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Coral reefs are foundational members of reefs and composed of many organisms including: animal host cells, protist algal symbionts, and bacteria. Here we present data from the MCR LTER on how coral microbiomes serve as sentinels for coral reef health and describe experiments we conducted in the field and lab to track the effects of multiple stressors on reefs and their microbiomes

1. Ecosystem Stressors Rarely Occur in Isolation.

Research on how multiple stressors & their severity is needed to accurately assess impact on coral hosts and reef habitats

Bleaching events in marine systems are the result of multiple stressors at one time including thermal stress, light stress, and nutrient cycling stress. While most research explores only one at a time, a multidisciplinary approach is needed.

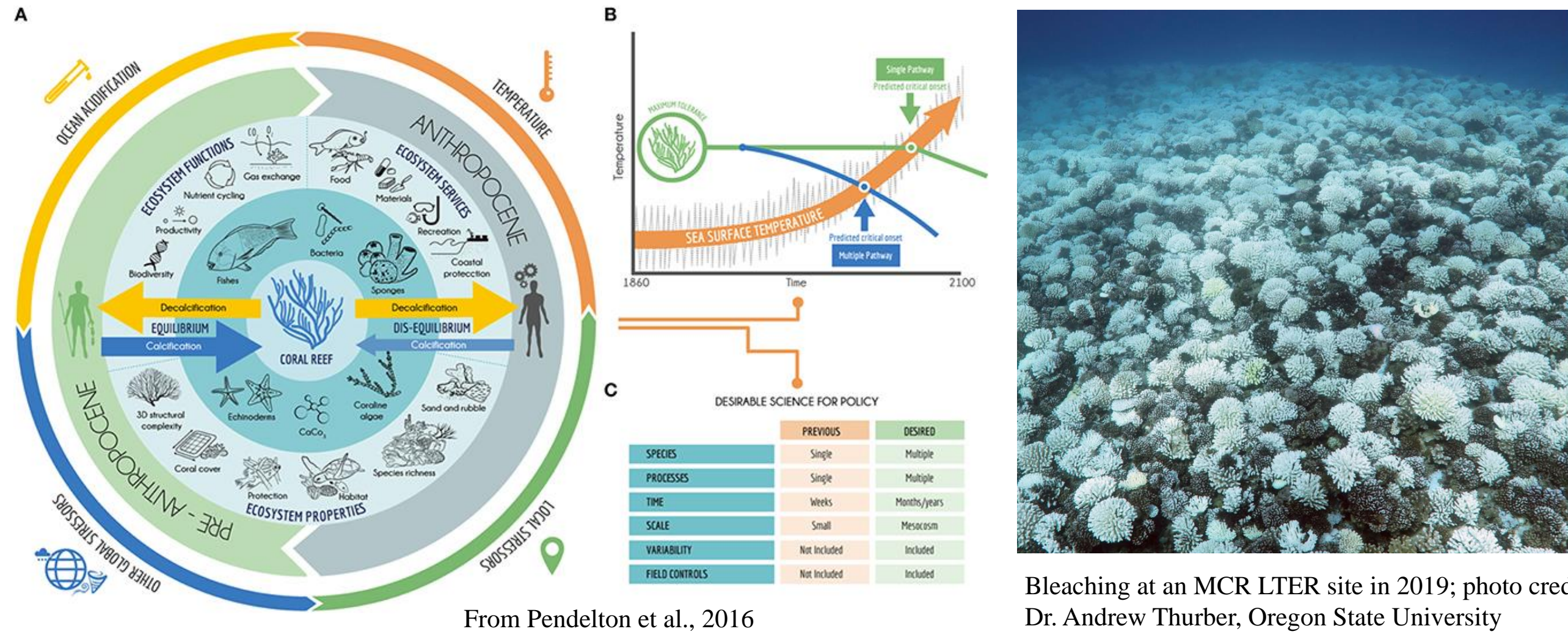


Fig. 1. Most coral reefs experience many local and global stressors acting together. These often result in coral bleaching that if prolonged can lead to coral mortality and a shift to macroalgal dominated states. To best assist in policy decisions research should explore multiple stressors and how they effect coral reefs and their abilities to resist and recover from stress.

2. Disturbance At Different Severities:

Resistance and Resilience to Coral Bleaching Likely Differs Based on Marine Heat Wave Severity

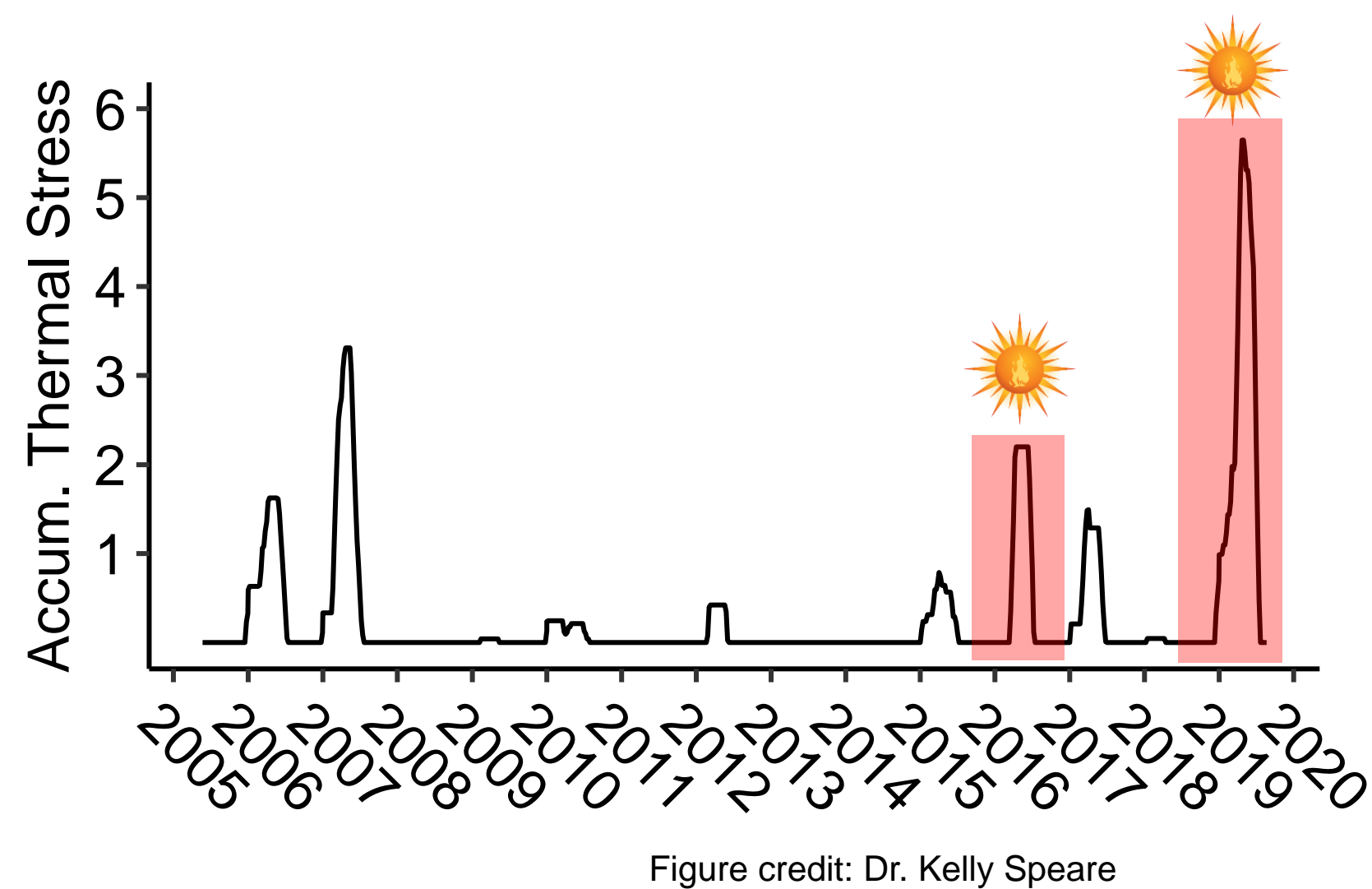
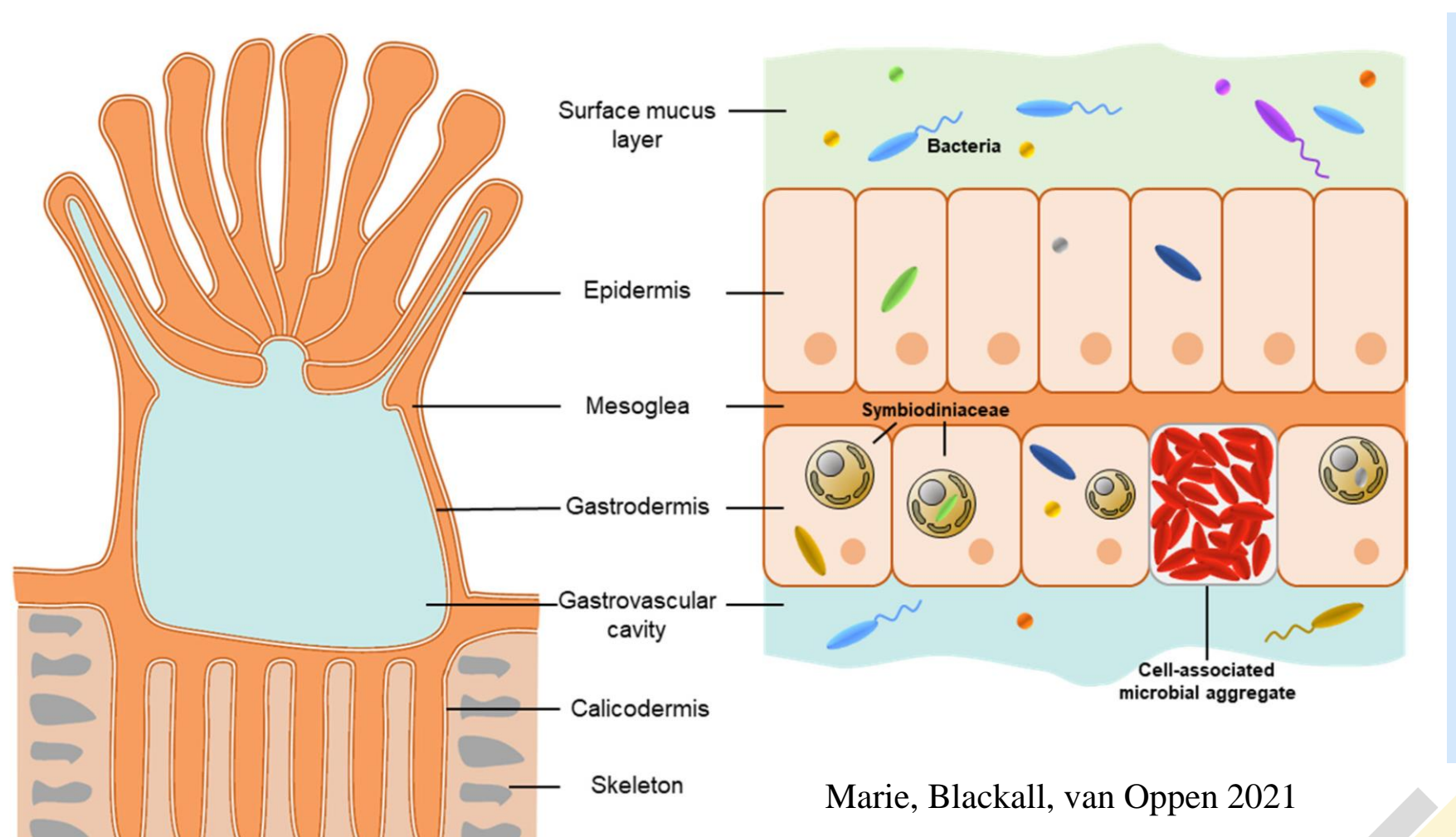


Fig. 2 Thermal stress events since the start of the MCR LTER vary in their severity. Accumulated thermal stress is a metric that quantifies heat stress that corals experience as function of the intensity and duration of a marine heatwave. In 2016 a moderate MHW occurred and in 2019 an extreme MHW was recorded. We conducted manipulative experiments to track the different effects of these 2 sequential MHW on corals and their microbiomes and determine if they are variably resistant or resilient to the stress.

Marine heat waves (MHWs) are occurring with more frequency and severity. How ecosystems and organisms respond to these different stressors is an essential question to understand the resistance and resilience of these important habitats.

2. Microbiomes Respond Quickly to Disturbances

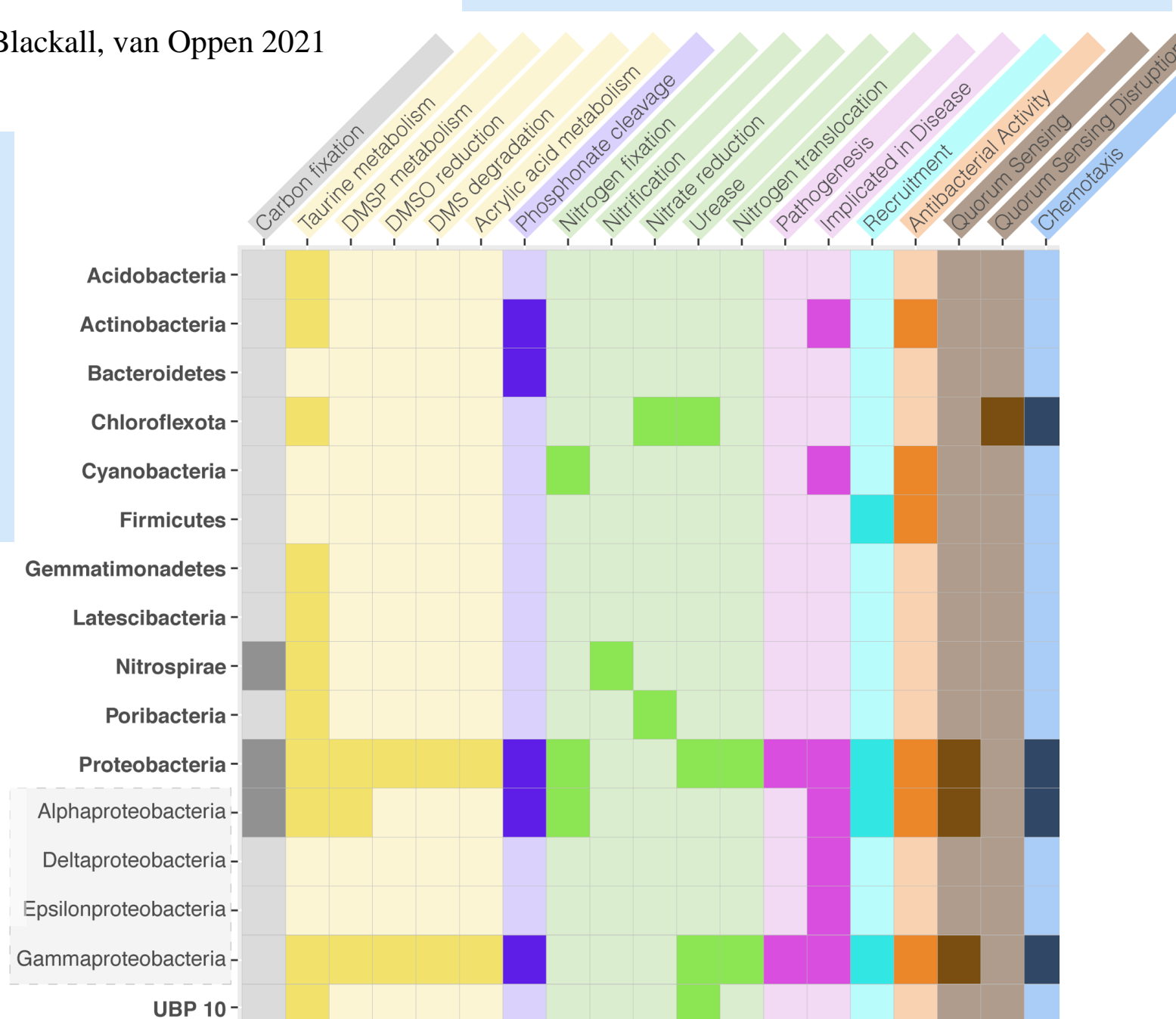
Microbiomes Can Serve as Early Reef Health Indicators



Corals are simple animals with 2 cell layers and a surface mucus layer. Within these layers are Symbiodiniaceae the protist symbiont of corals and a variety of intracellular or extracellular bacteria.

Coral microbiome members have unique functions from nutrient cycling to coral recruitment & settlement, and prevent or activate pathogenesis.

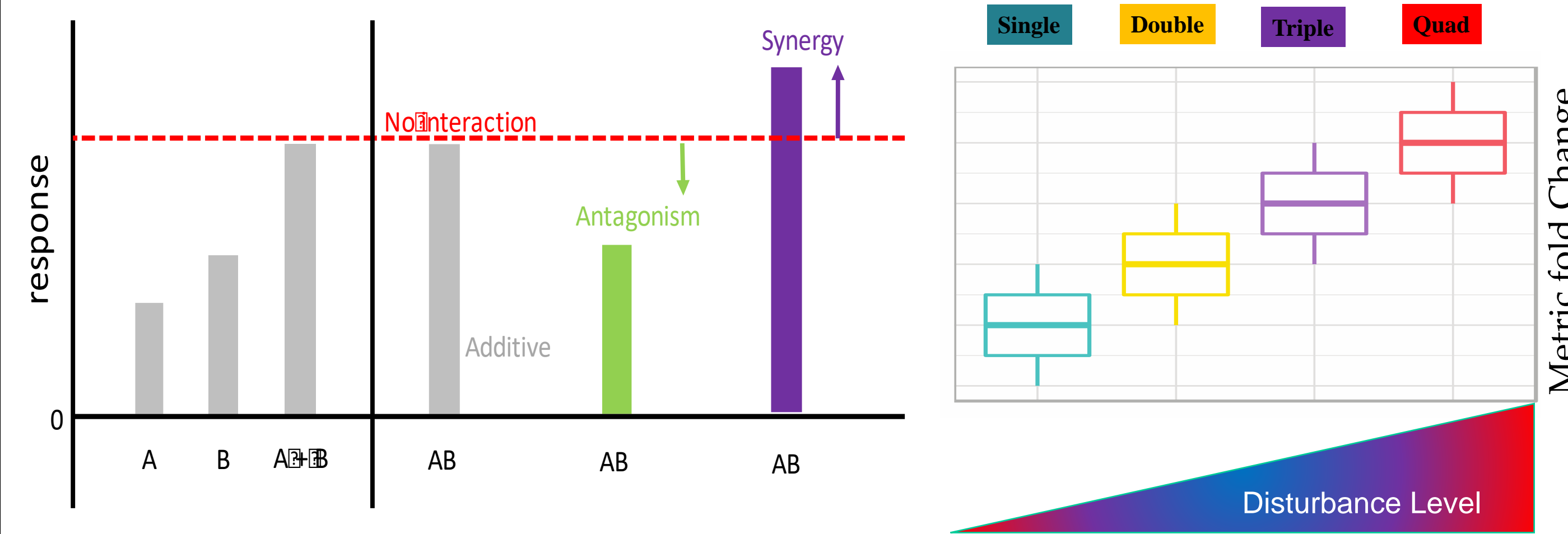
Fig. 3. We reviewed the coral literature and categorized different groups of coral bacteria into their hypothesized function for the coral holobiont. Most bacteria have roles in nutrient cycling like sulfur or nitrogen cycling but some exhibit aspects of pathogenesis. From Maher, Epstein, Vega Thurber 2022



3. Multiple Stressors Can be Antagonistic

Temperature alone alters microbiomes as much as multiple stressors

Theoretically, interactions can be additive, antagonistic, or synergistic but few studies fully examine the range of these possibilities



We conducted a multiple stressor aquarium experiment with *Pocillopora* coral microbiomes to test this theory with 12 individual and combinations of stress

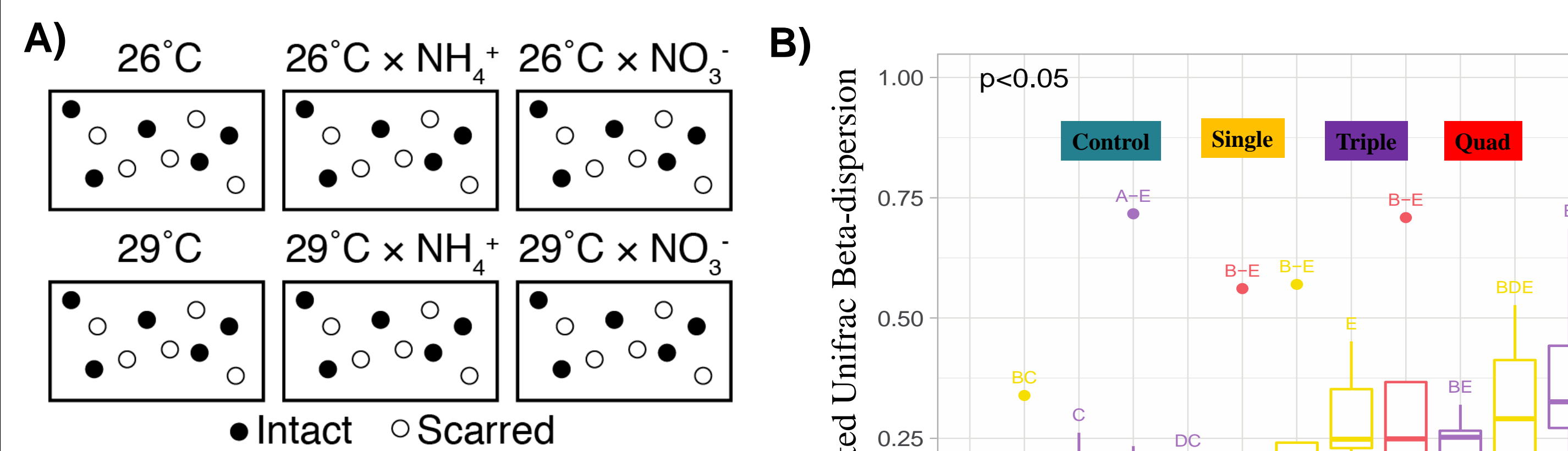


Fig. 4. A) Experimental design to test the effects of individual and multiple stressors on corals and their microbiomes. We conducted exposed corals to ambient or temperature stress individually or combined with different nutrient stressors and/or scarring the corals to mimic marine heat waves, nutrient pollution, and predation.

B) *Pocillopora* manipulations demonstrated that some single stressors (yellow boxes) like temperature and wounding cause more extreme alterations in microbiomes than triple or quadruple stressors alone. Thus, synergisms are rare and antagonisms tend to dominate these effects (Maher et al 2019).

4. Coral Microbiomes Exhibit Species Specific Resistance and Resilience to Mild Thermal Stress

Field and time series experiments during nutrient enrichment and thermal stress show similar effects to lab experiments

During the less severe 2016 MHW we tracked microbiome dynamics in 3 dominate coral genera: *Acropora*, *Pocillopora* and *Porites* to test for microbiome resistance and resilience to thermal stress and nutrient stress

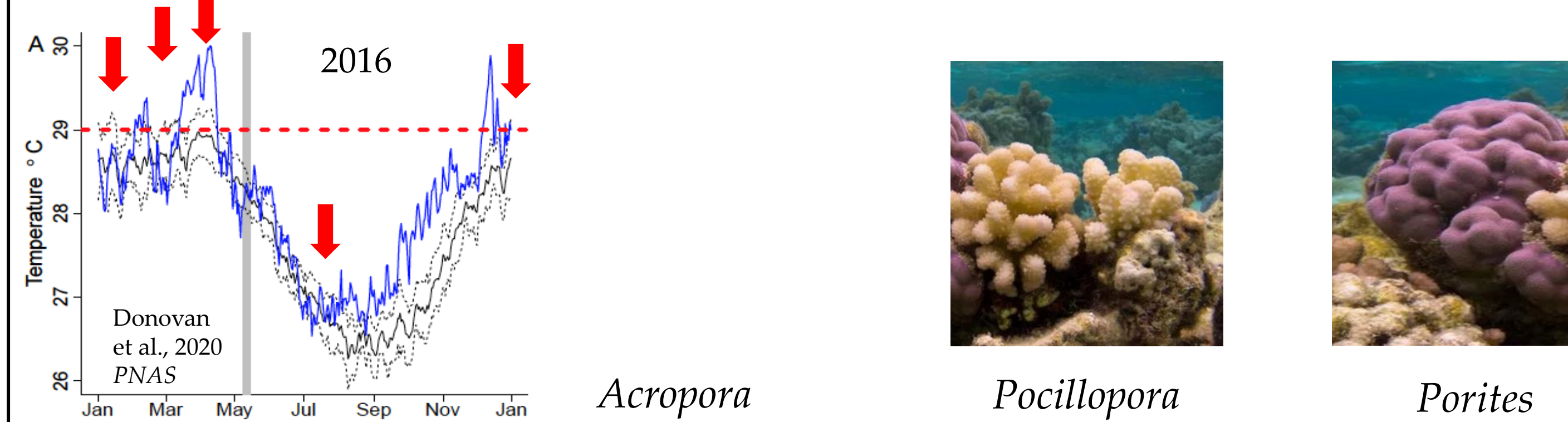


Fig. 5. We added 2 forms of nitrogen to corals during the above thermal stress event and sampled microbiomes (red arrows) before during and after the mild MHW. Like the lab experiments, nutrients did not act additively with temperature (data not show). Temperature alone had the largest effect is thus shown here.

Fig. 6. Above graph) We found that microbial α species diversity tended to either increase (*Acropora* and *Porites*) or decrease (*Pocillopora*) during the peak of temperature, but despite the differences, all returned to the initial diversity state 6 months (Jan 17) after the event (Maher et al 2020). Side) Overall community β -diversity also significantly changed during and most clearly after the event and all microbiomes became less variable after the event.

Microbiomes of three coral genera showed disruption in richness and composition during a mild bleaching event but then moved to a new state 6 months after the event.

5. Microbiomes of 3 Species Show Similar Response and Resilience to Disturbance

In a long term time series experiment from July 2018-August 2020 we tracked similar microbiome responses of the same 3 genera (*Acropora*, *Pocillopora* & *Porites*) to a more severe marine heat wave.

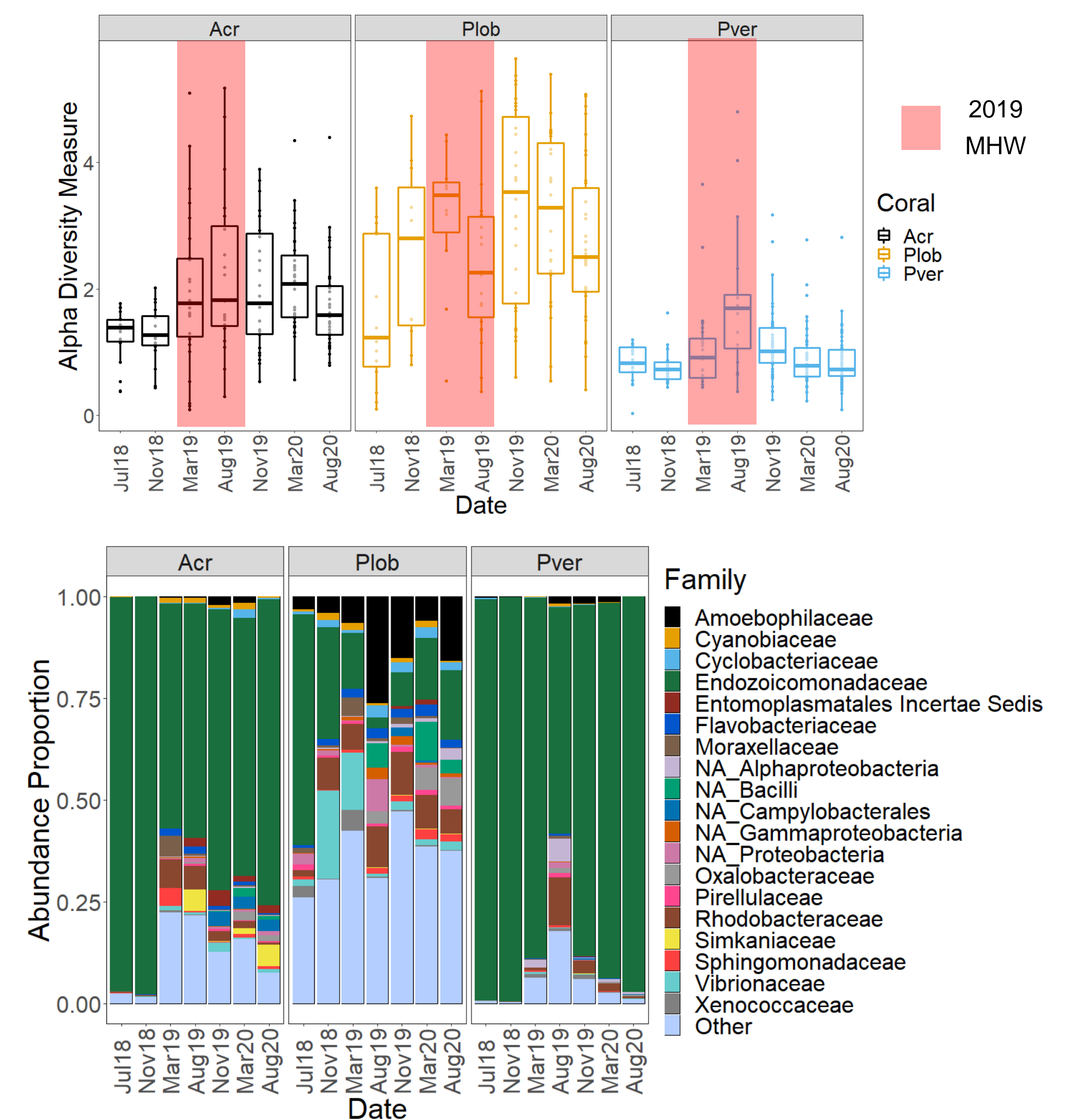


Fig. 7. We have been tracking the microbiomes of over 120 corals from 32 replicate plots on the forereef of Moorea from July 2018 to now. In this first analysis which occurred over the time period that included the severe marine heat wave in 2019 we found similar patterns in alpha diversity changes as we saw in 2016. A) Alpha diversity increased during the thermal stress event but returned to pre stress diversity within some months. B) Further, the composition of the coral bacteria was altered during the event but only returned to pre-disturbance composition in *Pocillopora*. *Acropora* microbiomes remained changed as did *Porites* microbiomes (Vompe et al., in prep).

The severity of coral bleaching and mortality declined with depth on the outer reef, resulting in greater declines in coral cover on shallower reefs compared to deeper reefs.

6. Models for Microbiome Responses to Disturbance

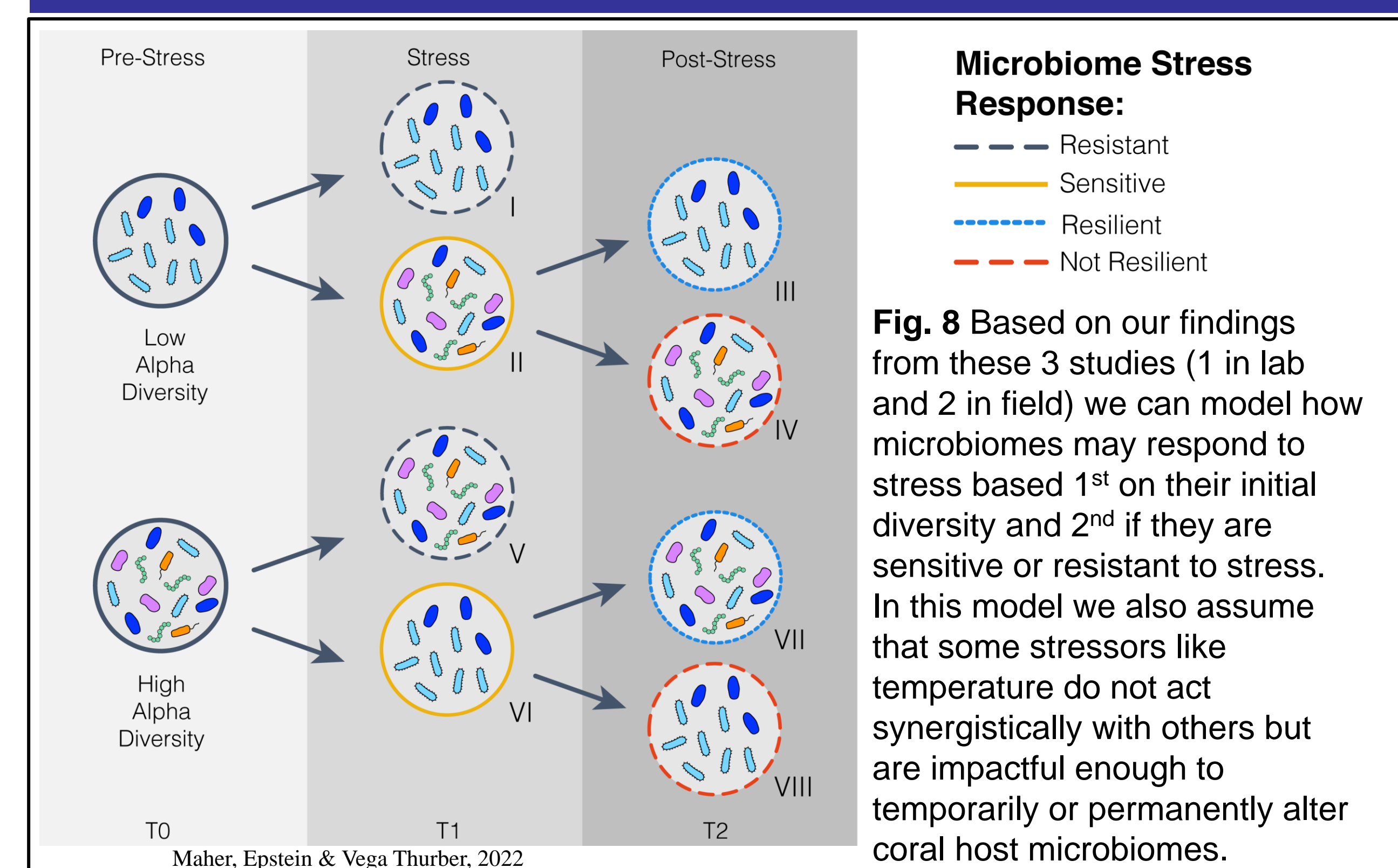


Fig. 8 Based on our findings from these 3 studies (1 in lab and 2 in field) we can model how microbiomes may respond to stress based 1st on their initial diversity and 2nd if they are sensitive or resistant to stress. In this model we also assume that some stressors like temperature do not act synergistically with others but are impactful enough to temporarily or permanently alter coral host microbiomes.

Summary: Disturbances vary in severity and can act together with other stressors simultaneously. Time series data and lab experiments can reveal patterns in microbial diversity that may exemplify a host's ability to acclimatize to disturbances. Coral microbiomes often demonstrate resilience but also exhibit alternate states after disturbance.

Question: Whether these microbiomes return to pre-disturbance states and what altered microbial states might mean for coral host resilience are outstanding questions in our field that are currently being investigated.



See related poster on material legacies



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