AN INVESTIGATION OF THE MICROBIOLOGICAL COMMUNITIES ASSOCIATED WITH THE DEGRADATION OF INVASIVE ALGAE IN A TERRESTRIAL ENVIRONMENT

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By
KEALOHI SABATE

Thesis Advisor

BRANDON YOZA

I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

THESIS ADVISOR

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BRANDON YOZA
HAWAII NATURAL ENERGY INSTITUTE

For my family, friends, and loved ones who have supported me through all my environmental endeavors

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Abstract

Coastal ecosystems worldwide have been significantly impacted by the overgrowth of invasive algae, leading to habitat loss and the decline of species diversity. There has been a number of success stories to remove invasive species in Hawaii. However, because algae are high in moisture content as well as contain carbohydrates that aren't typically found in the terrestrial environment, its disposal has become an ongoing issue. Macroalgae are known to be rich in nitrogen and carbon, suggesting that its use as a compost or fertilizing material may have agricultural benefits. The biological process for algae degradation in the terrestrial environment, however, remains undefined. From this study, we identified that the microbial communities associated with the degradation of invasive macroalgae, specifically *Gracilaria salicornia, Avrainvillea amadelpha,* and *Acanthophora spicifera,* are diverse and distinct compared to that of degraded terrestrial biomass. Contents of carbon, nitrogen, and phosphate within algae should be an important consideration for agricultural practices as these key nutrients have an effect on the decomposition process. Heavy metals such as iron and arsenic, which were present within these samples can also have an effect on the degradation process as well as have adverse human health effects. These are potentially important considerations for agricultural applications and future management practices.

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1.0 Introduction

Around the world and in Hawaii, invasive algae have a significant impact on marine ecosystems (Simberloff *et al.*2012, Smith *et al.* 2002, Magalhaes *et al.* 2014). As a result of these issues, environmental mitigation requiring the collection and/or removal of invasive algae have launched and resulted in the emergence of an atypical "green" waste that has yet to be understood for disposal purposes (Han *et al.* 2014). Macroalgae have complex structural polysaccharides and the process for biological degradation are poorly understood. For that reason, the determination of this process is an important consideration to effectively manage waste algae in a terrestrial environment.

There are three distinct groups of marine macroalgae; *Phaeophyta*, *Chlorophyta*, and *Rhodophyta*. All groups of algae are capable of becoming invasive when it settles in an environment that is not native to the defined geography and has a competitive survival advantage compared with the natural fauna. In other cases, native algae also have the potential of becoming invasive if there is a change in its natural environment. The competitive advantages of invasive algae include its ability to be more resilient to environmental change, which can lead to ecosystem shifts that allow for the invasive algae's rapid dissemination. Urbanization and resource utilization are the principal sources of anthropogenic-induced changes to the marine environment, which can have a significant effect on native species (Smith *et al.* 1999). Pristine marine environments are typically low in nitrogen and phosphorus, so the introduction of excess nutrients could lead to eutrophication in the nearshore environment, altering algal dominance and ecosystem biodiversity. Corals are the keystone species in most coastal ecosystems, with algae typically found in areas that have herbivory activity. Increases in anthropogenic disturbances can escalate nutrient loading which can reduce grazing rates through the process of eutrophication that can lead to rapid algae growth and proliferation (Smith *et al.* 2002). Changes in the natural fauna and algae overgrowth rates could also cause changes in the local food web (Simberloff *et al.* 2005).

In addition to coastal impacts, the removal of invasive algae can present management issues since its disposal is poorly understood in the literature. Currently there is little known about the natural capabilities of algae degradation in a terrestrial environment, and for that reason, important management strategies should be taken into consideration for algae wastes due to its high water content (Yokoya and De Oliveira, 1992; Han, Clarke and Pratt, 2014), high inorganic compositions (Hou and Yan, 1998), and distinct structural polysaccharides (Hoagland and Lieb, 1915) to that of terrestrial plant biomass. Presently, macroalgae have been investigated for potential biotechnological uses, as well as agricultural use as a compost or fertilizer. Biotechnological applications include the generation of fuel (Yoza *et al.* 2013), methane (Miura *et al.* 2014), the collection of rare earth metals (Jacinto *et al.* 2018), and other high-value products (Ruiz *et al.*, 2016). As for agricultural use, macroalgae as an organic substrate for mulch and the generation of composting materials have also been suggested as potential uses (Han, Clarke and Pratt, 2014). Availability and cost do, however, limit the advancement of these technologies (Lundquist *et al.*, 2010).

1.1 Invasive macroalgae

Invasive macroalgae is a global issue having negative effects on both coastal communities and other inland waterways (Martinez *et al.* 2012). Anthropogenic activities taking place near coastal regions are often cited as the most significant contributor to the introduction of non-native species, with a number of these sources being linked to urban development (Anderson *et al.* 2015). In Hawaii, invasive algae were introduced during the 1950s, however, the specific origins of many remain unknown. A total of 19 macroalgae species have found their way to the islands either accidentally through maritime activity or intentionally imported for aquaculture use (Smith *et al.* 2002). According to the Department of Land and Natural Resource's website, the invasive algae that are of concern within the State include; *Kappaphycus* (introduced), *Eucheuma* (introduced)*, Gracilaria* (introduced), *Avrainvillea*, *Hypnea* (introduced), and *Acanthophora* (DLNR, 2019). These algae are distributed throughout the island chain and are typically concentrated near industrial areas. In this study, the Maunalua Bay area that was sampled include the genus *Gracilaria, Avrainvillea,* and *Acanthophora.*

For many stakeholders, the main problem of invasive macroalgae removal efforts is its cost. Costs that are associated with management include planning, collection and disposal fees (Neilson *et al.* 2018). Some indirect expenses that are also involved as a result of invasion include tourism impacts, drops in land values (Hoagland *et al.* 2006) as well as environmental costs, including impacts and loss of natural diversity (Bax *et al.*, 2003), eutrophication, and coral reef loss.

Efforts to remove invasive algae have been in effect around the island for many years, but due to its competitive advantages in a tropical environment it is hard to manage. Thus far, manual, chemical, and biological techniques have been tested in hopes to control the problem, but the modes of removal are dependent on the goals of the effort, or wither it is for control or eradication purposes. Some management examples include cold shock, salinity treatments, dredging pumps, manual removal by hand, and biocontrol. The use of a single technique has shown to be ineffective at combating this issue, and the combination of manual removal and biocontrol are the most practical methods as it poses less of a risk to the surrounding ecosystems (Neilson *et al.*, 2018).

Today, if an algal bloom were to occur in an area under the right conditions, it could cost Hawaii millions of dollars within the tourism and fishery industries (Smith *et al.* 2003). Ecosystems benefit the communities with goods and services, and it is estimated that the oceans economically contribute to about 60% of these necessities (Katsanevakis *et al.*, 2014). In Hawaii, some areas are shown to have lost $20 million per year from a reduction in hotel development, property value, and associated costs for algae removal (Smith *et al.*, 2004). In 2008 for example, one of [China region]'s main water pipes became clogged with invasive algae, resulting in a severe drop in local fish populations in the area. The city removed about 150,000 tons of wet algal biomass which required over 10,000 workers to remedy the situation (Han, Clarke and Pratt, 2014). Long term consequences of these persistent algal dominated areas result in habitat loss, degraded reefs, and changes in community structures. In Hawaii, restoration efforts alone in Maunalua Bay (located on the south eastern side of Oahu) have managed to remove about 3 million tons of invasive algae per year within a 23 acre region, at the cost of $3.4 million (Kittinger *et al.*, 2016).

1.2 Invasive algae management

Strategies for the management of algae include biological, chemical, genetic, and environmental approaches. These management approaches vary based on place, and thus far not a single management method has been found to be universally effective (Anderson, 2004). Government interventions have worked towards facilitating the flow of materials of coastal regions and providing financial support in clearing these areas (Williams and Grosholz, 2008). Other techniques have introduced chemical loading as a means to reduce populations (Anderson, 2004). The implementation of eradication plans was used in California to control *C. taxifolia* through chlorine trapping to suppress growth (Anderson, 2005). Areas in Australia and New Zealand on the other hand, have introduced coarse salt as a way of reducing invasions, but this method has deemed to be very costly (Anderson *et al.*, 2015).

In Hawaii, the majority of invasive algae populations are mitigated through community restoration efforts and local government agencies. Maunalua Bay, for example, is an area that experience a lot of community attention through non-profit organizations that facilitate the manual removal of these invasive species (Kittinger, 2016). On average, the non-profit Malama Maunalua has removed around 3 million tons of invasive algae which spanned over a course of 23 acres (Macduff *et al.*, 2018). Other areas such as Kaneohe Bay have also worked towards algal removal through the use of the Super Sucker (Kittinger, 2016). This technique is used across aquatic communities where reefs are pumped with a machine that sucks off debris and pumps it on deck where it is manually sorted to ensure native species can be returned (Westbrook *et al.*, 2015). Manual removal is the most common of the mitigation techniques, but studies have shown the algae rapidly regrow once removed due to the disturbance of marine sediments (Weijerman *et al.* 2008). After these local collection efforts, the waste algae are typically deposited on land for potential agricultural use.

The use of biocontrol is a potentially less invasive method that can limit excessive algal growth. This is due to the fact that the introduction of selective herbivorous and grazing species are designed to feed selectively and control invasive algae populations. However, biocontrol techniques may create unintended alterations in ecosystem dynamics, potentially disrupting the trophic cascade in place. The use of biocontrol is a newly introduced idea that involves the introduction of other organisms to manage population sizes. Organisms such as sea urchins and mollusks are suitable applications of introduction to areas with invasive algae, but its effectiveness has only deemed successful in areas with low algal abundance (Neilson *et al.* 2018). As of 2014, a plan was devised to introduce 200,000 sea urchins in an effort to control invasive algae in Kaneohe Bay on the island of Oahu (Abercrombie, 2014). In conjunction with mechanical removal and community support, the effort has reduced the invasive algae population by 85 percent (Borunda, 2018), and it is hoped that this would control the invasive algae and result in favorable coral reef habitat recovery.

1.3 Macroalgae as a resource

Macroalgae can serve as a benefit for various reasons. As a source of biomass for the production of renewable energy, algae possess high photosynthetic capability, growth rate, and potential for offshore cultivation. Fossil fuels are limited resources that are in high demand for energy production, which pushed for other alternatives such as biofuels to be taken into consideration while planning for future source demands. Worldwide, biofuels derived from living matter account for about 10-14% of the total world energy supply (McKendry, 2002), and by further implementing this as a source of energy, it could have the potential to reduce greenhouse emissions by about 30% (Scharlemann, 2008). While biofuels offer an eco-friendly alternative reserve, the implementation of algae as a biofuel face challenges related to its composition, production, and economic feasibility (Milledge *et al.* 2014).

To increase economic viability the production of value-added products which are goods that serve to increase the worth/use of another product is a necessary consideration. Macroalgae are known to be capable of bioaccumulating metals to concentrations much higher than its surrounding environment (Gekeler *et al.*, 1988). Because of this, algae have been studied for use during remediation efforts and more recently for the collection of valuable metals (Jacinto *et al.* 2018). Another alternative includes its use for carrageenan. Carrageenan is a component of algae that is used as a food additive to thicken and preserve food. This polysaccharide is typically found in red algae and contributes to a $98.4 million industry used globally for various products across Europe, Latin America, North America, and the far East (Stanley, 1997). As an alternative, waste algae could potentially be used for the economic advantage of producing such a resource.

1.3.1 Macroalgae for the production of compost

Composting is defined as the optimization of decomposing organic material that is meant to stabilize organic matter in order to help improve soil fertility, amend porosity, reduce nutrient loss, and increase water holding capacity (Adugna 2016). To do this, soils are usually kept at a 25:1 or 30:1 ratio of carbon to nitrogen as well a 40-60% moisture level to optimize the degradation potential (Atalia et al. 2015). This process of decomposition is facilitated by fungi and bacteria that metabolize and breakdown organic matter for growth and reproduction (McClaugherty et al. 2001). Compost can differ in quality and stability depending on the raw material it is composed of (Azim et al. 2018). Not all microbes are capable of directly assimilating organic material and require certain enzymes to break down various polymers in order to decompose certain types of plant material (De Boer et al. 2005). Because soil microbes are vastly diverse based on host species and plant composition (Hattenschwiler et al., 2005), this can affect ecosystem processes that drive the decomposition of these plant materials (McGuire et al. 2010). Current understanding of the microbial communities related to algae are not well understood, so it is uncertain how this could affect soil decomposition capabilities.

Structurally, terrestrial plants are very different from macroalgae. This is in part because terrestrial plants are mostly comprised of cellulose, hemicellulose, lignin, pectins, callose, arabinogalactan-proteins, and extensions that can be broken down by the environment (Dehors *et al.* 2019, Doi *et al.* 2003, Makela *et al.* 2014, Zimmerman 1990). In contrast, macroalgae have complex structural and storage polysaccharide compositions that are different between divisions; *Chlorophyta, Rhodophyta, and Phaeophyta* (Xu *et al.* 2017). Compositional variability and sulfated polysaccharides are not typically associated with terrestrial biomass, therefore, may require a different degradation pathway that is not currently found in the literature. Although composting is a process that is highly influenced by microbial activity, not much research has been done to look at the influence of microbial communities with the introduction of algae.

Current studies show some peculiarities in the use for algae as a compost. Algae are high in moisture and salinity with relatively lower nitrogen contents, which complicate the composting process (Han *et al.* 2014). Additionally, due to its high mineral and moisture contents, this has made the process of landfilling problematic (Edmunson *et al.*

2013). Under certain moisture conditions, microbial activity is inhibited if it doesn't fall within a moisture range of 25%-70%, and algae could off set this balance since the moisture contents typically range between 50-80% depending on species (Silva et al. 2008). The composting process is largely influenced by the porosity of the pile which regulates the temperature and O_2 status within the pile. Temperature plays an important role in boosting the microbial activity within the decaying process, but too much activity can decrease microbial productivity which tends to complicate its implication. High salinity inputs can also inhibit microbial activity as the presence tends to lyse the cells of the microbes thus inhibiting its production.

Some compost piles have a higher fertilizer value than others, depending on its feedstock, influencing the overall net benefit for the soil quality. Generally, soils lack in nutrients such as nitrogen, phosphorus, and potassium, prompting many agriculture industries to invest thousands of dollars per ton on imported fertilizers to sustain their crops. Between 2006 to 2008, fertilizer costs jumped from $300 to $1000 due to the increase in nutrient poor soils from constant cultivation, which incentivized the demand for local farms to find alternatives to help lower costs (Radovich *et al.* 2012). Algae are anticipated to be rich in potassium, which is good for soil enrichment, however contents are highly dependent on the species (Radovich *et al.* 2012). It has been proposed that the algae in its organic form are essential in fixing atmospheric nitrogen for plant productivity and have antifungal properties that could make it a good bio fertilizer (Prakash 2014). This, however, is poorly understood, especially with algae in its whole form. Although algae can provide valuable nutrients to condition soils, there is little evidence that suggests the microbial communities within marine algae would be efficient in breaking down nutrients in a terrestrial environment. Organizations such as Malama Maunalua have taken waste algae removed from Maunalua Bay and transported it to local farms around the island to use as compost. To date, the organization has donated about 30 tons of waste algae to local farms where it is converted into either green waste or used as an organic fertilizer (Ranch, 1968).

The purpose of this study is to provide a better understanding of the microbial communities associated with the degradation process of marine algae in a terrestrial environment, in hopes to aid future management strategies. The structural makeup of macroalgae differ from terrestrial biomass, and descriptions of the microbiological degradation processes for macroalgae in a terrestrial environment are unavailable in the literature (Kraan, 2010). For that reason, it is assumed that the degradation processes should be different, and so, this study will evaluate the biological processes that are associated with macroalgae degradation in a terrestrial environment using a metagenomic approach. Another consideration that will be looked at is how effective the introductionof macroalgae will be in an agricultural setting using a compositional and tissue analysis. This information will be useful for the further development of technologies and also for the promotion of effective waste management strategies.

Hypothesis: The heterotrophic bacterial communities that are associated with macroalgae degradation in a terrestrial environment are different from those associated with terrestrial biomass, making it problematic for terrestrial management.

Figure 1. Washed up invasive algae in Maunalua Bay, located on the southeastern side of Oahu, Hawaii.

2.0 Methods

2.1 Sample collection

Six organic waste materials were collected which included 3 types of organic waste biomass and 3 types of invasive marine seaweeds (Figure 6). A mixed Algae Waste Biomass (AWB) sample was collected (May 2018) from a mound at Lang's Nursery in Hawaii Kai, with authorization (Figure 3). The mound consisted of invasive algae collected during a Maunalua bay clean up that was stock piled months prior for the intended use as a feedstock. This AWB sample was approximately 4m x 2m, and 1m in size (Figure 2), and although the age of the mound is unknown, the sample area was revisited a year later (Aug 2019) and did not exhibit significant visual changes. From there two distinct organic waste materials that were in close proximity to the AWB sample were collected (Jan 2019) as control samples that looked very distinct in appearance. Each of the control mounds consisted of organic material that was stock piled at a farm consisting of wood chips, leafy material, and other decomposed organic matter. One of these samples were collected 9m north of the AWB pile and consisted of mostly undecomposed woody material that was primarily wood chips and decomposed organic materials. This sample was classified as Waste Biomass 1 (WB1). Approximately 18m to the south of the AWB was a sample consisting of some decomposed woody material and featured various insects and worms unlike sample WB1. The soil within this sample was also darker in coloration compared to the WB1 sample, and therefore was classified as Waste Biomass 2 (WB2). A soil control of the AWB sample was also collected in an area which did not display any evidence of algae present, and this was found about 3m south of the AWB sample mound (Figure 3). From each of the terrestrial samples, approximately 0.5kg of sample was collected from the middle of each of the mounds to ensure the samples were relatively older in age. In addition to the terrestrial samples, fresh invasive algae samples of *Gracilaria salicornia, Avrainvillea amadelpha,* and *Acanthophora spicifera* (0.5kg) were collected from the nearshore area at Maunalua bay (Figure 6).

Figure 2. Algae waste biomass dumpsite near Maunalua Bay. Inset image is used to more clearly show the composition of the mound.

Figure 3. Area where the waste biomass samples were collected on the eastern side of Oahu.

Figure 4. Specific points along the area where the terrestrial samples were collected. These samples included the waste biomass 1, the algae waste biomass, the soil control, and the waste biomass 2.

Figure 5. Area in Maunalua Bay where the fresh invasive algae samples were collected. These samples were mostly consisted of *A. amadelpha, G. Salicornia,* and *A. spicifera.*

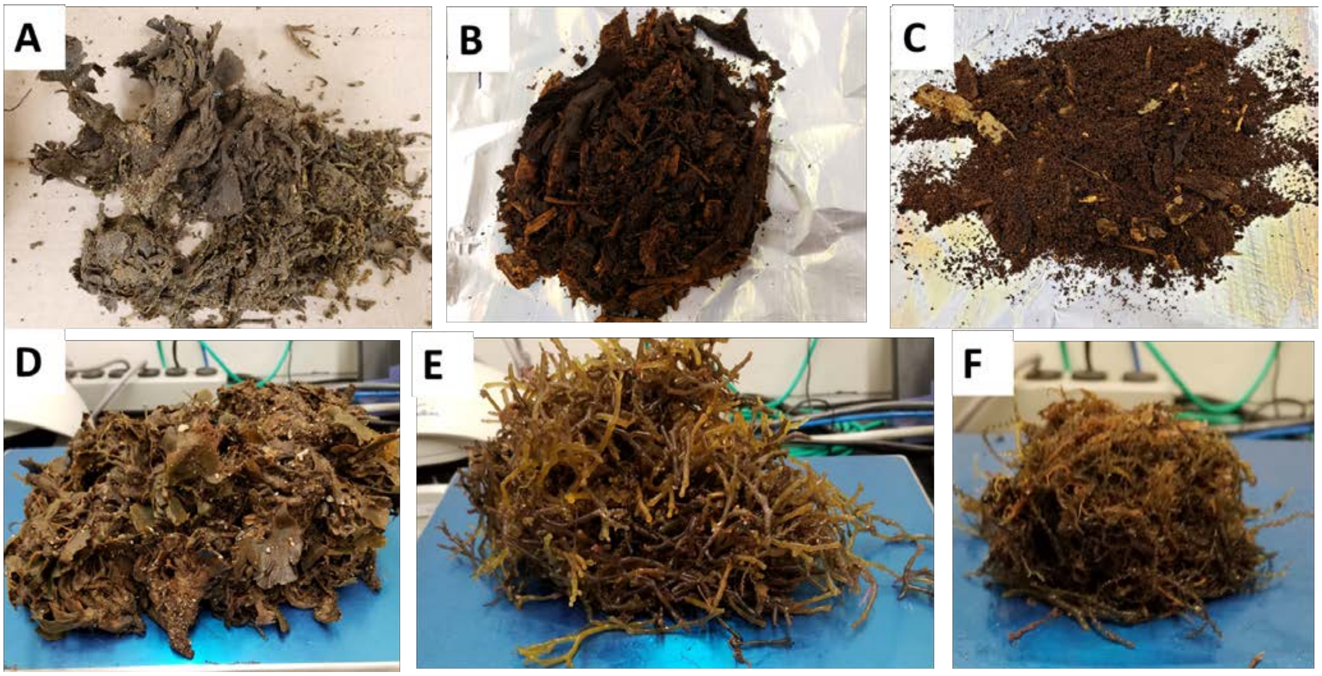


Figure 6. A) Algae Waste Biomass B) Waste Biomass 1 C) Waste Biomass 2 D) *A. amadelpha E) G. salicornia F) A. spicifera*

2.2 DNA extraction and sequencing

The purpose of the DNA extraction and sequencing was to observe the differences between the microbial communities within each of the samples. DNA was extracted from all 4 of the terrestrial materials sampled using a Qiagen DNeasy powersoil kit (Cat # 12888-100) to isolate microbial DNA from tough environmental samples. The waste biomass and algae subsamples (25g), were then ground up using a mortar and pestle with the addition of 10g of sterile silica sand (Fisher Cat #14808-60-7) to help distribute the microbial cells of each of the samples. From the ground materials, 0.25g were further subsampled and DNA was extracted using a Qiagen DNeasy powersoil kit (Cat # 12888-100).

For the three fresh algae samples collected from the nearshore area at Maunalua bay, including the *G. salicornia, A. amadelpha,* and *A. spicifera* samples (0.5kg), this required the Qiagen kit for the DNA extraction process. Each of these algae samples went through an initial cleaning to separate any large sediments and other algae that were attached to the three algae collected to obtain a homogenous sample. Further, the large organic components within these samples were manually separated from sand or visibly attached soil and set aside for use. Two grams of the separated algae were then gently washed three times using a sterile phosphate buffered saline to prevent the microbial cells from undergoing osmosis (PBS; NaCl, Fisher Scientific Cat # S271; KCl, Fisher Scientific Cat # P271; Na_2HPO_4, Fisher Scientific Cat # S373, KH_2PO_4, Mallinckrodt Cat #6810-137). The algae samples were then ground up in a mortar and pestle containing 1g of sterile silica sand with 0.25g of the grounded material being further subsampled and used for DNA extraction using the Qiagen kit. A metagenomic analysis was then applied to compare the microbial populations that are associated with the biological degradation of algae. In this experiment, microbial communities from the AWB, MB1, WB2, soil control, and fresh *G. Salicornia, A. amadelpha* and *A. spicifera.* were utilized.

Successful DNA extraction was determined using 16S rDNA primers, 1492R-27F, to study different species of herbivorous bacteria and archaea (Lane and Stackebrandt, 1991). Polymerase chain reaction (PCR) was performed to amplify the DNA sequencing using a Roche Taq DNA polymerase to amplify DNA fragments in a 25 µl reaction volume (0.1 µM of each primer, 1 ml of extracted DNA, 200 µM dNTP, 0.625 U Taq Polymerase and 2.5 µl of 10x buffer containing 1.5 mM $MgCl_2$). This test is typically used for dried mediums. The thermal program used for this amplification was an initial 2 minutes at 94°C; followed by 30°C cycles of 15s at 94°C, 1 minute at 50°C and 1 minute at 72°C; finished by a 7 minute final extension at 72°C. The PCR products were then run on a 1% agarose gel with SYBR green in TBE buffer which is a stain that quantifies double stranded DNA.

The extracted DNA samples were then sent to the University of Hawaii, (ASGPB) (http://www.hawaii.edu/microbiology/asgpb/) facility for Illumina MiSeq analysis, using short sequence primer pairs developed by Klindworth *et al.* 2012 to allow for the DNA sequencing to generate at a faster rate.

2.3 Soil and tissue analysis

The same three terrestrial waste biomass samples and the soil control were then utilized for the purpose of analyzing the difference in nutrient content to see how viable this algae would pose in an agricultural settings compared to other terrestrial soils. For the AWB sample, whole pieces of algae were separated from the existing soil and sand using a homogenous subsample (150g). The separated algae were then rinsed gently three times with distilled water to remove the sandy substrate and dried at 50°C for 1 week to be used for the tissue analysis. The interspersed sand and soil separated from the algae were used for the soil analysis and followed the same drying process as the tissue sample which occurred at 50°C for 1 week. These samples had not been physically washed because residual amounts of inorganic or degraded organic material can have an impact on the abundance of elements found within these samples. For the terrestrial waste biomass as well as the soil control, larger pieces of undecomposed organic matter were removed leaving a homogenous subsample (150g) to be dried at 50°C for 1 week. The terrestrial waste biomass samples were used for the tissue analysis, and the soil control was utilized for the soil analysis. The samples were then sent to the University of Hawaii, Agricultural Diagnostic Service Center (ADSC) (https://www.ctahr.hawaii.edu/Site/ADSC.aspx) with triplicates being done for the tissue and duplicates for the soil analysis. Duplicates had been done particularly on the soil analysis on the basis of funding restrictions.

2.4 Statistical Analysis

The analysis of the microbial communities was determined through the Geneious^{tm} software to look at the specific species within a community, and the Nephele mothur pipeline to do the statistical analysis of the microbiome data set. Analysis using Geneious^{tm} was performed using trimmed paired end reads and aligned using the DeNovo assembly tool to assemble short nucleotide sequences into longer ones in order to create a strand that is representative of the original DNA. Sequences were then analyzed using the NCBI Basic Local Alignment Search Tool (BLAST) to compare and identify species (Altschul *et al.* 1990). Using the Nephele analysis with the Mothur pipeline, this analyzed sequences of operational taxonomic units (OTU) for the purpose of classifying groups of individuals (Schloss *et al.* 2009) with untrimmed paired end reads and default settings (https://nephele.niaid.nih.gov/details_mothur/) to determine species richness, diversity, and relatedness.

3.0 Results

3.1 Illumina sequencing and analysis

Using the NIH Nephele and Mothur pipeline analyzed paired end reads, the soil control sample had a total of 1.6 x 10^5 paired end reads; AWB a total of 2.3 x 10^5; WB1 a total of 1.1 x 10^5; WB2 a total of 1.2 x 10^5; *A. spicifera* a total of 1.4 x 10^5; *G. Salicornia* a total of 1.3 x 10^5; and *A. amadelpha* a total of 1.1 x 10^5. The rarefaction analysis, which is used to display the species richness between each of the groups to determine expected abundance, indicates that the sequencing depth was adequate for the soil control, and all of the algae (Figure 7). Additional sequencing depth would have been beneficial for the WB1, WB2, and AWB since curves typically require an approach to steady state for sufficient results. The least number of Operational Taxonomic Units (OTU) which are groups that are closely related, was found in the control soil (202) while the greatest number of OTU's were found in the AWB (4560) then the WB1 (4539). The number of OTUs for the other samples were; WB2 (3391), *A. amadelpha* (1689), *A. spicifera* (773), and *G. salicornia* (495).

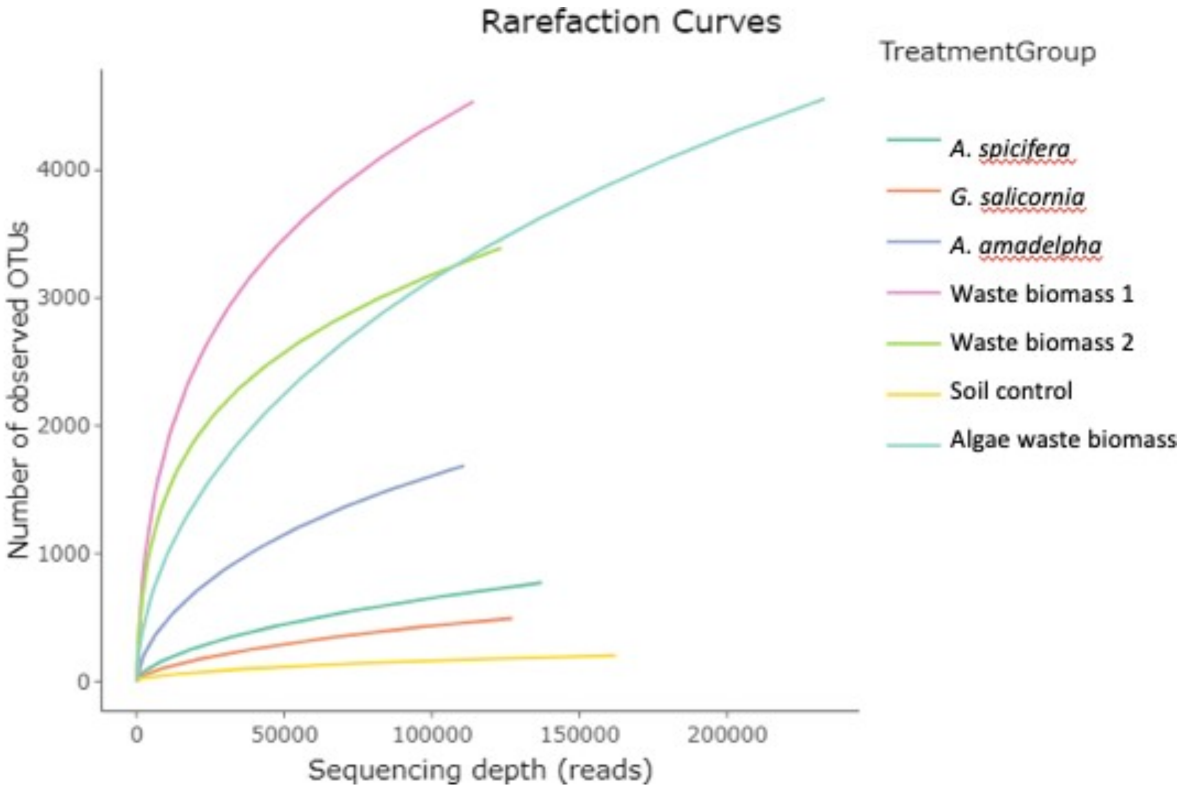


Figure 7. Biological rarefaction curves generated with Illumina 16S rDNA data analyzed using the Mothur pipeline and the total OTUs. The species richness of the Operational Taxonomic Units (OTU) ranged in descending order from the AWB (4560), WB1 (4539), WB2 (3391), *A. amadelpha* (1689), *A. spicifera* (773), *G. salicornia* (495) and finally the control soil (202). The *A. amadelpha*, WB1, WB2, and AWB samples require additional sequencing depth for a more accurate analysis.

A Shannon index was used to characterized species diversity within the communities. This signifies that the terrestrial biomass samples had the greatest diversity (WB2 6.7, WB1 6.07), followed by the AWB (4.97) (Figure 8). The Chao1 index shows the diversity relative to the sample abundance with the WB1 (6,596) and the AWB (5,515) displaying the highest diversity followed by the WB2 (4,789). The soil control site had the least diversity for both indices (Shannon 1.79 and Chao1 249).

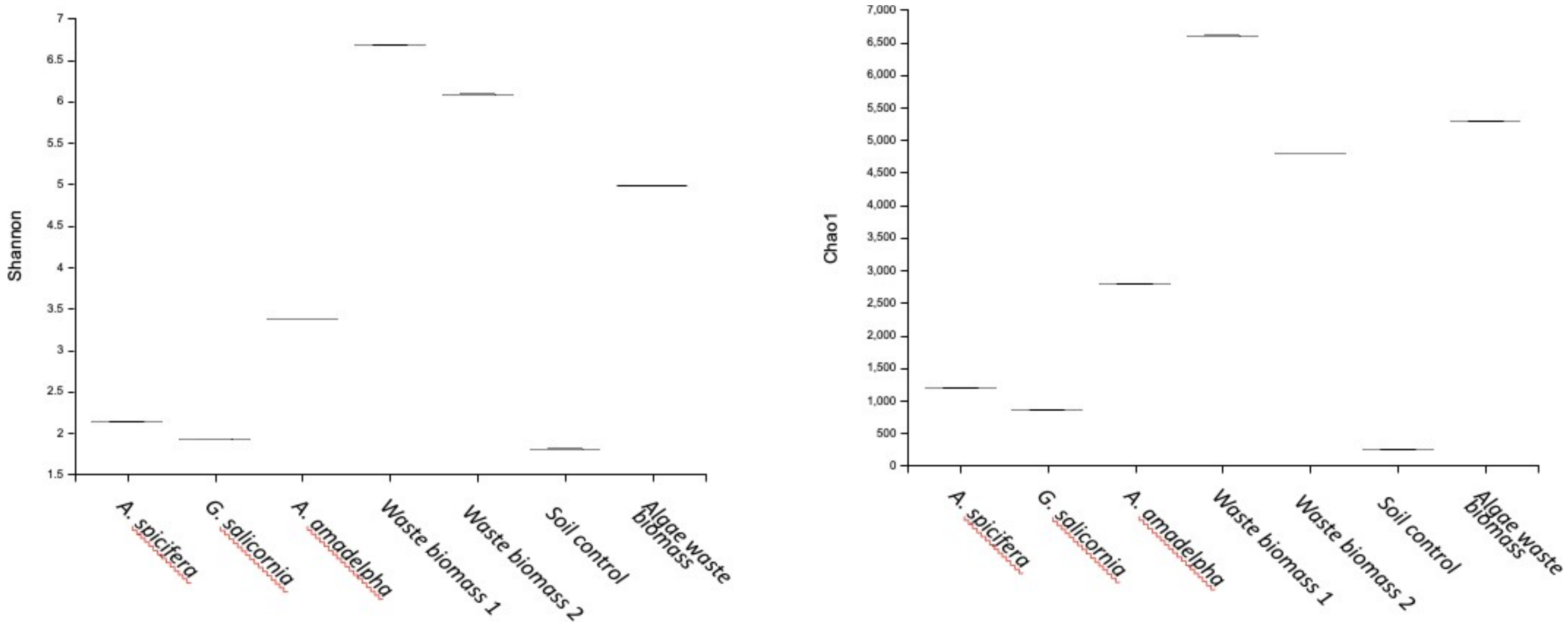


Figure 8. Shannon and Chao1 indices generated with Illumina 16S rDNA data analyzed using the Mothur pipeline are used to display sample diversity. The greatest diversity was displayed by the terrestrial biomass samples (WB2 (6.7), WB1 (6.07)), then the AWB (4.97). The Chao1 index show the diversity levels to be greatest in the WB1 (6,596) and then followed by the AWB (5,515), and the WB2 (4,789). The soil control site for both indices displayed the lowest diversity (Shannon 1.79 and Chao 249).

The metagenomic data includes null values (representatives are found in some of the samples and not in others) therefore a PCoA analysis using Bray-Curtis distances is applied in order to determine sample relatedness (Figure 9). This analysis is used to visually display similarities and differences between the data based on their OTU's. From the graph, these results indicate that the OTU's for the epiphytic algae are closely related while the populations for the terrestrial biomass samples are similarly related based on their proximity to each other on the graph. The epiphytic algae, terrestrial biomass, control soil, and the seaweed biomass however, show more distance between each other deeming them unrelated to one another.

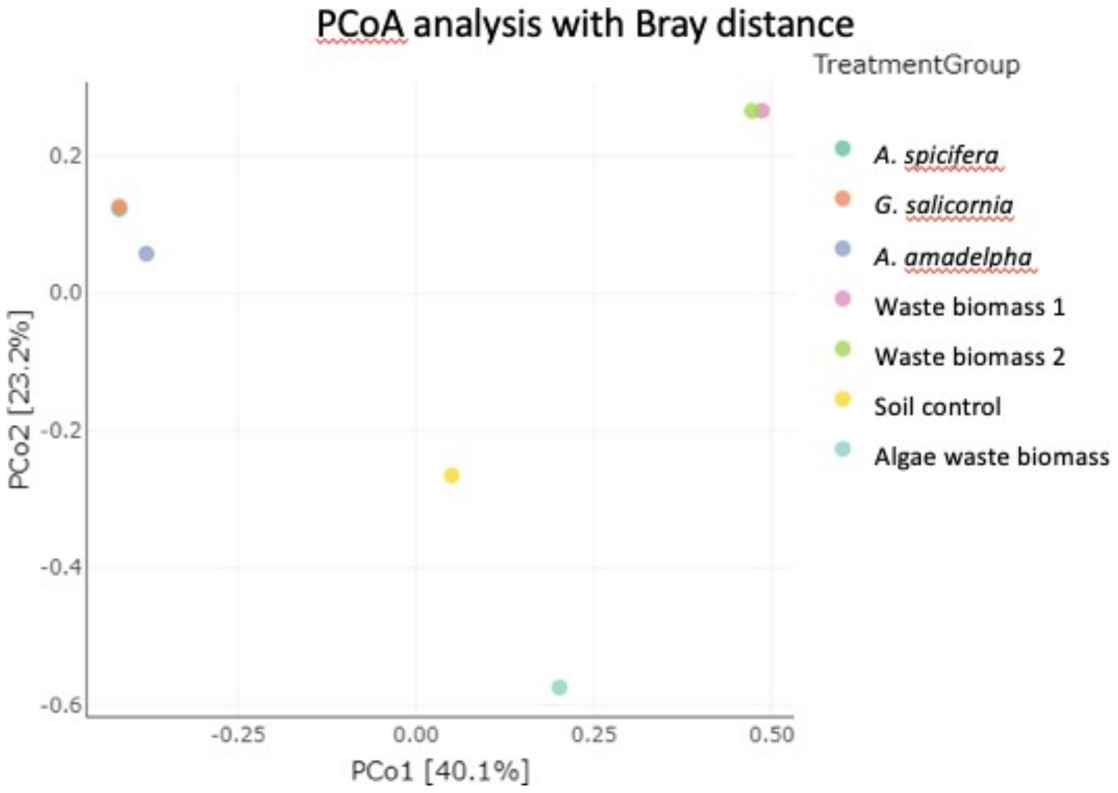


Figure 9. Principal coordinate analysis (PCoA) using Bray distance generated with Illumina 16S rDNA data analyzed using the Mothur pipeline. The epiphytic bacterial isolated from algae show a close relationship, as do the two terrestrial biomass samples. The terrestrial biomass and the algae biomass are however distinct from each other.

Using the Geneious software, this shows the distributions of species at the phylogenic family level (Figures 11 and 12). Looking at the raw data, it was identified that at the family level, AWB (235) had the largest amount of total families followed by WB2 (206) and WB1 (200). Of the total number of families found in each sample, both WB1 and WB2 had 147 families in common, and of those 147 families, AWB has 115 families in common with both of the waste biomass samples. Specifically, within the AWB sample, it was identified that 27% of the families had a strong marine origin with 61% of those species having degradation capabilities (Figure 10).

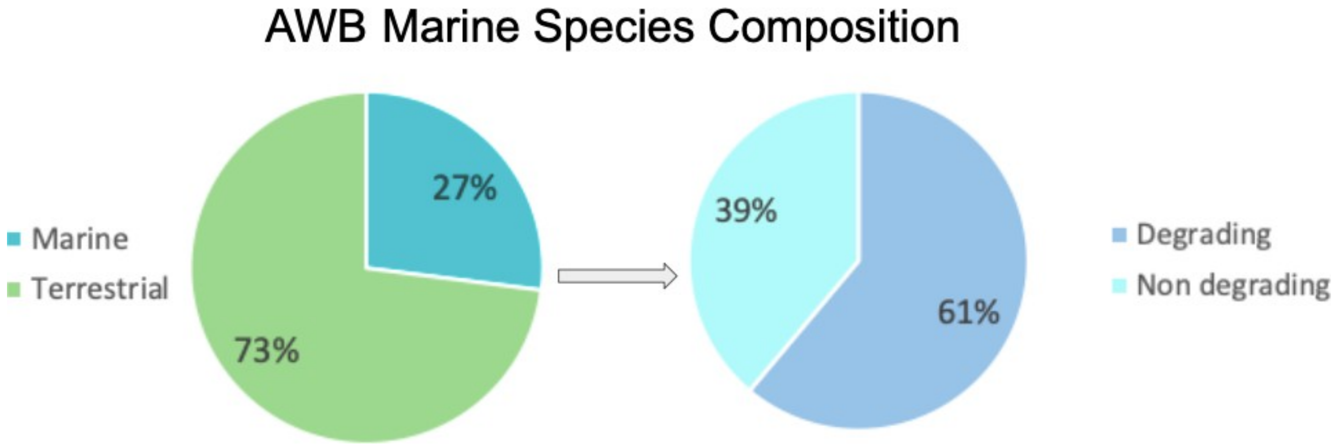


Figure 10. Composition of marine species with a strong marine origin found in the AWB sample at the family level. Of the 27% of families, 61% of them have degradation capabilities.

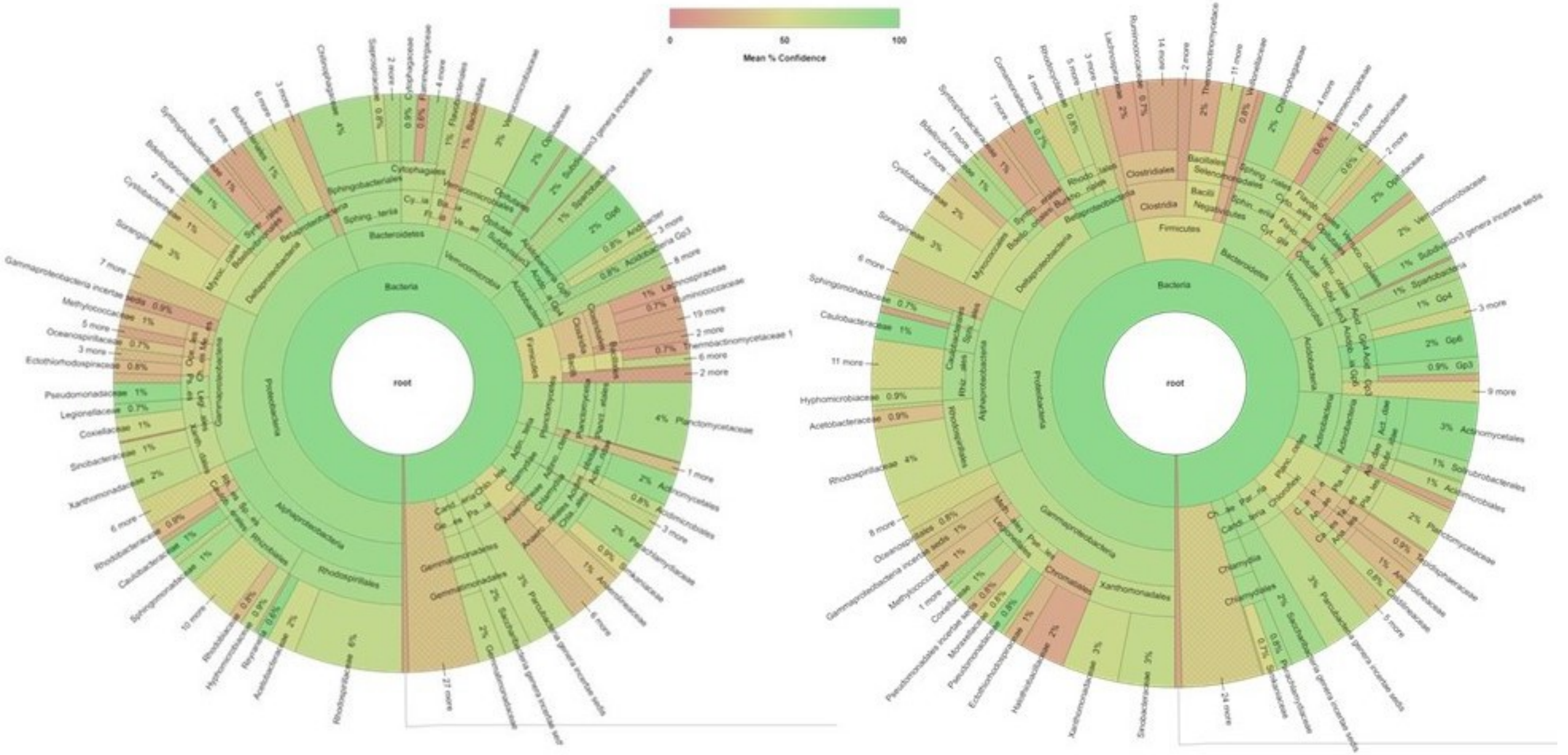


Figure 11. Using the Geneious software and Illumina 16S rDNA data, phylogenic distributions are illustrated at the family phylogenic level. WB1 is displayed on the left and WB2 on the right. This diagram depicts the species comprised of each of the microbial communities sampled from the organic decomposed waste piles. Specifically, the mean % confidence shows the confidence of the software in being able to identify the family.

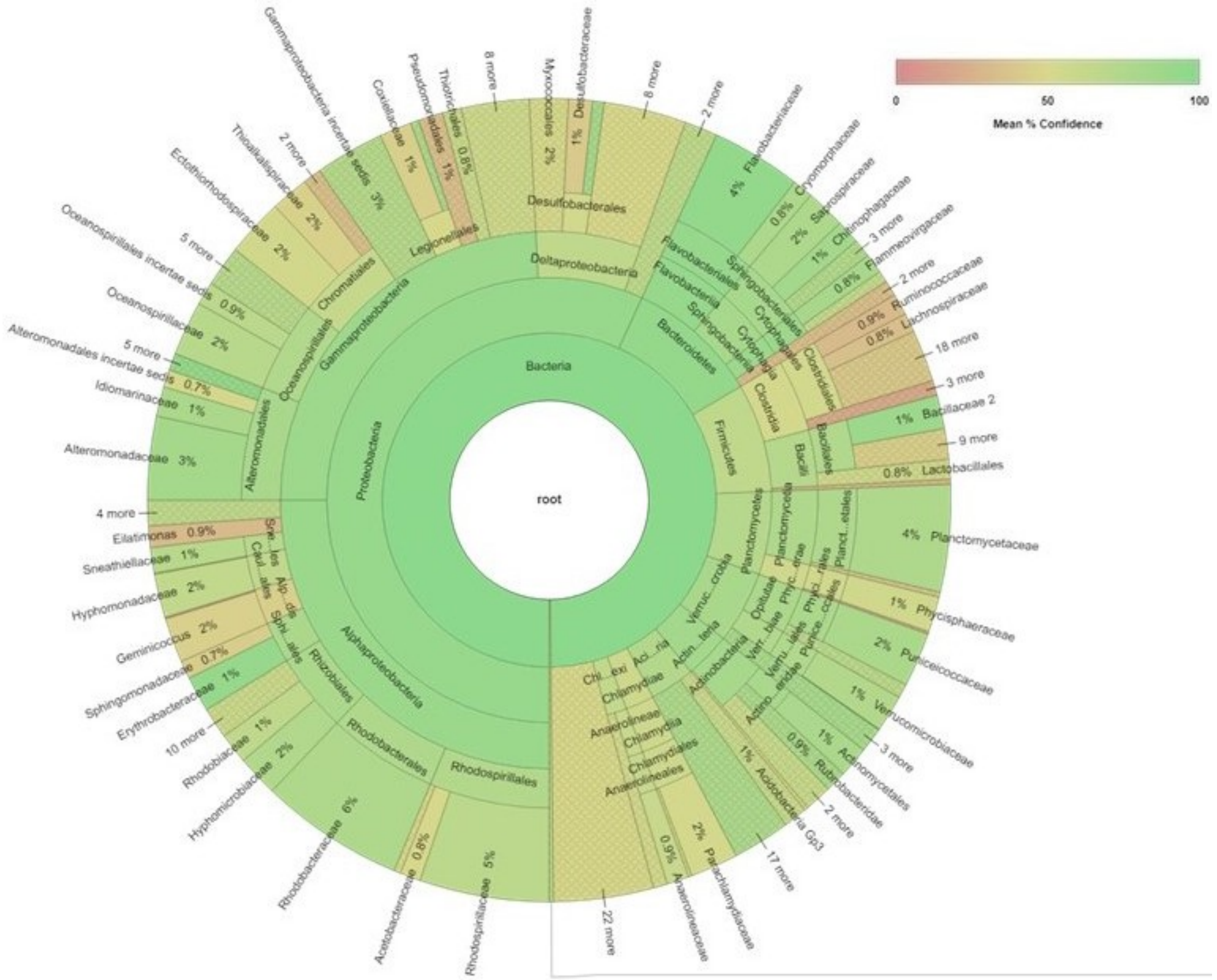


Figure 12. Using the Geneious software and Illumina 16S rDNA data, phylogenic distributions for the AWB sample is illustrated at the family phylogenic level. This diagram shows the specific microbe species comprised of the AWB sample. Specifically, the mean % confidence shows the confidence of the software in being able to identify the family.

3.2 Tissue and soil analysis

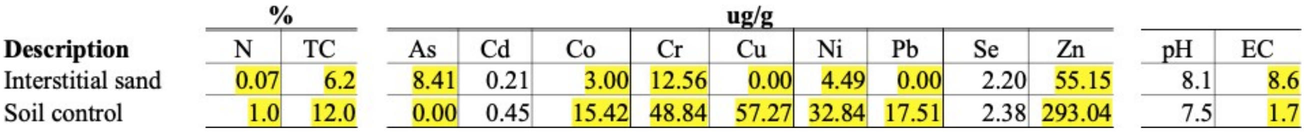
A tissue analysis was performed to compare the nutrient contents within the two terrestrial biomass samples in relation to the AWB sample (Table 1). Based on the results, the total percentages for both nitrogen and carbon were lower in the AWB sample in comparison to the other waste biomass piles. From this data, the carbon percentages in the terrestrial samples were approximately four times greater and nitrogen at least five times more than the AWB samples. In addition, the phosphate and potassium percentages in the AWB sample were at least half the concentration of the other waste biomass samples as well as the sodium content being at a five times greater abundance than both WB samples. A metals analysis further indicated that the AWB sample had accumulated higher concentrations of Fe, As, Co, Cr and Ni with the iron content in the AWB being more than 20 times greater than the terrestrial samples. Concentrations of arsenic, cobalt and nickel also showed a significant presence.

Table 1. A tissue analysis was performed using the different waste biomass samples. The highlighted values show content comparisons that are either significant to the microbial degradation process for the % composition or display significant differences for the heavy metal concentrations. The AWB had the lowest nitrogen and carbon contents and the highest amounts of iron, arsenic, cobalt, chromium and nickel.

	%							ug/g											
Description	N	C	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B	As	Cd	Co	Cr	Ni	Pb	Se
Waste biomass 1	1.59	42.79	0.44	0.96	4.24	0.71	0.10	406	235	255	9	55	0	1	1	1	2	1	3
Waste biomass 2	2.72	48.58	0.18	1.16	2.45	0.20	0.13	418	21	206	10	40	0	0	0	1	1	2	0
Algae waste biomass	0.20	10.92	0.08	0.56	3.03	1.52	0.61	9482	274	187	5	39	18	0	8	33	24	1	0

A soil analysis was performed with the interstitial sand between the algae and the control soil samples to look at the nutrient contents from the exterior (Table 2). The interstitial material possessed lower contents of nitrogen and total carbon when compared with the control soil collected from a nearby area. The soil control generally had higher contents of Co, Cr, Cu, Ni, Pb and Zn with the sandy soil displaying a significant amount of As, as similarly shown in the tissue analysis. A majority of these values are significantly different from each other, but still fall within reasonable environmental parameters. The Electrical conductivity (EC) is a value that displays salinity contents of the soils and overall can determine the health and productivity of soils. In this case, the interstitial sand exhibited a significantly higher concentration than the soil control.

Table 2. A soil analysis was performed comparing the interstitial sand with the control soil. Each of the highlighted values show contents that significantly differ between the two samples. N and TC represent the total nitrogen and carbon contents as well as EC showing the relative salinity within the soil. Arsenic was the only measured compound in the sand that had higher contents compared to the control soil.



	%		ug/g										
Description	N	TC	As	Cd	Co	Cr	Cu	Ni	Pb	Se	Zn	pH	EC
Interstitial sand	0.07	6.2	8.41	0.21	3.00	12.56	0.00	4.49	0.00	2.20	55.15	8.1	8.6
Soil control	1.0	12.0	0.00	0.45	15.42	48.84	57.27	32.84	17.51	2.38	293.04	7.5	1.7

4.0 Discussion

Invasive algae have been proposed as a soil amendment in the literature for farming practices, but effectiveness is highly dependent on the soil composition and algae used (Reppun 2016). Many of the benefits of its use as a compost or soil conditioner are associated with increased water retention (Stephenson 1968), better aeration (Myklestae 1964), and also the release of nutrients and trace minerals from biological degradation (Yvaraj and Gayathri 2017). Historically algae have been directly used as a compost or soil conditioner (Blench B.J.R 1966, Lopez- Mosquera and Pazos 1996) and more recently extracts have been applied to enhance agricultural productivity (Zodape 2001, Khan *et al.* 2009, Battacharyya *et al.* 2015). These investigations that use algae to improve plant productivity have generally shown that when applied to crops, it could increase yields. More recent investigations that utilize algae extracts are shown to be clearly effective, however are intuitively associated with higher costs that could reduce potential applicability (Gorka *et al.* 2018). These costs are typically associated with mechanical disruption as well as general or targeted extractions.

4.1 Metagenomic comparisons

The use of whole algae requires biological considerations that are not well defined in the literature. The structural composition of algae is dependent upon the algal classification which include the *Rhodophyta, Phaeaophyta,* and the *Chlorophyta* which have different structural polysaccharides (Hoagland and Lieb 1915). These structural components will require different metabolic pathways for successful microbial degradation, which may or may not exist in the terrestrial environment. Therefore, the biological process will directly influence the bioavailability of beneficial nutrients as well as the ability algae for algae to effectively degrade in a terrestrial environment.

4.1.1 Mothur Pipeline Analysis

To compare the microbial communities between each of the samples, the first thing that needs to be considered is the relative abundance and species richness within each of the samples. From the rarefaction curves generated through the Nephele with the Mothur pipeline, this showed the relative number of different OTU's represented amongst each community (Figure 7) . Typically, within these graphs, curves that show a steady state have a more accurate expected abundance, but this was not shown for the 3 biomass samples as well as the *A. amadelpha* suggesting that additional depth of sequencing may have been beneficial for the biomass analysis, which could result in some estimation error. Because these samples are so large, any sequencing errors that occurred within the raw data files can increase the error of the number of sequences read which could explain the lack of stability for the 4 top samples. Aside from the errors, reasons for the 3 biomass samples as well as the *A. amadelpha* showing the highest abundance amongst the other samples could be from the variety of materials within their initial composition. In general, the 3 biomass samples would be greater in species abundance than the fresh algae samples because their communities can possess microbes that can be found in both a marine and terrestrial environment, while the fresh algae samples are strictly marine based. A. *amadelpha* showed a significantly higher abundance of different species compared to all of the other algae samples most likely because that particular seaweed is known to trap in sediments, thus explaining the higher abundance compared to the other algae sampled. The soil control showed the lowest abundance out of all the samples which could be in part by its close proximity to the AWB sample. Although the soil control was not heavily saline within these results, soils exposed to high salinity contents over a long period of time can inhibit microbial activity, especially if most microbes are robust to change.

While communities can be very abundant, this does not always mean they are very diverse. By using a Shannon and Chao 1 index, this allowed for the observation of the relative diversity of a community compared to its abundance (Figure 8). In this case, the terrestrial biomass samples show to have the highest diversity followed by the AWB sample. Reasonings for this can again be explained by the salinity factor that was mentioned previously in the species richness section which can inhibit microbial activity and productivity. Especially for the AWB sample, most of the marine microbes found within this community are most likely tougher organisms that can withstand both terrestrial and marine environments, with a number of weaker organisms that may have died off over time.

Relatedness is another factor that plays a key role in the determination of community overlap. Using the PCoA analysis using Bray distances (Figure 9), this illustrates the differences in phylogenic community compositions. Based on these results, this showed that the three algae are closely related with each other, and the terrestrial waste biomass samples are closely related to each other, but each of these groups are distinct from the other populations. Both the control and AWB communities were very dissimilar to each other as well as the rest of the microbial communities. Reasonings for this wide spread distribution could be because land plants are structurally composed of cellulose and lignin, and the process of cellulose degradation is enzymatically mediated by microorganisms that produce the cellulase enzyme (López-Mondéjar *et al.*, 2016). Algae however, possess structural polysaccharides that are different from terrestrial plants and not typically found in the terrestrial environment (Hoagland and Lieb, 1915). In the literature, the descriptions of algal degradation of its polysaccharide and storage starch are typically associated with marine organisms (Daniel *et al.* 1999, Alderkamp *et al.* 2007), although some terrestrial bacteria have been shown to degrade algae specific polysaccharides (Gacesa 1992), including the shared cellulose contents (Abdallah *et al.* 2016). Though studies have demonstrated terrestrial degradation of marine algae is possible, due to its complex polymeric composition, algal cellulose decomposition is not necessarily effective (Bobim-

Dubigeon *et al.* 1999), and therefore the degradation of algae may not be efficient outside of the marine environment (Fleurence 1999).

4.1.2 Geneious analysis

Using the Geneious software, estimations of the metagenomic phylogeny are shown for the two terrestrial biomass samples and the AWB in figure 11 and 12. Using this data shows that there are about 115 of the 641 families that overlap between the 3 biomass samples. At the family level, the classification of species is still fairly broad which can explain the significant overlap between each of the samples. As species become more specific within the phylogeny tree, the smaller the similarities become.

Within the community of AWB, approximately 27% percent of this sample had phylogenic families that possess strong marine origins (*Alteromondaceae, Anaerolineaceae, Cryomorphaceae, Ectothiorhodospiraceae, Eilatimonas, Erythrobacteraceae, Flammeovirgaceae, Geminicoccus, Idiomarinaceae, Oceanospirialles incertae, Oceanosprillaceae, Phycisphaerae, Planctomycetaceae, Puniceicoccaceae, Saprospiraceae, Sneathiellaceae,* and *Thioalkalispiraceae*). Species of marine origin however should be potentially higher since these values don't account for species that can occur in both a marine and terrestrial environment (e.g. *Flavobacteriaceae, Rhodobacteraceae*). In comparison, WB1 possessed a 2% composition that had marine origins (*Oceanosprillales*, *Ectothiorhodospiraceae*) and WB2 a 6% composition that had marine origins (*Erythrobacteraceae, Sneathiellaceae, Ectothiorhodospiraceae, Oceanosprillaceae*). This could be due to the proximity of the area to the ocean which may account for either the natural presence of these marine organisms found here or may have been transported from the AWB sample from a series of rain events. While many algal polysaccharides are sulfated, the deltaproteobacteria, which include sulfate reducing bacteria (SRB), were not strongly represented within the sample proportionally (6%). The majority of the identified deltaproteobacteria were classified with those having anaerobic sulfate reducing metabolisms (5.5%). Due to high water contents that are associated with algae as well as its potential to retain moisture, anaerobic conditions are likely common. The samples collected from the middle of the algal mound show that sulfate reduction was occurring due to the black coloration associated with sulfide production (Figure 2). Comparatively, WB1 and WB2 contained similar contents of deltaproteobacteria, but had marginally lower proportions of those having sulfate reduction metabolisms (approximately 2.5 and 2%).

When comparing the Mothur pipeline and the Genious software, they differ based on community commonality. Using the same Illumina data analyzed with the Mothur pipeline, the Geneious software resulted in a total of 3,007 OTU accounted while the Mothur pipeline registered 12,725 OTU. This could be due to the fact that there are many different pipelines available for use, so results may vary depending on the settings and the reference database that is used (Siegwald *et al.* 2017). Nepehele uses the HOMD (Human Oral Microbiome Database) (http://www.homd.org/) or more likely the custom SILVA 97 database (https://www.arb-silva.de/), while Geneious utilizes Blast to read and analyze the dataset. Geneious in contrast however provides a friendly platform that allows for the estimation of phylogenic groups and is used here to assess population composition. Mothur on the other hand provides statistical analysis that is not available in Geneious.

4.2 Community overlap

To illustrate population differences, Venn diagrams were constructed using OTU sequence counts greater than 100 for the purpose of including OTU that were representative of the communities (Figure 13). The results here show that there is a larger overlap between the terrestrial groups as well as a sizable overlay between the marine groups, but when intersecting that with the AWB sample, there is very little commonality. This could again be due to the different structural carbohydrates found in algae that aren't normally identified in terrestrial plant compositions. All the samples except the WB1 had a common OTU, and the presence of this bacteria in most of the samples could have been attributed to the fact that the AWB and the control sample were collected in close proximity to one another. The total number of sequences that overlap in this OTU are 132 sequences making up a >1% cohesion.

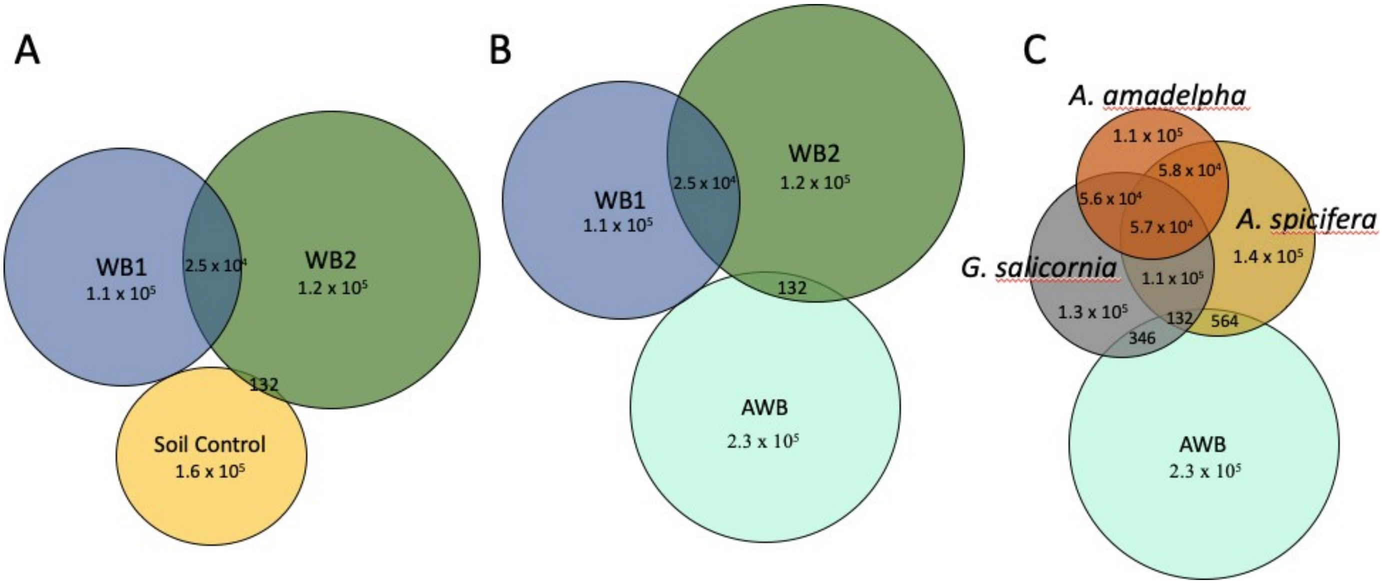


Figure 13. Venn diagrams showing the relationships between the different biological communities, based on sequence overlap for this analysis. A) Relationship between the two waste biomass samples and the soil control. B) Relationship between the 3 waste biomass samples C) Relationships between the invasive algae and the algae waste biomass.

4.3 Tissue and soil analysis

Looking at the contents of both the tissue and soil samples is important in understanding how well waste algae would do if they were implemented into an agricultural setting. It should be noted that the contents of heavy metals can be problematic for soil productivity because it can disrupt microbial activity due to community loss, structural modification, and overall microbe composition (Rajapaksha et al., 2004). In the AWB sample, *Avrainvillea amadelpha* and *Gracilaria salicornia* were identified as being compositionally significant with lesser amounts of *Acanthophora spicifera* present within this biomass pile. Although the AWB pile consisted of mixed algae, its exact compositions were not determined. Tissue analysis of the algae that was manually separated from the intermixed sand and soil show that compositionally it had; 0.2% nitrogen, 10.92% of carbon, 0.08% phosphate and 0.56% of potassium. For comparison, *A. amadelpha* and *G. salicornia* have been determined to contain; 0.9, 0.58% (N), 16.7, 15.7% (C), 0.6, 0.05% (P), and 0.2, 10.1% (K) (Radovich *et al.*, 2012). Compositional data was not found for *A. spicifera*, and from these results we can determine that the composition of the algae collected from the algae waste biomass are reasonable, especially since the algae has been subject to degradation. It is furthermore known that the contents of algae are species dependent and are also impacted spatially and temporally (Lourenco *et al.* 2002).

The tissue analysis for the two terrestrial biomass samples were; 1.59, 2.72% (N), 42.79, 48.58% (C), 0.44, 0.18%(P) and 0.96, 1.16%(K) (table 1). A benefit often stated for the use of algae as a fertilizer is its high N, P, and K contents (Michalak and Chojnacka 2013). The amount of N, C, and K in the examined AWB were lower compared with the terrestrial sourced biomass, which could be due to the addition of minerals present which could cause deficient soils that can favorably impact agricultural productivity (Moller and Smith 1998). Other things to consider include the fact that algae can differ in nutrient and carbon contents depending on the species, which suggests that these particular species from this sample site may not be as valuable as other species for agricultural implementation. C and N components are also important to consider if utilizing the algae as a source for compost because composting requires a 25:1 C:N ratio in order to maximize the rate of decomposition. While the use of waste algae can contribute to nutrient and mineral deficient soils, based upon this compositional analysis it is not favorable when compared with terrestrial biomass.

Heavy metal accumulation in invasive algae requires management considerations. Maunalua bay is in close proximity to a major highway as well as agricultural lands which could result in the coastline deposition of heavy metals. Near shore waters that are in proximity to agriculture are often impacted by runoff carrying Zn, Cu, Ni, and Hg (McMurtry, Wiltshire and Kauahikaua, 1995). Furthermore carbonaceous sands such as those found at Maunalua bay are capable of readily absorbing metals more voluntarily when compared with sands having different compositions (McMurtry, Wiltshire and Kauahikaua, 1995). Due to the frequency of recreational activities occurring in Maunalua bay, the presence of Pb could be attributed to the gasoline used for boating (Jaishankar *et al.*, 2014). Boating activity in this area, can also disturb sediments promoting metal uptake by algae (Anderson *et al.*, 2015). The Black point area on the South Eastern shoreline on Oahu is known to be impacted by ground water discharge that is gradually diffused along the coastline as the current moves West, suggesting another source for heavy metal input (Richardson, Dulai and Whittier, 2017).

Concentrations of Fe, As, Co, Cr, Pb, Zn, and Ni were different when comparing the terrestrial and marine biomass. Heavy metals can be introduced in to these environments from natural sources, industrial activities, runoff, and other anthropogenic sources which can contribute to high environmental contents in both soils (USDA, 2000) and in the near shore ocean environments (Sharifuzzaman *et al.*, 2015). Macroalgae can bioaccumulate heavy metals at concentrations up to 116% higher than its surrounding waters which explains why the tissue samples in the algae samples showed higher concentrations of heavy metals compared to the terrestrial samples (Giusti, 2001). Due to its bioaccumulation potential, algae have been investigated for use as a natural process in the remediation of metal contaminated sediments and waterways (Doshi *et al.*, 2008). Terrestrial plants are also capable of bioaccumulating metals from its surrounding environment and have been similarly applied (Michalak, 2006).

The use of algae as mulch or as compost are potentially problematic. Metals in agricultural soils have implications that require adequate consideration due to concentrations that have been determined to negatively influence the soil microbiome (Wang *et al.*, 2007) by causing structural damage to the cells (Afkar, Ababna and Fathi, 2010). Metals furthermore can reduce crop productivity (Okoronkwo, Igwe and Onwuchekwa, 2005), and have human health consequences when these crops are used as a food source and metals are in high enough concentrations (Wuana and Okieimen, 2014).

Health impacts are a major concern since many of these heavy metals present in the environment can have lasting health effects. Some plants for example are able to uptake Arsenic (Mir *et al.*, 2007), and contaminated agricultural soils could have serious human health implications that include; skin damage, circulatory problems and cancer risk

(Jaishankar *et al.*, 2014). Traces of As can typically be found in fertilizers, soaps, and dyes (Jaishankar *et al.*, 2014), and the residential use of these materials are the likely cause for its environmental presence. A study done by (Cutler *et al.*, 2013) indicates that arsenical herbicides were heavily used during sugar cane production on the Eastern side of the islands which suggests a possible reason for its presence within our samples. Algae are capable of assimilating As, but this is highly dependent on species, and overall contents that vary between 3-96% of the total wet biomass (Raab *et al.* 2005, Taylor and Jackson 2016). The results from this examination show that As was found in high concentrations in the sand relative to the terrestrial samples that was separated from the algae sediment at 8.41 µg/g and also in the algae biomass at 18 µg/g (table 2). According to (Sharifuzzaman *et al.*, 2015), concentrations exceeding 8 µg/g in marine sediments are considered heavily polluted and both marine samples surpass those limits. The terrestrial samples in comparison did not contain As (table 2). According to the Environmental Protection Agency, As in food crops are regulated and are allowed a maximum concentration of 75 ug/g in soils (EPA, 1996), which is still higher then what was found in the marine samples. Long term use of algae as a resource in an agricultural setting could however become problematic as metals could possibility accumulate in the soil.

Other metals should also be considered when trying to implement for agriculture use. From the tissue analysis, the nickel content was determined to be 24 µg/g in the algae and 1 µg/g in the terrestrial biomass. Although the algal samples had a higher concentration relative to the terrestrial biomass, the soil contents were higher for the terrestrial sample (32.84 µg/g). Interestingly the interstitial sand had a lower content (4.49 µg/g) when compared with the algae (table 2). While these results suggest that the algae had bioaccumulated Ni, this requires further investigation. As determined by the USDA, environmental Ni concentrations over 0.1 µg/g are a health risk in soils (USDA), and 50 µg/g in a marine environment (Sharifuzzaman *et al.*, 2015). The higher concentrations found in the soil could be the result from herbicides (McMurtry, Wiltshire and Kauahikaua, 1995). Nickle exposure have been known to be linked to cancer (Wuana and Okieimen, 2014). Chromium (Cr) is naturally present in the environment and was found in low concentrations in the soil (48.84 µg/g), allowable USDA recommendations are 3000 ug/g (USDA, 2000). Within our tissue analysis, only 1 µg/g was detected in the terrestrial biomass samples while the seaweed biomass possessed 33 µg/g which is still considered low. The content of zinc found in all of the examined samples were within typical environmental concentrations (10-300 µg/g) (USDA 2000, Noulas *et al.* 2018). The terrestrial samples showed similar concentrations with the soil control at 293.04 ug/g and in the terrestrial biomass samples at 255 µg/g and 206 ug/g in WB2. Lead was absent from the sand and in the soil, and tissue contents were low (1-2 µg/g). According to the EPA the regulatory limit for Pb is 420 µg/g, which is well beyond the contents found in our samples (USDA 2000). Fe is naturally found in the environment as a result of rock weathering (Wang *et al.* 2007), and are anthropogenically correlated to mining activities (Wuana and Okieimen, 2014). Iron toxicity typically occurs in plants with high concentrations of Fe (II) in soils (Sahrawat, 2004), and a study done on its affect with aquatic plants show that it can potentially inhibit plant growth (Jaishankar *et al.*, 2014). Iron concentrations exceeding 250 µg/g are considered to be toxic in soils (Genon *et al.*, 1994), and can inhibit the uptake of required plant nutrients including P, K, and Zn (Olaleye *et al.*, 2001). Overall, the presence of heavy metals can influence microbial community structures and soil productivity (Sandaa *et al.*, 1999) with microbes that have shown to impact plant growth and influence organic matter decomposition, nitrogen uptake, and enzymatic activities (Videmšek *et al.*, 2009).

5.0 Conclusion

Disposal of unused invasive algae is an ongoing worldwide problem and requires further investigation to better understand the degradation capabilities of algae in a terrestrial environment. From this experiment we were able to determine that the marine and terrestrial communities are in fact different which suggests that the degradation of algae may not be very efficient in a terrestrial environment. Within an agricultural setting, algae have been suggested for the use as compost or soil conditioner, but it has been furthermore suggested that agricultural benefits may not be as viable amongst certain species of algae. The chemical and biological degradation of algae is not well documented in the literature, but is fundamentally necessary for understanding how algae can be effectively utilized in the agricultural process as well as for other future management strategies.

In the current study, it was determined that the biological communities associated with the algae in the terrestrial environment are distinct and have a significant proportion of microorganisms that have marine origins and sulfate reducing potential. Furthermore, the nutritive and inorganic content of the algae would require consideration for agricultural use.

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