

COMMERCIAL DEPURATION OF THE CARPET SHELL CLAM (*Ruditapes decussatus*) USING HUMIC SUBSTANCES AS A MICROBIOME MODULATOR.

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Introduction

- Depuration is a vital in ensuring the safety and quality of commercial clams for consumption. As filter feeders, bivalves accumulate the contaminants found in their environment.
- Coadjutants in depuration can be used to improve safety and shelf-life of seafood products and may include chelating and microbiome modulating agents.
- Humic substances (HS) may enhance depuration by binding and purging pollutants, offering a cost-efficient and environmentally friendly solution.
- In this study, the potential use of HS as a depuration coadjutant is evaluated in respect to its ability to modulate bacterial communities of carpet shell clams (*Ruditapes decussatus*) during depuration.

Methods

- Two depuration systems were tested over a twenty-six-hour period, each with three replicate tanks:
 - Control (Cn): no coadjutant
 - Treatment (Hs): a water-soluble HS product (Humic Powder, FulviXcell) at final concentration of 2.5mgL⁻¹.
- Post-depuration, clams were stored at 5 ± 1 °C for 6 days to simulate *shelf-life* conditions.
- DNA was extracted from composite samples (n=4) of gastrointestinal tracts of clams sampled before depuration (St), after depuration (CnD and HsD) and after *shelf-life* (CnS and HsS).
- Bacterial composition was analyzed using high-throughput sequencing data of the hypervariable V3/V4 region of the 16S RNA gene.

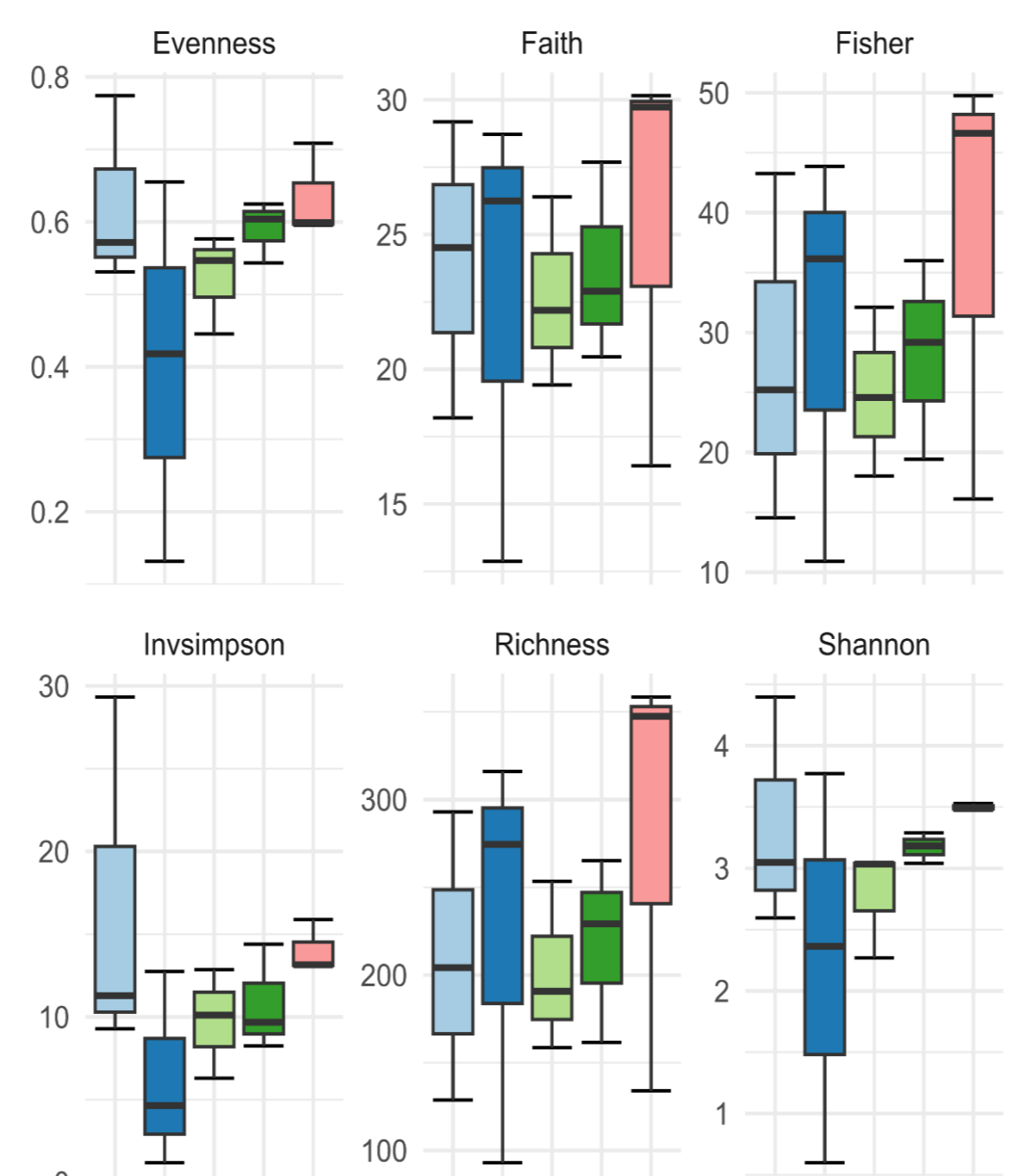


Figure 1 – Boxplot of α -diversity indexes: Shannon's H (Shannon); Inverted Simpson (Invsimpson) Pello's evenness (Evenness); Richness, Fisher's α (Fisher) and Faith phylogenetic distance index (Faith) of bacterial communities of clams before depuration (Start – St), after depuration (CnD), after depuration with humic substances (HsD), after depuration and after 'shelf-life' (CnS) and after depuration with HS and after 'shelf-life' (HsS).

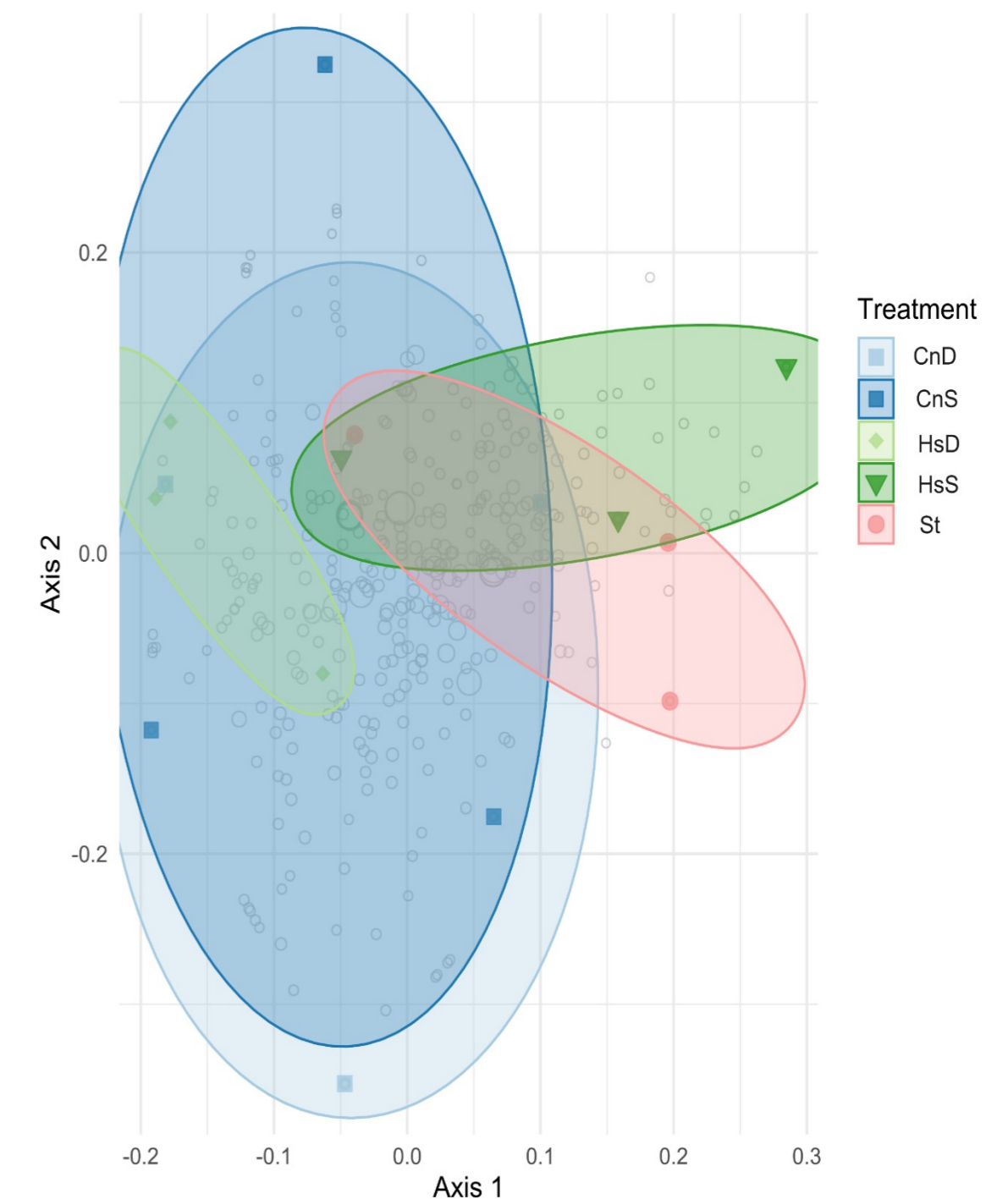


Figure 2 - First two axes of principal coordinates analysis (PCO) of ASV composition of bacterial communities of clams before depuration (Start – St), after depuration (CnD), after depuration with humic substances (HsD), after depuration and after 'shelf-life' (CnS) and after depuration with HS and after 'shelf-life' (HsS). Colored symbols are samples. Grey circles are weighted averages scores for ASV (size is proportional to abundance).

Results

- HS supplementation did not alter alpha-diversity parameters measured (Figure 1; Kruskal-Wallis: $P > 0.05$).
- Both HS addition and *shelf-life* storage were significant predictors of ASV composition (Figure 2, PERMANOVA: HS-addition: $R^2 = 0.154$ $P = 0.009$; *Shelf-life*: $R^2 = 0.079$; $P = 0.02$).
- Taxonomic composition of bacterial communities was dominated by the orders Mycoplasmatales and Rickettsiales (Figure 3).
- Random forest analysis detected classes Clostridia and Planctomycetia and families Fusobacteriaceae, Marinilabiliaceae and Metamycoplasmataceae as significant predictors of experimental variables.
- Abundance of Metamycoplasmataceae was highest in HS-depurated clams, but after *shelf-life* their relative abundance decreased to levels comparable to those observed in the control (Figure 4).
- Abundance of class Clostridia, which includes important food-borne pathogens, i.e. *Clostridium botulinum*, *C. perfringens*, and *C. difficile* and beneficial animal symbionts, i.e., *Epulopiscium* sp. and *C. butyricum*, was most differential in relation to timepoints with no difference in relation to HS addition.

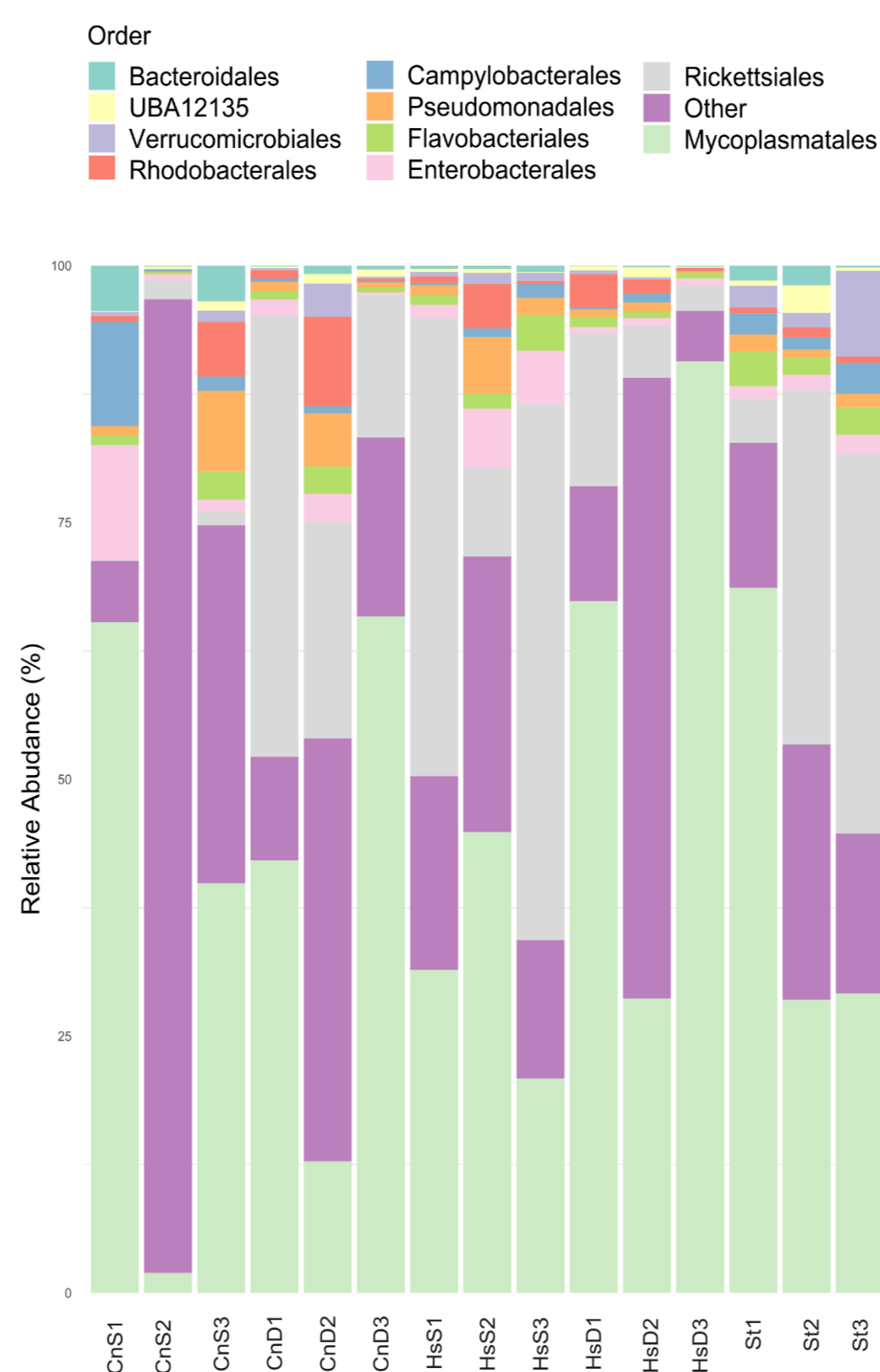


Figure 3 – Taxonomic composition at the order level of each sample of bacterial communities of clams before depuration (Start – St), after depuration (CnD), after depuration with humic substances (HsD), after depuration and after 'shelf-life' (CnS) and after depuration with HS and after 'shelf-life' (HsS).

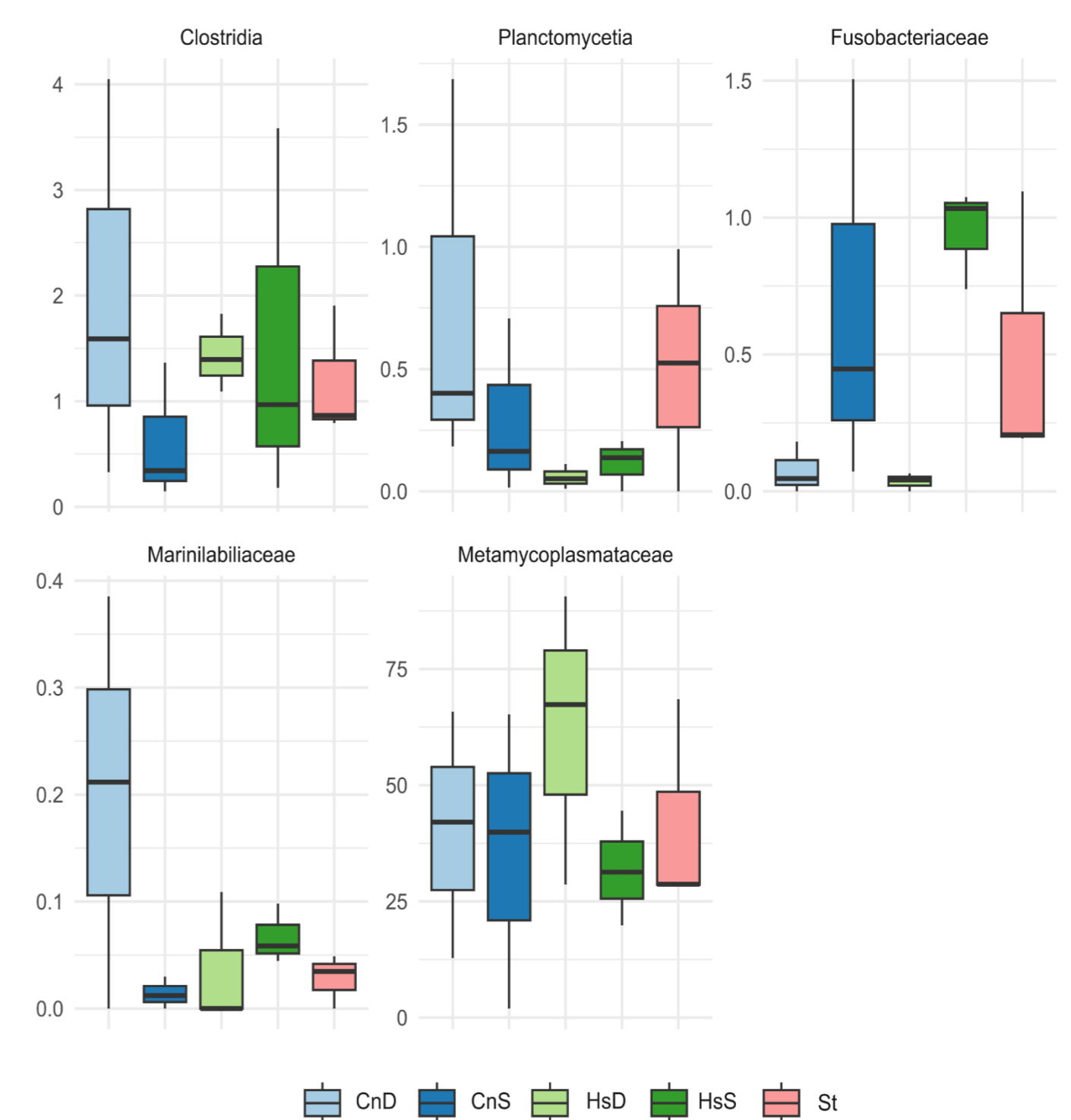


Figure 4 – Boxplot of relevant groups of bacterial communities detected in the random forest analysis of clams before depuration (Start – St), after depuration (CnD), after depuration with humic substances (HsD), after depuration and after 'shelf-life' (CnS) and after depuration with HS and after 'shelf-life' (HsS).

Conclusion

- HS-based depuration modulated clam bacterial communities.
- Both HS and *shelf-life* resulted in significant alterations to the ASV composition and to key taxonomic groups with importance to food quality.
- A higher abundance in family Metamycoplasmataceae in HS-depurated clams was detected, but their relative abundance decreased to values similar to the control overtime.
- Future studies should explore the relevance of HS in modulating clam bacterial communities, focusing on the implications for animal health and food security.

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