NITRATE ENRICHMENT & HEAT STRESS

impacts the **physiology** of the coral *A. kenti* and the composition of its associated **microbiome**

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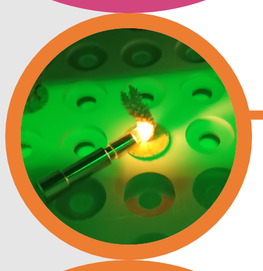
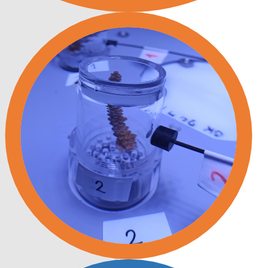
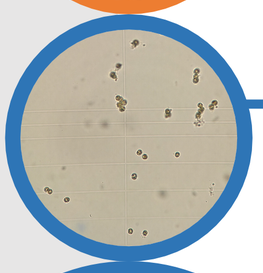
INTRODUCTION

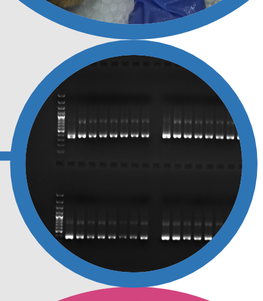
Corals survival in the face of environmental change relies heavily on the nutritional and functional relationship with their algal endosymbionts and their associated microbiome. Nitrogen underpins many aspects of coral holobiont functioning but the effect of its availability in its most abundant environmental form, nitrate (NO_3^-), on the coral response to stress is equivocal: while NO_3 sustains symbiont communities, it has also been reported to have adverse effects on the response to oxidative stress and to accentuate bleaching.

AIM : Identify the individual and combined effects of heat stress and NO_3 enrichment on the physiological performance and the microbiome of *Acropora kenti*.





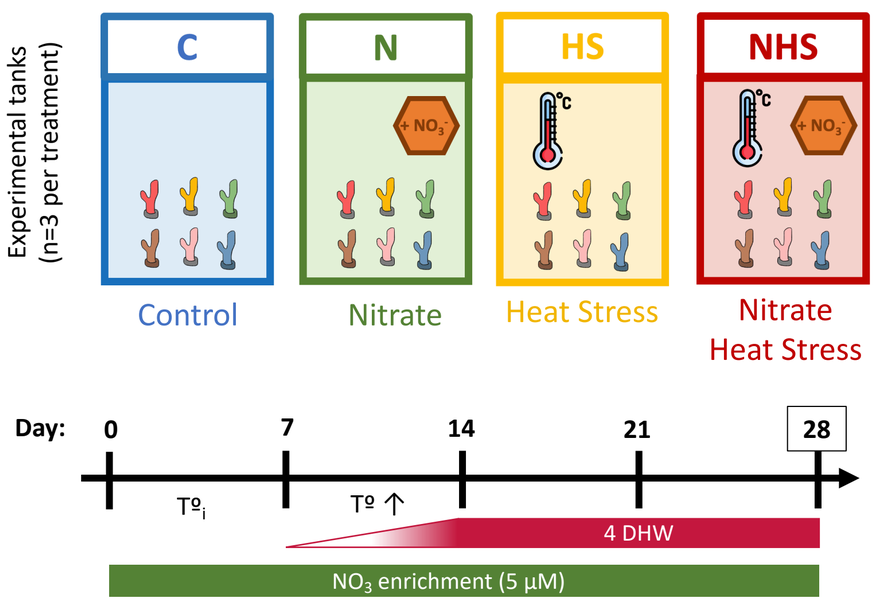




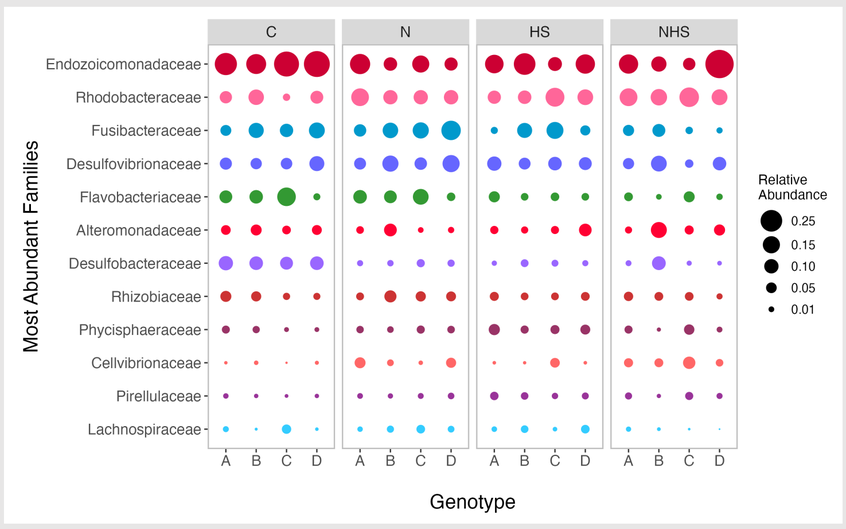
METHODS

Coral nubbins from 6 *Acropora kenti* colonies were distributed in 12 individual experimental tanks according to 4 treatments (C, N, HS, NHS) in a cross-factor design testing a NO_3 enrichment of 5 µM in combination or not with a heat stress of 4 degrees heating weeks (DHW). After 4 weeks, measurements were taken and corals sampled to assess the physiological response. DNA was extracted from coral tissues and the V4 region of the 16S rRNA gene was sequenced on the Illumina NextSeq 1000 P1.

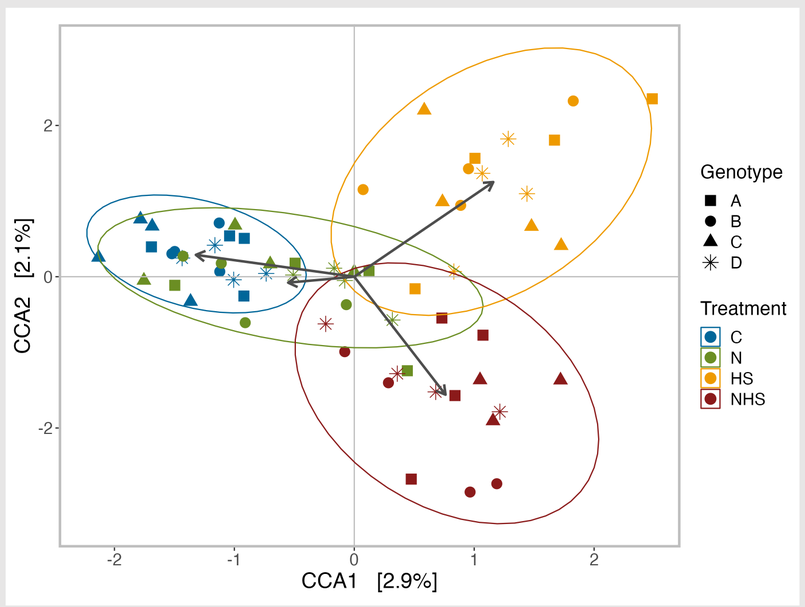
Experiment design & timeline :



RESULTS - MICROBIOME



Bubble plot showing the relative abundances of the 12 most abundant families across treatments and genotypes. Endozoicomonadaceae are dominant in *A. kenti*'s associated microbiome across treatments, although they present slightly decreased abundances in treated corals compared to controls. Other abundant families, such as Fusibacteraceae, Flavobacteriaceae, Desulfobacteraceae, and Cellvibrionaceae show different relative abundance patterns across treatments, indicating potential shifts in microbial community composition due to nitrate enrichment and/or heat stress.

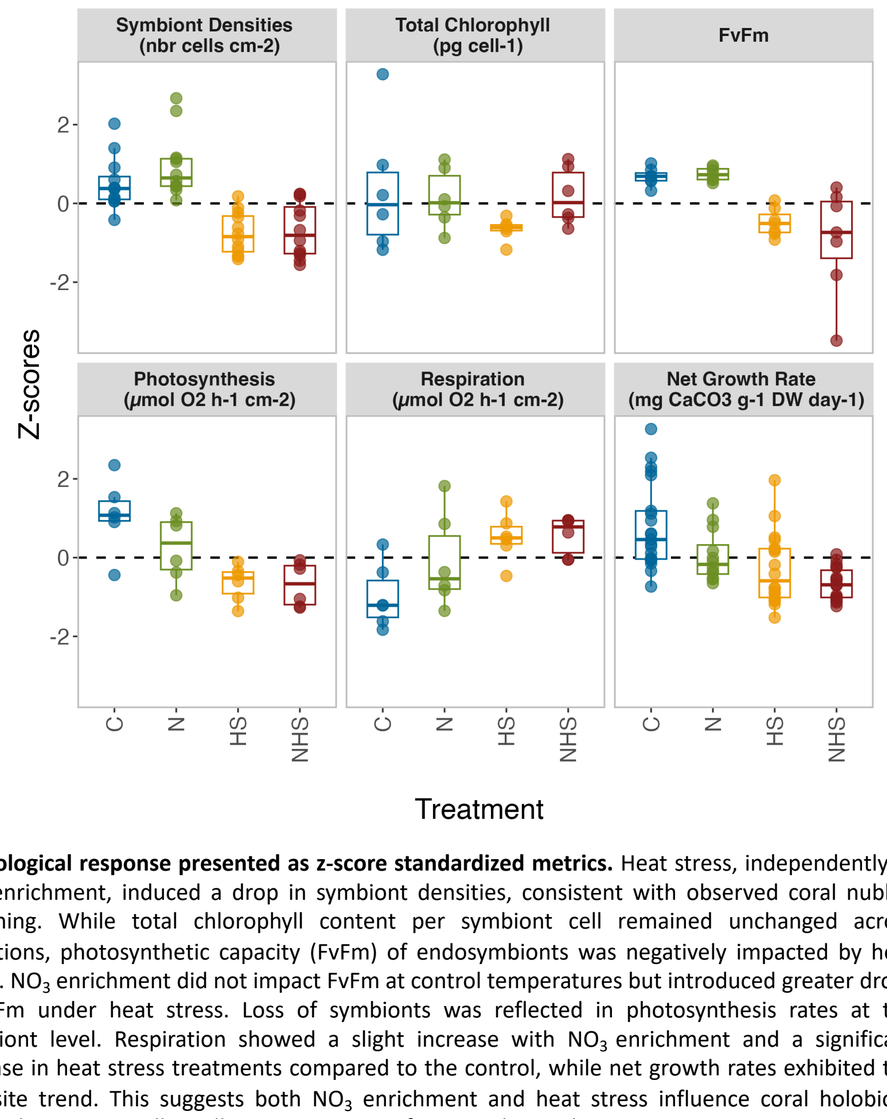


Effect of treatment on ß-diversity of *A. kenti* microbial communities. Constrained CCA ordination (Bray-Curtis distances) reveals distinct groupings of samples by treatments, with distinct centroids and dispersion. This suggests that a portion of the observed variance in microbial communities across samples is explained by the treatments. Heat stress and nitrate enriched communities show greater dispersion compared to control communities.

PERMANOVA results (9999 permutations)	R2	F	P
Treatment	0.117	2.68	0.0002 ***
Genotype	0.066	1.51	0.0284 *
Treatment * Genotype	0.135	1.03	0.3841

PERMANOVA test: Significant effects are observed for both treatment and genotype, indicating their individual contributions to the differences in community composition. Genotype, while significant, has a comparatively lesser impact. The interaction between treatment and genotype is not significant, indicating that treatment effect is consistent across genotypes.

RESULTS - PHYSIOLOGY



Physiological response presented as z-score standardized metrics. Heat stress, independently of NO_3 enrichment, induced a drop in symbiont densities, consistent with observed coral nubbin bleaching. While total chlorophyll content per symbiont cell remained unchanged across conditions, photosynthetic capacity (FvFm) of endosymbionts was negatively impacted by heat stress. NO_3 enrichment did not impact FvFm at control temperatures but introduced greater drops in FvFm under heat stress. Loss of symbionts was reflected in photosynthesis rates at the holobiont level. Respiration showed a slight increase with NO_3 enrichment and a significant increase in heat stress treatments compared to the control, while net growth rates exhibited the opposite trend. This suggests both NO_3 enrichment and heat stress influence coral holobiont metabolism, potentially reallocating resources from coral growth to stress response.

CONCLUSIONS

When subjected to heat stress, the coral *Acropora kenti* exhibited clear signs of disrupted metabolism. To a lesser extend, NO_3 enrichment also influenced coral metabolism without inducing visual signs of stress, such as bleaching. NO_3 did not enhance resilience to heat stress. Interestingly, all treatments significantly affected the coral-associated microbiome, which could play a vital role in coral resilience in the face of environmental stress.

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