# Temporal sampling of white band disease infected corals reveals complex and dynamic bacterial communities

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## Introduction

White band disease (WBD) is an infectious Caribbean coral disease affecting *Acropora cervicornis* and *A. palmata.* WBD is likely bacterial, but a pathogen has not been confirmed and very little is known about how the microbial community responds to infection. Previous work found enormous variablility in the microbiomes of *A. cervicornis* across both disease state and site. *Flavobacteriales, Rickettsiales, Rhodobacterales,* and *Vibrionales were* associated with diseased corals, but whether this association was a cause or effect of disease is unknown.

## Experimental



### Design

## Results

At T1, **Genotype** has a significant effect on the microbiome (PERMANOVA, $p=0.001$, $R^2=0.18$)

## Results

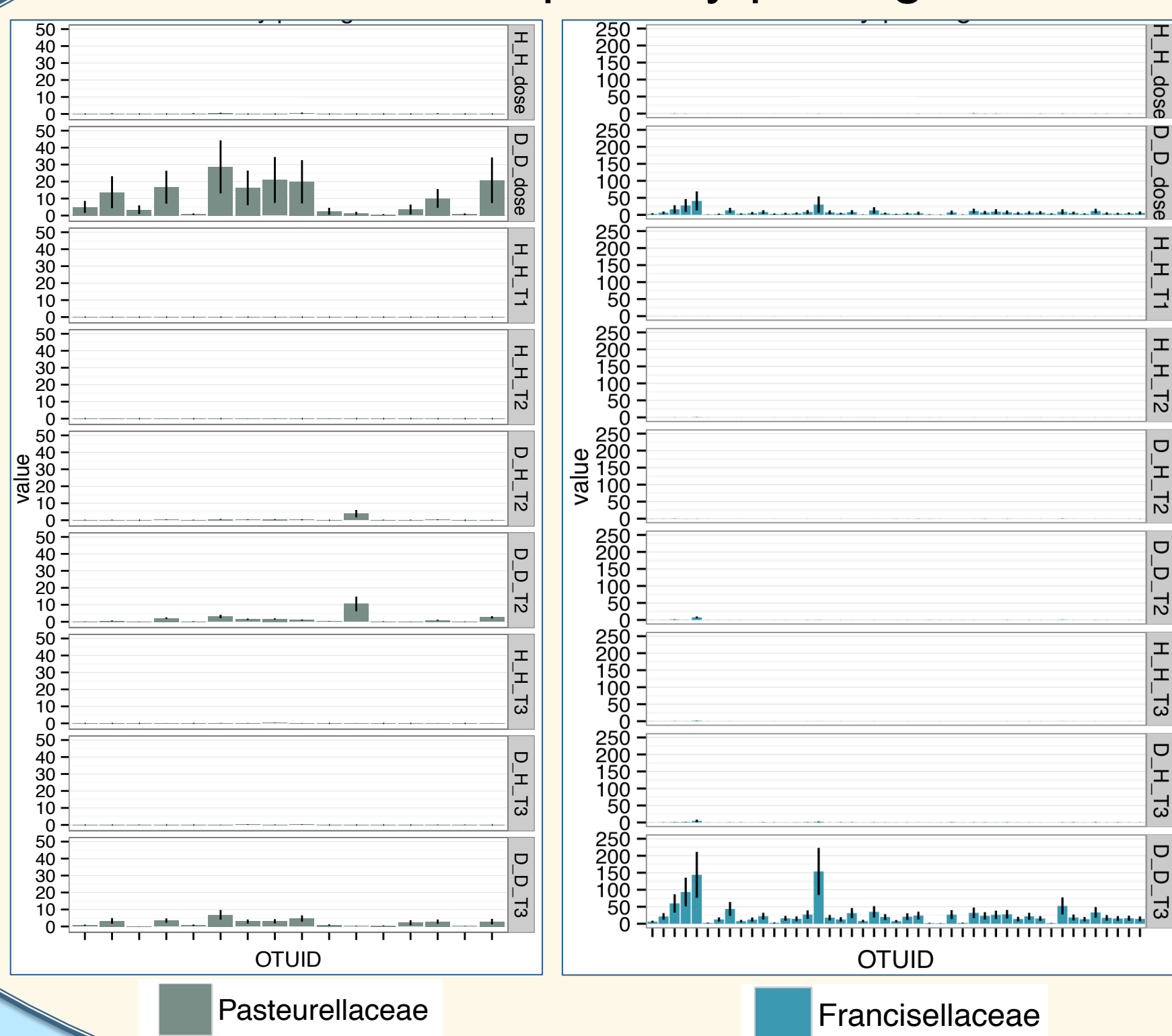What affects community structure at T2 and T 3?
PERMANOVA: significant effects

| Effect | $R^2$ | p |
|---|---|---|
| T3 disease state | 0.028 | 0.001 |
| Dose disease state | 0.012 | 0.001 |
| Timepoint | 0.043 | 0.001 |
| Dose site | 0.014 | 0.001 |
| Dose disease state x Timepoint | 0.012 | 0.001 |
| Dose disease state x Dose site | 0.017 | 0.001 |
| Timepoint x Dose site | 0.013 | 0.001 |
| Dose disease state x Timepoint x Dose site | 0.010 | 0.001 |

## Aim

We seek to better understand the roles coral-associated bacteria are playing by examining bacterial abundance over time.

## Methods

Corals were sampled for sequencing at 3 time points:
- **Time 1**—After collection
- **Time 2**—10 hrs after inoculation homogenate (D or H)
- **Time 3**—when corals displayed disease signs (healthy sampled at same time)

Amplified V6 hypervariable region of 16S
Sequenced on the Illumina Hiseq 2000

## Results
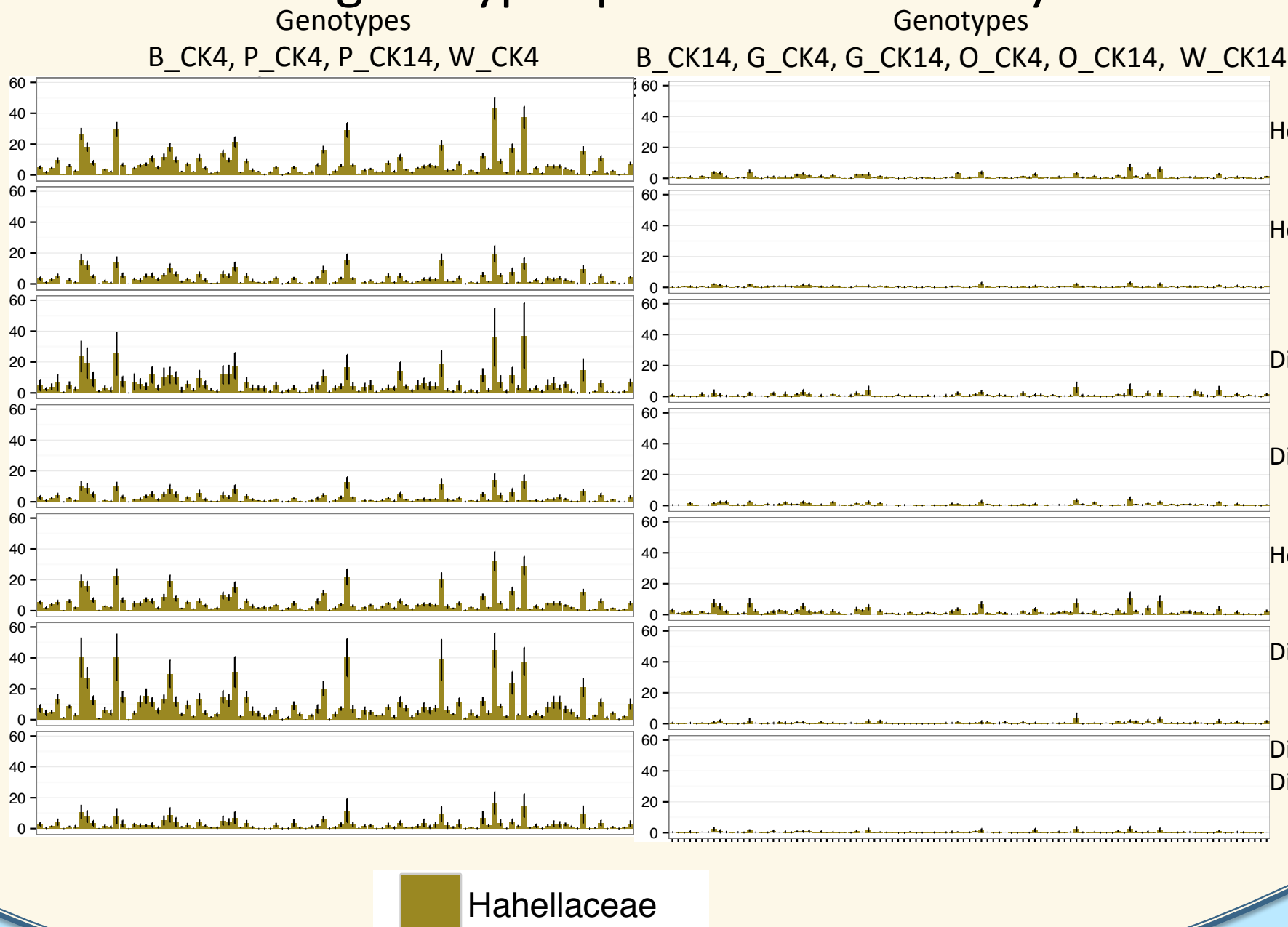
Putative primary pathogens:



T3 disease state: significant

Genotype, site, dose site, interactions: not significant

More abundant in diseased than healthy: doses, T2, and T3

250 OTUs

## Results

Secondary Colonizers
T3 disease state: significant
Dose disease state x Timepoint: significant
Up in T3

| Family | Freq |
|---|---|
| Campylobacteraceae | 132 |
| Shewanellaceae | 16 |
| Pasteurellaceae | 10 |
| Oceanospirillaceae | 8 |
| Pseudoalteromonadaceae | 8 |
| Enterobacteriaceae | 7 |
| Idiomarinaceae | 5 |
| Marinilabiaceae | 5 |
| Vibrionaceae | 4 |
| Alteromonadaceae | 3 |
| Flavobacteriaceae | 3 |
| Bacteriovoraceae | 2 |
| Hahellaceae | 2 |
| Methylococcaceae | 2 |
| Moraxellaceae | 2 |
| Rubritaleaceae | 2 |
| Caedibacter | 1 |
| Desulfovibrionaceae | 1 |
| Flammeovirgaceae | 1 |
| Francisellaceae | 1 |
| Oleiphilaceae | 1 |
| SB-1 | 1 |
| Staphylococcaceae | 1 |

## Methods

Reads were overlapped with FLASH, demultiplexed and quality filtered using a Python script. Sequences were clustered at 97% using QIIME and assigned taxonomy with BLAST against silva. PERMANOVA was run with the R package vegan (adonis). Quasipoisson GLMMs were run for each OTU using R packages MASS (GLMMPQL) and Car (Anova Type II SS). For time 2 and 3 corals: fixed effects: T3_disease_state + Site*Dose_disease_state*Timepoint*Dose_Site
Random effects: Tank/Timepoint
For time 1 corals: Fixed effects: Genotype Random effects: Tank
Plots made with ggplot2
Scripts available here: https://github.com /sagw

## Results

Hahellaceae are genotype specific beneficial symbionts



Genotype: Significant

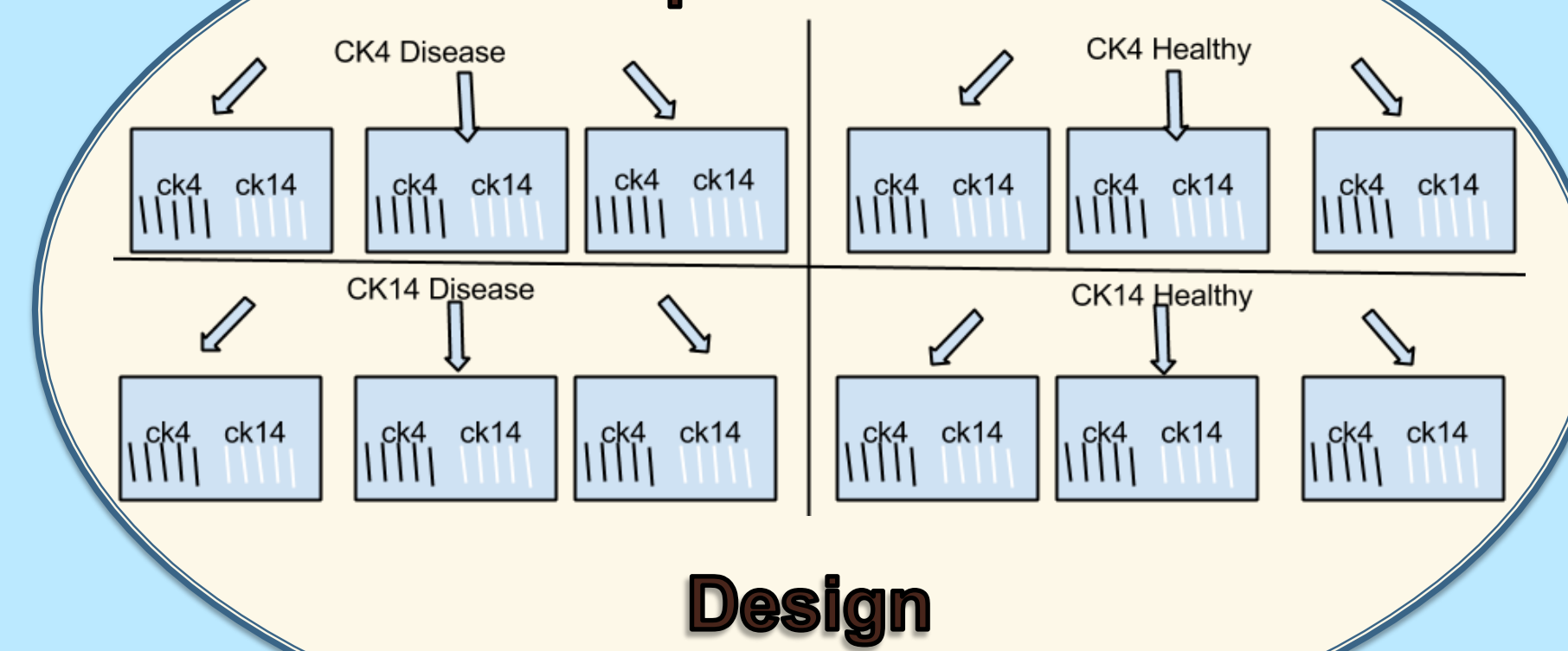T3 disease state: Significant

Healthy > Disease T1, T2, T3