IN SITU DOM REMOVAL AND NUTRIENT CYCLING BY DOMINANT EMERGENT SPONGES IN THE FLORIDA KEYS

A Thesis
by
JACQUELINE G KELEHER

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Abstract

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Coral reefs are biodiverse and productive ecosystems that are found in typically oligotrophic (low nutrient) environments. The focus of research on nutrient acquisition and partitioning among coral reef organisms has, historically, been focused on corals and their symbiotic zooxanthellae. For example, different clades of zooxanthellae are adapted to different irradiance levels which facilitates coexisting species of corals. Comparatively very few studies have asked if and how coexisting sponges and their symbiotic microbial communities partition nutrients (i.e., utilization of different nutrient pools across species). To address this question, this study set up an artificial reef off the coast of the Florida Keys using dominant emergent sponges found in the Caribbean and Florida Keys. Inhalant and exhalant water samples were collected using Vacusip, samples were filtered with a 0.2 μm supor filter then processed for dissolved nutrients using fluorescence dissolved organic matter (fDOM), total dissolved nitrogen (TDN), and dissolved organic carbon (DOC). This is the first

application of fDOM analysis to sponges. In this study, I found that the microbial abundance (i.e. the commonly used high and low microbial abundance (HMA/LMA) classification) was not an effective indicator for the way in which nutrients are processed by these sponge species. Additionally, the use of fDOM analysis indicated nuance in DOM utilization across species with differential consumption of fDOM components across sponge species. In summary, rather than microbial abundance alone, a combination of sponge species identity and the composition of the symbiotic microbial community members (e.g., presence of photosymbionts) appears to explain the most variation in nutrient processing by sponges. These results provide the first support for resource partitioning of dissolved nutrients across coexisting sponge species and provide support for the evolutionary importance of microbial communities in sponges.

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Dedication

This is for all the girls who aren't afraid to jump in the puddles, tromp through the woods, and swim in the tidepools, it isn't easy, but it is worth it.

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PURPOSE OF STUDY

Coral reefs are biodiverse and productive ecosystems despite being surrounded by oligotrophic seawater. Historically there has been a strong focus of studies on particular microfauna such as the corals, and fish that live in tropical reef systems, these studies have yielded insight into mechanisms of nutrient retention and partitioning within coral reefs. Specifically, tight nutrient recycling within the coral holobiont (coral plus zooxanthellae and associated prokaryotes) has been observed along with resource partitioning of light and/or limiting nutrients such as nitrogen by different zooxanthellae (algal symbionts) across coral species on a reef (Muscatine, Porter & Kaplan, 1989; Muscatine & Kaplan, 1994). Additionally, the presence of diverse fish communities generally supports a healthy reef (Beita-Jiménez et al., 2019) and there is often resource partitioning by coexisting fish species (e.g., Brandl, Casey & Meyer, 2020). Studies of sponges, by comparison, are relatively lacking, despite the prevalence of sponges on many coral reefs and there is limited understanding of the forces (e.g., nutrient availability, predators) that have shaped the present community assemblage of sponges. The primary purpose of this study is to examine resource partitioning in coexisting sponge species and more specifically, to better understand how sponge holobionts, the host sponges and their symbiotic microbial communities, differentially utilize and release dissolved organic matter across species. This project is a portion of a larger study that will provide a deeper understanding of the role of sponges in nutrient cycling on reefs in the Florida Keys and insight into ecological and evolutionary forces that have shaped sponge community assemblages in the Caribbean. Such data provide essential context for investigating the impact of current environmental stressors on reef structure.

INTRODUCTION

Coral Reefs

Historically coral reefs have been viewed as dominated by corals which are primary reef builders (Darwin & Fitzroy, 1842). Coral reefs are also commonly described as areas of high biomass and high species diversity in otherwise nutrient poor environments (Connell, 1978; Bellwood et al., 2019). This high diversity is due, in part, to the high rates of turnover of dissolved organic matter on coral reefs, tight recycling of nutrients by coral reef organisms, and nearshore enrichment from deep water nutrient sources (Wild et al., 2004; de Goeij et al., 2013; Gove et al., 2016; Morais & Bellwood, 2019).

Descriptions of reefs from early scientists like Darwin no longer fit the majority of the reefs that we see today (Darwin & Fitzroy, 1842; Bellwood et al., 2019). Over the past century there have been drastic changes in the structure and function of coral reef ecosystems (Bellwood et al., 2019). Specifically, coral cover has declined as ocean warming, coral bleaching events, and outbreaks of disease have increased, threatening the structure and stability of coral reef ecosystems (Bruno & Selig, 2007; Eakin et al., 2010; Hughes et al., 2017, 2018; Muller et al., 2020). While there have been advances in our understanding of some of the factors involved in the decline of many coral reefs (e.g., connections between algal communities and coral disease through the release of dissolved organic carbon), there are in fact fundamental questions yet to be addressed about the community structure and function of coral reefs, particularly involving the relatively under-studied organismal groups such as sponges.

Coral reefs found in the Caribbean and North Atlantic make up 14% of the world's coral reefs, including the Florida Keys which is one of the largest reefs in the world (Smith, 1978; Lapointe et al., 2020). Since the mid- to late- 1990's coral reefs in the Caribbean and Florida

Keys have been recognized as disease hotspots for corals because of how quickly diseases have emerged in these coral reefs (Weil, Smith & Gil-Agudelo, 2006; Muller et al., 2020). Stony coral tissue loss disease has ravaged the corals in the coral reefs in the Florida Keys often leaving entire colonies dead (Muller et al., 2020). Simultaneously, there has been a suggestion that sponges have increased on Caribbean reefs, particularly in regions of coral loss (Bell et al., 2013), although there is debate on this topic given the variable distribution of sponges (Wulff, 2001; reviewed in Bell et al., 2018) and variable outcomes of experimental stressors such as temperature and pH on sponge survival (e.g., Beepat et al., 2020).

The community composition of coral reefs is driven by species interactions, local environmental effects like nutrient input, occurrence of disease, and climate change (Muller et al., 2020; Page et al., 2021). Additionally, microbial symbionts, such as single-celled algae or bacteria or archaea, can influence species distribution by facilitating transfer and recycling of nutrients within a host organism such as corals or sponges (Wilkinson & Fay, 1979; Kvennefors et al., 2010; Nelson et al., 2011; de Goeij et al., 2013). The term 'symbiont' refers two or more organisms living together regardless of the outcome as beneficial or not, as defined by de Bary (Oulhen, Schulz & Carrier, 2016). The productivity from such symbiotic interactions supports the high species diversity of coral reefs and the resulting ecosystem services of coral reefs (Spurgeon, 1992). There is similar support for the role of microbial symbionts in expanding the metabolism in host sponges and thus, these symbionts may have an evolutionary impact on the species assemblages of sponge communities today, although empirical studies examining this hypothesis are limited and represent an important area of future work in coral reef ecology (Freeman, Easson & Baker, 2014; Morganti et al., 2016; Freeman et al., 2020a). Addressing this hypothesis is the goal of the research presented here.

Sponge Biology

Sponges are the oldest extant metazoans on earth, they belong to the phylum Porifera which is defined by sessile, filter-feeding organisms with complex aquiferous systems (Müller & Müller, 2003; Borchiellini et al., 2008; Van Soest et al., 2012). There are between 8,500-11,000 known species of extant sponges (Van Soest et al., 2012). They live in a variety of aquatic environments and grow in a variety of shapes and sizes, with some freshwater species being a few centimeters to some marine sponges being large enough for a human to sit inside (Downey et al., 2012; Pawlik et al., 2015). Sponges have been used by civilizations since prehistoric times, but were first described scientifically by Robert Grant in 1836 when he called them porifera, since then spongeology, or the study of the biology, ecology, taxonomy, and chemistry of sponges, has become an ever growing field (Voultsiadou et al., 2011; Van Soest et al., 2012). Despite their morphological simplicity, abundance in the world's oceans, and the growing number of spongeologists, they are not well studied, and there are still debates about how they should be classified in the animal kingdom (Dunn, Leys & Haddock, 2015; Simion et al., 2017; Feuda et al., 2017; Nielsen, 2019; Redmond & McLysaght, 2021).

Sponges have specialized cells with flagella called choanocytes that help pump water through their body. These choanocytes are packed in the inhalant and exhalant canals (ostia and osculum) of the sponge to help move water through the sponge. Most sponges have complex morphologies termed "leuconoid", while a few calcareous sponges have less complex systems termed "asconoid" or "syconoid"(Reiswig, 1975a; Manuel, 2006). Asconoid forms of calcareous sponges grow as a single tube with a short canal system and their choanocytes form in a single layer on the inner surface of the sponges spongocoel, which is the central cavity of the sponge

(Lavrov et al., 2022). Syconoid forms are similar but instead of being a single tube, their body is composed of several tubes, as if several asconoids were growing out of one base (Figure 1) (Asadzadeh et al., 2020). The vast majority of sponges on coral reefs are leuconoid with well-developed canals and choanocyte chambers for filtering seawater and belong to the class Demospongiaea. These demosponges exhibit different growth morphologies such as rope form, vase, or mound, and the aquiferous system also varies in extent across sponge species (i.e., density of choanocyte chambers). The extent of development of the aquiferous system may be influenced by the abundance of the symbiotic microbial community (e.g., Poppell et al. 2014) which is thought to be linked to whether a sponge species relies more on filtering seawater or relies on its microbial community as a source of nutrients.

Sponge Symbiotic Microbial Communities

Since the early 1900s spongeologists have known that sponges were hosts to microorganisms in their mesohyl, which is most of the sponge tissue except for the outermost layer of cells (Dosse, 1939; Gloeckner et al., 2014). Later it was seen that certain species had higher densities of microbes while others' mesohyl (inner layers of sponge tissue) seemed to be completely lacking in microbes (Reiswig, 1974; Vacelet & Donadey, 1977; Wilkinson, 1978). Reiswig (1974) referred to these two categories of microbial abundance as "bacterial sponges" and "non-symbiont harboring, normal sponges" but later the terms were changed to "high microbial abundance" and "low microbial abundance" (HMA and LMA respectively) (Reiswig, 1981; Hentschel et al., 2003). In general, HMA sponges have 10^8-10^{10} microorganism per gram of sponge tissue, which is 2-4 orders of magnitude more concentrated than seawater and is 20-35% of the biomass of the sponge while LMA sponges have 10^5-10^6 bacteria per gram of sponge

tissue, which is within the range of seawater microbial densities (Hentschel, Usher & Taylor, 2006; Taylor et al., 2007; Hentschel et al., 2012).

Sponges and their symbiotic microbial communities play a key role in the transfer of nutrients from pelagic to benthic food webs with some having the ability to selectively feed while others can change pumping rates to utilize available resources more efficiently (de Goeij et al., 2013; Reiswig, 1971; Hansen et al., 2009). Some differences in feeding strategy and morphology (e.g., choanocyte chamber density) can be seen between HMA and LMA sponges. For example, a study done on 8 Caribbean sponges showed HMA sponges had a lower pumping rate and removed less particulate organic matter (POM) when compared to LMA sponges (Weisz, Lindquist & Martens, 2008; Poppell et al., 2014; Mueller et al., 2014).

Historically, the difference in the acquisition of nutrients and morphological traits, has been used to support the idea that HMA and LMA species have completely different life strategies (Vacelet & Donadey, 1977; Weisz, Lindquist & Martens, 2008). However, as studies have more closely examined the microbial communities of sponges, nuances have emerged in the abundances of symbionts that supports the idea of a continuum of abundance and composition of sponge symbiotic microbial communities rather than two purely separate strategies, (Easson & Thacker, 2014; Thomas et al., 2016; Turon et al., 2018). This work has led to a shift in the paradigm of microbial symbiont abundances, setting up the hypothesis that there is evolutionary investment by sponges in their microbial communities and that these symbionts have had some role in shaping the trajectory of sponge assemblages on coral reefs (Freeman et al., 2020a).

Symbiotic Microbial Community Composition

Over the past few decades there has been an increased interest in the metabolites of sponge holobionts due to the potential uses they have pharmaceutically and industrially as well as to gain a better understanding of how their metabolites are utilized by sponges and other reef organisms (Faulknet et al., 2000; Marzuki, Kamaruddin & Ahmad, 2021; Freeman et al., 2013; Fiore, Jarett & Lesser, 2013; Freeman et al., 2020a; de Goeij et al., 2013). Microbial symbionts expand the amount of nutrients accessible to their hosts by breaking down complex molecules and making them bioavailable, especially in locations that would otherwise be nearly inhospitable due to a lack of available nutrients (Moran, 2007; Taylor et al., 2007; Hentschel et al., 2012; Rubin-Blum et al., 2019; Zhang et al., 2019). Different types of microbial symbionts utilize different fractions of dissolved and particulate organic matter in the water, for instance heterotrophic symbionts convert dissolved organic carbon (DOC) into microbial biomass and CO_2, while and autotrophic symbionts such as photosymbionts (primarily *Cyanobacteria*) and nitrifying symbionts may produce some DOC and likely influence the forms of bioavailable nitrogen within the sponge host (Weisz et al., 2007; Freeman & Thacker, 2011; Freeman et al., 2013). Nitrifying microbes are prevalent in marine sponges and may be part of a 'core' symbiont community (Thomas et al. 2016). These include the *Nitrososphaerota* (syn. *Thaumarchaeota*), which are archaea that oxidize ammonia to nitrite, and *Nitrospira* spp. which oxidize nitrite to nitrate.

Studies that have looked at metagenomes of sponge associated microbes have found similar features, or core functions, that are considered to be relevant to symbiosis (Thomas et al., 2010; Fan et al., 2012; Hentschel et al., 2012; Horn et al., 2016). These core functions (Table 1) have been found in sponges from around the globe and are not carried out by the same symbionts in each sponge, but rather by a variety of similar mechanisms (Ribes et al., 2012; Fan et al.,

2012). These core functions span a range of features, such as nitrogen cycling functions and the presence of eukaryotic-like domains in some proteins that allow sponge symbionts to interact with and adapt to the host sponge (Pita et al., 2018).

Recent studies looking at the community composition of sponge holobionts have found 41 bacterial phyla (including candidate phyla) with the most prevalent phyla being proteobacteria. Other primary phyla included *Actinobacteria*, *Chloroflexi*, *Nitrospirae*, *Cyanobacteria*, and *Poribacteria* (Thomas et al., 2016; Bayer et al., 2018; Pita et al., 2018). Sponges traditionally classified as HMA have been found to be enriched in *Chloroflexi*, *Acidobacteria,* and *Poribacteria* while those traditionally classified as LMAs are enriched in *Proteobacteria* and *Cyanobacteria,* the latter may be transient bacteria from the water column. However, despite the differences in community composition, the core functions carried out by the symbionts span the perceived dichotomy of microbial abundances (Pita et al., 2018). For example, there are many proposed pathways for which carbon, nitrogen, sulfur and vitamins may be exchanged between the model sponge species *Ircina ramosa* and its symbiotic microbes (Figure 2, Engelberts et al., 2020).

Dissolved Organic Matter

Natural organic matter (NOM) is a complicated mixture composed of organic compounds that come from decaying plant and animal tissue (Nebbioso & Piccolo, 2013; Sillanpää, Matilainen & Lahtinen, 2015). NOM is split into two categories, they are defined as matter that can pass through a 0.45-µm filter (dissolved organic matter, DOM) and matter that gets trapped on a 0.45-µm filter (particulate organic matter, POM) (Figure 3) (Thurman, 1985). Dissolved organic matter is a complex mixture of nutrients found in water and is relatively inaccessible to

most coral reef dwelling organisms (de Goeij et al., 2013; Rix et al., 2016b, 2018). DOM is primarily produced by benthic primary producers and is the largest organic resource on coral reefs (Tanaka et al., 2011; Haas et al., 2011; Atkinson & Falter, 2020). It is well established that corals and algae aid in cycling DOM on reefs and in the past decade sponges have also been found to take part in the cycling of DOM on reefs (de Goeij et al., 2013; Rix, 2015; Rix et al., 2016b, 2018). The "sponge loop" pathway (Figure 4), first described by de Goeij et al 2013, explains how sponges are able to facilitate the transfer of benthic DOM to higher trophic levels by converting it into biomass and then shedding cells as particulate organic matter (POM) (de Goeij et al., 2013).

DOM can be categorized in several ways, including how long it exists in the water under normal conditions, molecular weight, and composition (Benner et al., 1992; Moran et al., 2016). DOM reactivity, or the amount of time DOM exists in an environment under normal conditions, it is split into three categories: labile, semi-labile, and refractory. Labile DOM is DOM that does not exist in an environment for very long, it is consumed by microbes within a matter of hours or days. Semi-labile DOM is less reactive, meaning it lasts for weeks or years before it is degraded. Refractory DOM is the least reactive and can last in the ocean for millennia (Moran et al., 2016). When categorizing DOM by molecular weight there are two categories: high molecular weight and low molecular weight. High molecular weight DOM thought to generally be refractory and includes molecules like proteins and large humic molecules (Benner et al., 1992). Low molecular weight DOM is thought to include both more labile components like sugars, amino acids and vitamins and more recalcitrant molecules like complex humics and plant-derived lignin (Carlson et al., 1985; Benner et al., 1992). Low molecular weight DOM also makes up somewhere between 60-80% of DOM (Carlson et al., 1985; Benner et al., 1992; Ogawa & Ogura, 1992;

Amon & Benner, 1994). The primary subsections of DOM that this study is focusing on are: dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and fluorescent DOM (fDOM) (Figure 5).

Dissolved Organic Carbon

Dissolved organic carbon, DOC, is typically a complex mixture of all labile and refractory molecules and is considered a 'bulk' measurement of DOM (de Goeij & van Duyl, 2007). Reefs have enhanced levels of DOC compared to oceanic surface water because the production of DOC on coral reefs exceeds what reef organisms use (Johannes, 1967; Pagès, Torréton & Sempéré, 1997; van Duyl & Gast, 2001). DOC is released by algae and corals and consumed by a variety of organisms including bacteria (Johannes, 1967; Richman, Loya & Sloboclkin, 1975; Azam et al., 1983; Fenchel, 1988). Historically it was thought that sponges were purely suspension feeders, consuming plankton (Reiswig, 1971, 1975b; Vacelet & Boury-Esnault, 1995; Pile et al., 1997; Ribes, Coma & Gili, 1999; Perea-Blázquez, Davy & Bell, 2012). However, Reiswig hypothesized that they may consume DOC which was later confirmed by several studies (Reiswig, 1974; Yahel et al., 2003; de Goeij et al., 2008, 2013; Ribes et al., 2012; Mueller et al., 2014). Recently, it has also been shown that DOC is consumed and released by sponges and their microbial symbionts (Rix et al., 2016b; Hudspith et al., 2021).

In the early 1970s and 80s Reiswig showed that there was an incongruity with the metabolic demands of sponges and the amount of carbon that they consumed through particulate organic carbon (POC), this gap in carbon demand is filled by sponges ability to utilize DOC (Reiswig, 1971, 1974, 1981; Yahel et al., 2003). There was a hypothesis that due to their higher pumping rates and elevated retention of POM, that LMA sponges did not consume DOC (Wesiz 2008). However, it is now well documented that DOC is the primary source of carbon for

sponges, and it has been shown that sponges in both the LMA and HMA categories utilize DOC. Sponges use DOC to build tissue; for example, the sponge *Xestospongia muta* relies primarily on DOC for its carbon budget (96%) with the remainder being particulate organic carbon and detritus (Yahel et al., 2003; Hoer et al., 2018; Bart et al., 2021).

Role of Nitrogen on Coral Reefs and in Sponges

Nitrogen is the second most abundant molecule on earth, but it is primarily found as dinitrogen (N_2) which is not bioavailable to most organisms (Zehr & Capone, 2020). Because of this, nitrogen is a limiting factor in primary production of the ocean that influences the availability of nutrients to the system (Ward, Capone & Zehr, 2007; Fiore et al., 2010). Microbes are vital to making N_2 available to other organisms and even play a part in each of the 5 steps of the oceanic nitrogen cycle: fixation, nitrification, denitrification, anaerobic ammonium oxidation, and remineralization which control the available nitrogen in the ocean (Ward, Capone & Zehr, 2007; Zehr, 2009; Maldonado, Ribes & van Duyl, 2012).

Traditionally two types of *Cyanobacteria*, free-living *Trichodesmium* and diatom symbiont *Richelia,* were thought to be responsible for the majority of N_2 fixation, however, recent advances have found that other *Cyanobacteria* as well as other diazotrophs can fix nitrogen (Zehr & Capone, 2020). N_2 fixation occurs in a variety of environments in the ocean, from hydrothermal vents, sediments, surface ocean water, and in some corals and sponges (Mehta & Baross, 2006; Fiore et al., 2010; Knapp et al., 2016; Middelburg et al., 2016; Dekas et al., 2018; Zehr & Capone, 2020). Sponges host a variety of microbes that appear to only be found in sponges and are important in nitrogen cycling (Steindler et al., 2005; Taylor et al., 2007; Southwell, Popp & Martens, 2008; Southwell et al., 2008; Mohamed et al., 2008, 2010).

Interestingly, nitrogen cycling [nitrogen fixation, nitrification, denitrification, dissimilatory nitrate reduction to ammonia (DNRA)] has been observed as an important trait found in many sponge symbiotic communities that is present across sponge species and was considered less important for free-living microbial communities (Thomas et al., 2010; Fan et al., 2012). Additionally, there is support for transfer of nitrogen between symbionts and host sponge, which may vary across species more so than the transfer of carbon, indicating an important role of nitrogen in sponge-microbe symbiosis and that nitrogen may be important in nutrient partitioning in sponges (Freeman & Thacker, 2011; Fiore, Jarett & Lesser, 2013; Freeman et al., 2020b).

Colored Dissolved Organic Matter and Fluorescent Dissolved Organic Matter

Some DOM interacts with radiation and is called colored, or chromophoric (CDOM), and is defined as the DOM that absorbs light in the blue and UV spectra and can appear colored to the naked eye (Nelson, Siegel & Michaels, 1998; Coble, 2007). Within CDOM falls fluorescent DOM (fDOM) which is DOM that fluoresces when it absorbs light (Coble, 2007) When a molecule absorbs energy its electrons get excited which move them to a higher energy level, when that energy level decreases and the electron returns to its original energy level, or ground state, it lose energy in the form of emitting light, this process is called fluorescence. These excitations and emissions are measured in wavelengths and are unique for specific molecular structures and can be used to identify specific types of DOM and can be used to glean information about the origin and composition of DOM (Fellman, Hood & Spencer, 2010; Gonçalves-Araujo et al., 2016).

Spectroscopy is used to visualize these excitations and emissions. Spectroscopy uses a focused beam of light to excite the electrons in a controlled manner, the excitation is directed

towards a filter and on to a detector which is used to identify the molecule by the changes in its excitation and emission. The detector translates the energy gained and lost to a line on a graph which can be used to understand the molecule(s) being excited (Figure 6A). 3-D scans can also be created using the excitation, the emission to, and the intensity (or height of the peak), these are called excitation emission matrix (EEM) as seen in Figure 6B. EEMs are like fingerprints for each sample and provide information on the origin and composition of the sample they are testing (Coble, 2007; HORIBA Scientific, 2018).

There are 7 general types of peaks (Table 2) that have been identified in marine systems, they fall into two primary groups, humic-like and protein-, or amino acid-, like (Coble et al., 1990; Coble, 2007). The peaks that are included in the amino-acid like group are amino acids tryptophan (peak T), tyrosine (peak B), and phenylalanine; the peaks A (UVB excitation humic-like), M (UVA excitation marine humic-like), and C (UVA excitation humic-like) fluoresce at higher wavelengths and belong to the humic-like subgroup of peaks (Coble, 1996; Hudson, Baker & Reynolds, 2007; Jørgensen et al., 2011; Catalá et al., 2016). Humic-like fDOM is made of complex molecules, like lignin, which is resistant to degradation and is traditionally thought to be terrestrial in origin and sourced far from the location it was collected (Osburn & Bianchi, 2017; Maqbool et al., 2020). Amino acid-like fDOM is made of more simple molecules, like tryptophan, is easily consumed by microbes or altered by light, is traditionally believed to be sourced close to its collection location. Oceanic amino acid-like fDOM is believed to be created by bacteria on the surface of the water (Cory et al., 2007).

In the work described below, I address the hypothesis that sponge species will vary in the consumption of DOM, specifically bulk DOC and TDN and fDOM components. I further hypothesize that the symbiotic microbial communities have a role in the different consumption

of DOM across sponge species and that this symbiont effect will be detected by categorizing major groups of symbionts in sponges such as the presence of photosymbionts or nitrifying bacteria. These hypotheses were addressed with an artificial reef populated with the 10 most abundant sponge species in the Caribbean and these sponge species exhibit diverse symbiotic microbial communities.

METHODS

In Situ Experiment Setup

To examine differences in nutrient profiles across sponge species, an artificial reef was constructed with 5 rows, with 10 cinder blocks per row, the rows were arranged in a semi-circle, and rebar was run through each individual row and secured at the middle and ends to ensure that the rows would not move over the duration of the experiment. Species (n = 10 replicates of 10 species) were placed on the rows in roughly a Latin-square design to spread out any environmental variable that may exist on the artificial reef across replicates of species (Table 3). The depth of the reef was approximately 7 m and immediately surrounding the reef was sand on all sides. The rows of sponges were placed so that they are as equal as possible in exposure to light and water flow. Two pieces of 5mm polystyrene mesh were placed on top of each of the cinder blocks and secured with cable ties. Ten individuals of each of the 10 focal species (*Aiolochroia crassa, Aplysina cauliformis, Aplysina fulva, Amphimedon compressa, Iotrochota birotulata, Ircinia felix, Niphates digitalis, Callyspongia aculeata, Verongula rigida, and Xestospongia muta*) were selected and individuals were attached to the polystyrene mesh using cable ties and the sponges were allowed to acclimate for 4 months. The sponge species selected were based on prevalence in the Caribbean (Loh & Pawlik, 2014) and included four LMA

species (*Amphimedon compressa, Iotrochota birotulata, Niphates digitalis, Callyspongia aculeata)* while the remaining six species are considered HMA sponges.

In Situ Water collection

Samples for all nutrient analyses were collected using a modular vacuum set up (VacuSIP) which was implemented and modified from Morganti et al. 2016. The VacuSIP included poly ether ether ketone (PEEK) tubing (used commonly in HPLC instruments) that was placed over the sponge osculum (sponge exhalent seawater) or near the sponge (inhalant seawater) and positioned using tripods. The tubing was connected via syringe needle to a pressurized 250 mL amber glass bottles with Teflon septate caps (Figure 7). The 250 ml glass vials were pre-combusted within days of sampling (6 h at 450 °C) and pressurized manually to -15 psi and this pressure increased slightly at the depth of the artificial reef. VacuSIP lines, were acid rinsed in 10% HCl. Pumping was confirmed using fluorescent dye before each collection and the dye was allowed to clear before sampling. Tubing was then positioned directly above the pumping oscula for small sponges or inside the oscula close to the sponge for larger sponges. VacuSIP lines were then attached to the appropriate 250ml bottles in the crates by sticking the needles at the end of the line into the septa of the bottle. Tubing for inhalant water collection inserted into the appropriate 250ml bottles (Figure 7). The apparatus contained fewer lines than 250 mL collection bottles, so the apparatus was set up to fill half of the bottles in the collection crate (Figure 7) for 120 minutes, then the lines were moved to new bottles to fill the remaining bottles for 90 minutes. Once at the surface the 250 ml bottles for each individual sample were combined into 2L bottles, one for inhalant and one for exhalent water samples that were labeled and then stored on ice in coolers until they were filtered.

Water Filtration

A Cole-Palmer Masterflex L/S Intertek fitted with a Masterflex L/S easyload II head and Multichannel Pump Head Cartridges was used to filter the samples at a rate of 40ml/min through High-Performance Precision Pump Tubing, PharMed® BPT, L/S 15 with a 0.2µm supor filter into 1L acid-rinsed polycarbonate bottles that were covered with aluminum foil. The filters were archived for future microbiome analysis and the filtrate was stored in 40 mL amber vials with Teflon septa. Filtrate for DOC and TDN was acidified to ~pH 2 using 6N HCl and stored at 4°C. Samples for DOC and TDN were sent to the UGA Stable Isotope Ecology Laboratory for analysis using a Shimadzu TOC-5000A Total Organic Carbon Analyzer. Filtrate for fDOM was not acidified and stored at 4°C. The fDOM samples were shipped to a collaborator at the University of Hawaii (Dr. Craig Nelson) where they were stored until analysis. I worked with the Nelson lab at UH to process and analyze the fDOM as samples described below.

FDOM Sample Processing

Following the methods from Nelson et al. (2015) samples were analyzed with a Horiba Aqualog scanning fluorometer with 150 W Xe excitation lamp, Peltier-cooled CCD emission detector, and simultaneous absorbance spectrometer. Quartz cuvettes of 1 cm diameter, which were DIW-leached and rinsed were used to measure fluorescence. Samples were brought to room temperature while the Xe bulb warmed up. Excitation-emission matrices (EEMs), 3D contour plots of excitation and emission fluorescence, were measured from the 108 samples, starting with 4 DIW filled cuvettes as blanks at the beginning and end of the analysis. Scans were processed using a MATLAB script that use parallel factor analysis (PARAFAC) to identify peaks in the EEMs that correspond to previously characterized fluorescent components of humic-like and

amino acid-like components (https://github.com/zquinlan/fDOMmatlab/script.md) (Coble, 1996; Nelson et al 2015).

Microbial Community Abundance and Composition

Additional PCAs were implemented to gain a better understanding of potential impacts of the microbial community on sponge processing of nutrients, with the microbial communities categorized into three major groups based on the literature or coarse characteristics of certain microbial symbionts (e.g., presence of photoautotrophs). The defined groups included 1) microbial abundance, 2) microbial composition, and 3) species-specific microbiomes. The microbial community abundance was defined as either high microbial abundance or low microbial abundance (HMA/LMA) by published previous studies (Hentschel, Usher & Taylor, 2006; Taylor et al., 2007; Hentschel et al., 2012; Poppell et al., 2014). The microbial community composition was defined by the presence of both nitrifying bacteria and archaea and photoautotrophs ("both"), nitrifiers only ("nitrate"), or neither of these microbial groups ("neither") (data from Freeman et al., 2020).

Statistical Data Analysis

All statistical analyses were performed in RStudio (version 10.0.19044.1766). Data were normalized standardized by converting it to percent change to visualize the change in nutrient profiles by sponge species. Percent change was calculated by subtracting raw exhalant values from raw inhalant values then dividing that by inhalant values. Of the fDOM components, the categories of humics and amino acids were used either as a sum of amino acids and sum of humics (as in Nelson et al., 2015) or as their individual components (e.g., fulvic acid-like, phenylalanine-like) in the analysis. Fluorescent DOM measurements are known to correlate to

each other (Stedmon et al., 2003); thus, to visualize correlations between the measured DOM variables in this study as well as to build a better picture of correlation between the independent factors and DOM types (e.g., Chen et al., 2016), correlation matrices were created using z-scored inhalant and exhalant and percent change data using the package 'corrplot' which uses Pearson's correlation coefficient. To look at the impact that independent factors may have had on the data, principal component analyses were performed using the packages 'prcomp' and 'ggbiplot' inhalant and exhalant data were log-transformed and z-scored to approximate a normal distribution then standardized by z-score transformation for use in principle components analysis (PCA). Percent change data were standardized by z-score only prior to ordination by PCA. Permutational analysis of variance (PERMANOVA), a non-parametric multivariate test, was applied to assess the impact of sponge species, or microbial abundance, or microbial community composition (independent variables) on matrices of z-score standardized dissolved nutrient data (fDOM, DOC, TDN) from 1) inhalant seawater, 2) sponge exhalent seawater, and 3) percent change in nutrient concentrations (dependent variables). Permutations were set to 999 unless otherwise specified If there was a significant effect discovered in the PERMANOVA, then analysis of variance (ANOVA) or Kruskal-Wallis test was applied to individual nutrient components for a given category (sponge species, microbial abundance, microbial composition), followed by the Benjamini-Hochberg correction for controlling the familywise error rate (Benjamini & Hochberg, 1995) (*Figure 8*).

Assumptions of parametric analysis were assessed with the Shapiro-Wilk tests for normality of the data using residuals of the statistical model such as analysis of variance (ANOVA) and the Kruskal Wallis test for unequal variances (heteroscedasticity). Differences in a particular nutrient type across sponge species or categories of microbial abundance or

microbial composition were assessed with ANOVA or the non-parametric Kruskal-Wallis tests. ANOVAs were performed with log-transformed data followed by post-hoc Tukey honest significant difference (HSD) tests for significant ANOVA results using the functions 'aov' and 'TukeyHSD' in R respectively. Kruskal-Wallis rank sum tests were performed when the data did not fit a normal distribution or did not conform to homoscedasticity, followed by the Dunn test. For multiple tests, the Benjamini-Hochberg correction was implemented to adjust for a false discovery rate. Box and whisker plots were made using the percent change data to visualize the significant ANOVA and Kruskal-Wallis tests. Heat maps were performed using 'pheatmap' function to visualize how the independent factors and DOM values in the inhalant, exhalant, and percent change data using a Euclidean distance matrix.

RESULTS

Correlation Among Variables

Some correlations among the DOM components were observed (Figure 9). For inhalant, exhalant, and percent change total Raman units was strongly correlated with all fDOM values except phenylalanine. The majority of correlations between fDOM types were seen across inhalant, exhalant and percent change data. Most correlations occurred within like types of DOM, i.e. humic-like types were correlated with humic-like or amino acid-like were correlated with amino acid-like. The exceptions being tyrosine- like fDOM which were correlated with all of the humic-like fDOM signals for all three data sets (inhalant, exhalant, and percent change). The inhalant water samples were the only ones that showed correlations between fulvic-acid and DOC. The exhalant water samples showed positive correlations between TDN and marine humic-like and tryptophan-like fDOM, as well as strong positive correlations between tryptophan-like fDOM

for all other fDOM signals. The exhalant water samples were the only samples that showed negative correlations, and were only seen between DOC and marine humic-like, tyrosine-like, and lignin-like fDOM. The inhalant and exhalant samples showed strong positive correlations between lignin-like fDOM and ultraviolet, marine, and visible humic-like fDOM samples and a positive correlation between tyrosine and phenylalanine-like fDOM. The percent change data showed no negative correlations, this data set was the only one to show a positive correlation between tryptophan-like fDOM and DOC.

Overview of Nutrient Data

Principal Component Analyses (PCAs) were used to identify independent factors that may have an impact on sample analysis, such as the day the sample was collected, row on the reef, and neighbor. These PCAs used DOC, TDN, total Raman units (totalRU), humic-like fDOM components, and amino acid-like fDOM components as vectors to better understand how the DOM is influenced by the sponges filtering of the water.

There was no significant effect of day that a sample was collected for the percent change samples (PERMANOVA, $F_{5,46} = 1.3873$, $p = 0.1400$), however there was a significant effect of day that a sample was collected for the inhalant (PERMANOVA, $F_{5,46} = 4.7028$, adjusted-$p = 0.0030$) and exhalant (PERMANOVA, $F_{5,46} = 3.7072$, adjusted-$p = 0.0075$) (Figure 10). This significance was likely driven by day 5 collections (Figure 10).

There was no significant effect of the row that the samples were collected from for inhalant (PERMANOVA, $F_{4,37} = 1.1117$, $p = 0.304$), exhalent (PERMANOVA, $F_{4,37} = 0.8446$, $p = 0.601$) or percent change samples (PERMANOVA, $F_{4,41} = 1.0644$, $p = 0.386$) (Figure 11). There was no significant effect of neighboring species of sponge when looking at inhalant

(PERMANOVA, $F_{10,41}$ =1.2596, p =0.252), exhalant (PERMANOVA, $F_{10,41}$ =1.4175, p =0.166), or percent change (PERMANOVA, $F_{5,46}$ =1.0304, p-0.435*)*. High variability was observed for the percent change of measured components by *A. crassa* in particular (Figure 12).

Microbial Abundance

When looking at microbial abundance, the LMA sponges were more tightly grouped than the HMA sponges based on percent change of the dissolved nutrients between inhalant and exhalent seawater samples (Figure 13). The HMA sponges include, *Xestospongia muta*, *Aplysina cauliformis*, *Verongula rigida*, and *Aiolochroia crassa*, while the LMA sponge species include, *Amphimedon compressa, Iotrochota birotulata, Niphates digitalis,* and *Callyspongia aculeata*. There was not a significant effect of HMA and LMA status for sponges on the percent change of nutrients (PERMANOVA, $F_{45,9,1,34}$ = 0.4128, p = 0.645).

Microbial Composition

For the microbial composition of nitrifiers ("nitrate only"), photoautotrophs plus nitrifiers ("both"), or neither ("none"), there was some separation in the ordination of the nitrate group (Figure 14) which was significant (PERMANOVA $F_{2,35}$ = 2.2286, p = 0.038). The "neither" and "both" categories are relatively similar but there is an elongation of the ellipse for the "nitrate" group. Sponges that hosted neither photosynthetic nor nitrifying symbionts were more tightly grouped, while those that hosted both types of symbionts had a bigger spread. The spread of the data for each of these groups of samples were along the x-axis. Conversely spread of the data for the sponges that hosted only nitrifying symbionts was more vertical and which was primarily driven by the amino acid-like fDOM signals. Sponges that hosted only nitrate producing

microbial symbionts appeared to show higher variability in general for nutrient processing of seawater (Figure 14).

Sponge Species

There was little differentiation in the inhalant and exhalent seawater samples by species and this was supported by PERMANOVA analysis ($F_{9,32}$ = 1.1895, adjusted-p = 0.168, inhalant and $F_{9,32}$ = 1.4019, adjusted-p = 0.116, exhalent) (Figure 15). However, there was a significant effect of species for the percent change in the measured nutrients (PERMANOVA, $F_{9,32}$ =1.8521, adjusted-p = 0.036) (Figure 15). Additional ANOVA, Kruskal-Wallis models, and post-hoc tests were applied to discover which nutrient components are driving the significant effect adjusted. Individual fDOM components were visualized by PCA Figure 15. There was a significant effect of species on individual fDOM components in the PERMANOVA model even after p-value adjustment for false discovery rate ($F_{9,32}$ = 1.7387, adjusted p = 0.020).

Changes in DOM components: Dissolved Organic Carbon and Total Dissolved Nitrogen

The percent change of the dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were not significant for species, microbial abundance, or microbial community composition (Kruskal-Wallis, Table 5).

Changes in fDOM components: Total Raman Units (RU)

There was a significant difference in the percent change of the total RU consumed or released by the sponges that host nitrifying microbes when comparing sponges that host both nitrifying microbes and photosymbionts (Kruskal-Wallis, Table 5). However, the sponges that

host neither nitrifying microbial symbionts or photosynthetic microbial symbionts are not statistically different from the other two categories (Figure 17, Kruskal-Wallis, Table 5).

Changes in fDOM components: Humic-like

The consumption and release of visible humic-like fDOM signals that *A. cauliformis, A. compressa, and A. crassa* showed were statistically different from those of *X. muta*, while not statistically different compared to the rest of the sponges (Figure 18a, ANOVA, Table 4). The consumption and release of visible humic-like fDOM signals by the sponges that host nitrifying symbionts is different than the consumption and release of visible humic-like fDOM signals by sponges that host both nitrifying and photosymbionts with consistent net release of visible humic like components by sponges hosting nitrifiers (Figure 16ANOVA, Table 4). However, the sponges that host neither nitrifying microbial symbionts nor photosynthetic microbial symbionts are not statistically different from the two other categories (Figure 18b, ANVOA, Table 4).

Similarly, the consumption and release of ultraviolet like fDOM signals by sponges that host nitrifying symbionts was significantly different than those sponges that hosted both types of symbionts. However, sponges that hosted neither type of symbiont were not different from the other two categories (Figure 19, ANOVA, Table4). Post-hoc tests (Tukey's HSD) did not show significance between the species of sponge for processing other humic-like fDOM (Table 4). Lignin and fulvic acid-like fDOM were not found to have any significant differences between species, microbial abundance, or microbial composition (Kruskal-Wallis, Table 5).

Changes in fDOM components: Amino acid-like components

The percent change of the tyrosine-like fDOM signals released or consumed by sponges were statistically different for sponges that host nitrifiers when compared to sponges that host both nitrifiers and photosymbionts (ANOVA Table 4). However, the sponges that host neither nitrifying microbial symbionts nor those that host photosynthetic microbial symbionts are statistically different from the two other categories (Figure 20 and ANOVA Table 4).

Visualization of Nutrient Profiles for Sponge Species ("Sponge Species view")

Heatmaps were used to visualize the relative percent change in DOM and fDOM for each sponge species (Figure 16). Categories of microbial abundance or composition were also used to visualize potential similarities by these groups. *A. cauliformis* and *X. muta* consumed all of the types of DOM. The net production of DOC differentiates about half of the sponges (Figure 16). Amino acid release and production differentiate *A. crassa* and *I. felix* from other species (Figure 16). In contrast, humic-like release and production differentiated *I. birotulata*, *A. fulva*, *V. rigida*, and to some extent *A. crassa* and *I. felix* from other species (Figure 16). Total dissolved nitrogen (TDN) has variable production or is not consumed by these sponges except in *I. felix* (Figure 16). Most LMA species had a mixture of positive to neutral relative percent changes in the uptake of DOM with variability by sponge species observed across the defined groups of abundance and composition (Figure 16).

DISCUSSION

Early studies on sponge microbial ecology suggested that there are broad differences in physiology based primarily on microbial symbiont abundances, either high microbial abundance (HMA) or low microbial abundance (LMA), which lead to distinct ecological roles of sponges in

these groups (Vacelet & Donadey, 1977; Weisz, Lindquist & Martens, 2008). More recent work on holobiont processing of DOM have supported the idea that there is a combination of many factors that play into how sponges process nutrients (Easson & Thacker, 2014; Thomas et al., 2016; Turon et al., 2018). While it has been demonstrated that sponges do alter the DOM pool of nutrients as they filter water for food, there is a limited understanding of how sponges process DOM and what drives the differences in the which nutrients they consume or release (Fiore, Freeman & Kujawinski, 2017; Letourneau et al., 2020; Olinger et al., 2021). Gaining a better understanding of how sponges process nutrients will aid in better predicting potential changes in sponge dominated ecosystems as global climates change (Bell et al., 2013, 2018).

In this study I looked at the change of DOM in seawater after a sponge had processed it and compared these changes in DOM across sponge species. Through a combination of three-dimensional fluorescence excitation-emission matrix spectroscopy with PARAFAC analysis and complementary nutrient analysis I found that the variation in DOM processing by sponges was best explained by sponge species rather than by microbial symbiont abundance or broad categories of symbiont composition. However, there appears to be some significant effect of nitrifying and photoautotrophic microbial symbionts on DOM processing by sponges. This is the first study to apply fluorescent DOM analysis to sponge processing of DOM and provides new insight on how sponges process both the total amount of DOM (i.e., measurements of DOC and TDN) and broad categories of DOM based on fluorescence (i.e., humic-like and amino acid-like components of DOM).

Microbial abundance does not explain variation in DOM processing

Previous studies have suggested that differences in microbial abundance (HMA vs LMA sponges) were correlated with distinct differences in pumping rates and differences in the sponge

mesohyl composition such as density of choanocyte chambers, which contain the primary cells involved in pumping and filtration (Vacelet & Donadey, 1977; Weisz, Lindquist & Martens, 2008). For example, LMA sponges were found to have a higher pumping rate and more choanocyte chambers on average than HMA sponges (Mueller et al., 2014; Poppell et al., 2014). Additionally, LMA sponges are broadly considered to consume DOC and produce DON, while HMA sponges are broadly considered to consume both DOC and DON, potentially relying on LMA sponges as a source of DON (Reiswig, 1971; Vacelet & Donadey, 1977; Weisz, Lindquist & Martens, 2008; Mueller et al., 2014; Morganti et al., 2017; Bart et al., 2020). A connection between the presence of symbiotic microbes and the types of nutrients released has also been observed previously. For example, nitrate production from sponges with high densities of nitrifying bacteria and archaea (Southwell et al., 2008; Fiore, Jarett & Lesser, 2013; Archer et al., 2017). A lack of cohesion between the way that HMA and LMA sponges consume and release DOM was observed in this study. All LMA species were found to consume and release different portions of DOM, however, some HMA species only consumed DOM (*A. cauliformis and X. muta*), some consumed and released different portions of DOM (*A. fulva, V. rigida, and I. felix*) and *A. crassa* only released DOM.

Microbial composition may explain some variation in DOM processing

The results of this work provide support for differences in nutrient processing of sponges classified in broad categories of microbial composition. Microbial composition based on functional categories of the presence of nitrifying microbes and/or photosynthetic *Cyanobacteria* might be expected to shape how nutrients are processed by the sponge, particularly if these groups are abundant, as they have a distinct metabolic profile and have been shown to influence the rest of the microbial community composition in corals (Bourne et al., 2013). However, a lack

of influence of *Cyanobacteria* on the rest of the microbial symbiont community composition was also observed in another recent study with sponges (Britstein et al., 2020). In my study the specific type of DOM that sponges consumed and released was not consistently explained across the categories “neither”, “nitrate”, and “both”. However, some significant differences were observed, where nitrifying sponges were different from those containing both nitrifiers and photosymbionts, although these were not different from the sponges with neither of those symbionts. It should be noted that there were only 2 species in the group that hosted just nitrifying symbionts and further work will be necessary to gain a better understanding of how this grouping may be unique. These intriguing results point to a contribution by the phototrophic community or to a metabolism that is shaped by having both phototrophs and nitrifiers present that influence processing of the fluorescent components of DOM. There may also be important categories of microbes that are not captured by these broad categories (nitrate, both, neither) that influence how sponge holobionts process DOM (e.g., anoxygenic phototrophs, other chemoautotrophs, different types of heterotrophs). Alternatively, the sponge may be driving the processing of DOM with little influence by the microbial community. For example, sponge cells of at least two species have been shown to take up DOM directly (Hudspith et al., 2021), although more work is needed to determine how prevalent sponge uptake of DOM is across species, the rate of uptake, and if the proportion of uptake varies between sponge cells and symbionts cells across sponge species.

Support for sponge species-specific processing of DOM

While there is broad overlap in microbial phyla found in different sponges, studies using a variety of techniques for microbial analyses (including electron microscopy, fluorescence

microscopy, culture-based work, and Next Generation Sequencing) have demonstrated that sponge microbiome composition is primarily host specific and relatively stable over time, with some variation of the microbial symbiont community due to seasonal, environmental, and geographical effects (Erwin et al., 2012; White et al., 2012; Hardoim et al., 2012; Cleary et al., 2013; Reveillaud et al., 2014). Furthermore, Easson and Thacker (2014) found that microbial community variation was not related to the abundance of microbes a sponge hosted, which supports the idea that classifying microbial community structure based on microbial abundances alone is no longer a suitable method. Thus, if microbial symbionts do have some role in how sponges process DOM, there may be species-specific differences in nutrient profiles of sponge exhalent water that are driven, at least in part, by the species-specific symbiotic microbial community.

The current study supports the differentiation of DOM consumed by species and these results are broadly supported by studies that have shown nuances in the nutrient acquisition of sponge species that fit a spectrum rather than two rigid categories (Easson & Thacker, 2014; Thomas et al., 2016; Turon et al., 2018; Freeman et al., 2020b). While bulk DOC and TDN were not significantly different by species, microbial abundance, or microbial composition, there was high variability across species and the values were higher than expected (2-5x higher) and these results may change with further investigation into potential methodological issues with the analysis.

The visualization and statistical results of changes in fDOM composition by sponge filtration support a broad difference in nutrient acquisition based on sponge species. These results are in-line with the theory of resource partitioning of coexisting species that has been suggested previously for sponges (Freeman et al., 2020b; Freeman et al. 2021). Sponges

processed total Raman units, certain amino acid-like fDOM and certain humic-like fDOM (Tables 4 and 5). Sponges host several different taxa of symbionts that are unrelated but are able to break down large molecules, like polysaccharides that may derive from the sponge or from the seawater. This further explains how sponges are able to exist densely packed into one location (Robbins et al., 2021). Coral reefs are found in nutrient poor waters and nitrogen in particular is a limiting nutrient on reefs (Tanaka et al., 2011). Additional work has demonstrated that sponges in the Caribbean are likely not carbon limited but may be nitrogen limited based on C:N ratios of food sources and the prevalence of nitrogen cycling in sponges including transfer of nitrogen from symbionts to host (Robbins et. al., 2021). The findings from this project are supported by the competitive exclusion principle, which states that two species that compete for the same limited resource cannot co-exist (Hardin, 1960).

Marine sponges help recycle the nutrients on coral reefs. The results from this study, and others, suggest that differentiation in nutrient processing of sponges may support niche partitioning on coral reefs (Morganti et al., 2017; Freeman et al., 2020b). HMA sponges utilize nitrogenous waste that is excreted from LMA sponge species (Morganti et al., 2017). This allows HMA and LMA species to live in close proximity to each other, facilitating the dense assemblages of varied species of sponges that are often seen on coral reefs (Morganti et al., 2017). Furthermore, symbiont-derived benefit to host sponges have been shown to vary across species using stable isotopically labeled carbon and nitrogen compounds taken up by microbial symbionts (Freeman et al., 2020b). The ability to utilize different portions of DOM that we see in the results of this study could allow sponges to coexist on coral reefs in dense assemblages.

One hypothesis for how sponges process DOM is that sponges may broadly be net producers of humic-like compounds that are more recalcitrant to microbial degradation and tend

to accumulate in the ocean (Letourneau et al., 2020; Olinger et al., 2021). My work is the first to use the fluorescent component of DOM as a proxy for the broader profile of DOM and to gain insight into sponge-induced changes in humic-like and changes in amino acid-like compounds, which likely differ in their lability to coral reef microbes (Quinlan et al., 2018; Wegley Kelly et al., 2022). In general, differences in humic-like and amino acid-like fDOM components were observed across sponge species, suggesting that we may not be able to group all sponges together in terms of their impact on degradation of DOM.

Variation in the use of fDOM components by species was visible to some extent in the ordination with percent change of the fDOM components. In general, vectors for amino acid-like fDOM, humic-like fDOM, and total dissolved nitrogen seemed to drive some distinction by species as seen in Figure 15. High variability in the use of fDOM is well documented (Wurl, 2009; Nelson et al., 2015) and while there was not complete separation by species in the ordinations performed here, the results support differences in resource use across species and it may be that relatively minor differences in nutrient use is enough to facilitate the coexistence of sponge species, an observation made in other systems such as plants (Kulmatiski et al, 2020), fish (Knickle & Rose, 2014), and anurans (Cloyed & Eason, 2017).

Further differences in the processing of fDOM in these sponges can be seen in the heatmap which compared profiles of relative changes (i.e., changes in nutrient concentrations scaled between -1 and 1 for each species) in nutrient concentrations across species. Interestingly, the patterns that emerged from the nutrient profiles conflicted, to an extent, with the clear separations visible which may be a result of small changes in nutrients that are amplified when made into a relative profile. The results of these nutrient profiles, however, provide another view of fDOM processing across species and yielded four distinct patterns (see Figure 21). Pattern

group 1 includes *A. fulva*, *I. birotulata*, and *V. rigida* all tended produce humic-like signals (except lignin-like fDOM signals) with a mixture of consumption and release of amino acid-like fDOM. *I. birotulata* and *V. rigida* did show some separation by humics in the ordination, and the net release of humics may be an attribute of all three sponge species but to varying degrees. Pattern group 2 consists of *A. crassa* and *I. felix* and had similar humic-like trends to pattern group 1 but also had net production of lignin-like and amino acid-like fDOM components. Pattern group 3 included *N. digitalis, A. compressa, and C. aculeata* which are all LMA sponges that did not host either of the phototrophic or nitrifying symbionts, however, these three sponges tended to consume a broader spectrum of fDOM with low net production of some amino acid-like components. This is reflected to some extent the ordination where these three sponges align more with the vectors for amino-acid like components and notably *A. compressa* ellipse is distinct potentially and this species consumed relatively more DOM than the other two species in pattern 3. Pattern group 4 contains *A. cauliformis* and *X. muta*, this grouping had broad net consumption of all fDOM components to varying degrees. Sponges have been found to host symbionts that can break down complex molecules, such as lignin- and other humic-like fDOM which may explain the differences in humic-like fDOM consumption by species (Robbins et al., 2021).

Sponge processing of fDOM is likely shaped in part by the composition of fDOM in the surrounding seawater. The production of DOM in the ocean is thought to be driven by metabolic release by heterotrophic microbes, metabolic overflow by photoautotrophs, and viral lysis and predation of multiple plankton groups (e.g., bacterioplankton, zooplankton) (Carlson & Hansell, 2015). Marine picocyanobacteria are unicellular prokaryotic phytoplankton that are abundant and widely distributed throughout the ocean. *Synechococcus* and *Prochlorococcus* are two of the

most commonly found groups, including on coral reefs (Zhao et al., 2017). Despite their small size, they are major primary producers in the world's oceans and contribute to the marine DOM pool in the ocean's surface (Zhao et al., 2017). For example, picocyanobacteria-derived fDOM was recently shown to be an important source of fDOM at a global ocean scale, with similar optical properties and fDOM components between picocyanobacteria-derived fDOM and fDOM from the sea surface and deep-sea in the Sargasso Sea, a large expanse of the northern Atlantic Ocean (Sommer et al., 2015; Zhao et al., 2017; Fong, Ng & Ng, 2018). Importantly, picocyanobacteria produce more humic-like fDOM components than previously thought and may be a source of humic-like fDOM on coral reefs (Zhao et al., 2017). Therefore, lignin-like and other humic-like fDOM observed in the inhalant seawater in this study may have been present in the water due to phytoplankton and/or nearby reef. The collection site was relatively isolated and surrounded by a sand patch with scattered patch reefs comprised of sparse encrusting algae, sponges, and soft corals. Limited studies of DOM on coral reefs have also documented release of high concentrations of DOC from corals and from algae, although they composition of the that DOC and the fDOM components differ between them (Haas et al. 2013; Quinlan et al, 2018; Wegley Kelly et al., 2022). It is known that exudates from corals have been found to accumulate faster and are composed of higher portions of amino-acid like fDOM than algal exudates which do not accumulate and produce higher portions of humic-like fDOM (Quinlan et al., 2018; Wegley Kelly et al., 2022). This study indicated that sponges as a group do not align strictly with coral or algae based on the release of fDOM. Rather, there are differences in release of fDOM across species. The species-specific release of humic-like or amino acid-like compounds may have implications for understanding nutrient cycling on coral reefs and how nutrient dynamics may change as sponge populations change with environmental perturbations.

Limitations and Future Directions

There are some limitations to this study. Because of the location of the field site, which is approximately 5 miles from the Mote Marine Lab or 40 minutes by boat, the collection of data for this project was dependent on the weather and marine forecast. The collection of samples had to be prematurely stopped due to the impending tropical storm Fred and hurricane Grace (Pasch, 2022). This caused the sample size of the collections to be smaller (5-6 samples per species) than planned for (7-10 samples per species) with a larger sample size there may have been more identifiable differences. These impending storms also impacted the turbidity of the water, with some days being very turbid and others being less turbid. This in addition to possible natural variation across the artificial reef caused some differences by day in the inhalant water, but most of that appears to be mitigated when looking at the percent change. Indeed, given the natural changes in water flow, composition over the sampling period, and fewer than planned replicates, the observation of any signal by species is supportive of at least some resource partitioning of the DOM pool across sponge species. There are several correlations between the types of fDOM (Figure 9) for inhalant, exhalant and percent change seen in the results. Even though the amino acid-like and humic-like are comprised of material, fDOM is related which explains the correlations seen between several of the fDOM types (Coble et al., 1990; Jørgensen et al., 2011; Coble, 2013).

Additionally, sponges are generally sensitive, and many will stop pumping if touched, keeping that in mind, when the Vacusip apparatus was set up the lines were close to the pinacoderm (outer layer of cells of the sponge) without touching, however, this means that, inevitably, some non-filtered water contaminated each of the samples. Knowing this, the changes that are seen in the exhalant and percent change data are exciting and show the need for further

study. It is also possible that low concentrations of fDOM limited the amount of fDOM that was identified, since a 350nm absorption coefficient was used, other studies have used 280 in areas with decreased fDOM concentrations (Li et al., 2022).

Future directions of this work will be to look at the pumping rates, specific microbial communities of the sponges that were collected for this project, and fDOM indices. Understanding the pumping rates of these sponges will allow for better understanding of how much DOM is processed by volume and time which will aid in understanding how sponges will impact oceanic DOM in the future. Studies have found that the microbial composition of sponges is related to host species but that the primary attributing factor to the variation in community composition of symbionts was the presence or absence of photosymbionts in 11 invertebrates from the Great Barrier Reef (Bourne et al., 2013; Li et al., 2022). Understanding the specific microbial communities that these sponges host could be another piece to understand the puzzle of sponge nutrient processing. This will create a more detailed picture of the role sponges and their microbial communities play in processing nutrients on coral reefs and will aid in the understanding of how reefs will change in the future as global climate change progresses. Easson and Thacker (2014) found that the relatedness of hosts was a factor in the composition and abundance of sponge microbial communities. Future work could focus on including the relatedness of the sponge species with the microbial communities to investigate if that factor plays a role in how these sponges utilize DOM. Previous work with fDOM has looked at fDOM indices, such as humification index, biological index, and the fluorescence index, to understand the complexity of the DOM (Miranda et al., 2018; Wegley Kelly et al., 2022). Moving forward using to better understand the composition of the fDOM being processed could be done using these indices.

CONCLUSION

There are multiple factors that affect how sponges utilize and process DOM on coral reefs, including but not limited to, microbial community composition and physiology of the host species. Overall, there was a lack of support for differences in processing DOM by microbial abundance alone. Instead, there was support for distinct differences in DOM processing across sponge species and microbial compositions. These results support the idea that sponges exist on a spectrum of resource partitioning that are shaped by the host and the symbiont community rather than divided into discrete categories by microbial abundance or community composition.

This project is a portion of a larger study that will provide a deeper understanding of the role of sponges in nutrient cycling on reefs in the Florida Keys and insight into ecological and evolutionary forces that have shaped sponge community assemblages in the Caribbean. Such data provide essential context for investigating the impact of current environmental stressors on reef structure, create a fuller picture of the role sponges and their microbial communities in process nutrients on coral reefs, and aid in the understanding of how reefs will change in the future as global climate change progresses.

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TABLES

Table 1 Explanation of Core Functions of the sponge symbionts (Modified from Pita et al., 2018)

Category of core function	Core function	Interpretation	Refrence
Metabolic Features	emphasis on ammonia oxidation	environmental and host-derived nutrients	Reviewed by Webster et al 2016
	Carbon metabolism with emphasis on complex carbohydrates	Utilization of environmental and host-derived nutrients	Kamke et al 2013 and Slaby et al 2017
	Nitrogen and carbon metabolism utilizing creatinine	Utilization of environmental and host-derived nutrients	Moitinho-Silva et al 2017 and Fan et al 2012
	Vitamin synthesis (especially thiamine and vitamin B12)	Overproduction of vitamins that are then utilized by the sponge host	Thomas et al 2010, Fan et al 2012, and Fiore et al 2015
	Secondary metabolism	interaction, defense of the holobiont	
	Carnitine (vitamin BT) utilization	Utilization of host-derived component	Slaby et al 2017
Defense features	CRISPR-Cas systems	Defense against viruses/phages	Thomas et al 2010, Slaby et al 2017, and Horn et al 2016
	Toxin-antitoxin systems	Defense against foreign DNA	Thomas et al 2010, Slaby et al 2017, and Horn et al 2016
	Restriction modification systems	Defense against foreign DNA	Thomas et al 2010, Slaby et al 2017, and Horn et al 2016
	Eukaryotic-like protein domains	phagocytosis evasion	Thomas et al 2010, Liu et al 2012, Nguyen et al 2014, Díez et al 2017, and Burgsdorf et al 2015
	Modifications of the lipopolysaccharide	phagocytosis evasion	Burgsdorf et al 2015 and Wehrl et al
Other	Mobile genetic elements and transposases	Increased levels of horizontal gene transfer	Thomas et al 2010, Fan et al 2012, Horn et al 2016, and Gao et al 2014

Table 2 Common fluorescence regions modified from Coble (2007) by Stedmon and Nelson (Nelson & Gauglitz, 2016).

Peak	Region	Type	Excitation(nm)	Emission(nm)
α’	A	Humic (Visible, UVBExcitation)	260	400–460
γ	B	Tyrosine-like, protein-like (UVA)	275	305
α	C	Humic (Visible, UVAExcitation)	320–360	420–460
β	M	Marine humic-like (Visible, UVAExcitation)	290–310	370–410
	N	Protein-like (UVA)	280	370
δ	T	Tryptophane-like, protein-like or phenol-like (UVA)	275	340
	W	ECOCDOM Fluorometer	380	420

Table 3 In-situ reef design. Abbreviated species names are shown with the corresponding replicate number[§]. Blank spaces represent sponges that were not sampled.

Row 1		Row 2		Row 3		Row 4		Row 5	
Acra-1				Ndig-5	Aful-6	Cacu-7	Aful-8		
Acau-1	Acra-2	Ifel-2	Xmut-5	Vrig-5				Vrig-9	Xmut-10
Acom-1				Xmut-6	Vrig-6	Ndig-7		Ndig-9	
		Acau-3	Acra-3		Xmut-7	Vrig-7		Ifel-7	
Ndig-1		Acom-3	Acau-4	Acra-4			Vrig-8		
Ibir-1	Ifel-1		Acom-4				Xmut-9		Ibir-10
Ndig-2	Ibir-2		Aful-4		Acau-6	Acra-5	Ifel-6	Aful-9	Cacu-10
		Ibir-3	Ibir-4		Acom-6			Acra-7	Aful-10
Cacu-1	Vrig-2		Ndig-4		Cacu-6	Acom-7			
	Xmut-3	Ndig-3		Cacu-5	Ifel-5	Aful-7	Acom-8	Acau-9	Acau-10

[§]Acra = Aiolochroia crass, Acau = Aplysina cauliformis, Acom = Amphimedon compressa, Ndig = Niphates digitalis, Ibir = Iotrochota birotulata, Cacu = Callyspongia aculeata, Ifel = Ircinia felix, Aful = Aplysina fulva, Vrig = Verongula rigida, Xmut = Xestospongia muta

Table 4 ANOVA results from percent change of nutrient concentration from sponge processing for normally distributed data (marine humic-like, visible humic-like, ultraviolet, tyrosine-like, and tryptophan-like fDOM components. ANOVAS were used to understand if sponge species ("Species"), microbial community composition that the sponge hosts {"Microbial Composition), and/or the HMA or LMA classification of the host sponge ("Microbial Abundance") had significant impacts on the way that the sponges processed nutrients. Bolded text indicates statistical significance.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Marine Humic Like fDOM	**Species**	**9**	**19.29046**	**2.143384**	**3.060683**	**0.009673**
	Residuals	31	21.70918	0.700296		
	Microbial Composition	2	5.162158	2.581079	2.736828	0.077552
	Residuals	38	35.83748	0.943092		
	Microbial Abundance	1	2.037961	2.037961	2.039965	0.161173
	Residuals	39	38.96168	0.999017		
Tryptophan Like fDOM	Species	9	7.875072	0.875008	0.829359	0.594576
	Residuals	31	32.70627	1.055041		
	Microbial Composition	2	2.322137	1.161069	1.153202	0.326423
	Residuals	38	38.2592	1.006821		
	Microbial Abundance	1	0.261325	0.261325	0.25277	0.617959
	Residuals	39	40.32001	1.033846		
Tyrosine Like fDOM	Species	9	12.8287	1.425411	1.615766	0.154138
	Residuals	31	27.34786	0.882189		
	Microbial Composition	**2**	**6.344555**	**3.172278**	**3.563093**	**0.038172**
	Residuals	38	33.832	0.890316		
	Microbial Abundance	1	0.261325	0.261325	0.25277	0.617959
	Residuals	39	40.32001	1.033846		
Ultraviolet Humic Like fDOM	**Species**	**9**	**17.53837**	**1.948708**	**2.57499**	**0.024221**
	Residuals	31	23.46027	0.756783		
	Microbial Composition	**2**	**6.020748**	**3.010374**	**3.270472**	**0.048917**
	Residuals	38	34.97789	0.920471		
	Microbial Abundance	1	0.43548	0.43548	0.418698	0.521378
	Residuals	39	40.56316	1.040081		
Visible Humic Like fDOM	**Species**	**9**	**19.50464**	**2.167182**	**3.126269**	**0.008565**
	Residuals	31	21.48972	0.693217		
	Microbial Composition	**2**	**6.333967**	**3.166983**	**3.47213**	**0.041217**
	Residuals	38	34.66039	0.912115		
	Microbial Abundance	1	0.28728	0.28728	0.275233	0.602812
	Residuals	39	40.70707	1.043771		

Table 5 Kruskal Wallace results of percent change of nutrient concentration from sponge processing for non-normally distributed data (DOC, TDN, total Raman units, phenylalanine -like, fulvic acid-like, and lignin-like fDOM components). Kruskal Wallis tests were used to understand if sponge species ("Species"), microbial community composition that the sponge hosts {"Microbial Composition), and/or the HMA or LMA classification of the host sponge ("Microbial Abundance") had significant impacts on the way that the sponges processed nutrients. Bolded text indicates statistical significance.

		Chi-squared	Df	p.value
DOC	Species	9.010801	9	0.436278
	Microbial Composition	0.301473	2	0.860074
	Microbial Abundance	1.14966	1	0.28362
TDN	Species	7.286518	9	0.607316
	Microbial Composition	1.636614	2	0.441178
	Microbial Abundance	0.012134	1	0.912287
Total Raman Units	Species	14.85563	9	0.094979
	Microbial Composition	**6.261641**	**2**	**0.043682**
	Microbial Abundance	0.272865	1	0.601417
Phenylalanine Like fDOM	Species	12.03426	9	0.211385
	Microbial Composition	4.387393	2	0.111504
	Microbial Abundance	1.034769	1	0.309041
Lignin Like fDOM	Species	5.709988	9	0.768556
	Microbial Composition	1.265046	2	0.53125
	Microbial Abundance	0.170068	1	0.680051
Fulvic Acid Like fDOM	Species	12.55377	9	0.183858
	Microbial Composition	3.358489	2	0.186515
	Microbial Abundance	1.66969	1	0.1963

FIGURES

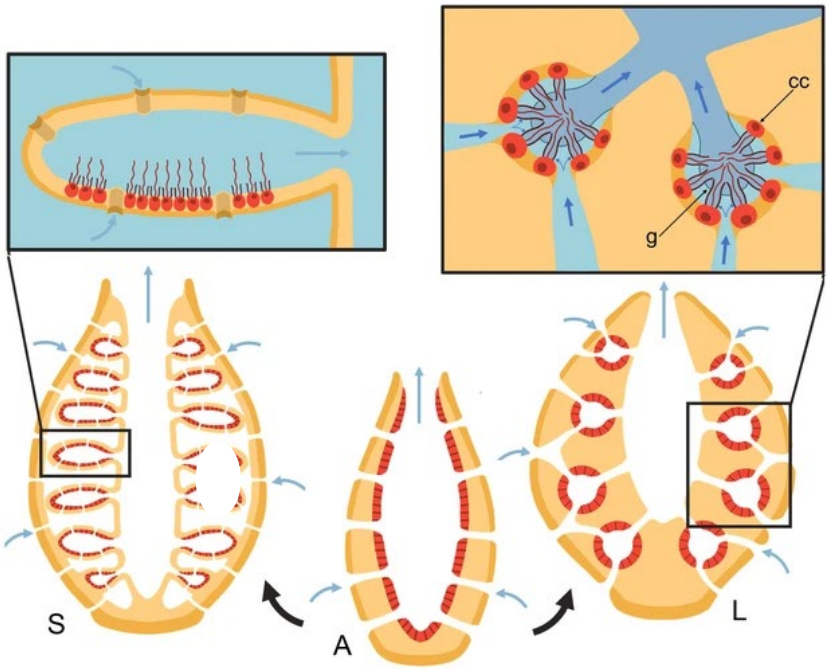

Figure 1 Sponge body types graphic ascon (A), sycon (S), and leucon (L). Most sponges are leucon, including the ones in this study. Insets show the path that water takes through the chambers of the sponge with choanocytes depicted in red. Used with author permission (Asadzadeh et al., 2020).

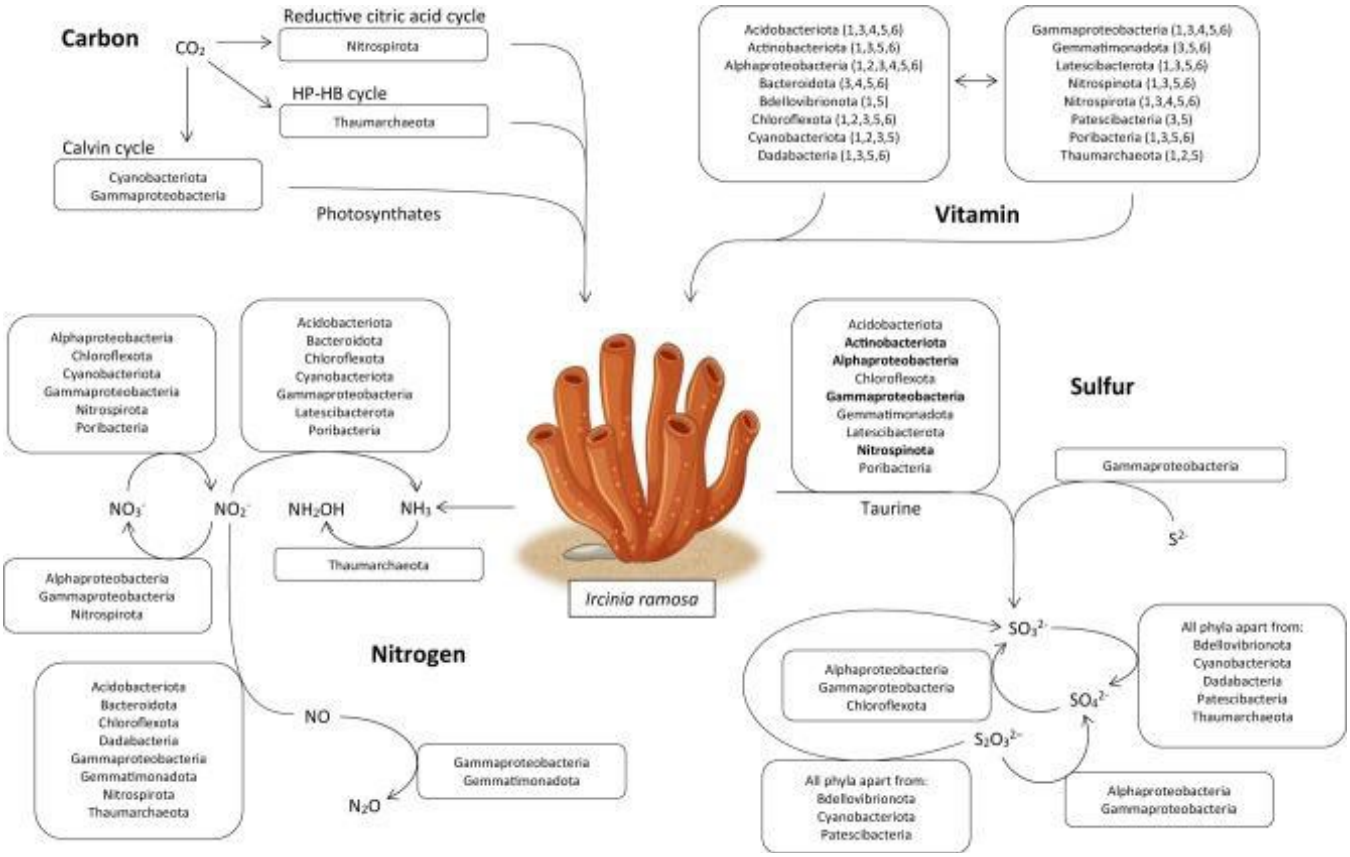

Figure 2 Metabolic reconstruction and the proposed exchange of 4 nutrient types between the model species Ircinia ramosa and its symbionts (Engelberts et al., 2020). The five major symbiont types found in *I. ramose (Actinobacteria, Chloroflexi, Nitrospirae, Cyanobacteria,* and *Poribacteria)* model are shown here, giving a general idea of what these symbionts may provide for their host. Used with author permission (Engelberts et al., 2020.

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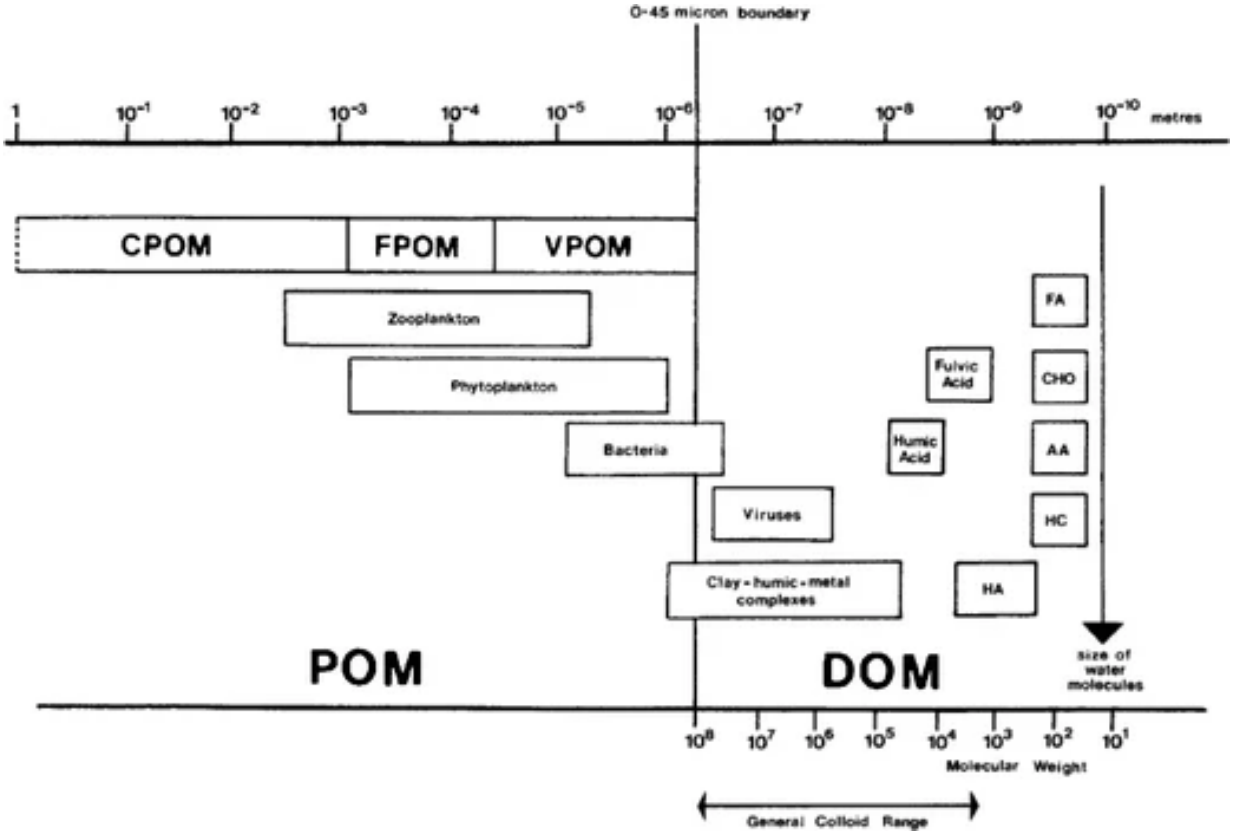

Figure 3 Size range of particulate and dissolved organic matter including other organic compounds found in water. Used with author permission (Nebbioso & Piccolo, 2013).

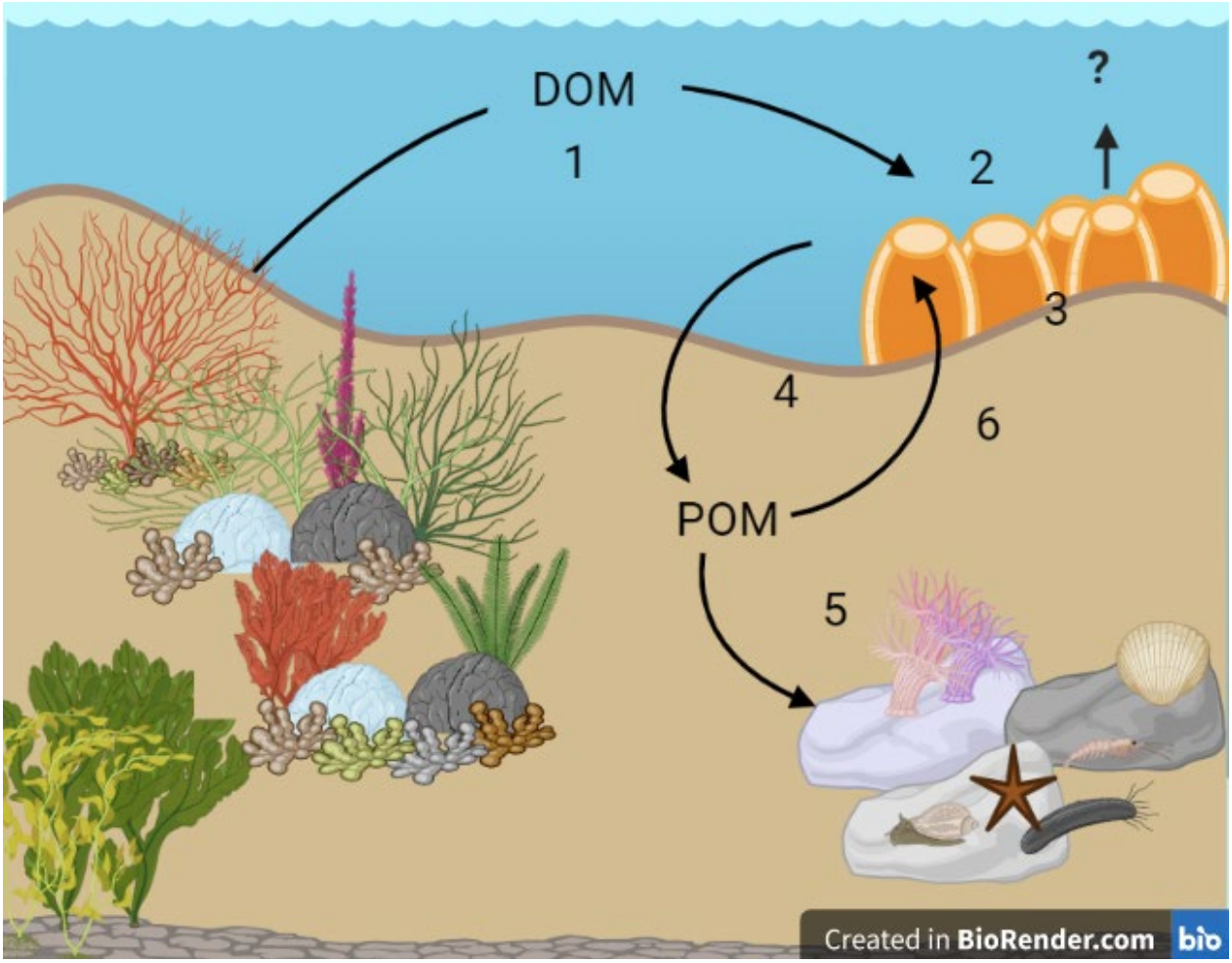

Figure 4 The sponge loop can be explained in a few steps (1) Exudates are released by primary producers like corals and algae into the water column in the form of dissolved organic material (DOM) (Naumann et al., 2010; Haas et al., 2011; Mueller et al., 2014). These primary producers are responsible for releasing up to 50% of their fixed carbon which is a transfer of nutrients and potential energy for the reef (Wild et al., 2004; Tanaka et al., 2008; Haas et al., 2010). (2)The DOM is taken up by the sponges and their symbionts, known as the sponge holobionts, (3)where they either assimilate the nutrients into their tissues, the (4) release particulate organic matter (POM) through sloughing off cells, or are (5) consumed detritivores ingest the sloughed cells or feed directly on the sponges(Rix et al., 2018). The arrows indicate trophic level transfer of nutrients, with the question mark denoting the unknown components of DOM released by sponges back into the environment, the composition of which may vary by sponge species. Image Credit: Keleher via BioRender

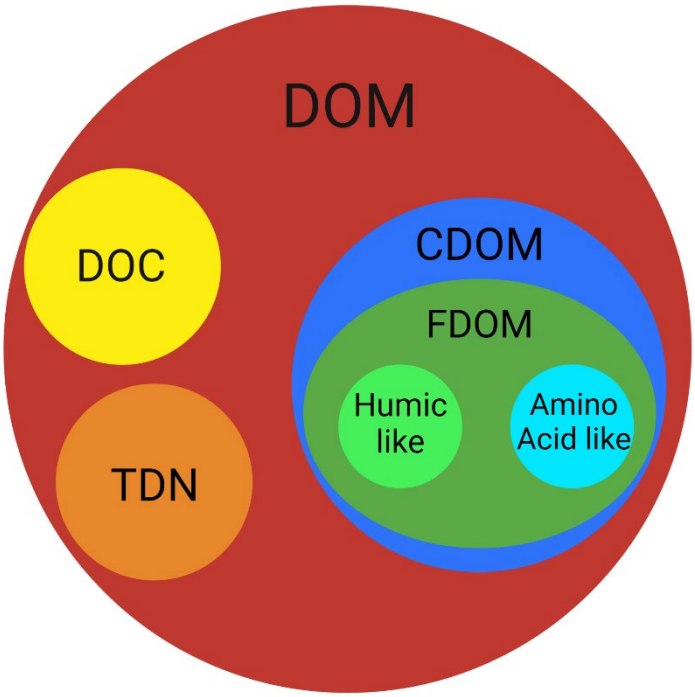

Figure 5 Schematic of dissolved organic matter. The pool of dissolved organic matter on reefs is complex and comprised of several different types of organic material. Dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and fluorescent dissolved organic matter (fDOM), which is a subsection of colored dissolved organic matter (CDOM), are the three types being focused on in this project. fDOM two primary subsections humic-like and amino acid-like. Image Credit: Keleher via BioRender

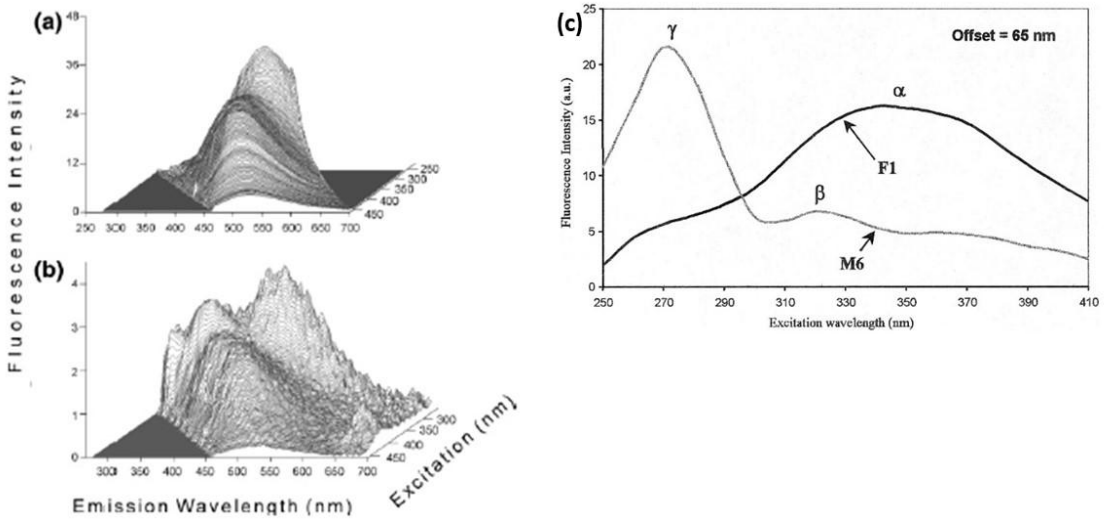

Figure 6 Example of EEM plots for samples from two environments characteristic of (a) rivers, (b) new marine productivity. The vertical axis is in relative fluorescence intensity for panel a, and fluorescence in QSE for panel b (Coble, 2007). (c) Example of a spectroscopy graph for a freshwater sample (F1) and a marine water sample (M6), both collected in April 1996 (Parlanti et al., 2000). The intensity and the wavelengths at which the peaks occur are what is used to determine the molecule. Used with author permission (Parlanti et al., 2000)..

Figure 7 In-situ Vacusip setup and artificial reef. The basket contained a series of pressurized 250 mL bottles for sponge inhalant and exhalent seawater collection. The tubing was stabilized using the red blocks shown on the cinder block near the sponge to be sampled and positioned over the sponge osculum to collect exhalent water. Tubing for inhalant water was positioned in the basket to collect surrounding seawater. The tubing was plugged into the 250 mL bottles via syringe to initiate a vacuum to pull in seawater at a rate of ~ 1 mL min^{-1}.

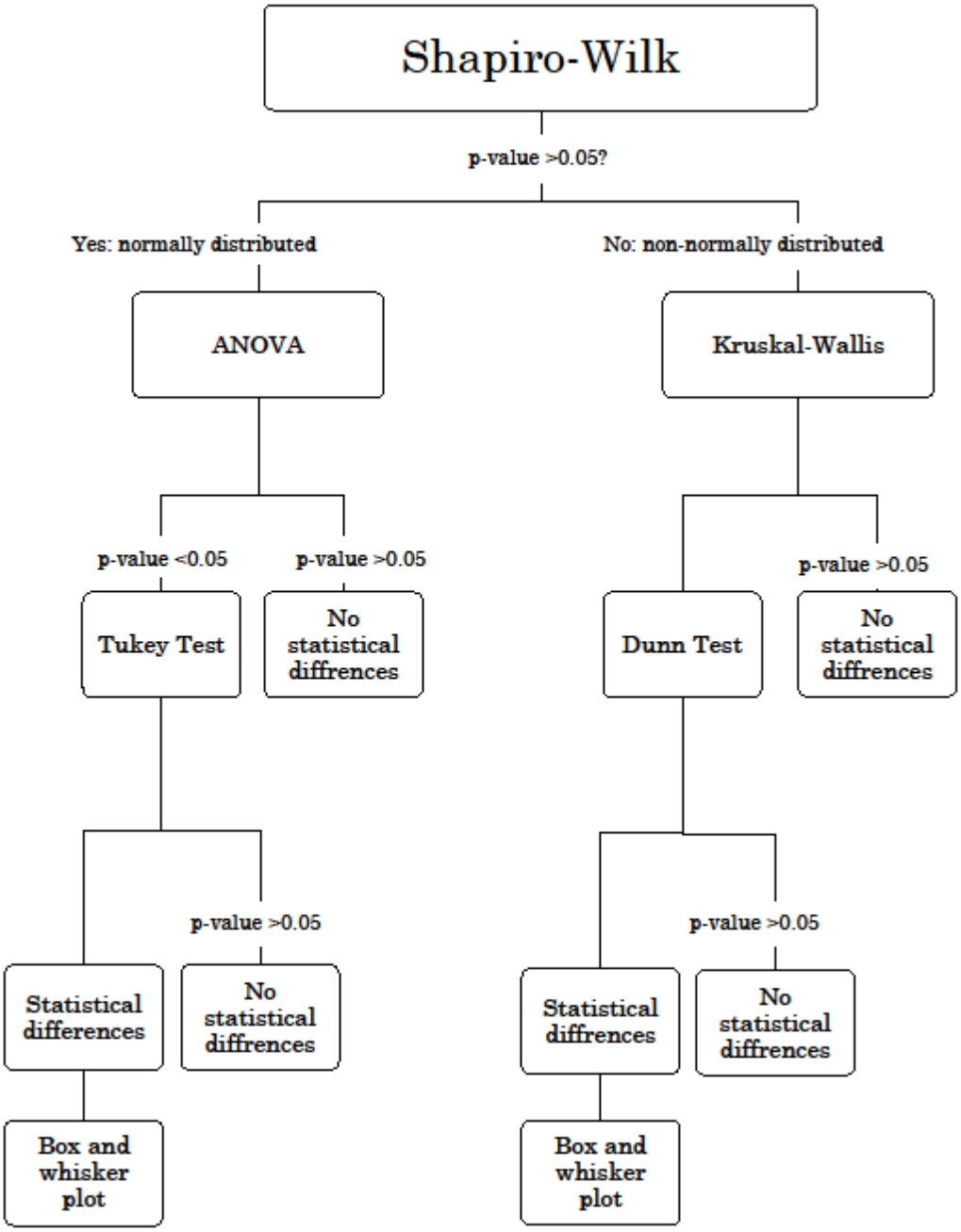

Figure 8 Flow chart to show steps taken during statistical analysis.

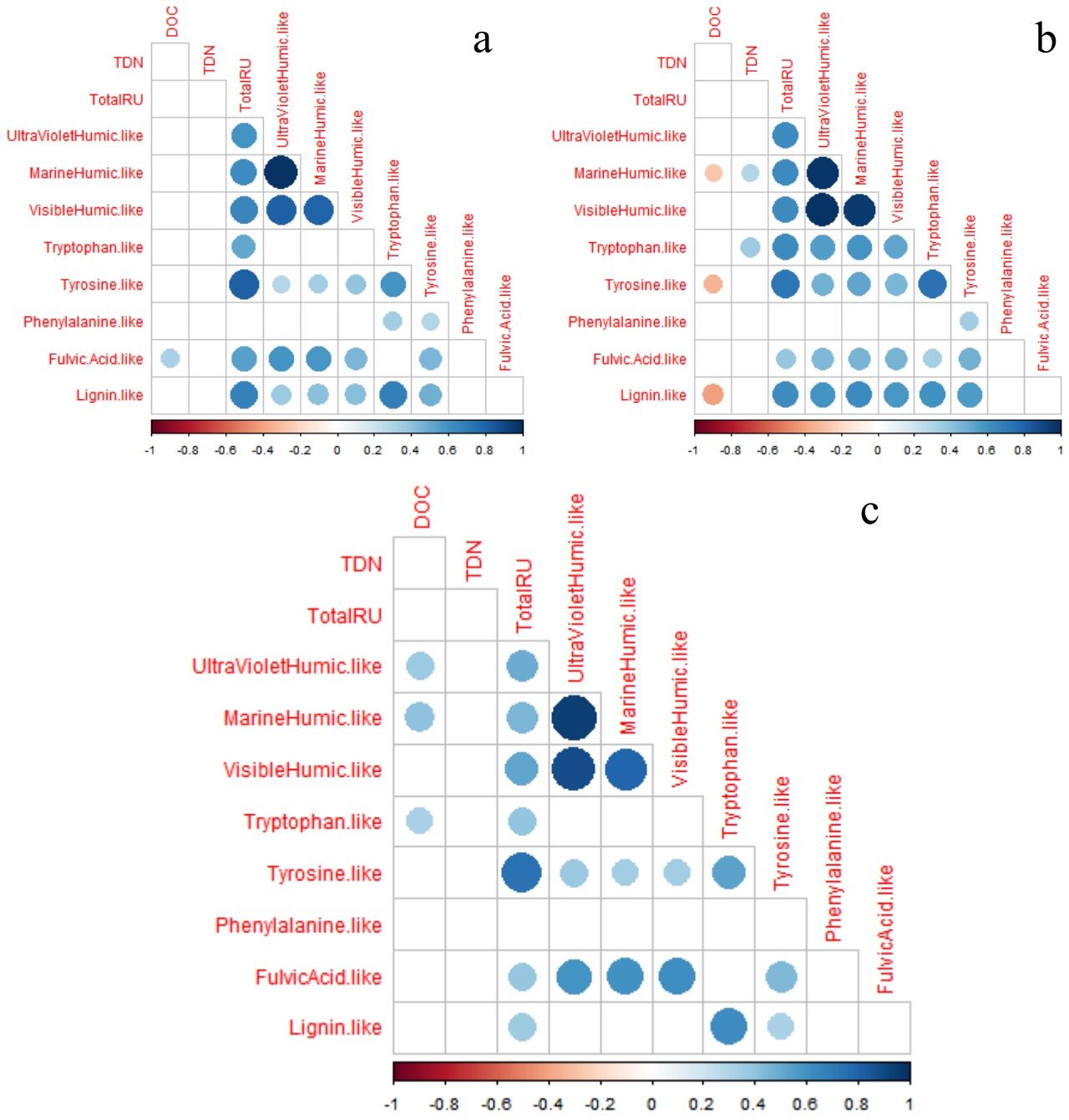

Figure 9 Spearman based correlation plot for Inhalant (a), exhalant (b), and percent change (c). Significant correlations are indicated by a dot in the figure. The darker and larger the dot, the stronger the correlation, additionally red represents negative correlations and blue represents positive correlations.

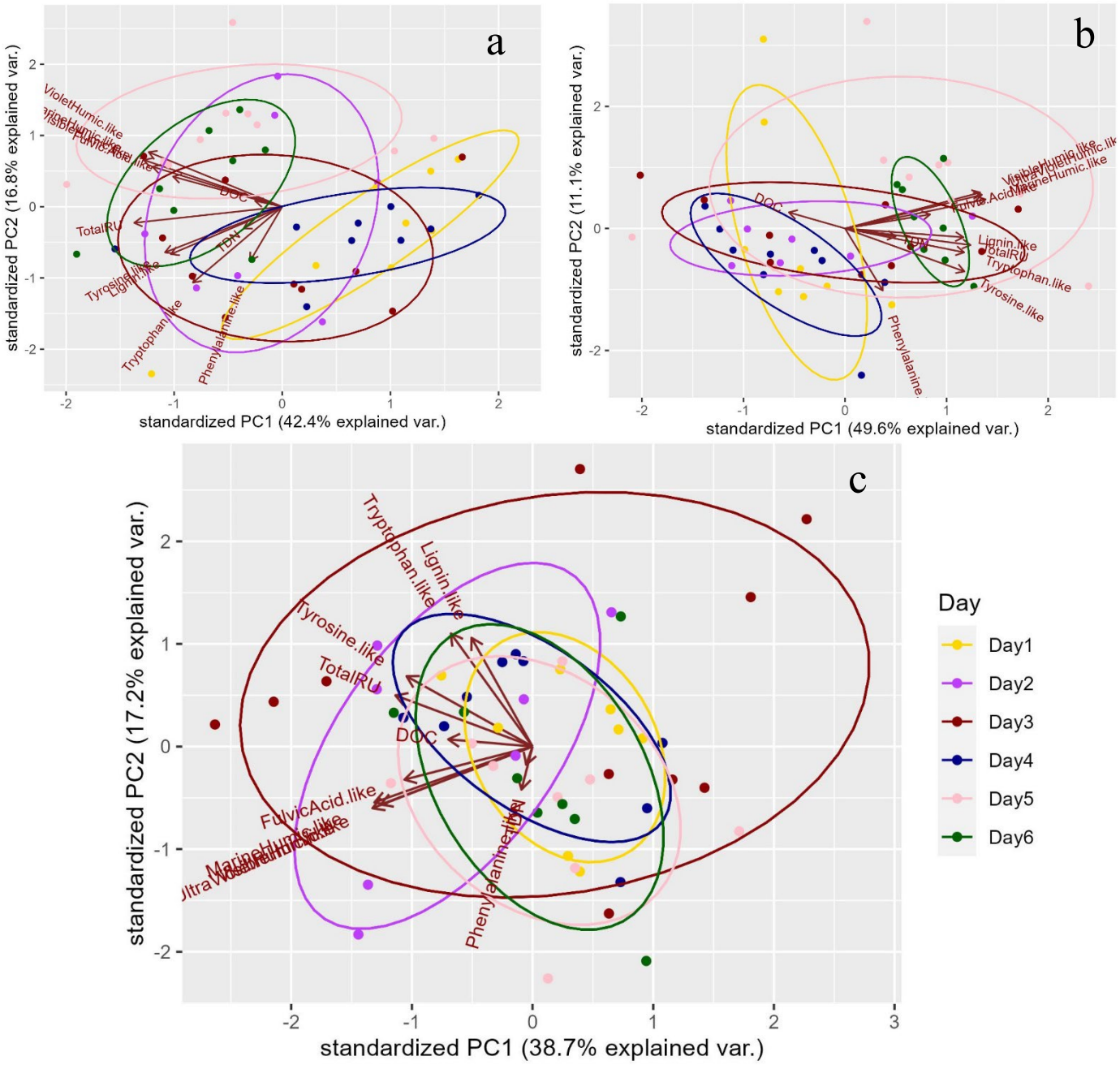

Figure 10 Principal Components Analysis (PCA) using z-score standardized data (a) inhalant *(PERMANOVA, F5,46 =4.7028, adjusted-p = 0.003), (b)* exhalant *(PERMANOVA, F5,48 = 3.7072, adjusted-p = 0.008),* and (c) percent change *(PERMANOVA, F5,46 = 1.3873, p = 0.14)* of the seawater samples. Colors correspond to the day that samples were collected.

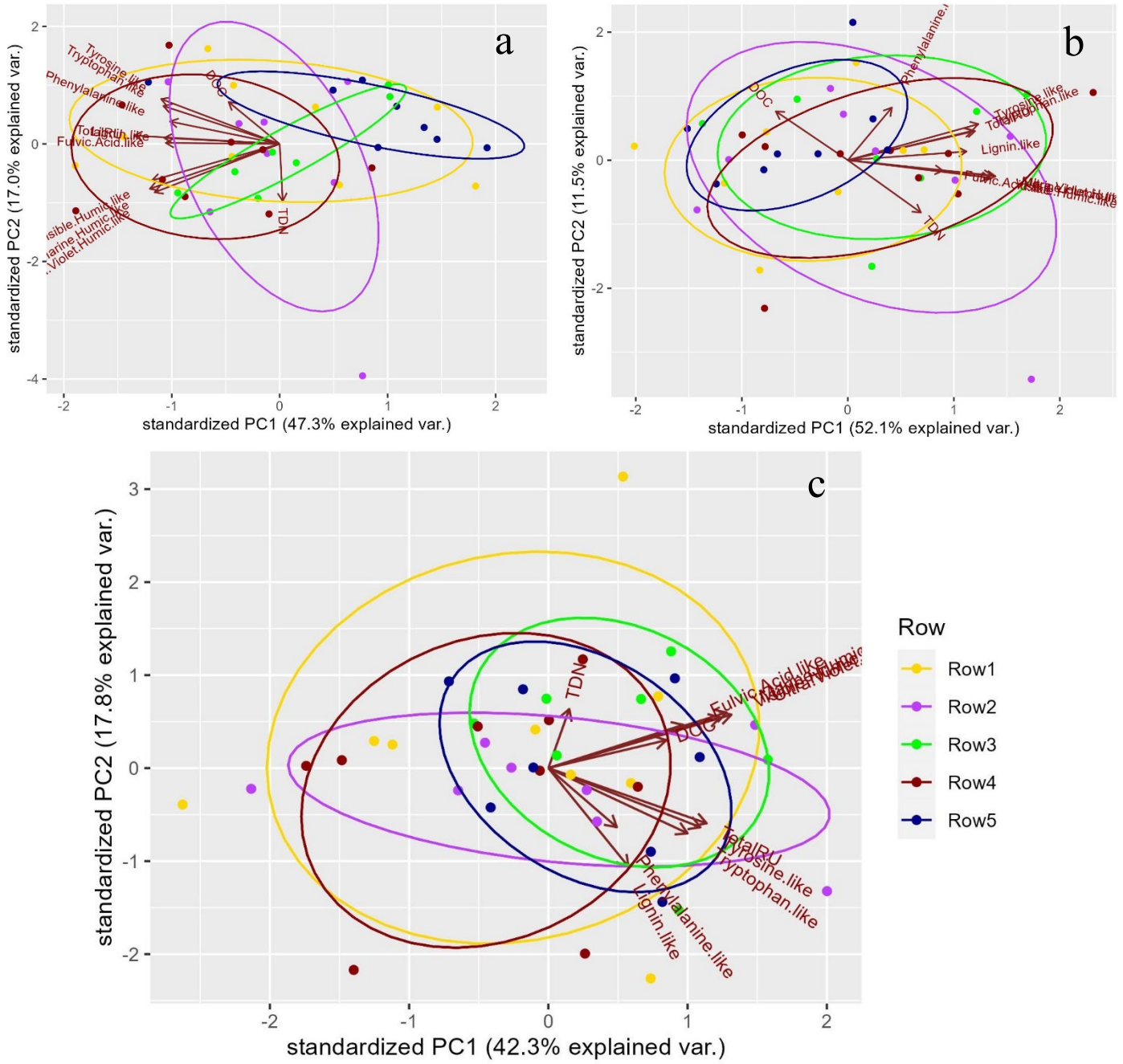

Figure 11 Principal Components Analysis (PCA) using z-score standardized data for (a) inhalant (*PERMANOVA, $F_{4,37}$ = 1.1117, p = 0.304*), (b) exhalent *(PERMANOVA, $F_{4,37}$ =0.8446, p = 0.601*), and (c) percent change (*PERMANOVA, $F_{4,37}$ = 1.0644, p = 0.386*) of the seawater sample. Colors correspond to rows on the artificial reef.

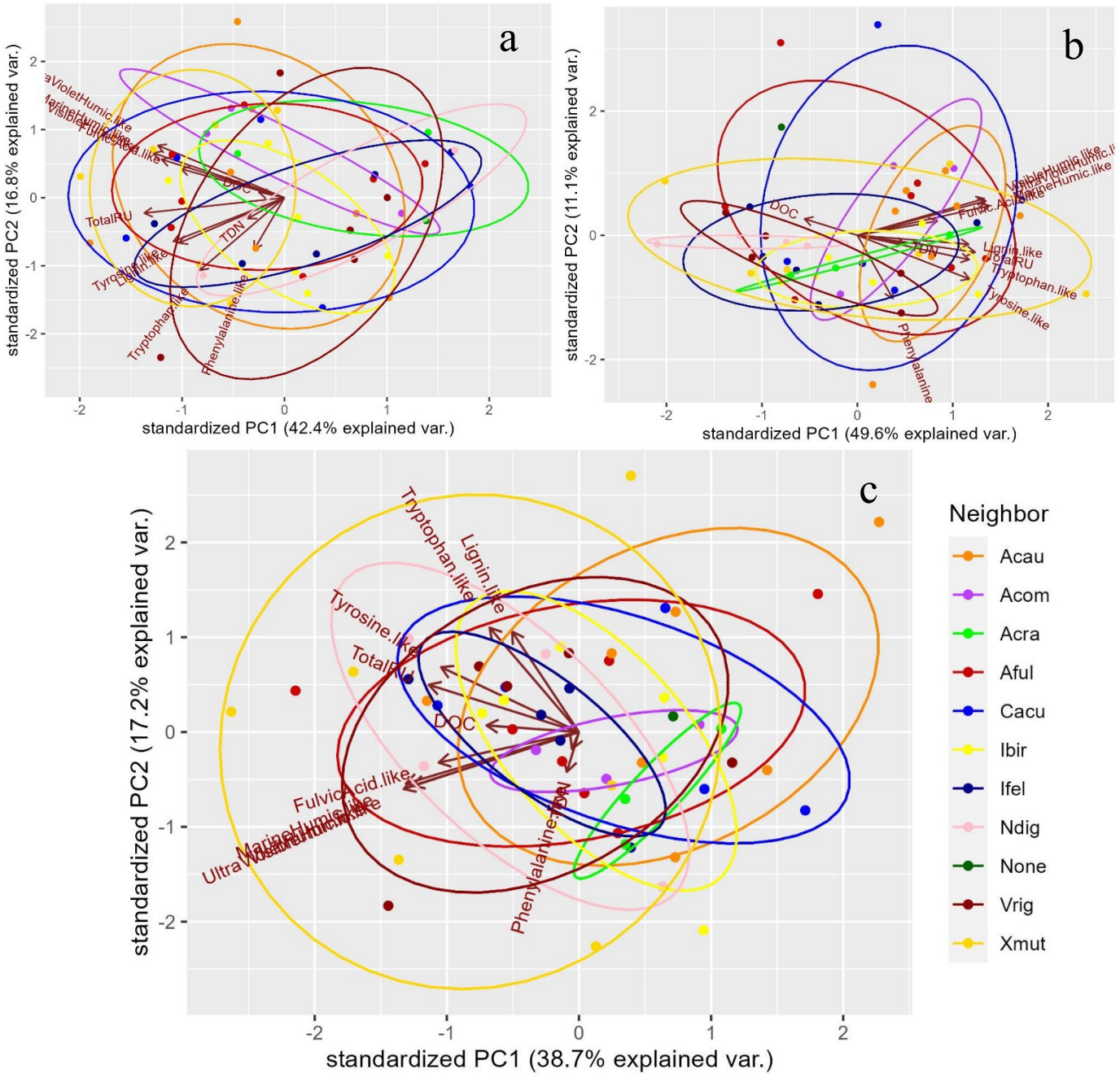

Figure 12 Principal Components Analysis (PCA) using z-score standardized data for (a) inhalant (*PERMANOVA,* $F_{5,46}$ *=4.7028,* p *=0.001*), (b) exhalent (*PERMANOVA,* $F_{5,46}$ *=3.7072,* p *=0.005*), and (c) percent change (PERMANOVA, $F_{5,46}$ =1.3873, p-0.14) of the seawater samples. Colors correspond to the species neighboring the individual that was sampled.

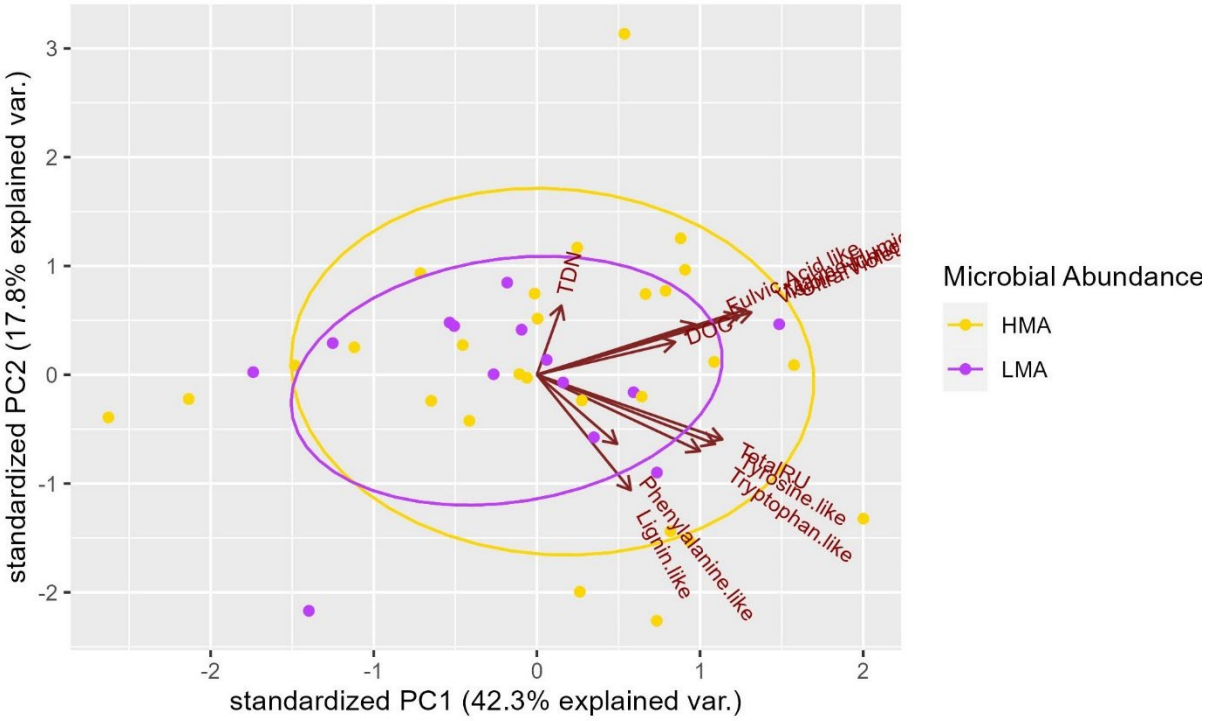

Figure 13 Principal Components Analysis (PCA) using z-score standardized nutrient data (percent change in nutrients) using individual fDOM components. Colors represent microbial abundance (HMA or LMA) (PERMANOVA, $F_{1,40}$ =0.6164, p-0.646).

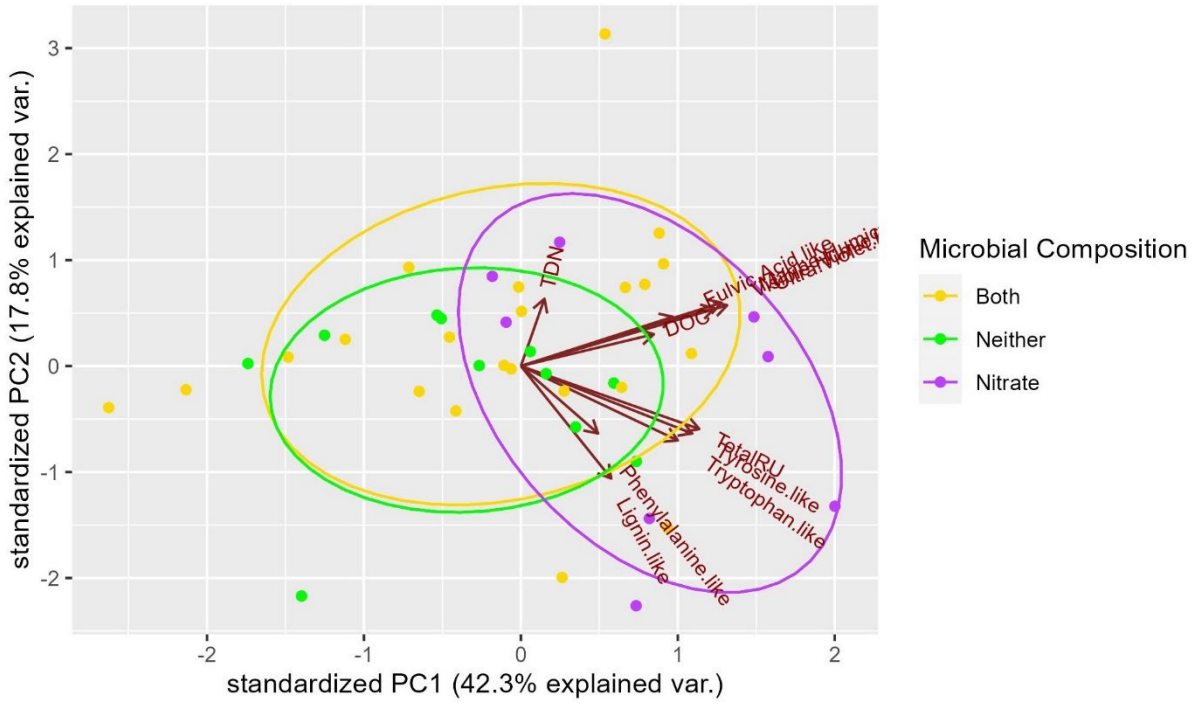

Figure 14 Principal Components Analysis (PCA) using z-score standardized nutrient data (percent change in nutrients) using individual fDOM components. Colors represent microbial composition. *(PERMANOVA, $F_{2,39}$ =2.3286, p-0.028)*

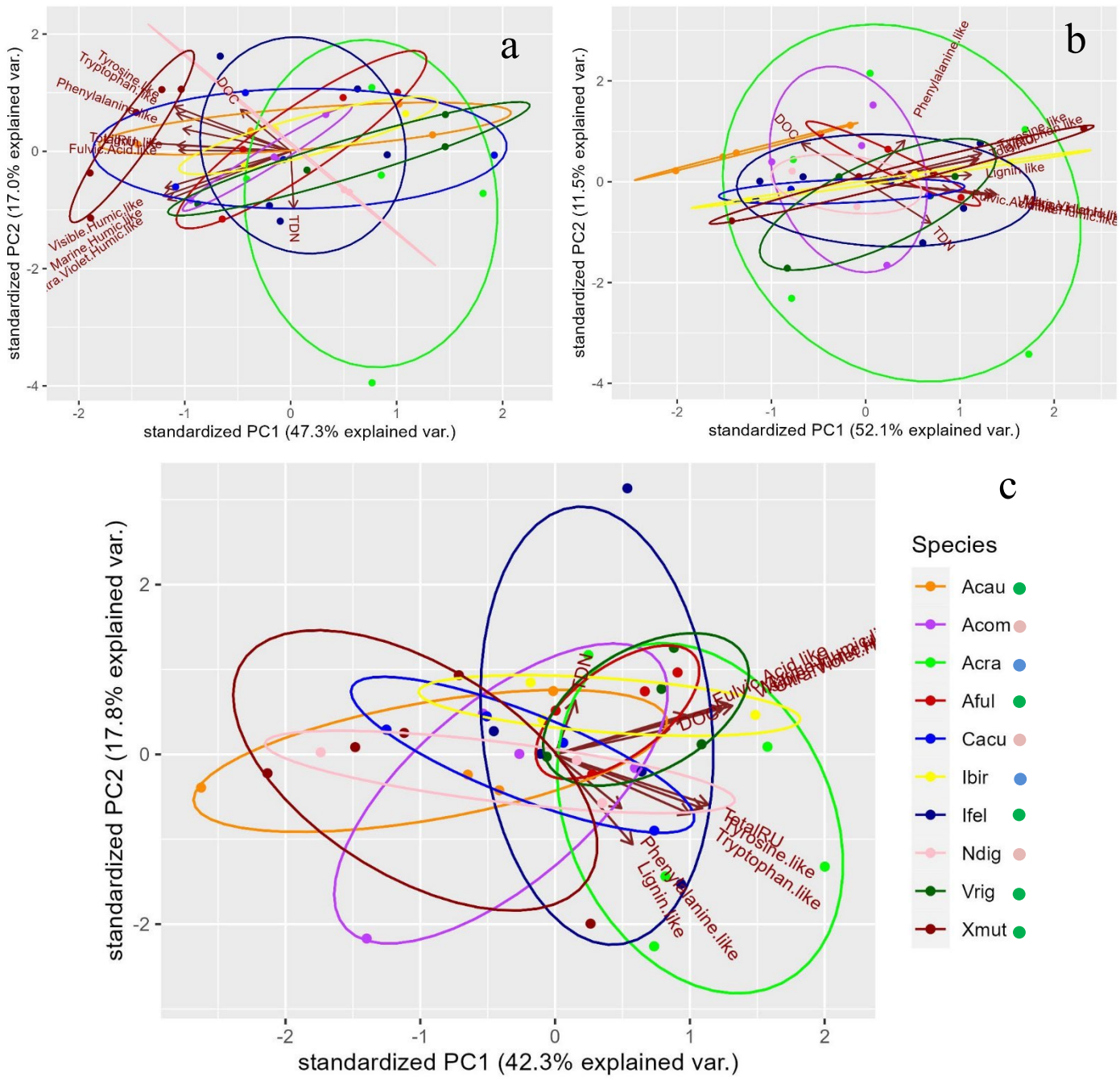

Figure 15 Principal Components Analysis (PCA) using z-score standardized data for (a) inhalant (*PERMANOVA, $F_{9,32} = 1.1895$, adjusted-p = 0.168*), (b) exhalent *(PERMANOVA, $F_{9,32} = 1.4019$, adjusted-p = 0.116*), and (c) percent change (*PERMANOVA, F9,32 =1.8521, adjusted-p = 0.04*) of the seawater samples. Colors of data on the PCA plot correspond to sponge species. Colors next to species in key correspond to microbial composition with green indicating sponges that have both nitrifying and photoautotrophic symbionts, pink indicating species that have neither type of symbiont, and blue indicating sponges that have nitrifying symbionts but not photoautotrophic symbionts. Vectors in panel C that are overlapping are fulvic acid-like, visible humic-like, and ultraviolet-like fDOM.

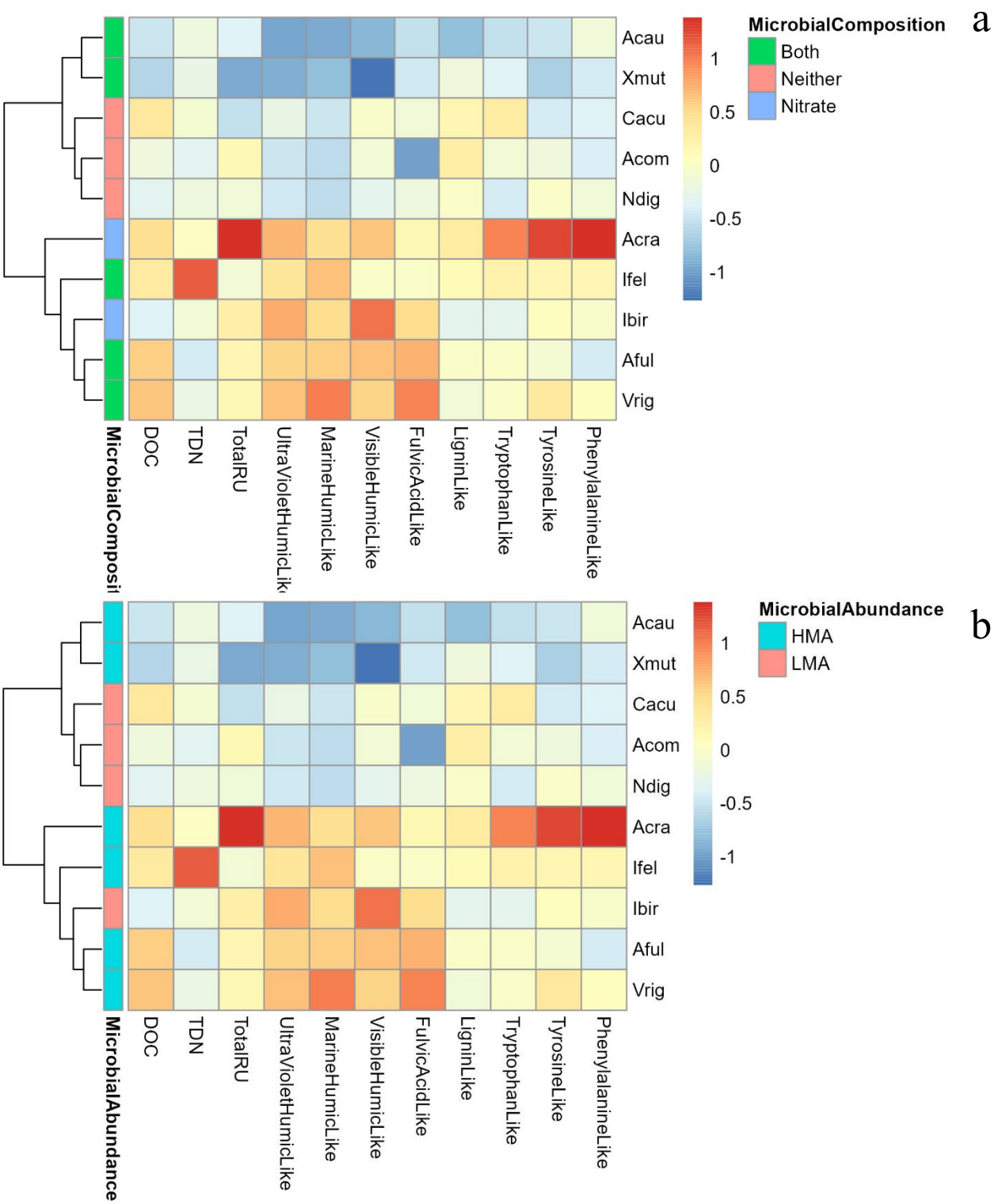

Figure 16 *Heat map with hierarchical clustering of relative change in nutrients across sponge species. Sponge species are listed on the right and types of DOM on the bottom colored by known microbial community composition (a) or (b). Nutrient values were log-transformed and converted to relative values for each sponge species.*

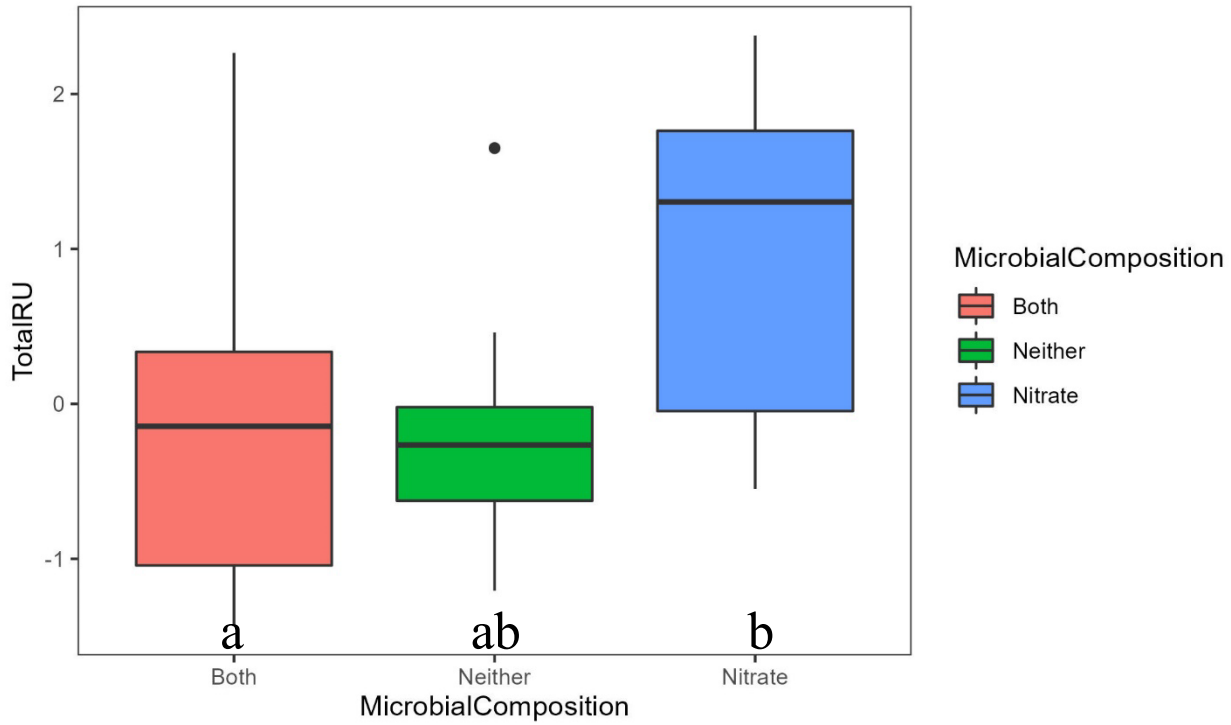

Figure 17 *Percent change of total Raman units microbial composition*. Pairwise comparison using Dunn's tests indicated a significant difference between the Both and Nitrate groups (*p-0.047*). No other differences were statistically significant.

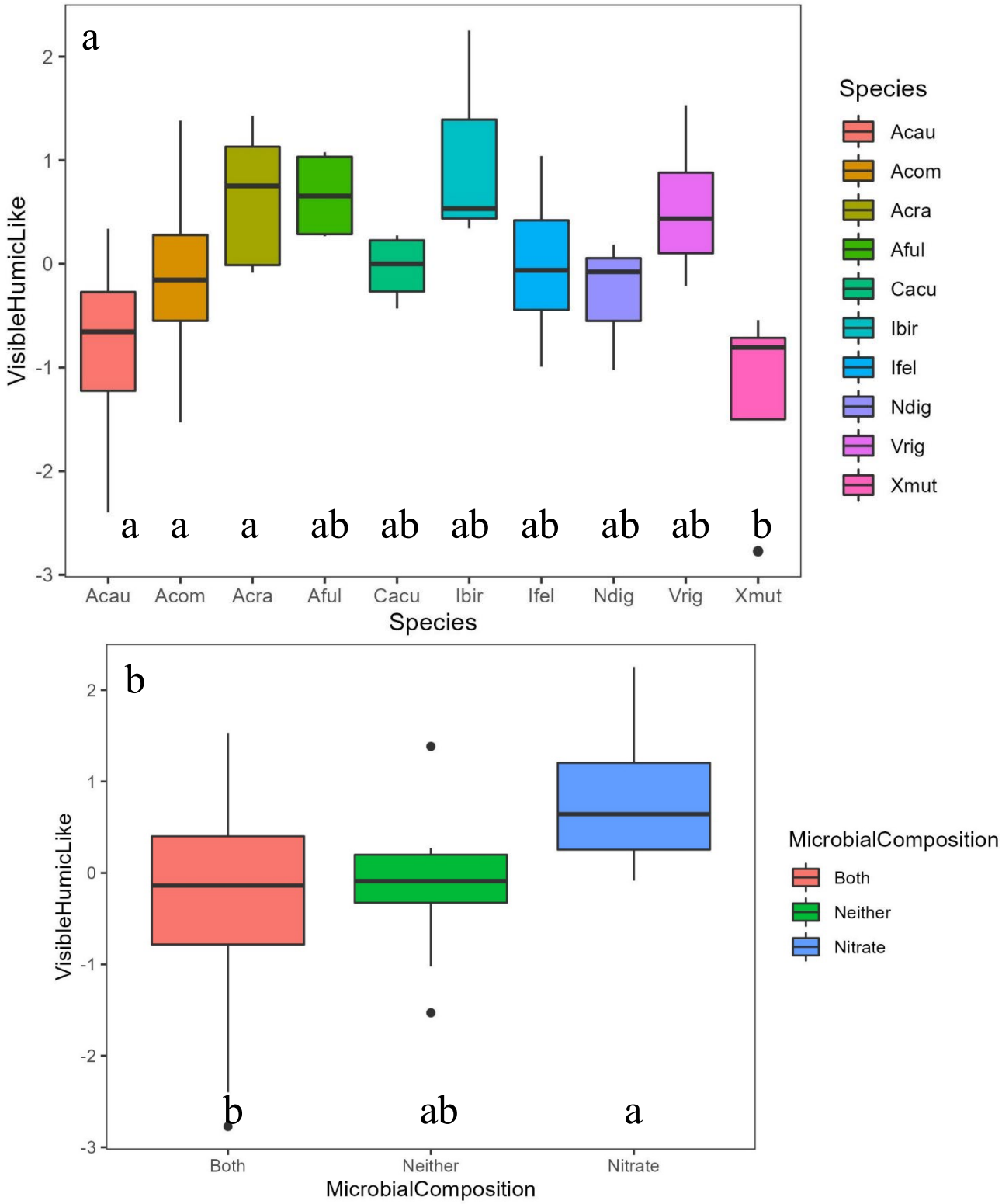

Figure *18 Percent change of visible humic-like fDOM by sponge species (a) (Pairwise comparison using Tukey's tests indicated a significant difference between Xmut* and *Acra (p= 0.029), Xmut* and *Aful (p=0.044)*, and *Xmut* and *Ibir (p=0.019).* No other differences were statistically significant between species*.) and microbial composition (b)* (Pairwise comparison using Tukey's tests indicated a significant difference between the Both and Nitrate groups *(p= 0.037)*. No other differences were statistically significant within the microbial composition.).

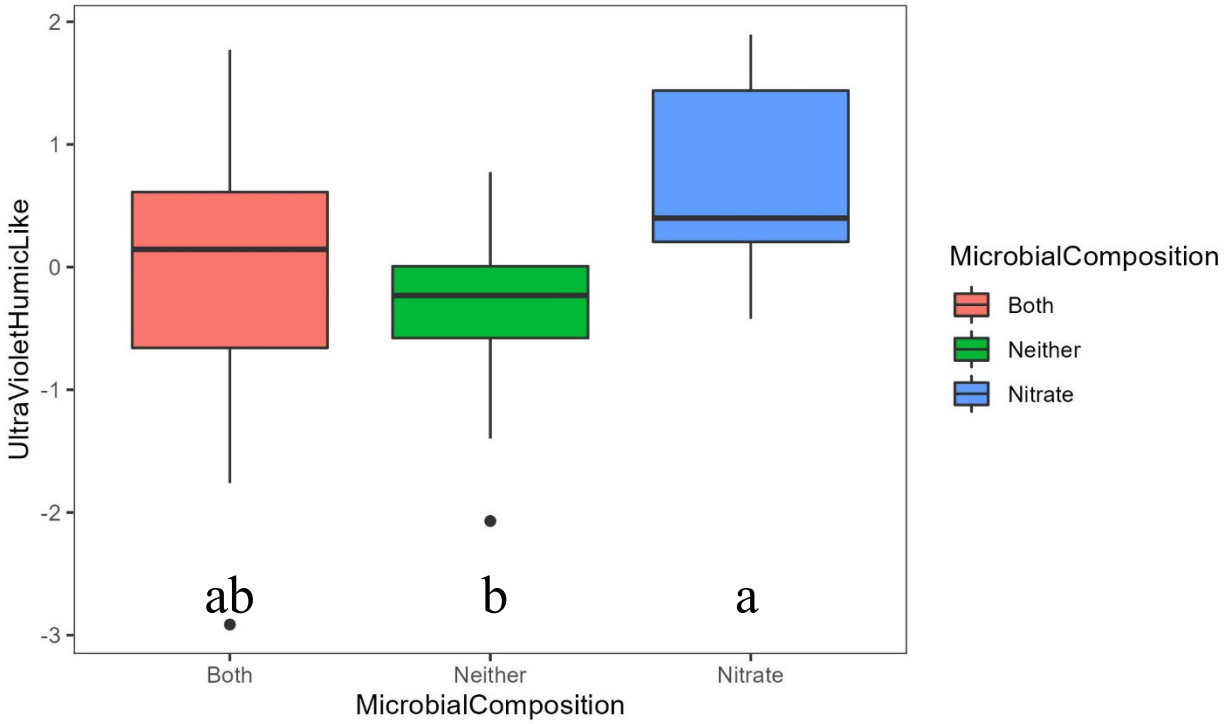

Figure 19 *Percent change of ultraviolet humic-like fDOM compounds by microbial composition* (Pairwise comparison using Tukey's tests indicated a significant difference between the Neither and Nitrate groups *(p= 0.042)*. No other differences were statistically significant within the microbial composition.).

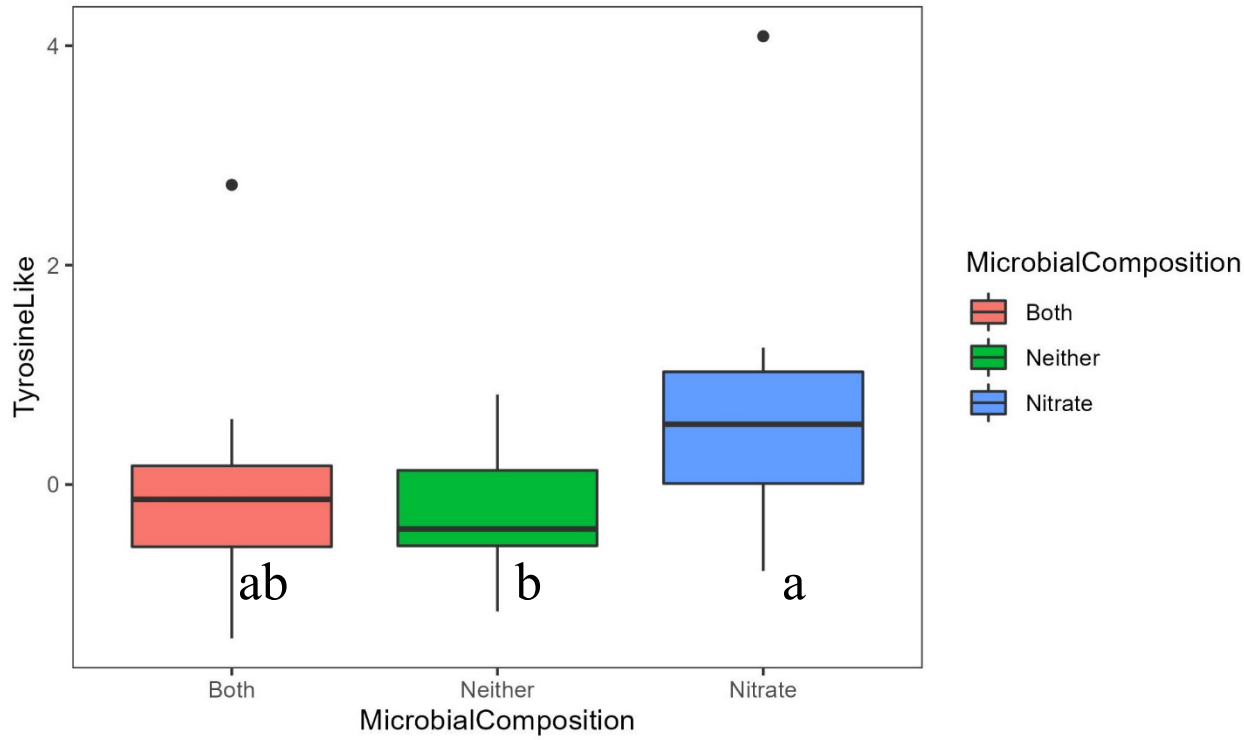

Figure 20 Percent change of tyrosine-like fDOM by sponge species (a), microbial composition (b), and microbial abundance (c). Kruskal Wallis (Pairwise comparison using Tukey's tests indicated a significant difference between the Neither and Nitrate groups *(p= 0.045)*. No other differences were statistically significant within the microbial composition.).

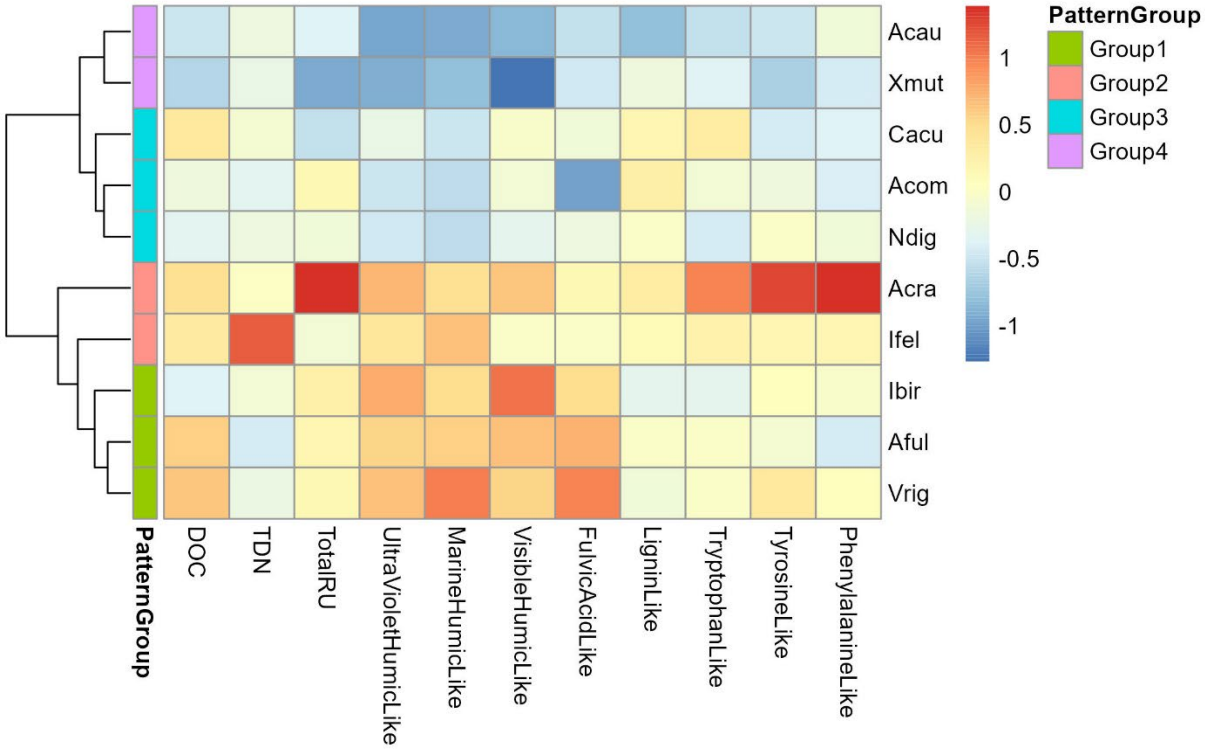

Figure 21 Nutrient profiles seen in the form of this heatmap provide another view of fDOM processing across species and yielded four distinct patterns. Pattern group 1 *(A. fulva, I. birotulata and V. rigida)*, Pattern group 2 *(A. crassa and I. felix),* Pattern group 3 *(N. digitalis, A. compressa, and C. aculeata)*, Pattern group 4 *(A. cauliformis and X. muta).*

Vita

Jacqueline Grace Keleher was born in New Hampshire to Dan and Carole Keleher. She graduated from Texas A&M University at Galveston in August of 2018 where she was awarded Bachelor of Science Degrees in Marine Biology and Marine Fisheries with a minor in Diving Technology and Methods. She began pursuing a Master of Biology from Appalachian State University in August of 2019. The M.S. was awarded in December of 2022.

Ms. Keleher currently resides in Washington State with her husband. She is working as a Dive Program Aide at the Point Defiance Zoo and Aquarium, where she is able to combine her passions for conservation, science communication, and diving.