University of Nevada, Reno

First investigations of meiofauna in Lake Tahoe and impacts of Asian Clam remediation on community structure

A thesis submitted in partial fulfillment
of the requirements for the degree of

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by

MORGAN P. RICCI

Dr. Jeffrey Baguley, Ph.D, Thesis Advisor

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MORGAN P. RICCI

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Dr. Jeffrey Baguley, Ph.D, Thesis Advisor

Tamara Valentine, Ph.D., Director, Honors Program

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Abstract

Meiofauna are very small animals that are found in the sediment of marine and freshwater environments such as Lake Tahoe. This study was designed in order to provide a first look at the meiofauna that inhabit Lake Tahoe. Another aspect to the study was to observe the effects that the remediation methods to remove the invasive clam species *Corbicula fluminea* have had on the meiofauna. Sediment core samples were collected from Lake Tahoe at the locations that clams have invaded and the remediation treatment locations. The two remediation methods were suction dredging to remove sediment and large mats places on the lake floor. The taxa composition varied between the two sites Lakeside and Marla Bay, which may be due to grain size, extent of clam invasion, or enrichment effect. The mat treatment was found to not be significantly different from the control while the suction treatment was significantly different from both with a beneficial effect on the meiofauna.

Acknowledgements

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Introduction

Meiofauna are very small animals found in freshwater and marine benthic environments. Five phyla are entirely meiofaunal, all are microscopic with a size ranging between 0.500 mm and 0.045 mm (Giere 2009). Of the 36 recognized animal phyla 22 have some meiofauna-sized representatives. Nematodes and harpacticoids dominate community structure. Meiofauna have high metabolic rates, fast generation times, and high production to biomass ratios in comparison to macro- and megabenthos (Giere 2009). Ecologically, meiofauna may have equal or greater functional importance than macrofauna due to high production to biomass ratios and therefore high community turnover rates (Gerlach 1971). Meiofauna have been identified as food for higher trophic levels such as macrobenthos, juvenile fish, and even zooplankton, providing a mechanism for meiofauna secondary production to be transferred up the food web (Coull 1990).

While studies of marine meiofauna are numerous, freshwater meiofauna investigations are rare, and there is little or no record of meiofauna community structure or function in Lake Tahoe. Lake Tahoe is an oligotrophic lake at a subalpine elevation of 1898m, has a surface area of 497 km^2, a maximum depth of 501 m, and temperature in the littoral zone ranges from 5 to 28 °C (Wittmann et al. 2012b). The entire water column stays oxygenated throughout the year and the macrobenthic invertebrate community is dominated by oligochaetes, amphipods, and ostracods (Wittmann et al. 2012b). In similar oligotrophic mountain lakes in Canada it was found that meiobenthic nematodes, rotifers, gastrotrichs, and harpacticoids were of relative importance to overall benthic community structure (Anderson and De Henau, 1980), so presumably these major taxa are also significant members of the Lake Tahoe benthic invertebrate community.

Native benthic communities in Lake Tahoe are now threatened by many invasive species including the Asian clam, *Corbicula fluminea*. *C. fluminea* is a clam native to southern Asia, Africa and eastern Australia in temperate or tropical waters (U.S. Geological Survey 2012). *C. fluminea* was first introduced in the United States in 1938 and has since spread throughout the country to 42 states (U.S. Geological Survey 2012). *C. fluminea* was first seen in low densities in Lake Tahoe in 2002 and were found to have reached nuisance levels in 2010 (>10,000 m^{-2}) (Wittmann et al. 2012b). The clam has proven to be a very successful invader due to its effective adaptability, short life span, and high fecundity (McMahon 2000). The effect of the clam is typically harmful to those endemic species through means such as filter and deposit feeding which would remove organic nutrients from the sediment and increase inorganic nutrients by excretion of ammonia (Vaughn and Hakenkamp 2001). *C. fluminea* has other effects on the abiotic environment through their sediment reworking and contribution of feces and pseudofaeces which also increases inorganic nutrients in the environment (Vaughn and Hakenkamp 2001, Dame 1996). The increase in nutrients has been linked to an increase in algal blooms as well (Wittmann et al. 2008). It has also been found that clam mortality and degradation of their shells creates sediment porewater calcium concentrations to be twice that of the ambient lake level (Wittmann et al. 2008).

Many methods have been devised to remove this invasive species, two of which are diver assisted suction dredging and bottom barriers. The suction method is a non-chemical approach that is able to initiate a short-term reduction in the number of clams

(1500 individuals m^{-2} before treatment to 60 individuals m^{-2} 14 days after treatment), but suction treatment also removes the native species and alters the sediment structure (Wittmann et al. 2010). Suction treatments in Lake Tahoe involved a diver using a suction dredge apparatus with a 4 cm hose and the sediment was removed to a depth of 13 cm at Marla Bay and 8 cm at Lakeside (Wittmann et al. 2012a). Dredging to remove *C. fluminea* significantly reduced benthic macroinvertebrates abundances, but effects on meiobenthos were not quantified (Wittmann et al. 2012a).

Bottom barriers are intended to create anoxic conditions resulting in clam mortality. This is also non-chemical but still impacts native species and the habitat (Wittmann et al. 2010). Anoxic conditions reduced non-target macroinvertebrate abundances and caused variation in their recolonization rates (Wittmann et al. 2012b). Mats were 9 m^2 ethylene propylene diene monomer (EPDM) sheets and were found to reduce dissolved oxygen concentration to 0 after 72 hours and cause 100% *C. fluminea* mortality after 28 days (Wittmann et al. 2012b).

Here, first investigations of meiofauna community structure are presented for the south shore of Lake Tahoe. A secondary purpose was to understand the effects that *C. fluminea* remediation methods have had on the meiofauna community.

Methodology

Hypotheses: H_{01} – Abundance and diversity are not significantly different between Marla Bay and Lakeside, and not affected by suction dredging to remove Asian Clams.

H_{02} – Abundance and diversity at Marla Bay are not significantly affected by suction or mat remediation methods to remove Asian Clams.

Field: Collaboration took place with Sudeep Chandra to access the locations that have been previously set up with the remediation methods. 2 different locations in Lake Tahoe were sampled: Marla Bay and Lakeside. Both Marla Bay and Lakeside have an established population of *C. fluminea* with a high density in Marla Bay (average abundance ~2000 m^{-2}) and a lower density in Lakeside (average abundance~500 m^{-2}) (Wittmann et al. 2012a). On September 14th, 2013 three replicate control cores and three replicate suction treatment cores were taken at Lakeside. On September 21st, 2013 three replicate control cores, three replicate suction treatment cores, and three replicate mat treatment cores were taken at Marla Bay. Suction treatments were performed in March 2009, about 3 and half years prior to our sampling. Mat treatments were performed in July 2010, a little over two years prior to our sampling.

All core samples were acquired through diver-assisted coring using a 2 cm inner diameter core tube. The 6-centimeter sediment cores were divided into 3 strata, 0-2 cm, 2-4 cm, and 4-6 cm. Each strata was put into a corresponding labeled 100 ml plastic jar and preserved in a solution of 70% ethanol and 4% glycerol. Rose Bengal, a protein stain, was added in order to dye the living organisms (Montagna 2002). The sample jars were then sealed with electrical tape and transported back to the University of Nevada, Reno. Upon arrival at the university the samples were stored in a freezer until sieved.

Lab: In order to extract the meiofauna from the sediment the sample jars were poured through a 0.045 m sieve, which retains the meiofauna and larger sand and sediment. Fine sediments should pass through the sieve while larger particles remain (Montagna 2002). The samples were divided evenly into two centrifuge tubes and silica sol Ludox HS 40 (solubilized silica) was added (Burgess 2001). Each tube was vortexed for 5 minutes, which separates the meiofauna from sediment, and then isopycnic centrifugation was performed to divide the contents using density gradients (Burgess 2001). During centrifugation, sediment is forced toward the bottom of the tube due to a higher density, and meiofauna float toward the top due to a lower density than the Ludox. The top layer of about 30 ml was then sieved off and transferred to a 100 ml beaker. The isopycnic centrifugation process was repeated, which has been shown to extract greater than 95% of all meiofauna taxa (Burgess 2001). The sediment that remained at the bottom of the centrifuge tubes after both centrifuges was combined into one tube and preserved in a 70% ethanol/4% glycerol solution. The samples were enumerated using a ward zooplankton wheel and a Leica S8APO stereo microscope. When animals could not be identified under the stereoscope, they were slide mounted and viewed at higher magnification under a Leica DM2500 compound microscope equipped with differential interference contrast (DIC) optics.

Statistical Analysis: Taxa abundances were recorded on laboratory abundance data sheets prior to digitizing in a Microsoft Excel database. Univariate and multivariate data analyses and hypothesis testing were carried out in SAS version 9.3 and Primer version 6.0. A SIMPER analysis in PRIMER provided the percent contribution of each taxa for each site and treatment. Data was log transformed to the fourth root for the ANOVA tests. A two-way ANOVA was run to test for the interaction between the two variables, site (Marla Bay and Lakeside) and treatment (suction and control). A one-way ANOVA was run to test for the effects of the treatments and then another one-way ANOVA tested for the effects of the locations both tests involved abundance and diversity. A one-way ANOVA was used to test for treatments differences (suction, mat, and control) at the Marla Bay site.

MDS tests were run in PRIMER to ordinate sites and treatments based on the underlying taxonomic similarities. MDS provides a visual representation of similarity using distance in two or three dimensions. The total core abundances per m^2 were calculated by taking each core's total organisms and multiplying by 1513.

Results

Overall 4,715 meiofaunal individuals were counted throughout all the samples representing 14 taxa. The total abundance for the Lakeside control was 568 individuals, Lakeside suction had 1851 individuals, Marla Bay control had 284 individuals, Marla Bay suction had 785 individuals, and Marla Bay mat had 281 individuals. The mean abundance per core for Lakeside control was 189.3 ±73.1, Lakeside suction was 617 ±76.3, Marla Bay control was 94.7 ±32.4, Marla Bay suction was 257.67 ±84.83, and Marla Bay mat was 93.7 ±37.9. The SIMPER test revealed that Lakeside control (Figure 1) was composed primarily of nematoda at 32%, harpacticoid at 22%, nauplii at 13%, annelida at 19%, both ostracoda and rotifer at 4%, and both tardigrada and bivalvia at 3%. Lakeside suction (Figure 2) was composed of nematodes at 28%, harpacticoids at 15%, nauplii at 12%, annelida at 13%, rotifer at 13%, bivalvia at 10%, and cladocera at 9%. The percent contribution for Marla Bay in the control (Figure 3) consisted of harpacticoids at 26%, tardigrada at 16%, annelida at 15%, acarina at 15%, nematoda at 12%, nauplii at 12%, and rotifer at 4%. Marla Bay mat treatment (Figure 5) consisted of harpacticoid at 28%, nauplii at 23%, acarina at 22%, tardigrada at 14%, annelida at 5%, and both rotifer and nematoda at 4%. Marla Bay suction (Figure 4) was composed of harpacticoid at 17%, nematoda, nauplii, and acarina all at 14%, rotifer at 12%, both cladocera and tardigrada at 10%, and annelida at 9%. The abundance per meter squared was calculated for each core as found in Table 1 and displayed in Figure 6.

The abundance between Marla Bay and Lakeside was significantly different (p=0.0039) (Table 2, Figure 10). The abundance between the suction treatment and control was significantly different (p= 0.0005)(Table 2, Figure 11). The abundance for the interaction of site (Marla Bay and Lakeside) and treatment (control and suction) was not significantly different (p=0.5515)(Table 2, Figure 9). The diversity between Marla Bay and Lakeside was significantly different (p=0.0001)(Table 3, Figure 13). The diversity between the control and suction treatment was significantly different (p=0.0168)(Table 3, Figure 14). The diversity between the interaction of the site (Marla Bay and Lakeside) and the treatment (control and suction) was also significantly different (p=0.0092)(Table 3, Figure 12). The similarities between all the treatment and site combinations are displayed in Figure 15.

For Marla Bay the abundance among the three different treatments (control, suction, mat) was significantly different (p=0.0221). In Marla Bay the diversity among the three treatments was significantly different (p=0.0041). The abundance means at Marla Bay for control and mat treatment were not significantly different from each other but both were significantly different from the suction treatment (Figure 7). For the diversity means at Marla Bay again the control and mat treatment were not significantly different from each other but were both significantly different from the suction treatment (Figure 8).

Discussion

The results revealed that Marla Bay was dominated by harpacticoids and Lakeside was dominated by nematodes. This could possibly be explained by the higher abundance of *C. fluminea* at Marla Bay (average abundance ~2000 m^{-2}) and a lower density in Lakeside (average abundance~500 m^{-2}) (Wittmann et al. 2012a). Generally nematodes are the dominant taxa, they are believed to be the most abundant benthic animals in freshwater (Strayer 1986). Nematodes feed on such things as bacteria, fungi, algae, and higher plants (Nicholas 1984 cited in Giere 2009). Since *C. fluminea* has been found to reduce the abundance of phytoplankton this could explain the higher abundance of nematodes at Lakeside where there were fewer clams to remove their food supply (Cohen et al. 1984).

The different taxa composition between Lakeside and Marla Bay could also be influenced by the organic enrichment. Lakeside receives more organic enrichment due to its proximity to South Tahoe, Edgewood Golf course, run-off, etc. In enriched habitats nematode dominance increases (Raffaelli et al. 1981). This reasoning could explain why nematodes contribute a higher percentage at Lakeside as compared to the other taxa and to Marla Bay. Since Lake Tahoe is oligotrophic, maybe nematodes are not the dominant taxa most of the time.

Another factor that could explain the diversity and abundance distinction between Marla Bay and Lakeside is the grain size. Grain size determines the amount of interstitial space available for the meiofauna to inhabit, so the coarser the grain size the greater the volume of space (Nybakken and Bertness 2005). The surface area of the grain particles is another determinant of meiobenthic life through establishment of the area available for attachment (Giere 2009). The size of the space created by the sediment has been found to have a direct correlation to the body size of the meiofaunal animals in experiments (Williams 1972). Finer sediments are preferred by most nematodes while harpacticoids generally favor the coarser sediment (Coull 1985 cited in Giere 2009). The grain size at the Lakeside site was classified as coarse to medium sand with a median sediment particle size, Me = 0.375 mm and very coarse sand at Marla Bay, Me = 1.180 mm (Wittmann et al. 2012a). This explanation of grain size could be the reason of a higher abundance of nematodes in Lakeside where the sediment is finer and more harpacticoids in Marla Bay where the grain is very coarse.

The results demonstrate that the suction treatment methods had a significant effect on the diversity and abundance of the meiofauna. The suction treatments increased the abundance of the meiofauna at both sites, Marla Bay and Lakeside. It has been found that the recolonization of meiofauna occurs much quicker from intensive disturbances than macrofauna (Schratzberger et al. 2006; Bolam et al. 2006 cited in Giere 2009). In sandy habitats, like much of Lake Tahoe, the recovery tends to be quicker than in mud and nematodes proved less affected than harpacticoids (Giere 2009). The mat treatment was not statistically different from the control at Marla Bay. This could be due to the different timelines for the remediation methods, the suctions were set up in March 2009 while the mats were set up in July 2010. Based on the data the suction methods appears to be a beneficial remediation method for meiofauna as it increased the abundance at both sites and the diversity at Marla Bay.

All the samples collected for this study were taken where clams have already invaded. Future investigations will have true controls taken from an area of Lake Tahoe where *C. fluminea* has not yet invaded. A detailed study of meiofauna community structure and function should provide an understanding of the potential trophic linkages.

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Sample	Abundance per m^2
LS1 Control	158865
LS2 Control	344964
LS3 Control	355555
LS1 Suction	921417
LS2 Suction	824585
LS3 Suction	1054561
MB1 Control	116501
MB2 Control	113475
MB3 Control	199716
MB1 Suction	473569
MB2 Suction	260236
MB3 Suction	453900
MB1 Mat	205768
MB2 Mat	95319
MB3 Mat	124066

Table 1. Core abundance per m^2. This table displays the calculated abundance per meter squared for each core.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Site	1	0.3521	0.3521	16.00	0.0039
TRT	1	0.7051	0.7051	32.04	0.0005
Site*TRT	1	0.0085	0.0085	0.39	0.5515

Table 2. ANOVA test between the sites and treatments based off abundance. Sites (Marla Bay and Lakeside) and treatments (control and suction).

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Site	1	18.7432	18.7432	49.45	0.0001
TRT	1	3.4377	3.4377	9.07	0.0168
Site*TRT	1	4.4104	4.4104	11.64	0.0092

Table 3. ANOVA test between the sites and treatments based off diversity. Sites (Marla Bay and Lakeside) and treatments (control and suction).

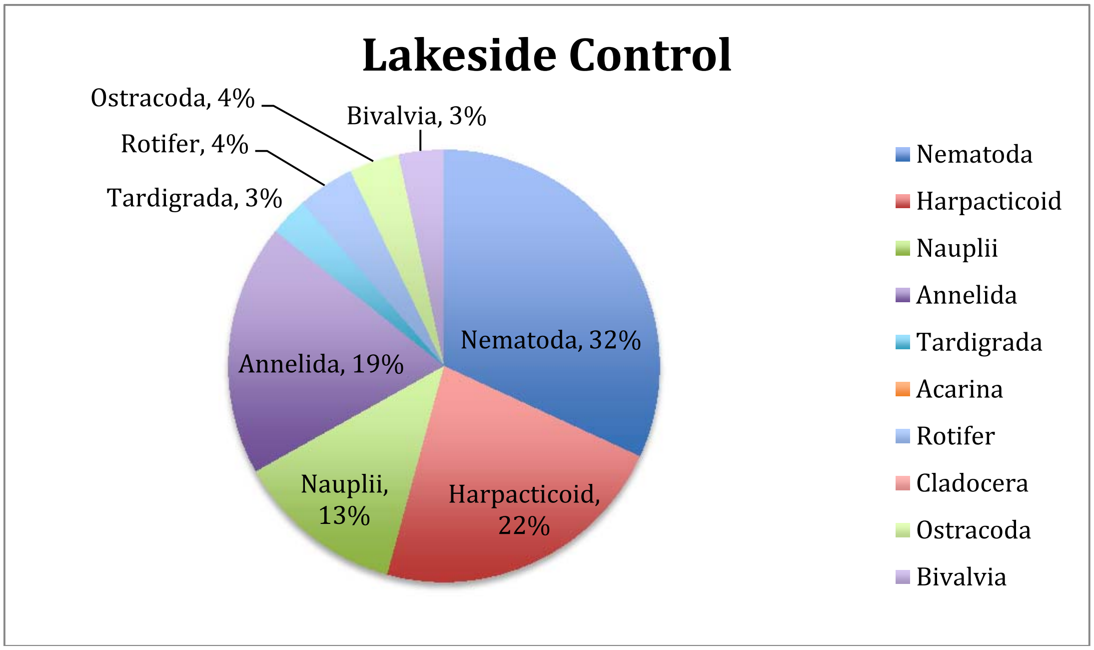

Figure 1. Percent contributions of Lakeside control taxa. This pie chart displays the percent distribution of taxa found at the Lakeside control samples.

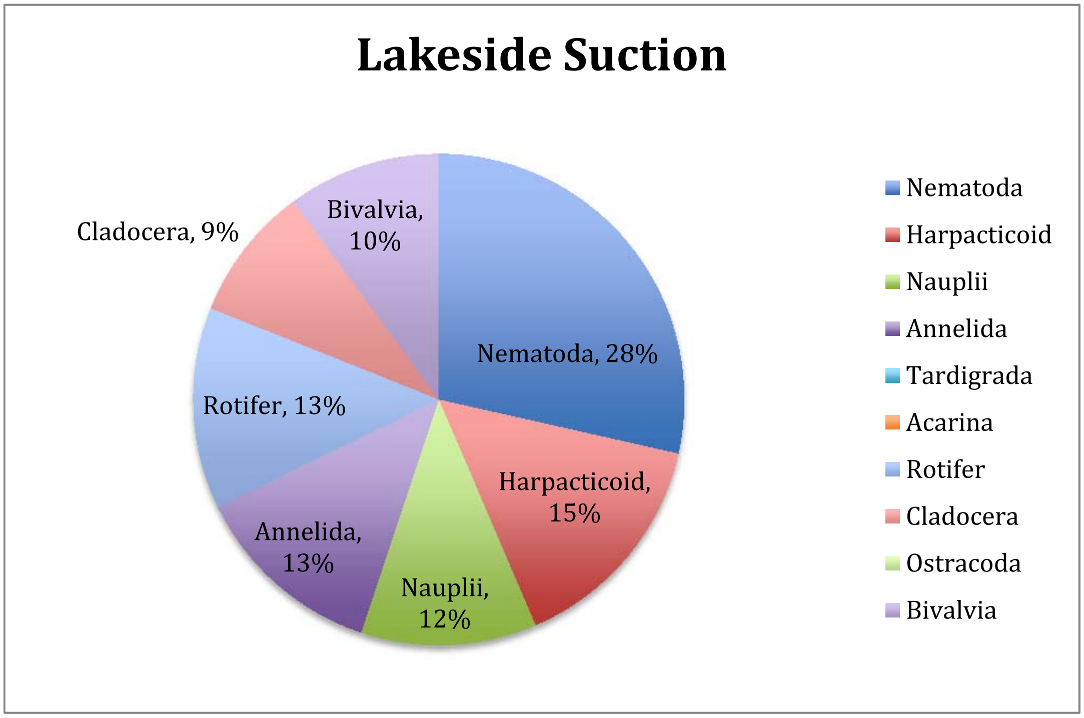

Figure 2. Percent contributions of Lakeside suction taxa. This pie chart displays the percent distribution of taxa found at the Lakeside suction treatment samples.

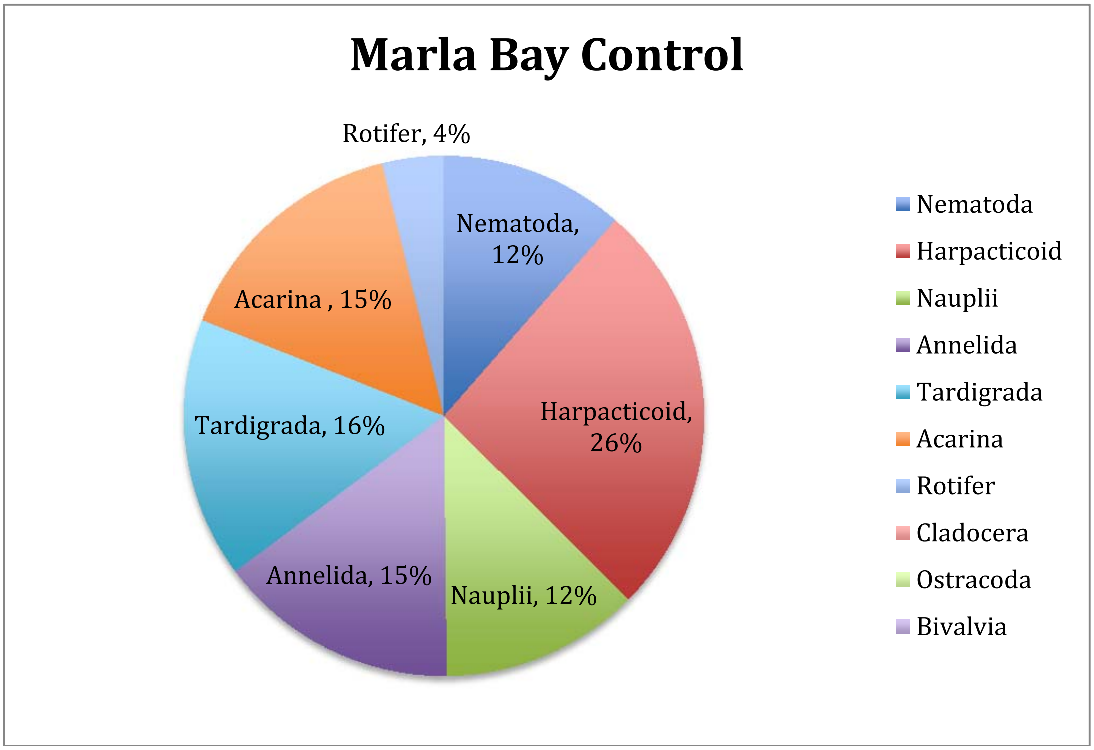

Figure 3. Percent contributions of Marla Bay control taxa. This pie chart displays the percent distribution of taxa found at the Marla Bay control samples.

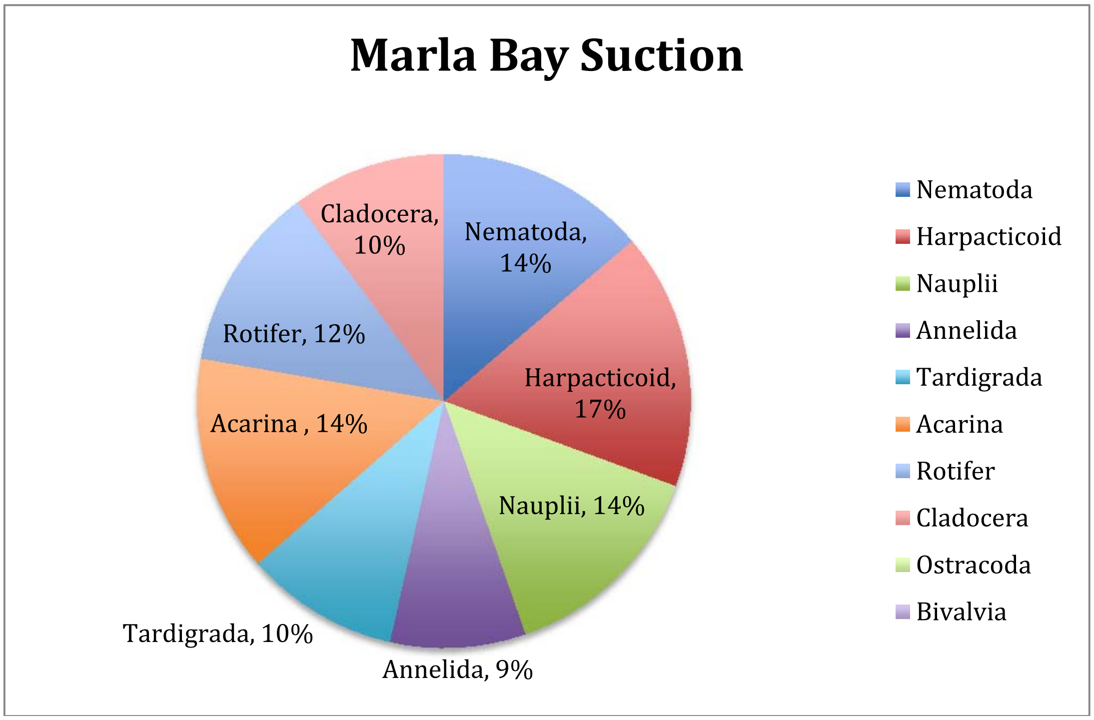

Figure 4. Percent contributions of Marla Bay suction taxa. This pie chart displays the percent distribution of taxa found at the Marla Bay suction treatment samples.

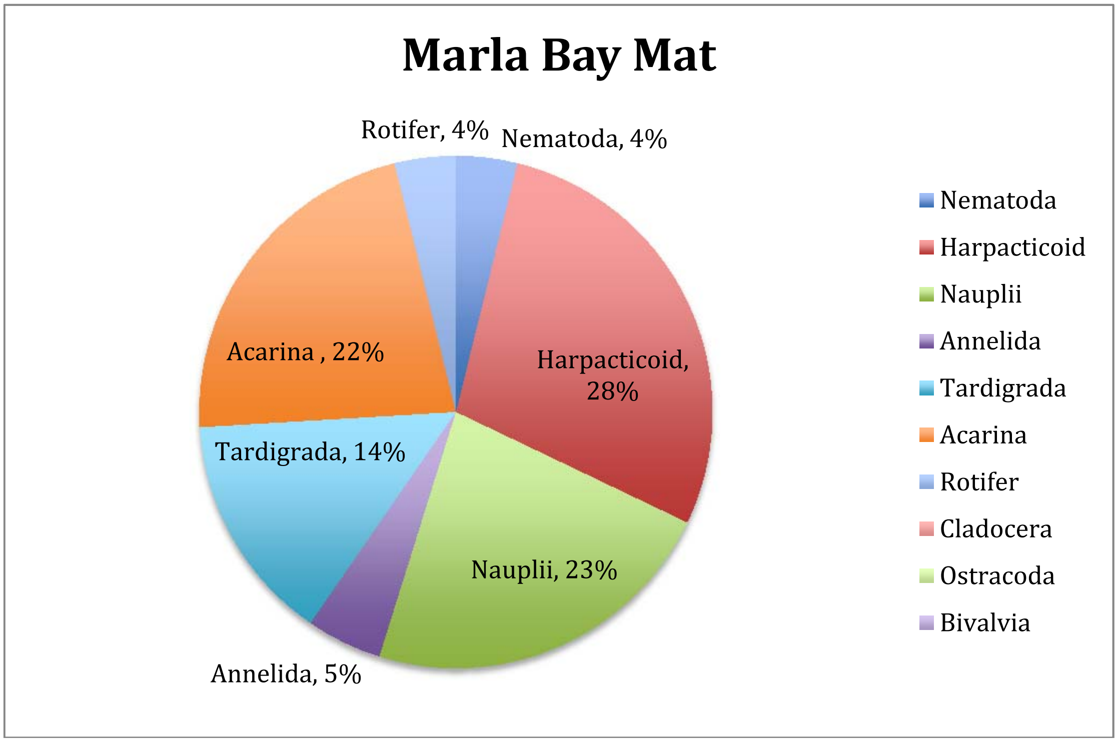

Figure 5. Percent contributions of Marla Bay mat taxa. This pie chart displays the percent distribution of taxa found at the Marla Bay mat treatment samples.

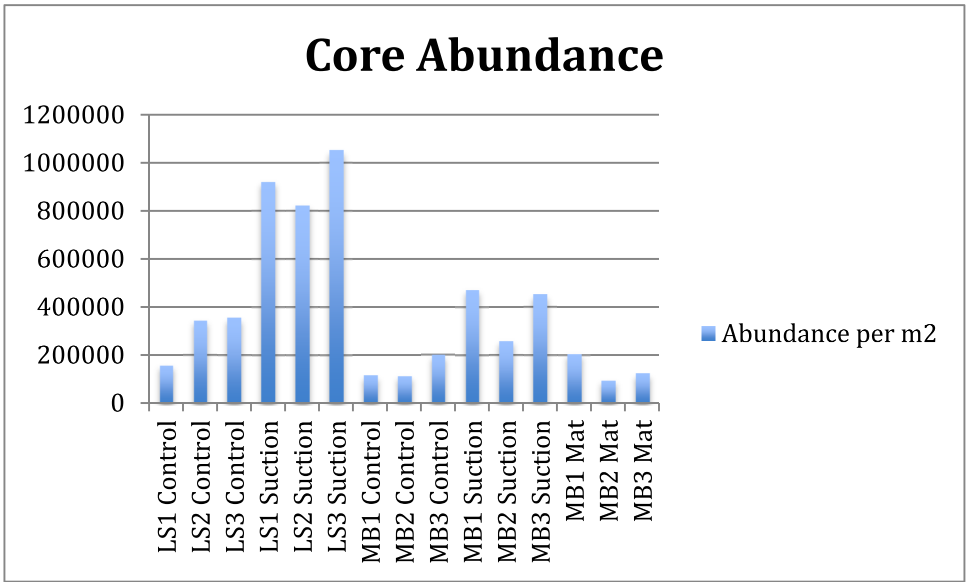

Figure 6. Core abundance per m^2. This is a graphical representation of the calculated abundance for each core.

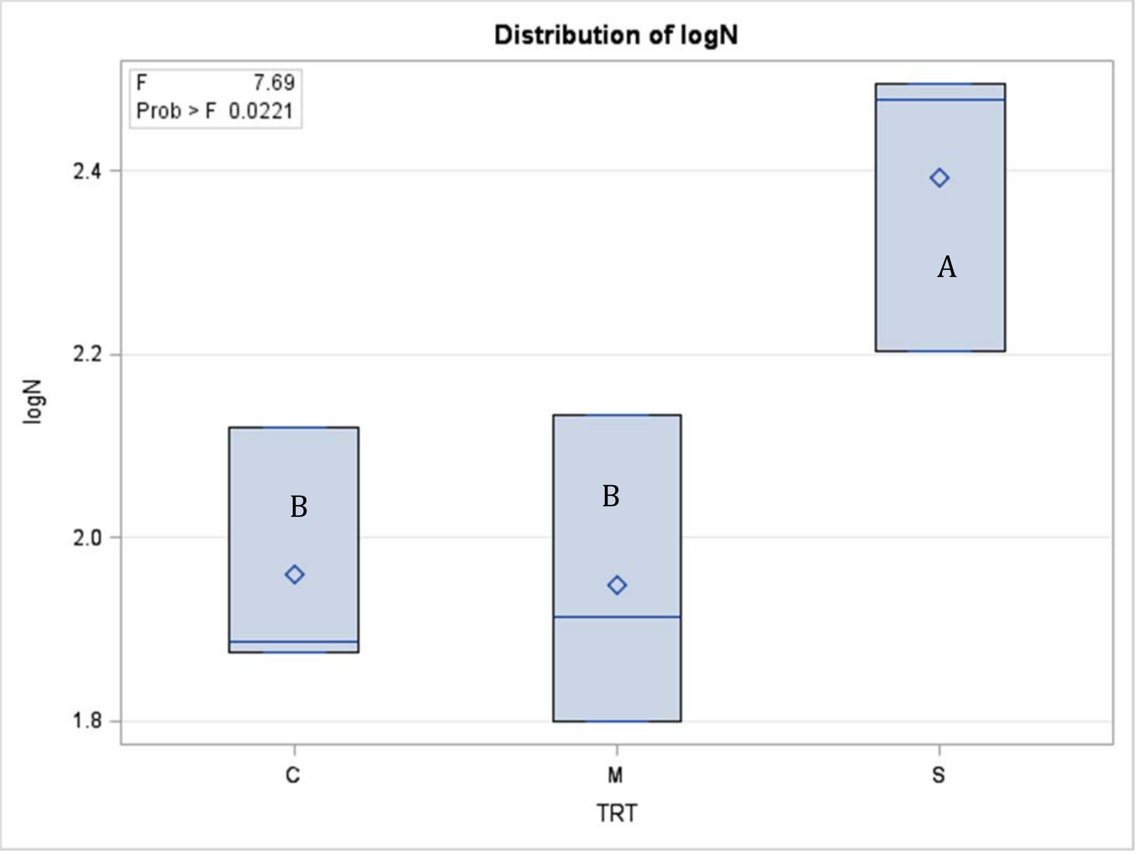

Figure 7. Abundance for treatment methods at Marla Bay. The A and B labels indicate that those with the same letter are not significantly different and those with different letters are significantly different.

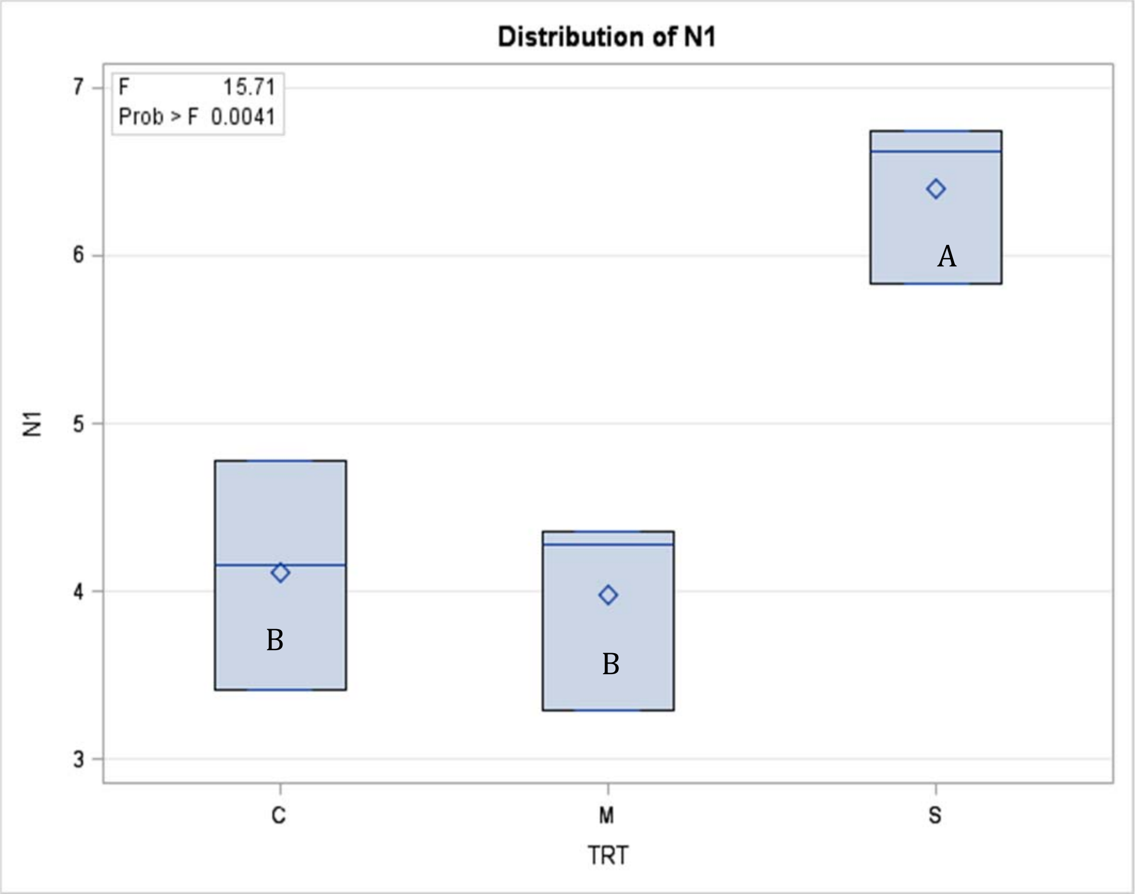

Figure 8. Diversity for treatment methods at Marla Bay. The A and B labels indicate that those with the same letter are not significantly different and those with different letters are significantly different.

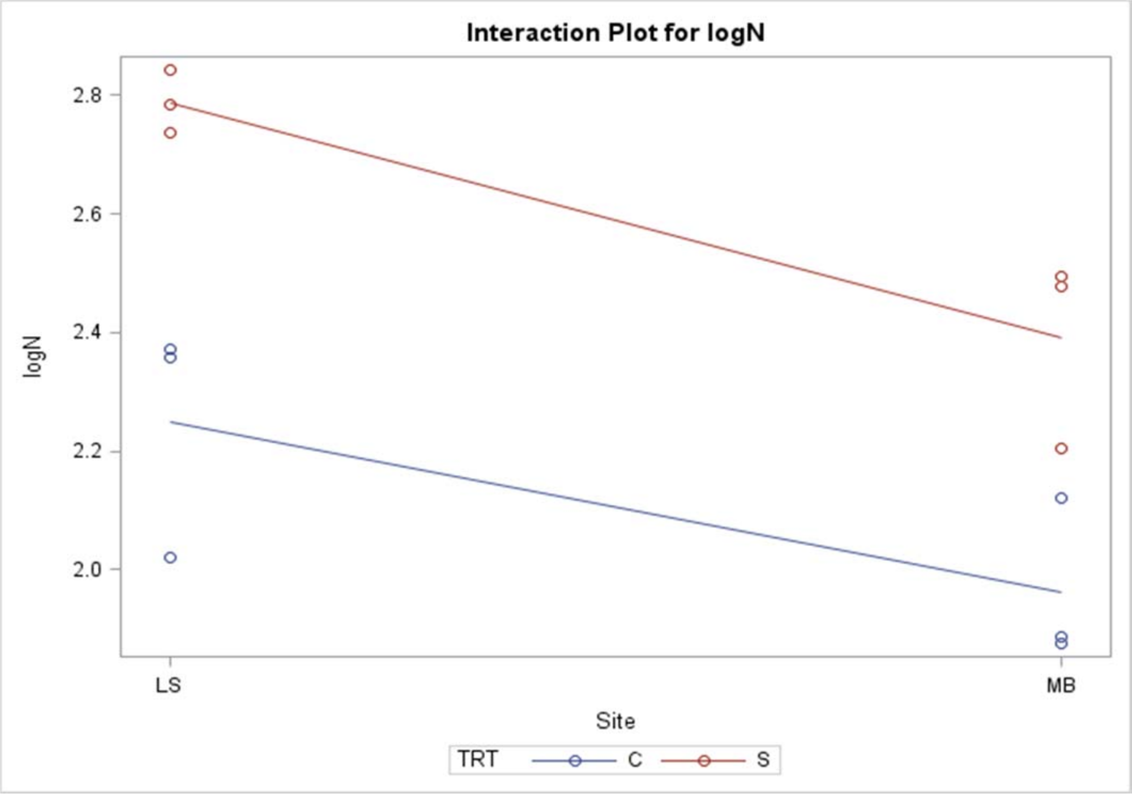

Figure 9. Interaction of the sites and treatments based on abundance. Marla Bay and Lakeside have differences between the suction and control with the suction treatments having higher abundances than the controls.

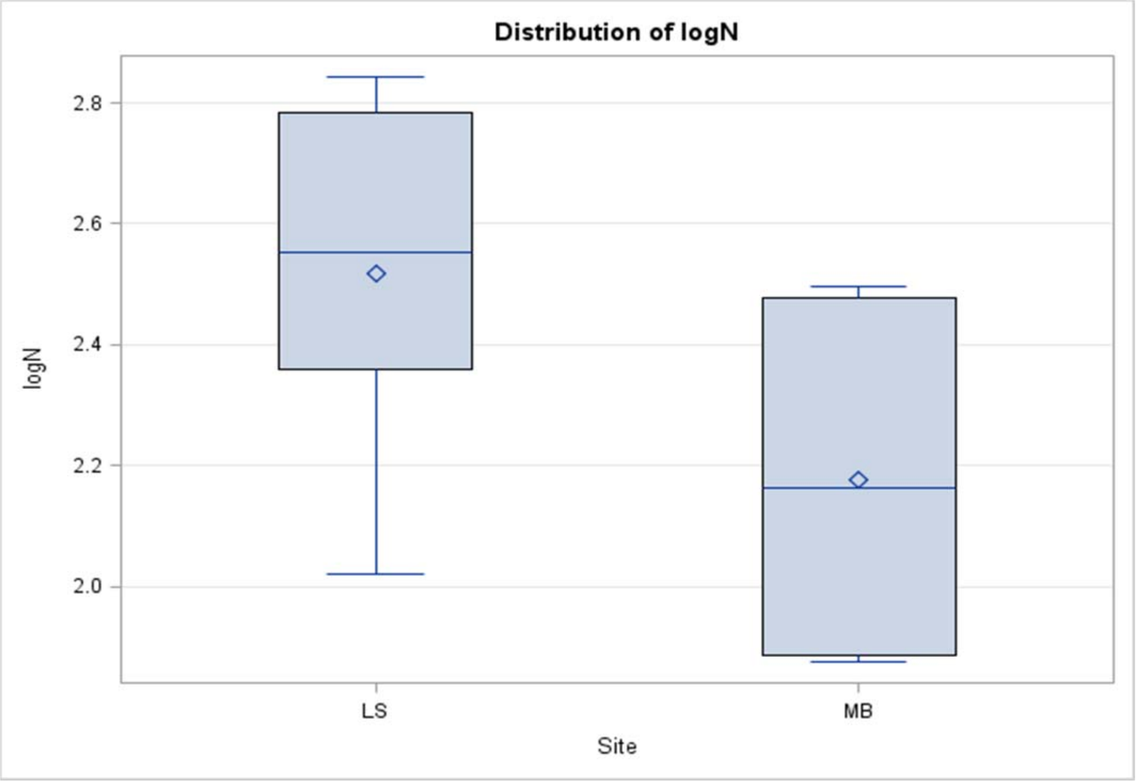

Figure 10. Abundances for Marla Bay and Lakeside. Displays standard deviation, median, and mean.

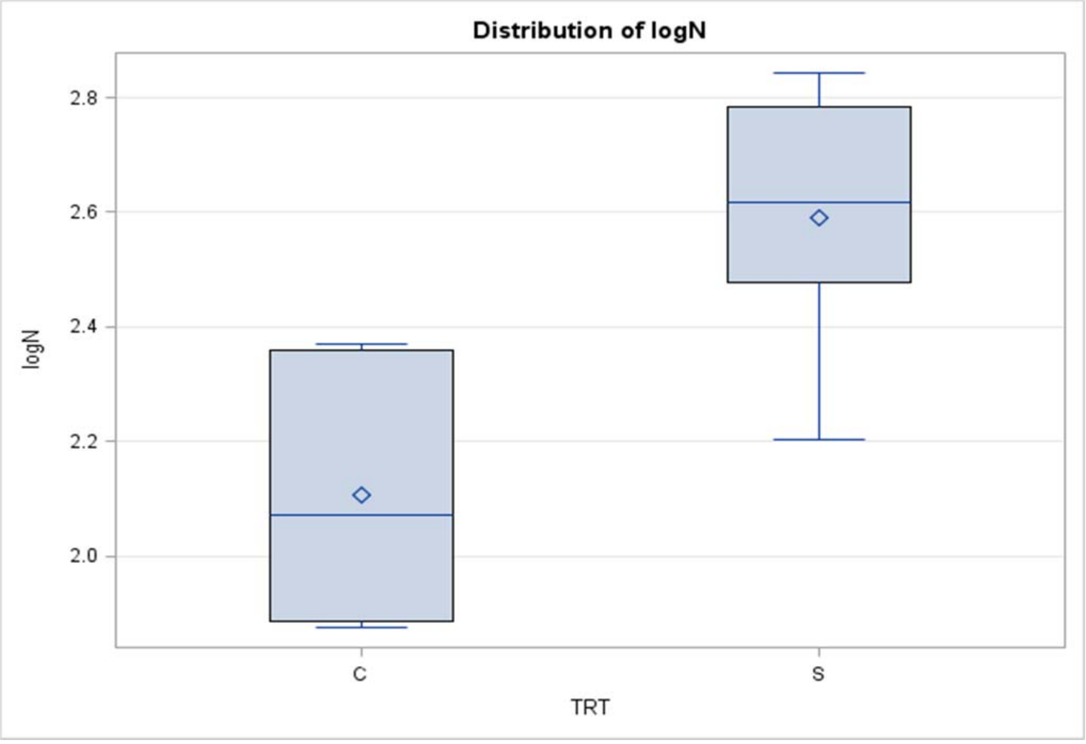

Figure 11. Abundances for control and suction treatments. Includes standard deviation, median, and mean.

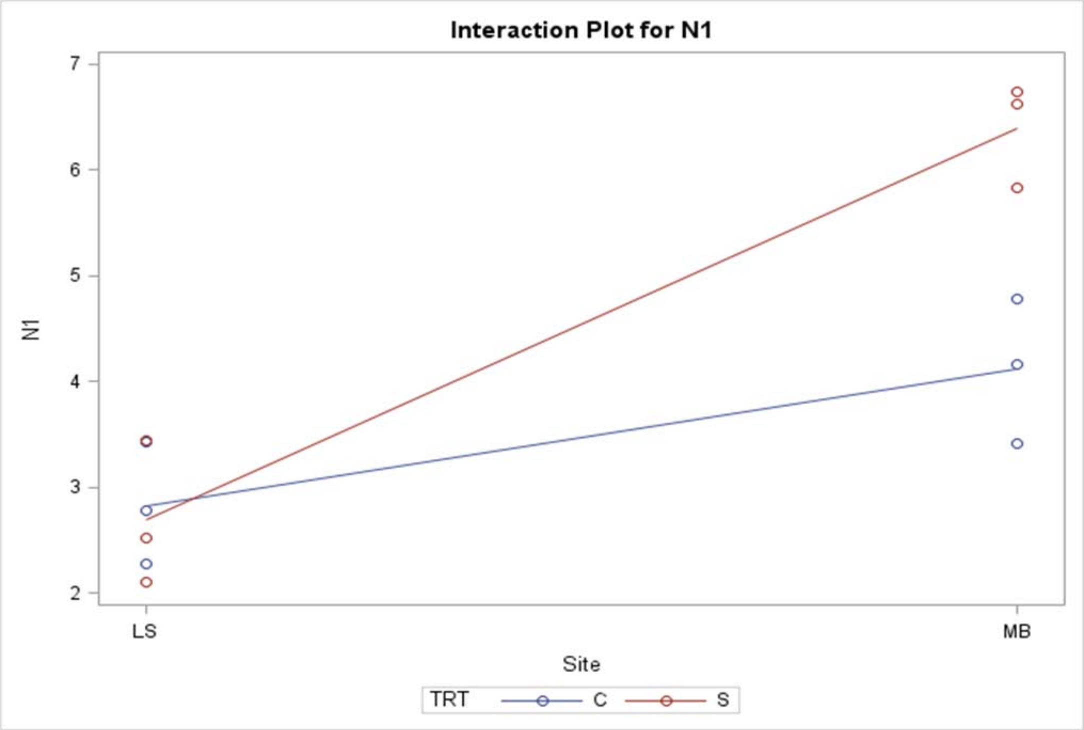

Figure 12. Interaction of the sites and treatments based on diversity. Marla Bay has a difference between the suction and control with the suction having a higher diversity than the control. Lakeside however has similar diversity for both the control and suction.

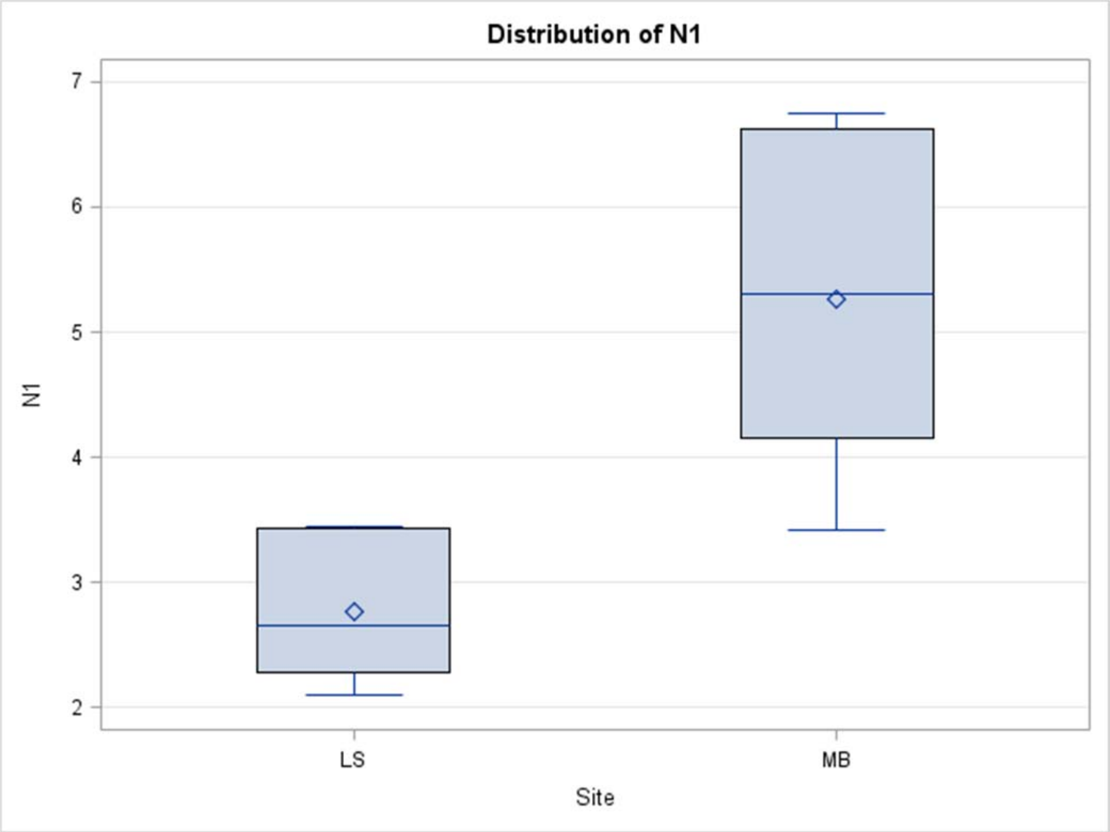

Figure 13. Diversity for Marla Bay and Lakeside. Displays the mean, median, and standard deviation.

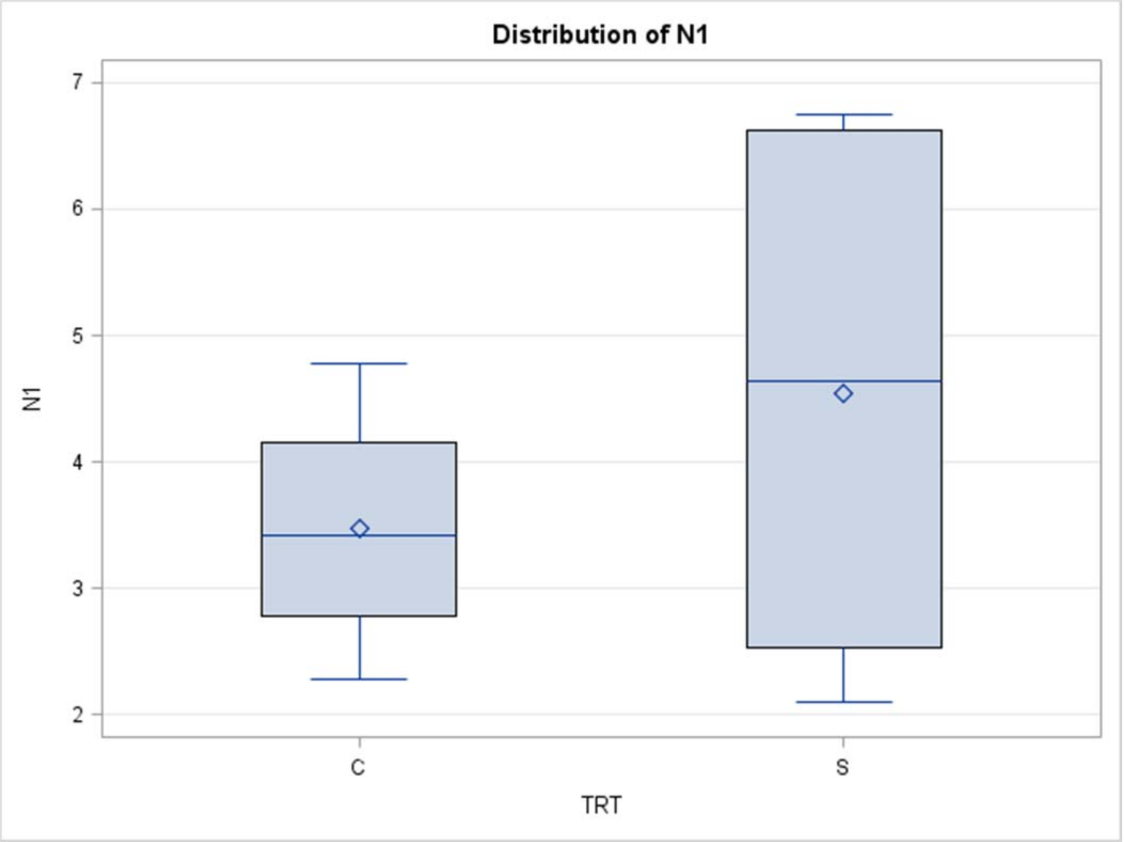

Figure 14. Diversity for control and suction treatments. Displays the mean, median, and standard deviation.

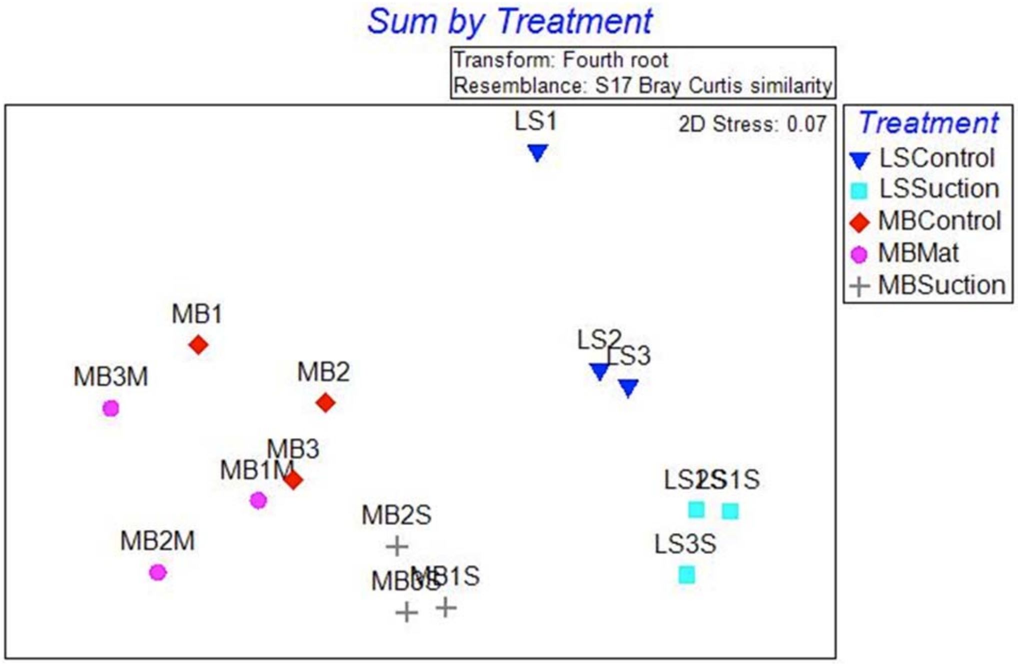

Figure 15. MDS plot of sites and treatments. Displaying the similarities of the different site/treatment combinations through distance.