nitrogen fixer *Leptospirillum ferrodiazotrophum* sp. nov. from an acidophilic microbial community. *Appl. Environ. Microbiol.* **71**: 6319–6324.
- Urrine, J.M., Colman, B.P., Bone, A.J., Gondikas, A.P., and Matson, C.W. (2012) Biotic and abiotic interactions in aquatic microcosms determine fate and toxicity of Ag nanoparticles. Part 1. Aggregation and dissolution. *Environ. Sci. Technol.* **46**: 6915–24.
- De Vargas, C., Audic, S., Henry, N., Decelle, J., Mahe, F., Logares, R., et al. (2015) Eukaryotic plankton diversity in the sunlit ocean. *Science (80-.)*. **348**: 1261605.
- Vellend, M. (2010) Conceptual synthesis in community ecology. *Q. Rev. Biol.* **85**: 183–206.
- Veraart, A.J., Faassen, E.J., Dakos, V., van Nes, E.H., Lürling, M., and Scheffer, M. (2012) Recovery rates reflect distance to a tipping point in a living system. *Nature* **484**: 404–404.
- Vitousek, P.M., Mooney, H.A., Lubchenco, J., and Melillo, J.M. (1997) Human Domination of Earth' s Ecosystems. *Science (80-.)*. **277**: 494–499.
- Werner, J.J., Knights, D., Garcia, M.L., Scalfone, N.B., Smith, S., Yarasheski, K., et al. (2011) Bacterial community structures are unique and resilient in full-scale bioenergy systems. *Proc Natl Acad Sci U S A* **108**: 4158–4163.
- White, P.S. and Pickett, S.T. a. (1985) Natural Disturbance and Patch Dynamics: An Introduction. In, *The Ecology of Natural Disturbance and Patch Dynamics.*, p. 472.
- Widenfalk, A., Bertilsson, S., Sundh, I., and Goedkoop, W. (2008) Effects of pesticides on community composition and activity of sediment microbes--responses at various levels of microbial community organization. *Env. Pollut* **152**: 576–584.
- Worden, A.Z., Follows, M.J., Giovannoni, S.J., Wilken, S., Zimmerman, A.E., and Keeling, P.J. (2015) Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. *Science (80-.)*. **347**: 1257594.
- Yang, C., Hamel, C., Vujanovic, V., and Gan, Y. (2011) Fungicide: modes of action and possible impact on nontarget microorganisms. *ISRN Ecol.* **2011**:
- Yannarell, A.C., Stepe, T.F., and Paerl, H.W. (2007) Disturbance and recovery of microbial community structure and function following Hurricane Frances. *Env. Microbiol* **9**: 576–583.
- Yeo, S.K., Huggett, M.J., Eiler, A., and Rappe, M.S. (2013) Coastal bacterioplankton community dynamics in response to a natural disturbance. *PLoS One* **8**: e56207.

- Yin, L., Colman, B.P., McGill, B.M., Wright, J.P., and Bernhardt, E.S. (2012) Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants. *PLoS One* **7**: e47674.
- Yung, C.-M., Vereen, M.K., Herbert, A., Davis, K.M., Yang, J., Kantorowska, A., et al. (2015) Thermally adaptive tradeoffs in closely related marine bacterial strains. *Environ. Microbiol.* **17**: 2421–2429.
- Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D., et al. (2014) Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. *Proc. Natl. Acad. Sci. U. S. A.* **111**: E836–45.

Biography

Christopher Spencer Farinholt Ward was born in Baltimore, Maryland on July 15, 1985 to Kathryn S. Farinholt and Michael T. Ward. After graduating from high school at Baltimore Polytechnic Institute, he attended Carleton College in Northfield, Minnesota where he graduated cum laude with a Bachelor of Arts in Chemistry and a concentration in Biochemistry in June 2007. During a two and a half year associate position in the lab of Sabrina Ronen at University of California-San Francisco, he conducted research leading to five peer-reviewed papers: (1) "HDAC inhibition induces increased choline uptake and elevated phosphocholine levels in MCF7 breast cancer cells", (2) "Reduced phosphocholine and hyperpolarized lactate provide magnetic resonance biomarkers of PI3K/ Akt/mTOR inhibition in glioblastoma", (3) "17-allylamino-17-demethoxygeldanamycin treatment results in a magnetic resonance spectroscopy-detectable elevation in choline-containing metabolites associated with increased expression of choline transporter SLC44A1 and phospholipase A2", (4) "Hyperpolarized ^{13}C spectroscopic imaging informs on hypoxia-inducible factor-1 and myc activity downstream of platelet-derived growth factor receptor", (5) "Noninvasive detection of target modulation following phosphatidylinositol-3-kinase inhibition using hyperpolarized ^{13}C magnetic resonance spectroscopy". In 2010, he began a doctoral degree at Duke University in Marine Science and Conservation and the Integrated Toxicology and Environmental Health Program. During his time in the lab of Dana Hunt, he was a National Science Foundation Graduate Research Fellow and contributed to three peer-reviewed papers: (1) "A network-based approach to disturbance transmission through microbial interactions", (2) "Thermally adaptive tradeoffs in closely related marine bacterial strains", (3) "Dramatic variability of pH in the coastal ocean is regulated by physical and biogeochemical processes on multiple timescales".