EFFECTS OF NOVEL "LIGHTS OUT" MULCHING AND FERTILIZATION TURFGRASS RENOVATION TECHNIQUE ON ARTHROPOD, WEED, AND NEMATODE COMMUNITIES

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DEDICATION

To my mother, who told me to never be embittered by circumstance, and to never give up.

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Chapter 1. Introduction: A review of the novel Lights Out mulching technique as an alternative to chemical control in warm-season turfgrass systems

Abstract

"Lights Out" mulching is a pesticide-free method of weed and pest management that can reduce weed pressure when replanting turf, switching grass types, or completing a total renovation of a designated grassy area. In this two-year study, we addressed the feasibility of using black, geo-textile weed mats made of woven polypropylene plastic where: 1) the black plastic mulch significantly reduced light from reaching the soil surface for repeated coverings, 2) a fertilization amendment followed by irrigation on de-thatched ground stimulated weed seed germination to effectively flush the weed seed bank, and 3) prolonged periods of covering eliminated old turf and weed presence before re-establishing new turf. This introduction provides a justification for pursuing this management method, provides a comparison to traditional methods of control, and addresses the gaps in knowledge about Lights Out mulching. A comprehensive literature review is also provided to elaborate on what is known about the effects of plastic mulching on arthropod, weed, and nematode communities.

Key Words: Lights Out mulching, turfgrass renovation, geo-textile weed mat

Introduction

70% of the population of Hawai′i lives on Oahu and 62% of visitors spent part of their stay on Oahu. This makes the island home to many recreational turfgrass sites (Kawate et al., 2015). Many of these warm-season turfgrass systems in Hawai′i including golf courses, athletic fields, recreational areas, and even home lawns are valuable economic resources. The golf economy alone has a size of $1.4 billion, indicating that turf is a significant part of Hawai′i's tourism-driven economy (PGA Aloha Sector, 2009). Recreational surfaces also play a crucial role in the landscapes that surround the native residents. The grass system itself is unique because unlike temperate climates, it receives abundant solar radiation, rainfall, and maintains robust soils that support high rates of biodiversity year round. Among a variety of turfgrass pests, many annual weeds in temperate climates are able to compete for resources as perennials in tropical conditions. Some of these weeds grow uncontrollably with turfgrass, presenting a major obstacle for turf managers. In other situations, turfgrass mismanagement can encourage weed growth and cause severe infestations (Murphy, 2004). This often requires a complete renovation to re-establish the desired grass species (Cheng and DeFrank, 2014).

Hawaiian turfgrass landscape

Almost all turfgrasses used in Hawai′i are warm-season turfgrasses. Most of these grasses are C4 plants. They photosynthesize more effectively than C3 plants, generally require less water, and thrive in hot conditions (Kawate et al., 2015). The grasses will become dormant below 10°C, which makes them excellent choices for turf that will be used year-round at tropical elevations that generally stay above 15°C for the entire year. Some of the most common cultivars that are used in Hawai′i include Common Bermudagrass (*Cynodon Dactylon*), Zoysiagrass (*Zoysia japonica*), St. Augustinegrass (*Stenotaphrum secundatum*), and Seashore

Paspalum (*Paspalum vaginatum*). Bermudagrass is an excellent grass choice for turf that receives a lot of annual "wear and tear". It is the most commonly used turfgrass on golf courses and athletic fields in Hawai′i. It establishes quickly, and has a moderate tolerance of drought stress, and thrives in the sun. However, it is somewhat prone to thatch buildup, which can compromise the integrity of the grass surface if not properly managed (Kawate et al., 2015). Bermudagrass is also somewhat vulnerable to certain lepidopteran larva, including webworms (*Herpetogramma* spp.) and armyworms (*Spodoptera* spp.) Other Bermudagrass cultivars include Sunturf, Tifway, and Tifgreen varieties. A Riviera Bermudagrass cultivar was selected for the Lights Out mulching project because of these lucrative properties.

Zoysiagrass is another common turfgrass used in Hawai′i. It is slower to establish than Bermudagrass, but is more heat and drought resistant. Zoysiagrass varieties are more difficult to mow, and extra precaution should be used when mowing to prevent increased vulnerability to scalping and disease (Kawate et al., 2015). It is more common to establish Zoysiagrass varieties using sod, sprigs, or plugs. If proper irrigation occurs, strong roots will form and will yield a well-developed stand. Webworms are important pests of Zoysiagrass, and managers should look out for typical signs of damage. Some common cultivars include El Toro, Z-3, and Emerald.

St. Augustinegrass is also a popular turfgrass species in Hawai'i, but has a shallow root system that is not as resistant to drought as the other two turfgrasses. Like Bermudagrass, it is also prone to thatch buildup. It also has higher rates of pest problems. However, it is a good choice for areas that have abundant shade (Kawate et al., 2015).

Seashore Paspalum is tolerant to a wide variety of environmental stressors, especially increased salinity (Kawate et al., 2015). Seashore Paspalum is a lucrative choice for turfgrass managers because there is high species diversity, and it can be irrigated with non-potable water.

However, the grass can also be very sensitive to herbicides, so it may have limited uses on surfaces that are treated with chemicals frequently (Kawate et al., 2015). Each grass species has its own advantages and disadvantages. It is up to discretion of the turf management team to choose the most effective variety, which depends on how the surface will be used.

Traditional methods of control

Non-selective herbicides such as glyphosate have traditionally been used as the first step of weed control (Stier, 2000). The dead weeds are then mechanically removed or incorporated into the soil using tillage. However, the use of non-selective herbicides to renovate turf is somewhat contentious in the turfgrass industry. There has been an elevated public concern of exposure to chemicals. There have also been several bans and restrictions on the number and variety of pesticides that can be used on turfgrass (Cheng and DeFrank, 2014). These types of obstacles necessitate the flexibility and creativity of the turf community to develop alternative methods of control.

Lights Out mulching as an alternative

One non-chemical alternative is light exclusion (McCarty and Murphy, 2004). "Lights Out" mulching uses light exclusion rather than chemical introduction as a method of weed control in turfgrass systems, and has many practical applications for turf and landscape management. Dark, geo-textile weed mats made of woven polypropylene are placed over an affected area to exclude light over a prolonged period of time. This limits common problem weeds in Hawai′i from proliferating in turfgrass systems. The surface underneath the mat also reaches high temperatures (106-130°F) (Chauhan, 2015), which may kill weeds, nematodes, and insects in the top layer of soil (Klein et al., 2012). The heat and darkness drastically reduce the ability of the weed to survive, and may facilitate plant decomposition over time (Cheng and

DeFrank, 2014). A fertilizer amendment is applied in order to stimulate weed seed germination after covering and to flush the weed seed bank. Potassium nitrate and ammonium sulfate have been found to work well for this type of approach (IPNI, 2015). These newly germinated weeds will also be covered, reducing weed pressure for when the new grass is planted. Mulching provides turf managers with an alternative to traditional control strategies in a variety of warm season turfgrass including Bermudagrass (*Cynodon dactylon*), Zoysiagrass (*Zoysia* spp.), and St. Augustinegrass (*Stenotaphrum secundatum*) (Kawate et el., 2015).

Integrated Pest Management

Integrated Pest Management (IPM) could be considered the overarching 'umbrella' for the combination of traditional, alternative, and cultural methods of control in turf systems. IPM is a great approach for turf managers to incorporate into general maintenance and renovation because it reduces the use of controversial pesticides, reduces potential environmental harm, and increases the overall effectiveness of control tactics (Kawate et al., 2015). Some of the most important principles of a well-rounded IPM program include maintaining a robust, healthy turfgrass system (Stier, 2000). This ensures that the grass is treated properly with chemicals, mowers, and irrigation, which reduces weed and insect pressure. One similar approach to Lights Out mulching that falls under the IPM umbrella is soil solarization. This method used clear, polyethylene plastic of various thicknesses and colors to reduce unwanted pests, weeds and nematodes (Elmore et al., 1993, Ferris et al., 2001, Gill, 2010; Golzardi et al., 2015; Livingston, 1998; Stapleton, 2000;). IPM doesn't completely eliminate the use of chemicals on turf, but it allows managers to maximize the health of the grass, and use any necessary chemical treatments more effectively (Kawate et al., 2015).

Gaps in knowledge and potential outcomes

There are limitations in knowledge that must be addressed in order to fully assess the potential of Lights Out Mulching. Due to the novel nature of the approach, little information was available on the most effective protocol to achieve effective turf renovation, or what factors should be analyzed before the study began. To combat this gap in knowledge, a class project was completed in Spring 2014 using a small turf plot that was heavily infested with weeds. The results of using the lights out approach were comparable to the traditional chemical method (Roundup and Fusilade T&O) used on the same plot (Cheng and DeFrank, 2014). This called for further investigation in the form of a formalized study, which was partitioned into two years to develop a better understanding of how to perform the novel technique in the most effective way. Year 1 of the study served as a preliminary trial to develop a basic protocol that would be refined for use in year 2. This included but was not limited to determining what densities of weed mat, as well as what fertilizer rate and type to use in year 2. It also included making predictions on how these factors would affect the insect, weed, and nematode communities. We were able to make formal conclusions about the results from year 1, address the effects of mulching on the biotic community, and make projections about the feasibility of Lights Out mulching as a turf renovation technique. Data were collected in both year 1 and year 2, and analyzed based on these generalized objectives below:

1. Assess the ability of woven polypropylene weed mats to control weeds and restore turf.
2. Determine the most effective fertilizer amendment to stimulate weed seed germination and flush the weed seed bank.
3. Determine changes in the biotic community including insects and nematodes as a result of Lights Out Mulching.

Conclusions

The overall outcome of this project determined the efficacy of Lights Out Mulching as a viable turf renovation technique. The study addressed the unknowns of "Lights Out" technology for turf renovation, and presented a generalized mulching protocol that has potential use in the turf industry. It is necessary to evaluate the potential impact and benefits of "Lights Out" technology on the natural ecosystem that has been altered for recreational purposes. Turfgrass, whether on a golf course or a lawn, is not just a cultivated surface. It is also a dynamic ecosystem teeming with life. Lights Out mulching provides a practical alternative to the current glyphosate application and verticutting method used for turf or lawn maintenance, which can be rather disturbing to the soil ecosystem. It is hypothesized that the Lights Out technique for turf renovation would provide a more sustainable approach to protect the soil and ecosystem health of landscapes in Hawai′i.

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CHAPTER 2 Part I. Effects from year 1 of the Lights Out mulching study

Abstract

The effects of Lights Out mulching were studied in year 1 in order to: 1) assess the ability of a double-layer weed mat to suppress weeds and old turf, 2) evaluate the best combination of fertilizer treatments to stimulate weed seed germination, and 3) understand changes in the nematode and arthropod community. Weed coverage was reduced significantly on average from approximately 90% pre-cover, to 60% after weed mat removal and fertilization, to less than 5% after the final weed mat removal in September, and less than 15% in December after 2 months of uncontrolled Bermudagrass growth (ANOVA, $P = 0.026$). There were no differences in weed seed germination between fertilizer treatments (ANOVA, $P = 0.065$). Fertilization also had no effect on the arthropod or nematode community (ANOVA, $P > 0.05$). Results indicated that arthropod diversity and abundance decreased after the second covering, and the majority of the arthropods that were collected were identified as neither pests nor beneficial insects (i.e. isopods and amphipods). The nematode maturity index and total abundance were significantly reduced (ANOVA, $P < 0.05$) over time, but nematode structure and enrichment indices remained in-tact. Results benefit the scientific community as well as turf renovators because they offer new information on how "Lights Out" mulching affects biotic factors in weed and pest management. They also greatly benefit the set-up and predictions for year 2 of the study.

Key Words: Lights Out mulching, non-chemical weed control, fertilizer amendment

Introduction

Effects of Lights Out mulching

A generalized method for "Lights Out" mulching was designed before the study began that effectively suppressed weeds. Preliminary comparisons to traditional (chemical) methods of control demonstrated that light exclusion had the potential to successfully restore turf and control weeds (Cheng and DeFrank, 2014). There is a paucity of available literature that addresses any experiment with the exact Lights Out mulching protocol developed in Spring 2014. However, justifications were made using the literature of closely related soil solarization trials in agricultural systems.

Solarization requires at least 4 weeks of covering with a weed mat to suppress weeds (Golzardi et al., 2015). The original Lights Out mulching protocol suggests covering for an undisclosed period of time, but a 4-week minimum was determined to be acceptable and was incorporated into both years of the study. It was generally unclear whether a low, medium, or high density of black plastic would stimulate the most weed seed germination. However, it was hypothesized that higher density fabrics would be the most effective at trapping heat and excluding light, because a double layer weed mat was used in the initial class demonstration (Cheng and DeFrank, 2014). More data generation in both years of the study gave more insight on this observation.

Maintaining high temperatures is crucial to the success of Lights Out mulching. Thus, changes in soil temperature during covering were taken into account when executing the experiment. In previous studies, soil solarization was found to significantly increase soil temperature (Chauhan et al., 2015). In both years of our study, temperature and humidity data were analyzed using HOBO loggers in the soil and above ground to ensure soil surface temperatures underneath the weed mats were high enough to facilitate plant decomposition.

Effects on the arthropod community

High soil surface temperatures from plastic light exclusion practices may limit the ability of surface arthropods to survive (Cheng and DeFrank, 2014). While it would be beneficial if problematic insects were removed, it would not be a positive outcome if non-target or beneficial insects were negatively affected. Transparent plastic used in soil solarization that effectively limits weed growth was shown to limit numbers of plant-feeding insects. Other arthropods such as spiders, ants, grasshoppers and crickets, were shown to be unaffected by solarization treatments (Gill, 2010).

Numbers of collembola, which play an important role in soil decomposition, were shown to decrease as a result of solarization. However, they were able to recover when organic mulch was present in conjunction with the plastic (Gill, 2010). The Lights Out method may have a similar result, but it is unclear to what extent the prolonged high temperatures will affect the insect community. Black plastic mulch has also been shown to be moderately effective in controlling thrips and aphids, but these insects are not common pests in turfgrass systems (Livingston, 1998). These results simply demonstrate that black mulch can deter insects that harm plant communities, and affected insects may return to the system naturally. Therefore, Lights Out mulching has the potential to reduce numbers of turf pests such as webworms and armyworms without chemical intervention.

Weed suppression

Annual and perennial weed suppression using clear polyethylene plastic has been practiced in turf management (Elmore et al., 1993). It has been shown to effectively remove undesirable Bermudagrass and renovate the turf landscape, and is an excellent method of control for small-seeded annuals. Solarization can be less effective with large perennial weeds with

rhizomes, but cultural control can supplement the solarization process (Elmore et al., 1993). Cultural methods such as mowing, verticutting and hand weeding were implemented to ensure a stable turf environment. The Lights Out process demonstrates the importance of incorporating cultural control into turf protocols. Additionally, the preliminary class demonstration also revealed that Lights Out Mulching can be used to successfully renovate a small turfgrass area when compared to traditional control methods. The black mulch material was effective at suppressing old turf and newly germinated weeds that grew after the post-cover fertilizer amendment (Cheng and DeFrank, 2014).

Changes in the nematode community

Nematodes are the most abundant metazoa found on earth, and are ubiquitous in soil environments (Ferris et al., 2001), and beneficial bacteria and nematodes have been shown to persist through the plastic mulching process (Stapleton, 2000). Nematodes were not only able to persist, but also recover from the solarization process (Stapleton, 2000). These observations support the notion that a prolonged increase in soil temperature can significantly affect nematode communities, but some nematode species are able to recover over time. Species such as ring nematode (*Criconemoides* spp.*)* did not show significant variation in number, but maintained low values during the solarization process (Gill, 2010). In terms of control of harmful nematodes, soil solarization has been shown to be effective at suppressing root-knot nematodes in the upper 20cm of soil above 40°C (Klein et al., 2012). Additionally, combining Lights Out mulching with other IPM methods makes it a more desirable approach, and may help restore beneficial nematode composition more quickly (Kawate et al., 2015). More exploration of the biotic components of soil must be completed to provide a complete picture of the soil profile for managers that desire to incorporate the Lights Out approach into their IPM strategy.

Fertilizer review

Nitrogen exists as two main forms in the environment; nitrate and ammonium. The plant takes up the majority of nitrogen as nitrate (IPNI, 2015). It has a negative charge and interacts easily with water, which gets taken up by the plant. Nitrate is prone to leaching in the soil as well as surface runoff. It is metabolized in the leaves, where it is converted to ammonium and used to make proteins (IPNI, 2015). Ammonium is metabolized in the roots, where it can be converted directly into amino acids to make proteins. It has a positive charge, which allows it to bind effectively to the soil and prevent loss of nitrogen through leaching. However, if ammonium remains in the soil for too long, it may be converted to nitrate and lost through leaching (IPNI, 2015). Incorporating ammonium into the soil with proper irrigation will help reduce the chance of leaching. Both types of nitrogen are commonly used in turfgrass management, which is why they were chosen as the two treatments.

Because ammonium is metabolized in the roots and benefits the early life stages of the plant, we hypothesized that ammonium sulfate at 90 kg N/ha of 21-0-0 NPK would provide enough nitrogen as ammonium to the new root systems of germinating weeds. This would maximize weed seed germination, and effectively flush the weed seed bank. Visual observations from the class demonstration justified the incorporation of this hypothesis into the formalized study. A low rate, and a moderate rate were also used to serve as a comparison in year 1. The low and high rates of each fertilizer type were used the year 2 because the study area was too small to accommodate more than two fertilizer treatments. Overall, proper fertilization techniques are a requirement for a healthy, resilient turfgrass system.

Use of pre-study class demonstration

A small-scale experiment was completed for a turfgrass pest management course at University of Hawai′i Manoa in Fall 2014. This "micro" renovation served as a demonstration for the novel Lights Out mulching technique, and helped to outline a general protocol for use in the larger year 1 trial. The observations that were made during the class demonstration allowed us to make predictions about year 1 outcomes. Based on the class demonstration, it was concluded that: 1) 21-30 days was sufficient time for each covering to eliminate old turf and weeds and 2) the highest rate of ammonium sulfate fertilizer would be the most successful at stimulating weed seed germination (Cheng and DeFrank, 2014).

In the class demonstration, students renovated a weedy Zoysiagrass plot over a period of 4-5 months. The affected area was covered for the first time for 21 days, eliminating the old, weedy turf. The plots were uncovered and were de-thatched to remove the dead debris. All plant material was removed, and then a fertilizer amendment was added. 6 medium-sized plots were treated with 22.5, 45, and 90 kg N/ha of ammonium sulfate ($(NH_4)_2SO_4$)) and potassium nitrate (KNO_3), respectively. There were no additional replications. Weeds were allowed to grow for 2-3 weeks and were then re-covered to eliminate the new growth. This was a sufficient amount of time to eliminate the newly emerged weeds (Cheng and DeFrank, 2014).

Riviera Bermudagrass was selected for planting at a rate of 1.4 kg seeds/92m^2. The seed was covered in a hydromulch cap and irrigated in order to maximize grass seed germination. Thirty-three days after planting, a combination of Bermudagrass and weeds filled in all of the plots. Post-emergence herbicides were then used on half of the plots. A tank mix of Manor (0.4 oz/ac) and Monument/Blade (0.3 oz/ac + .25% NIS) was applied to three plots at a 1x, 2x, and 4x rate, respectively. Three plots remained untreated. After 54 days, visual observations were made to compare the untreated plots, as well as the treated plots. Visual observations revealed that the

90kg N/ha seemed to be more effective than the lower rates. Additionally, the ammonium sulfate seemed to be more effective than potassium nitrate. The newly planted Bermudagrass seemed to tolerate post-emergent herbicides well after 33 days. There were visual differences between each rate, 90 kg N/ha of ammonium sulfate demonstrating the greatest ability to stimulate weed seed germination. The Riviera Bermudagrass assimilated well into the system, and the portion of the lawn was successfully renovated in less than six months (Cheng and DeFrank, 2014).

Incorporating class demonstration into year 1

This demonstration provided a timeline for the year 1 protocol. We were able to reasonably conclude that 21-30 days of Lights Out mulching with a weed mat would be sufficient time to eliminate old turf and existing weeds. However, we did cover plots for a slightly longer period of time during the first covering, because the year 1 area was more heavily infested when compared to the lawn used for the demonstration. We also concluded that ammonium sulfate at 90 kg N/ha would be the most effective at stimulating weed seed germination. Finally, the same 6 treatments from the class study were replicated 4 times and randomized to minimize bias and error throughout the experiment. We also incorporated a third covering, but this was only due to a technical delay where new weeds had time to emerge before seeding. The class demonstration provided an excellent foundation for year 1 experimentation and analysis.

Materials and Methods

Experimental design

There are six basic steps needed in order to complete a Lights Out mulching process. The steps are listed here to provide a concise overview:

1. Cover existing weedy turf area for a certain period of time (3-6 weeks) to eliminate all above ground portions.

2. Remove cover, and then remove dead weeds and turf (and re-establish the desired site grade if necessary).

3. Fertilize to stimulate weed seed germination.

4. Allow for maximum weed seed germination and growth with overhead irrigation.

5. Cover the site a second time to eliminate newly emerged young weeds.

6. Remove the cover for second time and re-plant new turfgrass.

Year 1 (April 2015 to December 2015) research was conducted at the Magoon Research Facility in the Manoa Valley of Oahu, Hawai′i. A weedy turf area on the Magoon premises was divided into 24 plots that were 1.5 m × 2.4 m each. Plots were arranged in a randomized complete block design (RCBD.) The study area was gated, and in close proximity to an old house foundation with coarse, gravely soil surrounding it. From visual observation, it was described as an extreme soil environment to complete a turf renovation. A preliminary survey indicated that the area was comprised of morning glory (*Ipomoea obscura*), garden and graceful spurges (*Euphorbia* spp.), creeping indigo (*Indigofera spicata*), various amaranths (*Amaranthus* spp.), guinea grass (*Panicum maximum)*, and other common Hawaiian weed species. The untreated plots were covered with a double-layer (2x), geo-textile weed mat on April 21, 2015 to shade out the aboveground weeds and old turf. The weed mat was the 1859 style fabric from Belton Industries (Exacta Sales, INC). The weed mat was held in place using large, commercial fire-fighting hoses that were pressurized with irrigation water and placed in a rectangular format extending to the outside corners of the plots.

Temperature underneath the mats was recorded using a HOBO data logger monitoring device (Onset Computer Corporation) that collected data every 60 seconds. One data logger was placed on the south side of the turf area, and one at the north side. Temperature data from 5 days of each covering was recorded. The weed mat was removed after 48 days of covering on June 8, 2015 and the dead weeds were cleared from the plot by hand weeding and the use of two Redmax SGCZ2460S commercial Reciprocators (Husqvarna Group) on June 15, 2015. Thatch and debris were removed in order to allow light to penetrate and maximize weed seed germination. The fertilizer treatments were randomized and added to the plots. After one month, the weed seeds had sufficient time to germinate. The site was covered for a second time on July 14, 2015 with the same double-layer weed mat to eliminate the newly emerged weeds. The plot was uncovered after 27 days on August 10, 2015. There was a technical delay with the hydro-capping machine, so a third covering was required to eliminate newly emerged weeds. The plots were covered for a third time on August 27, 2015. They were un-covered for the final time on September 3, 2015. Then on September 17, 2015, each plot was coated with Riviera Bermudagrass seed at the maximum rate of 151 kg/ha. The newly spread seed was then hydro-capped with shredded, recycled paper materials and a surfactant to prevent birds from removing seed, increase contact with the soil, and encourage rapid germination and growth. Observations occurred on a monthly basis after the fertilizer treatments were added.

On June 15, 2015 the fertilizer amendment was added. The amendment was made using a 2×3 (fertilizer type x fertilizer rate) factorial design arranged in RCBD with 4 replications (Table 2.1). Ammonium sulfate ($(NH_4)_2SO_4$) and potassium nitrate (KNO_3) fertilizers were the fertilizer types tested after the first covering. Six 1.5 m ×2.4 m plots received ammonium sulfate and six plots received potassium nitrate in each of the two large study plots. The fertilizers were applied

at rates of 22.5 kg Nitrogen/ha (N/ha), 45 kg N/ha, and 90 kg N/ha (Table 2.1). A control was not included in the year 1 study because it created a 7-plot block. The study area could not accommodate that block size because it was tightly enclosed with a weed-covered fence. All plots were irrigated twice a day at 8AM and 2PM for ten minutes to stimulate weed germination, and to stimulate turf growth after Riviera Bermudagrass seed was applied.

Data Collection

Three pitfall trap collections were completed on the same days as the soil samples. One 250 ml cup was filled approximately halfway with tap water and placed in a small hole that was dug randomly in each plot. The excess space between the outside of the cup and the hole was filled with the remaining soil to ensure no specimens fell beneath the trap. It was left in place for two days. The specimens in the water were collected in small Tupperware containers and refrigerated. They were analyzed within 48 hours of collection to ensure that the specimens did not decompose in the water and become unidentifiable. The specimens were identified, recorded, and analyzed for diversity and abundance.

Soil samples were taken on April 12, 2015 before weed mat covering and fertilization to determine the composition of the nematode community before mulching. Soil samples were also taken after the first covering on June 8, 2015 and second covering on August 12, 2015 using the same method that was used before covering. Four random soil core samples were collected from each plot and made into one composite sample. In the lab, each composite sample was gently mixed and 10 grams of soil was removed from the plastic bag and was added to a Baermann funnel with tap water. After 3 days in the funnel, 50 ml of the solution was collected in a vial and labeled with the plot number. Samples were allowed to settle overnight in the refrigerator. The following day, 45 ml of water was removed slowly with a 10 ml pipette so the maximum number

of nematodes remained at the bottom of the vial for analysis. 5 ml of boiling water was added to kill the nematodes, creating a 10 ml sample. The samples were refrigerated for at least 24 hours before analysis. The vials were removed and the contents placed in a Petri dish with grid-lines. An inverted microscope was used to identify and count the nematodes to genus level along each grid-line. The individual counts were entered into an excel spreadsheet.

Several parameters were analyzed including: nematode maturity, enrichment, and structure indices for free-living nematodes (Okada and Kadota, 2003). The combined maturity index, plant-parasitic index, number of individual nematodes, richness (number of genera), and ratio of free-living to plant-parasitic nematodes were calculated as well. The nematodes *Tylenchus* spp. and *Filenchus* spp. were analyzed initially as plant feeding nematodes (PF2s). They were analyzed again as fungal feeding nematodes (FF2s) to serve as a comparison (Okada and Kadota, 2003).

Turfgrass quality was rated on April 9, 2015 before the first covering. It was rated again on July 14, 2015 after the fertilization but before the second covering. Then it was rated once every month after the third uncovering starting on October 2, 2015. A $1ft^2$ quadrant was used to determine percent turf coverage, percent weed coverage, weed species abundance, and weed species richness. The frame was distributed randomly, landing within the perimeter of each plot. Three monthly ratings were completed until the experiment was terminated in December, 2015. Shannon-Weiner diversity index was calculated. An analysis of variance (ANOVA) was used to identify any significant variance among the factors. A Tukey HSD multiple comparison procedure was used to identify differences between treatments.

Statistical analysis

Data were subjected to a one-way ANOVA originally using JMP 12 software (SAS Institute Inc., Cary, NC).

Results

Arthropod community

Analysis of the Shannon-Weiner diversity (SWD) index using a one-way ANOVA indicated that there were significant differences in arthropod diversity from April to August ($F = 31.03$, $DF = 2$, $P < 0.0001$). Average index values were 0.77 before the first cover in April and 0.5 after the first cover in June (Fig. 2.1). A multiple comparison procedure revealed that arthropod diversity in April was greater than June (Tukey-Kramer HSD, $P = 0.0017$). The fertilizer treatment occurred in July, but there were no significant differences in arthropod diversity in August due to the fertilizer treatment ($F = 0.1754$, $DF = 5$, $P = 0.968$) (Fig. 2.1). These diversity index ratings ranged from approximately 0.18-0.21 +/- 0.048 SE. The medium rate of ammonium sulfate had the largest average diversity rating of 0.22 when compared to the low and high rates of ammonium sulfate (0.19 and 0.20, respectively). Moreover, the medium and high rates of potassium nitrate seemed to have slightly larger average arthropod diversity, but the average range between the highest index value (medium of potassium nitrate) and the lowest index value (high of ammonium sulfate) was only 0.05 on the Shannon-Weiner scale (Fig. 2.1). The August sample also had lower arthropod diversity when compared to June (Tukey-Kramer HSD, $P = 0.0002$) (Fig. 2.1), indicating that all pairwise comparisons in terms of sample time were significant.

There were significant differences for the absolute abundance of arthropods ($F = 3.56$, $DF = 2$, $P = 0.034$) (Fig. 2.1). A multiple comparison procedure revealed a significant increase when comparing the abundances from April and June (before and after the first covering)

(Tukey-Kramer HSD, $P = 0.0254$). There were no significant differences in arthropod abundance based on treatment when August data (after fertilization) was analyzed ($F = 0.322$, $DF = 5$, $P = 0.8932$) (Fig. 2.1). However, all rates of ammonium sulfate had larger arthropod abundance when compared to potassium nitrate, but the differences were not considered statistically different (Fig. 2.1). Additionally, there were no significant differences between the arthropod abundance in August when compared to the beginning of the experiment in April (Tukey-Kramer HSD, $P = 0.373$), or between the abundance numbers recorded for June and August (Tukey-Kramer HSD, $P = 0.389$). Many of the arthropods were neutral in nature, and could not be classified as a beneficial insect or a pest to the turfgrass system. The overwhelming majority of the arthropod families that were identified were of the orders Amphipoda (*Porcellio* spp.*),* Isopoda (*Talitrus* spp. and *Talitroides* spp.), and Diplopoda (*Paradoxosomatidae* spp.*, Blaniulidae* spp.) Other arthropods that were identified and determined to be neither beneficial nor deleterious belonged to the insect orders Hymenoptera (Formicidae), Coleoptera (Tenebrionidae, Coccinellidae,) and Blattodea (Blattidae) (Table 2.2.). Very few spiders and centipedes were collected, but they did appear in a small number of samples (less than 10 in all of year 1).

Weed growth and suppression

Weed diversity was calculated before the first covering in April, and after the fertilization amendment in July using the Shannon-Weiner diversity index in a one-way ANOVA analysis. As expected, weed diversity was significantly greater in July after the fertilization treatment when compared to diversity before covering in April ($F = 28.5$, $DF = 4$, $P < 0.0001$). When differences in weed diversity based on treatment were compared for July data only, the highest rate of ammonium sulfate exhibited the highest average diversity (1.48 +/-0.098) (Fig. 2.2).

However, this difference was not statistically significant (F = 2.55, DF = 5, $P = 0.065$) (Fig. 2.2). Weed diversity was also analyzed on a monthly basis after the new Bermudagrass seed was sown and allowed to grow after the unexpected and short third covering in early September. Weed diversity ratings from October, November, and December were all significantly lower than July (Tukey-Kramer HSD, $P < 0.0001$ for all pairwise comparisons), but not when compared to April. The only month with coverage that was significantly lower than the April rating was October (Tukey-Kramer HSD, $P < 0.0001$). Furthermore, there was a significant increase in weed diversity from October to November (Tukey-Kramer HSD, $P < 0.0001$) (Fig. 2.2). November maintained the highest diversity of the three monthly ratings, and had nearly equivalent diversity to the April rating (Shannon-Weiner indices were both 0.69) (Tukey-Kramer HSD, $P = 1.000$). There were no other significant pairwise differences in weed diversity for the monthly ratings (Fig. 2.2).

Percent weed coverage data was analyzed using a one-way ANOVA. There were significant differences in weed coverage (F = 159.3, DF = 4, $P < 0.0001$). Weed coverage percent was lower after the first covering (Tukey-Kramer HSD, $P < 0.0001$), indicating that the first covering helped eliminate some initial weed pressure (Fig. 2.2). When July data was compared for differences in coverage due to treatment, no significance was found (F = 0.1891, DF = 5, $P = 0.963$) (Fig. 2.2). However, the medium rate of ammonium sulfate was noticeably greater than the other treatments, with approximately 60% weed coverage. All of the monthly ratings after the final covering had significantly lower percent coverage when compared to April and July data (Tukey-Kramer HSD, $P < 0.0001$ for all pairwise comparisons). However, no significance was found in terms of differences in percent coverage from October-December. When the three monthly ratings were compared independently from the April and July data,

there were significant differences in percent coverage (F = 3.67, DF = 2, *P* = 0.031). December had the greatest weed coverage percent at 13.25%, and was significantly greater than the October rating (Tukey-Kramer HSD, *P* = 0.026). This was low with respect to the April (pre-cover) average weed coverage of 90% (Fig. 2.2). There were no other significant pairwise differences between coverage percent at each sampling time for the monthly ratings. Some of the most common weeds that were present throughout the ratings were *Ipomoea obscura*, *Eleusine indica*, *Euphorbia hirta*, *Oxalis corniculata*, and *Eragrostis tenella* (Table 2.3).

Nematode community

Several parameters were analyzed with respect to the nematode community, including Maturity Index, Plant-Parasitic Index, and Combined Maturity Index (MI, PPI, CMI). Nematode abundance of free-living and plant-parasitic nematodes (nFLN, nPPN), ratio of free-living to plant-parasitic nematodes (FLN/PPN), and the number of genera were also analyzed. Finally, enrichment and structure indices were analyzed (EI, SI). A one-way ANOVA was used to compare each factor with the changes due to covering or fertilization. There were no significant differences in Maturity Index due to covering (F = 2.71, DF = 2, *P* = 0.073) (Fig. 2.3). There were significant differences for PPI due to covering (F = 4.52, DF = 2, *P* = 0.014). PPI decreased after the first covering (Tukey-Kramer HSD, *P* = 0.0123), but no other pairwise comparisons were significant (Fig. 2.3). There were also no significant differences due to covering for CMI (F = 2.58, DF = 2, *P* = 0.083) (Fig. 2.3). MI did not change due to the fertilizer treatment in July (F = 1.49, DF = 5, *P* = 0.24) (Fig. 2.4). PPI and CMI were also unaffected by the fertilizer treatment (PPI, F = 0.49, DF = 5, *P* = 0.78) (CMI, F = 0.92, DF = 5, *P* = 0.49) (Fig. 2.4).

There were significant differences in nFLNs over time (F = 12.95, DF = 2, *P* < 0.0001), but not by fertilizer treatments. April had the highest abundance of free-living nematodes when

compared to July samples (Tukey-Kramer HSD, $P = 0.0001$) (Fig. 2.5). There were also significant differences in nPPNs (F = 3.75, DF = 2, $P = 0.0283$). PPN abundance decreased after the first covering (Tukey-Kramer HSD, $P = 0.025$). There were no significant differences in nFLNs due to the July fertilizer treatment (F = 1.03, DF = 5, $P = 0.429$). There were also no differences in nPPNs due to the fertilizer treatment (F = 2.11, DF = 5, $P = 0.11$) (Fig. 2.6). Numbers of FLNs were noticeably greater with the higher rates of both ammonium sulfate and potassium nitrate, but this trend was not statistically significant ($P = 0.429$ for FLNs and 0.111 for PPNs) (Fig. 2.6). The numbers of free-living nematodes from the August sample were significantly lower when compared to data from before covering in April (Tukey-Kramer HSD, $P = 0.0001$). In contrast, plant-parasitic numbers appeared to increase slightly after the second cover, but this increase was not statistically significant when compared to numbers from April before the first cover (Tukey-Kramer HSD, $P = 0.157$). (Fig. 2.5).

Furthermore, there were no significant differences in the ratio of FLNs to PPNs due to covering (F = 1.12, DF = 2, $P = 0.3326$) or fertilizer treatment (F = 1.1, DF = 5, $P = 0.39$) (Fig. 2.6). There were significant differences in nematode richness (number of genera) (F = 6.59, DF = 2, $P = 0.0024$), with a significant decrease after the first covering compared to before covering (Tukey-Kramer HSD, $P = 0.0016$) (Fig. 2.5), but no other pairwise significance was observed. Number of genera remained unaffected by fertilizer treatment (F = 1.64, DF = 5, $P = 0.20$), but was noticeably greater with the high rate of ammonium sulfate (Fig. 2.6). Plots that received the low rate of ammonium sulfate had lower average number of genera than other treatments including potassium nitrate (5.75 compared to approximately 10.0), but this difference was not significant. There were no differences in the enrichment index (F = 2.88, DF = 2, $P = 0.063$) or the structure index (F = 1.11, DF = 2, $P = 0.336$) over time. There were also no differences in

fertilizer treatments for enrichment index (F = 0.311, DF = 2, *P* = 0.899) or the structure index (F= 2.67, DF = 5, *P* = 0.057). The significance value for difference in the structure index due to fertilizer treatment (*P* = 0.057) was considered marginally significant, and a Tukey HSD test did reveal a difference between the medium rates of potassium nitrate and ammonium sulfate (treatments 2 and 4) (*P* = 0.047) (Fig. 2.7).

The nematode community data was originally calculated with both *Tylenchus* spp. and *Filenchus* spp. counted as PPNs with a CP value of 2. Due to their high numbers relative to other species, their role in the soil food web was re-analyzed. It was determined that counting them as fungal feeding FLNs with a CP value of 2 (FF2) would be acceptable. The analysis yielded similar results with similar significance values (Table 2.4). However, there were significant differences in CMI between sampling times (F = 4.03, DF = 2, *P* = 0.022). The pairwise comparison for this combined index pointed to a significant increase from June to August (Tukey-Kramer HSD, *P* = 0.0225). The change in nematode rating was also associated with a significant difference in the FLN/PPN ratio between sampling times (F = 5.29, DF = 2, *P* = 0.0077). The June samples (after the first covering) had a lower ratio than the August samples (after the fertilization and second covering) (Tukey-Kramer HSD, *P* = 0.0057). No other pairwise significance was found.

The same parameters counting *Tylenchus* and *Filenchus* as FLNs were compared to fertilizer treatments using a one-way ANOVA analysis. The results were not statistically significant, with near similar *P*-values to the original analysis (Table 2.4). As expected, PPN numbers remained low by comparison because they were no longer dominated by *Tylenchus and Filenchus*. Overall, it appeared that regardless of how *Tylenchus* and *Filenchus* were classified, the results in year 1 remained very similar (Table 2.4). Aside from *Tylenchus* and *Filenchus,* the

nematodes identified in year 1 comprised a wide variety of genera, including *Acrobeles, Aphelenchus*, *Cephalobus*, *Eudorylaimus*, *Rhabditis*, and others (Table 2.5). These nematodes compromised a variety of feeding types including fungal-feeding (FF), plant-feeding (PF), and omnivorous (OM) with colonizer-persister (CP) values 1-5 (Table 2.6).

Discussion

The significant decrease in arthropod diversity over time indicate that covering plays a role in reducing the number of arthropod species that can persist through prolonged high temperature and light exclusion (Fig. 2.1). This result contrasts previous findings that many groups of arthropods actually remain unaffected from prolonged soil solarization (Gill, 2010). The Gill study did report that some species (collembola) are initially reduced and then recover from solarization, but collembola did not contribute to the diversity ratings in year 1 because they were not found in any of the pitfall traps. Berlese funnels will be used in year 2 to identify any potential effects on microarthropods that may go undetected in pitfall traps.

The fertilizer treatments did not negatively affect arthropod diversity, but did not appear to benefit arthropods either (Fig. 2.2). This result contrasts previous findings that fertilization amendments make a beneficial contribution to soil biodiversity (Ying-Hua et al. 2013). However, the Lights Out results may support a different observation that response to fertilizer treatments is species-specific (Garrat et al. 2010). Some herbivorous insect species (*Metopolophium dirhodum)* respond to increased robustness of plant tissue for consumption, while others (*Rhopalosiphum padi*) are sensitive to a change in the availability of nutrients. The majority of the arthropods collected during year 1 were not classified as herbivorous grass pests (i.e. *Herpetogramma* spp.), so it is possible that species of amphipods and isopods that were found are more resistant to any negative effects from fertilization. This disparity suggests that fertilization not only affects plant growth, but is also scalable to higher trophic levels regardless

of nutritional specificity and should be well-understood when using a fertilizer amendment during the Lights Out process.

In contrast to the decrease in arthropod diversity, there was an increase in arthropod abundance between the first and second covering (Fig. 2.2). One study reported that sampling time and seasonal variation in soil physicochemical properties play a role in determining insect abundance (Ying-Hua et al. 2013). This study reported that insect abundance was highest in June, where soil moisture was also greater when compared to other months. The fertilizer treatment with nitrogen and phosphate (NP) maintained the highest insect abundance during this time, and the nitrogen potassium (NK) maintained the lowest. Although soil moisture was not quantified during the Lights Out experiment, it is possible that the June sampling date had significantly great arthropod abundance due to the sampling time. Other studies have found evidence that contrasts the Lights Out fertilization results (Hancock et al., 2012). In this study, fertilization was shown to reduce insect abundance, but the results appeared to be based on tri-tropic interactions of plants, insect herbivores, and their associated parasitoids (Hancock et al., 2012). This tri-trophic structure was not observed during the Lights Out mulching investigation, but supports the scalable, species-specific response found by Garrat et al. (2010) It also provides more context for potential outcomes in turfgrass ecosystems that are more diverse or contain more herbivorous insects than the year 1 Lights Out study area.

The fertilizer amendment showed greater weed diversity values amongst the low and high rates of ammonium sulfate (Fig. 2.2), but these trends were not considered statistically significant. This indicates that there was no statistical difference between fertilizer treatments in terms of stimulating weed diversity to flush the weed seed bank. Weed diversity values were relatively small before the first covering, increased significantly as expected directly after the

fertilization, and then decreased after the second and third coverings (Fig. 2.2). This finding indicates that even though there were no significant differences between fertilizer treatments, the use of fertilizer is a crucial step in Lights Out mulching because it flushes the weed seed bank and reduces additional weed germination over time. Weed diversity did increase significantly in November, but maintained a Shannon-Weiner index less than 0.5 by the end of the experiment in December, indicating an overall reduction in weed presence (Fig. 2.2). These findings were consistent with the class demonstration completed in Spring, 2015, as well as the preliminary results from 2014 (Cheng and DeFrank, 2014). In an actual renovation, other cultural methods and selective herbicides may be used to manage any persistent weed growth that arises after the last covering. The experiment plots were simply allowed to grow over the three months to more accurately identify weeds to the species level. Overall, diversity values were relatively low across all times and treatments, indicating that the turf system may not have had a variety of weed species in the weed bank.

Weed coverage was approximately 90% across all plots in April. It was reduced significantly to an average of less than 25% after the last covering (Fig. 2.2). This indicated that the coverings significantly reduced weed pressure by reducing total percent coverage over time. Additionally, weed coverage was reduced to 5% in October after the second and third covering. It was significantly greater in December, but the monthly increase never reached more than 15% coverage. Furthermore, weeds were not able to fully re-establish after the first covering or the fertilization event. These findings are consistent with the results from the 2014 study (Cheng and DeFrank, 2014). There were no differences in coverage percent between fertilizer treatments (Fig. 2.2). These findings were consistent with review papers about the benefits of using each type of fertilizer (IPNI, 2015), where any clear advantage of using one fertilizer over the other

was unclear. This provided enough reason to use low and high rates of both fertilizers in year 2, since they showed values that were noticeably greater directly after fertilization in either weed abundance or coverage percent. Controls were used in year 2 to compare differences in weed coverage and diversity between fertilizer treatments and traditional methods of control using glyphosate.

Additionally, visual observations indicated that the coated Riviera Bermudagrass variety assimilated into the system well, and had good tolerance of the surrounding environmental pressures. However, visual observations revealed that turf greenness became less apparent as winter approached, and its growth rate seemed to slow. The final rating was taken in December, and the plots were left to grow without further treatment as year 1 came to an end. After the experiment, additional visual observations were made to assess the longevity of the treatments. Many weeds began to establish indiscriminately throughout the plots. Since the new Bermudagrass seed had already germinated, additional coverings would not have aided the renovation process. It was clear that a post-emergence herbicide or glyphosate amendment would be needed in order to restore a weed-free environment and allow the Bermudagrass to establish more dominance. The need for cultural and chemical controls throughout the renovation process is consistent with established reports on IPM and turf renovation (Murphy, 2004), but the Lights Out renovation approach significantly reduced the reliance on chemical inputs.

Soil food web diversity was considered minimal before the experiment began because of the relatively low Shannon-Weiner values reported in April. This was thought to be in part caused by the presence of a crumbling house foundation that had a clear impact on the texture and homogeneity of the soil. Nevertheless, there was still a reduction in the average numbers of nematodes that was associated with covering. The reduction of PPNs (Fig. 2.5) after the first

covering may have benefitted the establishment of the young weeds after the fertilization amendment because the root systems were not threatened (Fig. 2.6). Other similar studies support this conclusion that solarization does reduce harmful nematode species over time (Chellemi et al. 1997, McGovern and McSorley 1997, McGovern et al. 2002, Stapleton and Heald 1991). The reduction in free-living nematodes is troubling, because they play an important role in soil biodiversity. A fourth sample in year 1 would have disrupted the establishment of the newly renovated turf, but it would have been an interesting addition to the study to understand how the nematode community changed during the monthly turfgrass and weed ratings. It may also have determined whether numbers of free-living nematodes recovered after the Bermudagrass established. It did not appear that either free-living or plant-parasitic numbers were affected due to difference in fertilizer treatment, which may suggest that fertilizer can be used to stimulate weed seed germination and flush the weed seed bank without disturbing nematode abundance.

The overall structure and enrichment appeared to remain in-tact (Fig. 2.7). This indicates that while average numbers may have been reduced, the community was able to endure the heat pressure. The change in nematode rating from PF2 to FF2 demonstrates that *Tylenchus and Filenchus* dominated the total number of nematodes collected after the first covering. For example, both ratings yielded significant values for number of plant-parasitic nematodes affected over time (Table 2.4). but there were no significant differences between observations from after the first and second coverings for number of PPNs when they were rated as PF2s. This difference was highly significant when they were rated as FF2s ($P < 0.0001$). It's clear that when *Tylenchus and Filenchus* were removed from the PF2 rating, it caused this highly significant difference. However, it is unclear why these nematode groups were more abundant than others in the sample

after the first covering. It is possible that less plant material supports less organic matter, which aids in fungal decomposition. The same methodology was repeated in year 2 in order to establish a more conclusive result in the context of the Lights Out mulching protocol.

Moreover, this turf renovation successfully removed old turf and suppressed weed dominance without the use of chemical pesticides and without tillage. It was originally hypothesized during the class demonstration that 21-30 days would be effective for each covering to eliminate old turf and weeds. The plot was covered for 48 days and resulted in a completely eliminated turf. The plots may have been covered for a longer period of time than the smaller class-demonstration plots because it was a larger area shaded by two trees, and was exposed to less sunlight. The first covering also took place in early spring rather than late summer/early fall, when temperatures are not as high and days are shorter. Notwithstanding, 48 days was sufficient time to eliminate the entire turf using a double-layer weed mat.

The outcome of this experiment benefits several actors in the scientific community. It benefits researchers by providing a comprehensive overview of the soil profile that includes some important biotic changes as a result of "Lights Out" mulching. It also benefits IPM managers seeking to diversify their control methods with a new management protocol. Plastic mulching is a proven method of weed control in agricultural systems, home lawns, and more recently, recreational turf systems. It can be used in tandem with other control methods to achieve maximum preservation of a healthy soil system. However, Lights Out mulching alone may require managers to spend more time on renovation. It may also interfere with the soil food web, including arthropod and nematode abundance. Agencies that fund turf and landscape research initiatives are more likely to allocate funding towards projects if there is an increase in data that supports "Lights Out" mulching as a valid and valued method of turfgrass management.

Repetition in year 2 will provide more data to confirm how turfgrass insects are affected, if the loss in nematodes was due to mulching or another factor (i.e. soil composition), and if weeds need to be treated with a post-emergence herbicide in tandem with Lights Out mulching in order to stymie the re-establishment of weeds.

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Table 2.1. Fertilizer randomization for year 1 of the Lights Out mulching experiment. Treatments include four replications of 22.5, 45, and 90 kg N/ha of potassium nitrate (KNO_3) and 22.5, 45, and 90 kg N/ha of ammonium sulfate ($(NH_4)_2SO_4$).

Treatment ID	Treatment	Rate (Kg N/ha)	Application (grams)
1	KNO_3	22.5	60
2	KNO_3	45	120
3	KNO_3	90	240
4	$(NH_4)_2SO_4$	22.5	40
5	$(NH_4)_2SO_4$	45	80
6	$(NH_4)_2SO_4$	90	160

Table 2.2. List of arthropods identified in year 1 of the Lights Out mulching study. Arthropods were collected from pitfall traps and identified to family, sub-family, or genus and species depending on the quality of the specimen.

				April 2015 Total	June 2015 Total	August 2015 Total
Class	Order	Family	Sub-family/Genus spp.			
Arachnida	Araneae	Aranidae		1	1	4
Diplopoda	Julida	Blaniulidae	*Proteroiulus fuscus*	1		
	Polydesmida	Paradoxosomatidae	*Asiomarpha coarctata*	10	88	1
	Polydesmida	Paradoxosomatidae	*Oxidus gracilis*	4		15
Insecta	Blattodea	Blattidae			2	4
	Coleoptera	Coccinellidae	*Adalia bipunctata*	1		
		Tenebrionidae	*Gonocephalum* spp.	16	2	7
	Dermaptera	Forficulidae			2	7
	Diptera	Culicidae			1	1
		Fannidae	*Fannia* spp.	1	1	
		Muscidae				1
	Hemiptera	Reduviidae		5		
	Hympenoptera	Formicidae	*Dolichoderinae*	1		
		Formicidae	*Pheidole megacephala*	61	35	
		Formicidae	*Ponerinae*		1	38
		Pompillidae	*Cercopales* spp.			1
		Xylocompinae	*Xylocopa* spp.	1		
	Lepidoptera	Pyralidae			1	1
	Orthoptera	Gryllotalpidae				3
Malacostraca	Amphipoda					
	Isopoda	Porcellionidae	*Porcellio spp.*	192	497	370
Total				294	631	453

Table 2.3. Common and scientific names of weeds identified during year 1 of the Lights Out mulching experiment.

Common Name	Scientific Name
Ageratum	*Ageratum conyzoides*
Alsike Clover	*Trifolium hybridum*
Amaranth, Slender	*Amaranthus viridis*
Amaranth Weed	*Amaranthus* spp.
Cinderella Weed	*Asclepias* spp.
Creeping Indigo	*Indigofera* spp.
Filarees	*Erodium* spp.
Garden Spurge	*Euphorbia hirta*
Goosegrass	*Eleusine indica*
Graceful Spurge	*Euphorbia glomerifera*
Hairy Crabgrass	*Digitaria sanguinalis*
Hairy Indigo	*Indigofera hirsuta*
Haole Koa	*Leucaena leucocephala*
Honohono	*Commelina diffusa*
Kyllinga	*Kyllinga brevifolia*
Love Grass	*Eragrostis tenella*
McCoy Grass	*Cyperus gracilis*
Morning Glory	*Ipomoea obscura*
Oxalis Weed	*Oxalis corniculata*
Panama Paspalum	*Paspalum fimbriatum*
Pitted Beardgrass	*Andropogon pertusus*
Prostrate Spurge	*Euphorbia prostrata*
Purple Nutsedge	*Cyperus rotundus*
Radiate Finger grass	*Chloris radiata*
Sensitive Plant	*Mimosa pudica*
Sida	*Sida cordifolia*
Sprawling Horseweed	*Calyptocarpus vialis*
Unidentified Grass	*Gramineae*

Table 2.4. A factorial analysis of variance (treatment × sampling time) of nematode community indices with *Tylenchus* spp. and *Filenchus* spp. calculated as Plant-Feeding (PF2s) or Fungal-Feeding (FF2s) in year 1. Numbers are *P* values for each parameter.

	Treatment		Time	
	Tylenchus and *Filenchus* as PF2s	*Tylenchus* and *Filenchus* as FF2s	*Tylenchus* and *Filenchus* as PF2s	*Tylenchus* and *Filenchus* as FF2s
MI[a]	0.2406	0.6089	0.0733	0.0556
PPI[b]	0.7775	0.3752	0.0143	0.0017
CMI[c]	0.4904	0.5820	0.0834	0.0222
FLN/PPN[d]	0.3921	0.1297	0.3326	0.0077
Genera[e]	0.1996	0.1996	0.0024	0.0024
nFLN[f]	0.4298	0.5020	0.0001	0.0005
nPPN[g]	0.1110	0.6089	0.0283	0.0001

[a] Maturity Index of free-living nematodes
[b] Plant-Parasitic Index
[c] Combined Maturity Index of free-living and plant-parasitic nematodes
[d] Ratio of free-living to plant-parasitic nematode abundance
[e] Number of genera (richness)
[f] Absolute abundance of free-living nematodes
[g] Absolute abundance of plant-parasitic nematodes

Table 2.5. Nematode genera identified during year 1, feeding group code, and total absolute abundance from each sampling time.

Nematode Genus	Feeding Group	April 2015	June 2015	August 2015
Acrobeles	**BF2**	21	4	6
Acrobeloides	**BF2**	66	40	18
Aglenchus	**PF2**	0	0	0
Alaimus	**OM4**	4	0	0
Aphelenchoides	**FF2**	128	39	32
Aphelenchus	**FF2**	83	56	148
Aporcelaimellus	**OM5**	0	0	0
Cephalobus	**BF2**	78	18	118
Cervidellus	**BF3**	4	0	2
Chiloplacus	**BF2**	1	0	0
Criconemoides	**PF3**	0	0	0
Diplogaster	**BF1**	32	27	49
Discolaimus	**OM5**	0	0	0
Dorylaimus	**OM4**	77	30	88
Eucephalobus	**BF2**	176	36	72
Eudorylaimus	**OM4**	79	21	60
Filenchus	**PF2**	113	55	89
Helicotylenchus	**PF3**	76	44	64
Heterodera	**PF3**	3	0	0
Hoplolaimus	**PF3**	0	0	0
Longidorus	**PF5**	0	0	0
Malenchus	**PF2**	6	0	0
MesoCriconemoides	**PF3**	0	0	0
Mesodorylaimus	**OM4**	0	0	0
Monhystera	**BF1**	61	10	21
Mononchus	**PR4**	0	0	5
Nygellus	**OM4**	0	0	0
Panagrolaimus	**BF1**	0	0	0
Paratylenchus	**PF2**	0	6	30
Pelodera	**BF1**	1	0	0
Plectus	**BF2**	3	6	7
Pratylenchus	**PF3**	60	77	20
Psilenchus	**PF2**	2	0	0
Pungentus	**OM4**	2	0	13
Rhabditis	**BF1**	102	65	13
Rotylenchulus	**PF1**	96	13	2
Rotylenchus	**PF3**	0	1	0
Telotylenchus	**PF2**	0	0	0
Turbatrix	**BF1**	0	0	0
Tylenchorynchus	**PF3**	0	0	2
Tylenchus	**PF2**	133	47	83
Unknown		550	0	82
Wilsonema	**BF2**	0	0	0
Xiphinema	**PF5**	0	0	4

Table 2.6. Nematode feeding type related to each feeding group code, and the CP values that were associated with each group during year 1.

Feeding Type	Code	Colonizer Persister (CP) Value
Bacterial Feeding	BF	1, 2,
Plant Feeding	PF	1, 2, 3, 5
Fungal Feeding	FF	2
Omnivorous	OM	4, 5

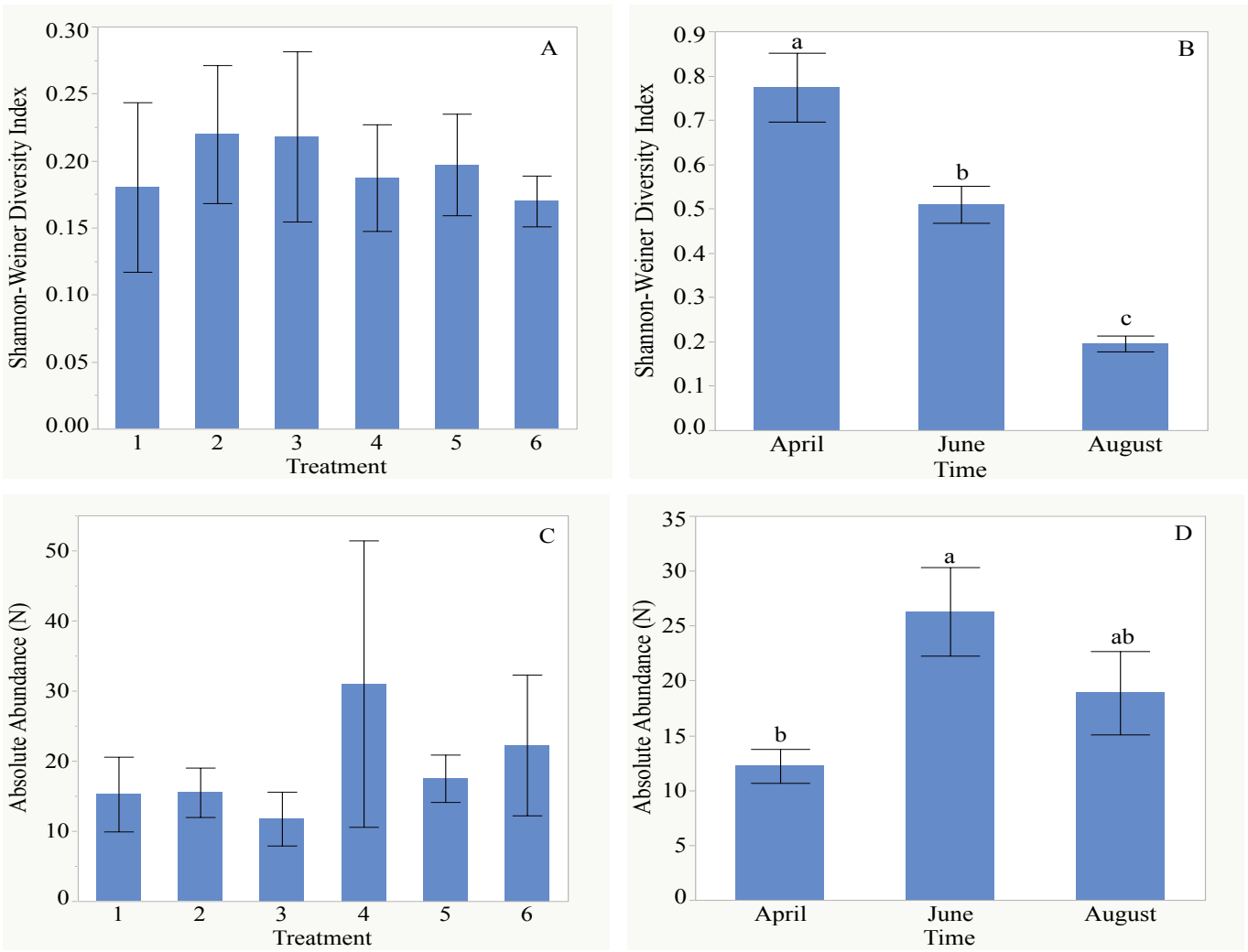

Fig. 2.1. Change in arthropod diversity due to A) treatment and B) sampling time, as well as arthropod abundance due to C) treatment and D) sampling time during year 1. Treatments 1-3: low, med, high rates of KNO_3 and treatments 4-6: low, med, high rates of $(NH_4)_2SO_4$. Error bars indicate one standard error from the mean. Bars with different letters indicate a significant difference.

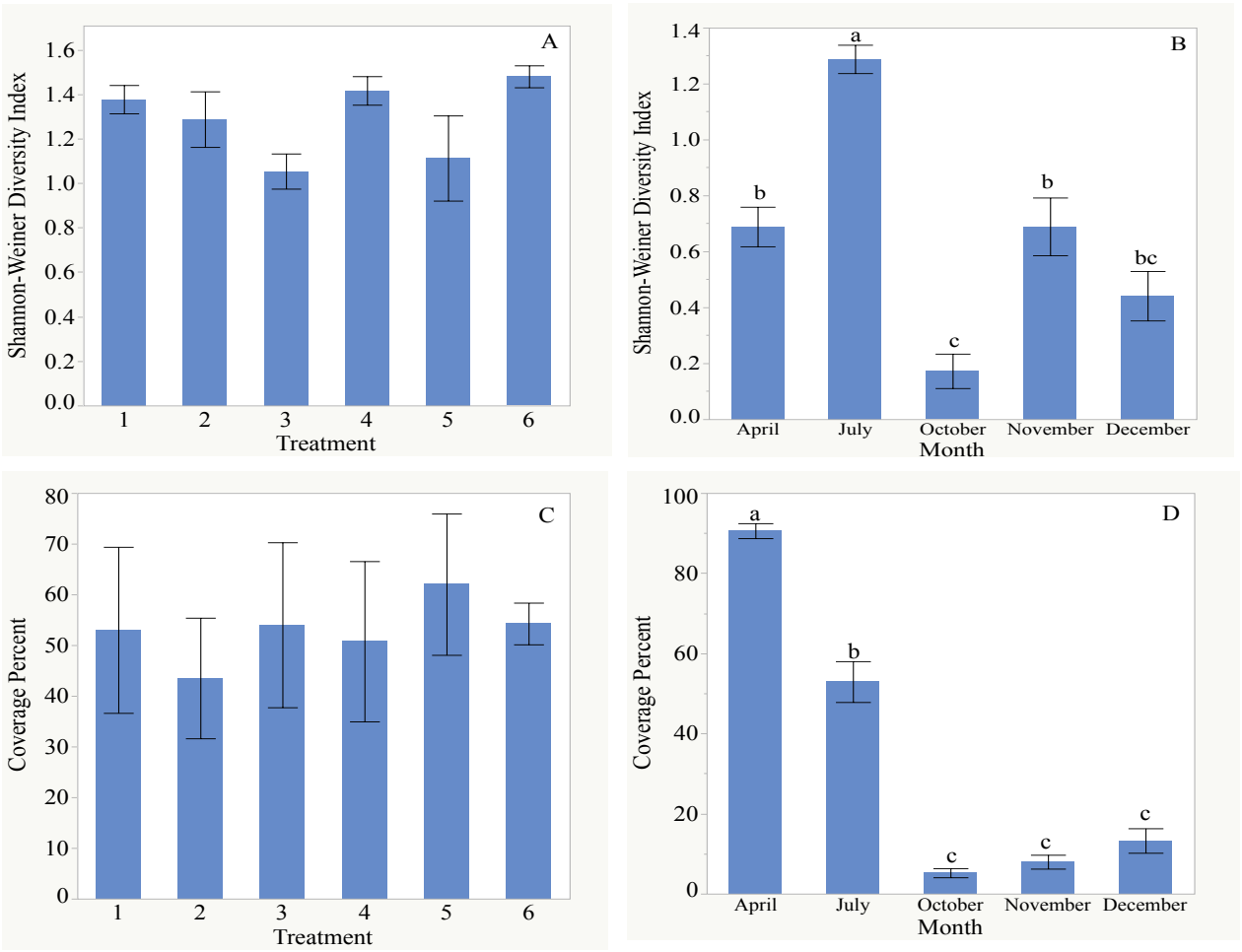

Fig. 2.2. Change in year 1 weed diversity due to A) treatment and B) sampling time, as well as coverage percent due to C) treatment and D) sampling time. Treatments 1-3: low, med, high rates of KNO_3 and treatments 4-6: low, med, high rates of $(NH_4)_2SO_4$. Error bars represent 1 standard error from the mean. Bars with different letters indicate a significant difference.

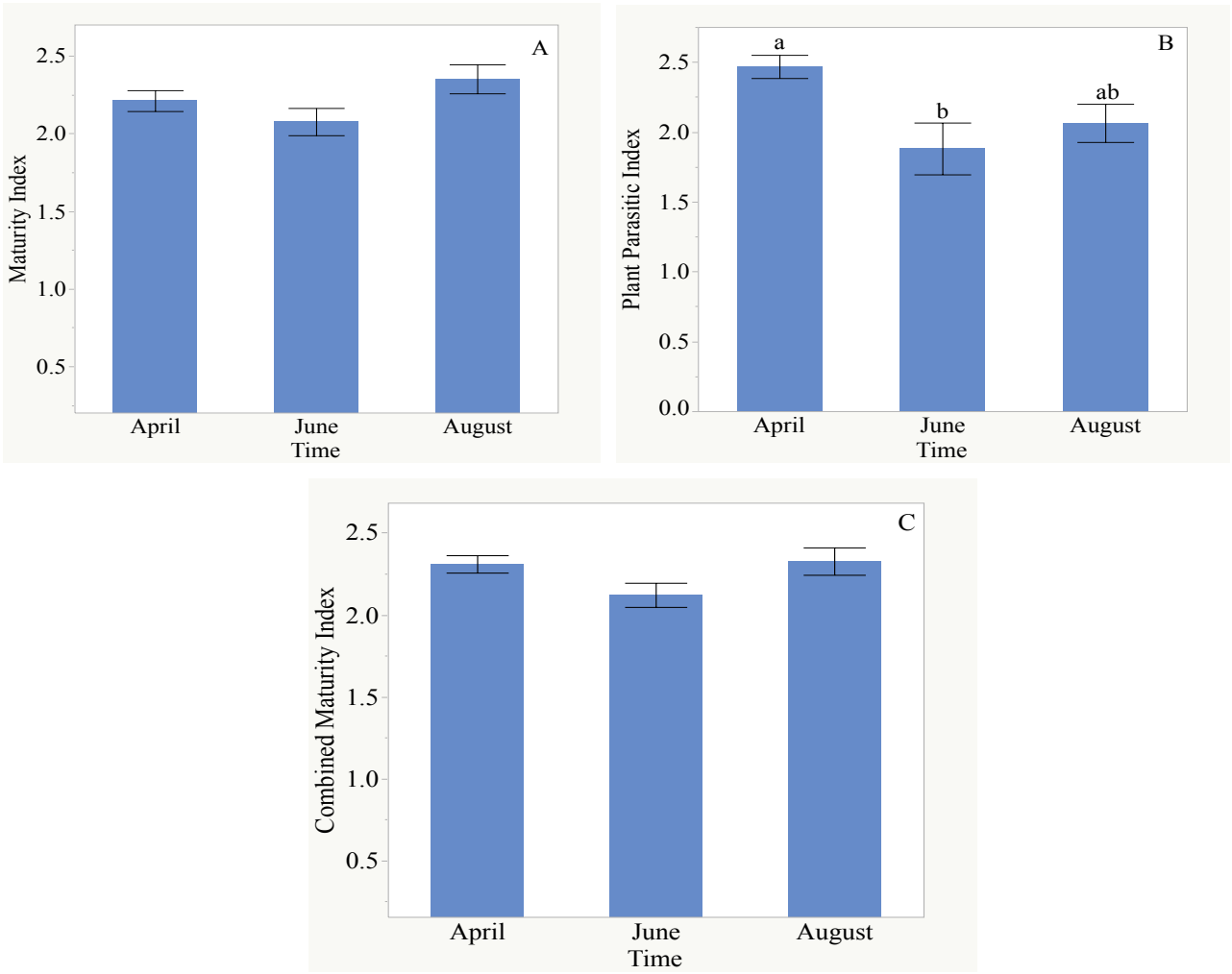

Fig 2.3. Year 1 change in A) Maturity index B) Plant-Parasitic Index, and C) Combined Maturity Index before and after the first and second cover. Error bars are one standard error from the mean. Different letters indicate significant differences in mean index values.

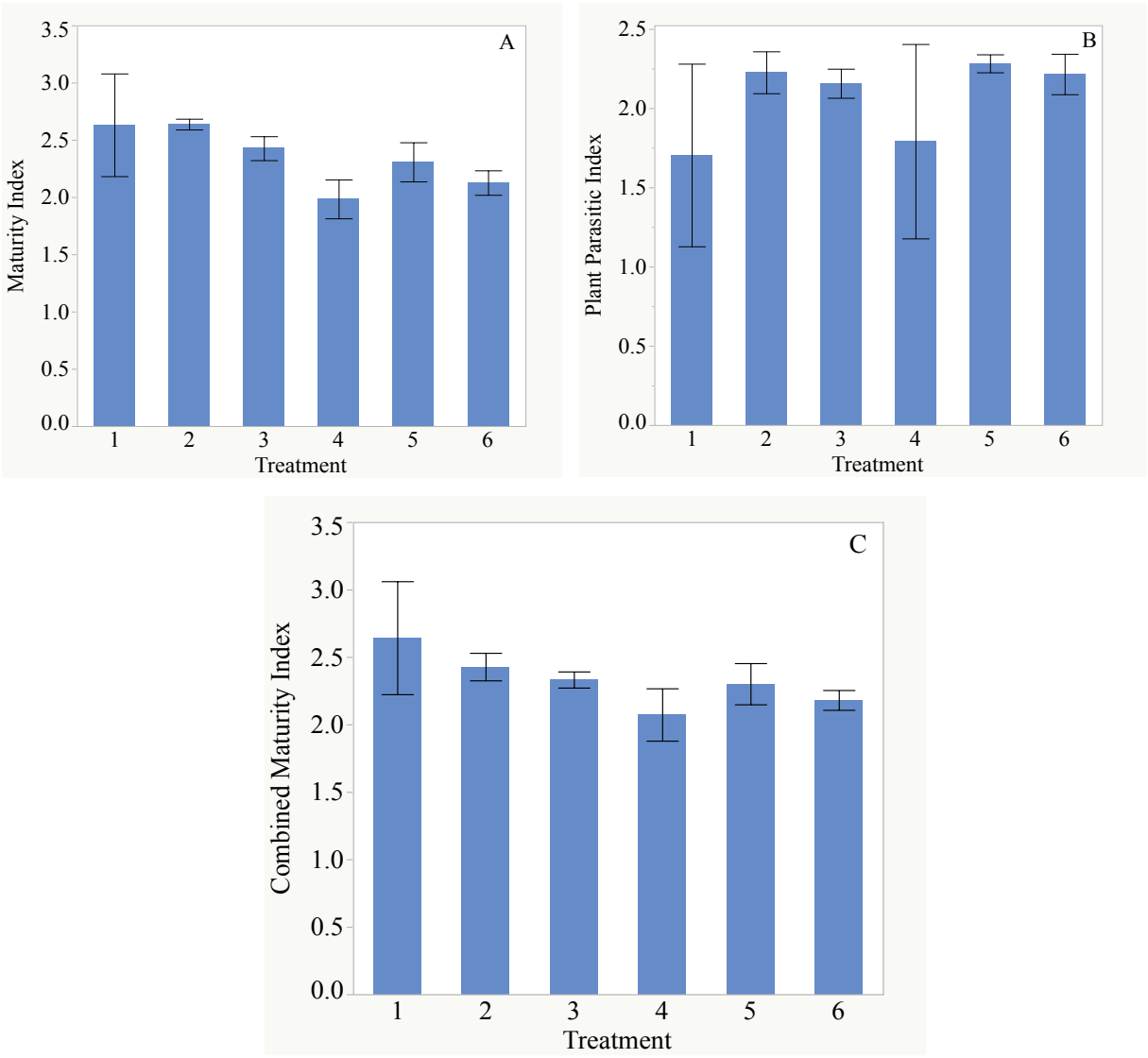

Fig 2.4. Year 1 change in A) Maturity Index due to treatment for free-living nematodes B) Plant-Parasitic Index, and C) Combined Maturity Index. Treatments 1-3: low, med, high rates of KNO_3 and treatments 4-6: low, med, high rates of $(NH_4)_2SO_4$. Error bars are one standard error from the mean. Different letters indicate significant differences in mean index values.

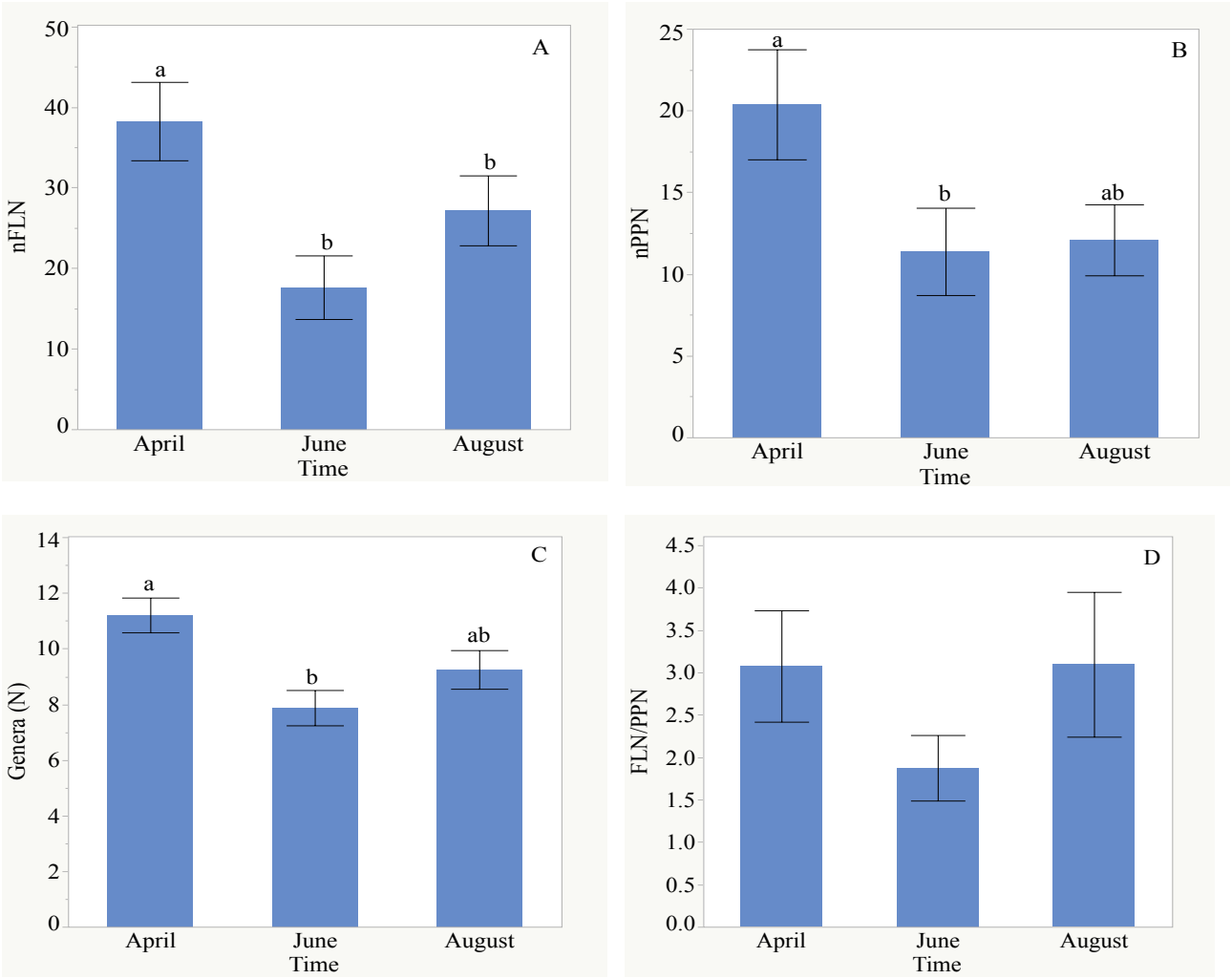

Fig 2.5. Year 1 difference in absolute abundance before and after the first and second cover for A) free-living and B) plant-parasitic nematodes, C) number of genera, and D) ratio of free-living to plant-parasitic nematodes. Error bars are one standard error from the mean. Bars with different letters indicate a significant difference.

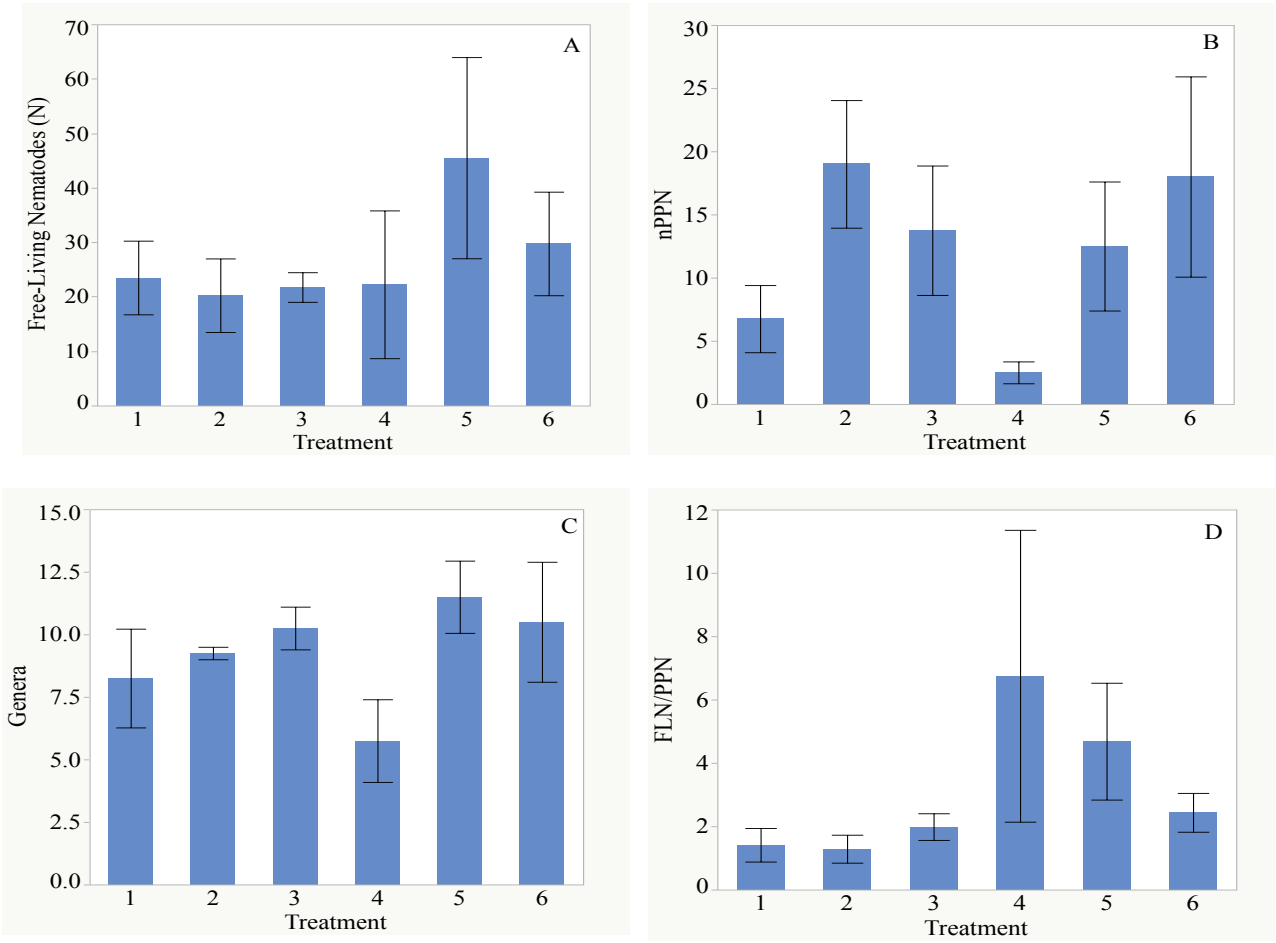

Fig 2.6. Year 1 difference in nematode abundance due to treatment for A) free-living and B) plant-parasitic nematodes, C) number of genera, and D) ratio of free-living to plant-parasitic nematodes. Treatments 1-3: low, medium, high rates of KNO_3 and treatments 4-6: low, med, high rates of $(NH_4)_2SO_4$. Error bars are one standard error from the mean. Different letters report significant differences in mean index values.

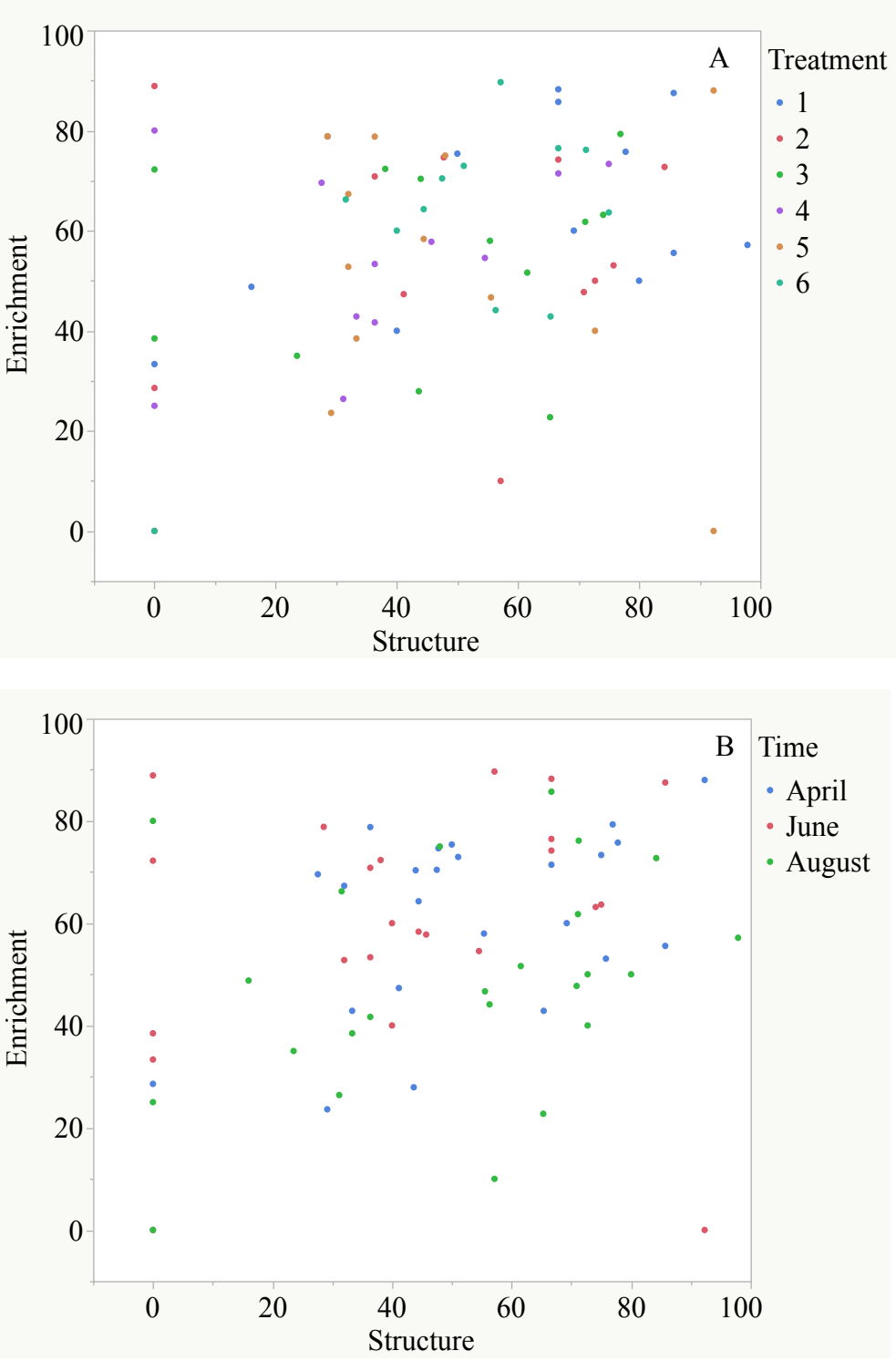

Fig 2.7. Year 1 change in structure and enrichment indices due to A) treatment and B) time with *Tylenchus* and *Filenchus* spp. rated as PPNs. Treatments 1-3: low, med, high rates of KNO_3 and treatments 4-6: low, med, high rates of $(NH_4)_2SO_4$.

Chapter 2. Part II. Effects from year 2 of the Lights Out mulching study

Abstract

Year 2 of the study began in January, 2016 and ended in September 2016 at Magoon Research Facility. A more detailed methodology was developed in order to further explore the effects of Lights Out mulching on insect, weed and nematode communities. The refined objectives included 1) determining the most effective weed mat density to suppress weeds 2) determining the most effective fertilizer treatment (fertilizer x rate) to stimulate weed seed germination and flush the weed seed bank and 3) understanding changes in the biotic communities in the soil including nematodes and insects**.** Results indicated that the Lights Out method was effective, with all cover types providing better weed suppression than control plots treated with glyphosate (Kruskal-Wallis Test, $P < 0.05$). There were no significant differences between fertilizer treatments for weed diversity or coverage percent one-month post-fertilization (Wilcoxon MCP, $P < 0.05$). However, all plots treated with fertilizer had significantly less weed diversity and coverage after the Bermudagrass was planted (Wilcoxon MCP, $P < 0.05$), indicating that fertilization plays a crucial role in flushing the weed seed bank. The arthropod and nematode communities were significantly different after each sampling time, indicating that covering and fertilization play a role in shaping the diversity, abundance, maturity, structure, and enrichment of the soil food-web (Kruskal-Wallis Test, $P < 0.05$).

Key Words: Lights Out mulching, Fertilizer amendment, nematode community

Introduction

Year 2 justification

While the class demonstration and year 1 provided promising results, we wanted to further understand the effects of Lights Out mulching. Many of the first year analyses of variance revealed noticeable changes in insect, weed, and nematode composition over time, but only a few were significantly affected by fertilizer treatments. The changes were noticeable enough that repeating the study with greater numbers of replications and a refined protocol seemed appropriate in order to find more conclusive answers to the original questions about Lights Out mulching.

Year 2 commenced with refining research objectives and revising methodology in order to study the effects of Lights Out mulching on insects, weeds, and nematodes more thoroughly. Objectives were refined in order to accommodate a new factor of weed mat density. The methodology of the experiment as a whole was significantly improved to address the feasibility of home-lawn and commercial usage. A new type of data collection was also added in order to understand changes in the insect community more effectively. These improvements added to year 1 results, addressed the new questions that arose at the end of year 1, and addressed the original gaps in knowledge surrounding Lights Out mulching. This led to increased awareness about Lights Out mulching as an alternative to chemical weed control and turf renovation technique.

Refined objectives

The Lights Out mulching study was initiated with the intent of assessing weed mat density as a factor of weed suppression. Year 2 was chosen to study this factor to give more time to develop the protocol throughout year 1 and assess preliminary results. The study area was also

larger, and could accommodate a larger number of weed mats. Furthermore, the highest rates of potassium nitrate and ammonium sulfate treatments were most effective at stimulating weed seed germination in year 1, but the differences between the two were not statistically different. Therefore, the low and high rates (22.5 and 90 kg N/ha, respectively) were used once again in year 2 to assess any differences in visual results in weed seed germination, as well as diversity and coverage percent data. The middle rate of 45 kg N/ha was not used because there were no noticeable changes or significant differences on the response. Finally, Berlese funnels were added to the study as an additional method in order to assess the diversity and abundance of soil microarthopods. Some insects and arthropods (for example, Collembola) remain more encapsulated in the soil compared to more mobile insects, and cannot be captured and identified using pitfall traps with ease. Berlese funnels offer an in depth look into the changes in abundance or diversity of any smaller soil-dwelling insects and arthropods.

Taking these observations into account, year 2 objectives were as such:

1) Determine the most effective weed mat density to suppress weeds and old turf;
2) Determine the most effective fertilizer treatment (fertilizer x rate) to stimulate weed seed germination and flush the weed seed bank;
3) Understand changes in the biotic communities in the soil including nematodes and insects affected by Lights Out mulching

Protocol revisions

Year 2 experimentation was completed using several infrastructure improvements. The study area was a singular 9.14 m × 15.24 m plot of homogenous, maintained turf on the front lawn of the Magoon Research Facility. The site offered a more condensed and accessible work area that was easy to fence off from the general public. The irrigation system was built from the

ground up, and incorporated a pressure gauge and water meter to calibrate water output. Five sprinkler heads were angled and positioned to ensure each plot received an equal amount of irrigation. The weed mats were held into place using metal clip bases and wiggle wire, which were zip-tied to a pressurized nylon hose. These was more secure than the fire hose weights that were used in year 1. These improvements to the Lights Out mulching system helped to standardize the set-up, ensuring calibrated irrigation as well as weed mat security. It also made the study area look more professional. The pressurized hoses created a grid that differentiated between the blocks and provided a well-defined study area.

Expected outcomes

The results from year 1 showed that the double layer weed mat would effectively suppress old turf and weeds over time, and the highest rate of ammonium sulfate fertilizer would be the most effective at stimulating weed seed germination. The new incorporation of weed mat density as a factor was added to the year 2 hypothesis. It was expected that because a higher weed mat density will exclude more light and trap more heat, it would be more effective at removing old turf and suppressing weed growth over time. Additionally, it remains equivocal whether the highest rate of ammonium sulfate is the most effective at stimulating weed seeds. There were noticeable increases in response between fertilizers based on treatment in year 1, but the differences were not statistically significant. Therefore, the original hypothesis about fertilizer treatment remained the same. Moreover, the highest density weed matt will be the most effective at suppressing weeds over time, and the highest rate of ammonium sulfate fertilizer will help stimulate weed seed germination and flush the weed seed bank.

Year 2 built upon the results from the class demonstration and year 1 of the study. The objectives were modified to address new questions about Lights Out mulching, the hypothesis

was refined to incorporate the modifications, and the well-defined study area improved upon the existing protocol and execution of the study. These improvements will continue to fill the gap in knowledge about alternative methods of turfgrass management.

Materials and Methods

Experimental design

A 9.14 m × 15.24 m mixed turfgrass study area at the Magoon Research Facility in Manoa was divided into 16 2.13 m × 3.05 m plots during the first two weeks of January, 2016. The study area was comprised of mostly St. Augustinegrass (*Stenotaphrum secundatum*) and some Bermudagrass (*Cynodon dactylon*). Black, geo-textile fabric was cut at 3ft width and secured using metal stakes around the study plots. It was also cut at 1ft width and secured in between plots to serve as a treatment buffer. This created a walkable perimeter around each plot. The area was enclosed with orange construction fencing using large, metal rods.

Throughout the rest of January and February, an irrigation system was built using black irrigation tubing that ran the entire perimeter of the plots. A smaller irrigation tube was attached the large irrigation tube to the sprinkler head. The sprinkler heads were zip-tied to the top of a vertical, 1-inch PVC pipe. They were angled evenly to ensure even water distribution, and were calibrated using small trays that collected water from the sprinkler heads for 10 minutes. A pressurization system was also built in order to hold the weed mats in place for each treatment. Nylon reinforced tubing lined the perimeter of the plots next to the irrigation tubing. The pieces were cut and attached with PVC irrigation fittings that were individually sanded by hand to fit properly with the tubing. A metal clip base was attached to the tubing using three zip ties. Once the clip bases were in place, the nylon tubing was sealed using metal clamp rings and twist ties.

Additional silicone tape was required because the fixtures were too large for the tubing. The system was checked for leaks, and was then filled with water.

The plots were measured so that each weed mat would reach the clip base at the perimeter of the plot. The weed mat materials were shipped in large rolls, and were cut according to the plot measurements. The lowest density fabric was an LP200 woven fabric from Belton Industries (Exacta Sales, INC). The medium density fabric was an LP315 woven fabric from Belton (Exacta Sales, INC). The high density fabric was an 876 style woven fabric from Belton (Exacta Sales, INC). They were labeled the 1x, 2x, and 4x treatments, respectively. Each cover was approximately 3.66 m × 4.57 m, accommodating for the extra length needed in order to reach the clip base. The rolls were measured and cut using a propane blow-torch to melt the fabric away from the roll. They were arranged by treatment and folded for storage. Each treatment fabric was tagged with a different colored spray paint to ensure that they were placed correctly at the field site. They were transported to a nearby outdoor storage area until the covering date. They were attached to the perimeter of the plots using the clip base and a piece of wiggle-wire on February 25, 2016.

Plots were arranged vertically at the site in order to accommodate a slope gradient. Each block contained four treatments that included no cover (0x), low-density weed mat (1x), medium density weed mat (2x), and high density weed mat (4x). Cover duration remained constant amongst all plots for the first covering. On February 29, the control plots were sprayed using a 40-gallon capacity, CO_2 powered research spraying tank and a 3 nozzle boom with an 8004 LP TeeJet spray tip operating at 20 PSI. The tank was filled with 37.5 ml of a standard rate of Roundup Pro Concentrate (2 qts/ac). No surfactants or wetting agents were used. Plots were

covered initially for 4 weeks. We elected to wait for two more weeks to ensure that all of the old turf and weeds were suppressed.

Temperature was recorded in the north and south ends of the plot using a HOBO data-logger monitoring device. Light penetration under each covering was quantified using a PAR Quantum Light Meter (Spectrum Systems, INC.). The nylon tubing was de-pressurized so the mats could be lifted and pulled back with ease. The meter was placed fully underneath each cover type and three recording were taken and averaged.

The weed mats were removed after six weeks on April 13, 2016. On April 25, 2016, the dead weeds and old turf were cleared from the plot using verticutters. The 12-day gap in between un-covering and verticutting was not necessary, but was due to rainy weather conditions that made using the verticutters difficult. A fertilizer amendment was added on April 26, by dividing the larger 2.13 m × 3.05 m plots into 4 smaller 1.07 m × 1.52 m plots. The potassium nitrate fertilizer was a powder composition with a Nitrogen-Phosphorus-Potassium (NPK) rating of 13.7-0-43. The ammonium sulfate was a granular composition with a rating of 21-0-0. The 0x + Roundup Pro plots served as a control. Each of the four plots was selected randomly to receive either a low or high rate of potassium nitrate or ammonium sulfate arranged in RCBD. There were 12 treatments per block with 4 blocks, making a total of 48 treatments. The 16 control plots did not receive the fertilizer amendment. Irrigation resumed at the same rate and schedule as before. New weeds were allowed to germinate for approximately one month. On May 26, the plots were re-covered. A small portion of the weed mat was pulled back each week to rate the turf, take photographs, and make visual observations regarding the ability of each density to control new weed growth. They were uncovered on June 29. The dead weeds did not create enough debris to warrant use of the verticutter.

On July 11, 2016, the plots were coated with Scotts Turf Builder Grass Seed, Bermudagrass variety at the maximum rate of 151 kg/ha. On July 13, ammonium sulfate fertilizer was added at the rate of 148.79 grams/plot to encourage germination. The newly spread seed and fertilizer were capped using a hydromulch that contained shredded newspaper and a surfactant to prevent birds from removing seed and increase surface contact. Turf growth and weed presence were monitored weekly for the remainder of the experiment. The study area was broken down over the last two weekends in September. Irrigation lines were removed. Black mulch used as a perimeter was removed. Plots were hand-weeded and large weed overgrowths were pulled from the roots. Weed mats were brushed off, rinsed, and saved for future use. The stripped study area was mowed to restore it back to its original state.

Data collection

Soil samples were taken on January 24, April 14, and July 6, 2016. For each sample date, four random soil core samples were collected from each plot and made into one composite sample. In the lab, each composite sample was gently mixed and 10 grams of soil was added to a Baermann funnel with tap water. After 3 days in the funnel, 50 ml of the tap water was collected in a vial and labeled with the plot number. The samples were refrigerated overnight to allow the nematodes to settle to the bottom of the vial. The following day, 45 ml of water was removed slowly with a 10 ml pipette so the maximum number of nematodes remained at the bottom of the vial for analysis. 5 ml of boiling water was added to kill the nematodes, which created a 10 ml sample. The samples were refrigerated for at least 24 hours before analysis. The vials were removed and the contents placed in a Petri dish with grid-lines. An inverted microscope was used to count the number and genera of nematode along each grid-line. The individual counts were

entered into an excel spreadsheet. Similarly, soils samples were collected after the first covering and after the fertilization to measure nematode community data and other parameters.
Soil samples were collected in order to measure changes in the nematode community. More specifically, several parameters were analyzed including: nematode maturity, enrichment, and structure indices for free-living nematodes. Combined maturity, number of individual nematodes, number of genera, and ratio of free-living to plant-parasitic nematodes were calculated as well. Data from before and after the first covering were compared using a non-parametric Kruskal-Wallis test and if found to be significant, was followed with a Wilcoxon multiple comparison procedure.

Pitfall traps were set up on January 24, April 14, and July 6, 2016, which were on or near the dates the soil samples were taken. If they were not taken on the same day, it was usually due to a weather delay or a time delay from only one person in the field setting traps. One 250 ml cup was filled halfway with tap water and placed in a small hole that was dug randomly in each plot. The excess space between the outside of the cup and the hole was filled with the remaining soil to ensure no specimens fell beneath the trap. The pitfall traps were left in place for two days. Then, the contents were placed in small Tupperware containers and refrigerated shortly after collection. They were analyzed within 48 hours of collection to ensure that the specimens did not decompose in the water and become unidentifiable. The specimens were identified, recorded, and analyzed for abundance. Diversity was calculated using the Shannon-Weiner Diversity Index.

The three collections of soil from before and after the first covering, as well as after the fertilization were also used for Berlese funnel analysis. 6 small Berlese funnels were set up in the laboratory with small collection beakers placed to collect any soil microarthropods. The beakers

were filled 1/3 of the way full with 90% EtOh and placed underneath the funnel. 50 grams of soil was removed from each sample and placed onto a double layer of mesh inside the funnel. Heat lamps were turned on for 24 hours to allow any microarthropods to move down through the funnel and into the beaker. Beakers were collected and viewed under a dissection microscope. Individual counts were entered into an excel spreadsheet.

Turfgrass ratings

After the second covering, Turfgrass was rated using a 1ft x 1ft frame. The frame was randomly distributed in each small plot. Weeds were identified in the field to the family level (and species level whenever possible). If weeds could not be identified a sample with the roots and seed head in tact were taken to the laboratory for identification. Species richness and evenness were assessed in the field, and the Shannon-Weiner diversity index was calculated once data was compiled in an Excel spreadsheet. Additional information on turf greenness and coverage percent was also taken each week. Ratings were completed on a weekly basis from July 26, 2016 until August 29, 2016. However, there were two weeks in between the August 9, 2016 rating and the August 23, 2016 rating. The weekly ratings gave a more complete picture of how weeds progressed over time after the last covering.

Biomass collection

The weeds took over the stand almost completely by September 1, 2016. On September 15, 2016 a 1ft by 1ft square of plant biomass was randomly selected in each plot. The new Bermudagrass and its surrounding weeds were removed together and placed into a bag labeled with each plot number. Weeds and grass dried in the greenhouse at Gilmore Hall on campus of the University of Hawaii. The grass and weeds were then separated and weighed in the lab to determine any differences in dry biomass due to long term effects of fertilizer treatment.

Statistical analysis

Data analysis was completed using JMP 12 software (SAS Institute, Cary, NC). A multivariate model was developed to analyze interactions between weed mat density and fertilizer rate. Since the control did not receive the fertilizer amendments like the other treatments did, and the data distributions of the observations were not normally distributed and had no homogeneity of variance, data was then subjected to one-way, non-parametric analysis with supplemental non-parametric pair-wise comparisons.

Results

Arthropod community

The year 2 experimental design called for analysis of Berlese funnel data. Preliminary samples indicated little to no insect presence from any collection date, which resulted in too few observations to make statistical inferences about the soil microarthropods using the Berlese method (Table 2.7). Data collected from pitfall traps was the source of inference for insect diversity and abundance, and The Kruskal-Wallis rank sum test was used to detect any systematic differences in any of the plots based on treatment and density before the first covering. Insect diversity appeared uniform across all plots based on weed mat density (χ^2 = 3.45, DF = 3, P = 0.658) and fertilizer treatment (χ^2 = 2.43, DF = 4, P = 0.326). Insect abundance was also observed to be uniform for weed mat density (χ^2 = 4.65, DF = 3, P = 0.199) and fertilizer treatment (χ^2 = 0.462, DF = 4, P = 0.977) (Fig. 2.8). This indicated that there were no systematic differences between blocks for insect diversity and abundance before the experiment was executed. In April after the first cover, insect diversity was reduced in plots covered with the low density weed mat (χ^2 = 6.61, DF = 3, P = 0.0854) (Wilcoxon MCP, P =

0.0079). There were no differences between cover types for insect abundance ($\chi^2 = 2.81$, DF = 3, $P = 0.421$) (Fig. 2.9).

There were significant differences in insect diversity due to the fertilization treatment that occurred after the first cover ($\chi^2 = 12.32$, DF = 4, $P = 0.0151$). Treatment 3 ($(NH_4)_2SO_4$-20) had significantly greater insect diversity when compared to controls (Wilcoxon MCP, $P = 0.0060$) (Fig. 2.10). Treatment 3 also had greater insect diversity when compared to Treatment 2 (KNO_3-80) (Wilcoxon MCP, $P = 0.0484$), but this was the only pairwise difference (Fig. 2.10). There were no differences in insect diversity after the second cover due to weed mat density ($\chi^2 = 5.74$, DF = 3, $P = 0.1251$). There were also no significant differences in insect abundance due to the fertilizer treatment ($\chi^2 = 2.36$, DF = 4, $P = 0.6694$) or weed mat density ($\chi^2 = 1.97$, DF = 3, $P = 0.5775$) (Fig. 2.10).

Besides analyzing the effects of different cover types and treatments within each sampling time, each sampling time was compared to address any changes that occurred over the course of the experiment. Notably, there were significant differences in insect diversity (Shannon-Weiner Diversity Index) ($\chi^2 = 58.13$, DF = 2, $P < 0.0001$) and insect abundance ($\chi^2 = 88.94$, DF = 2, $P < 0.0001$) over the three sampling times (Fig. 2.11). Insect diversity was the highest after the first covering in April, and was significantly greater than before the first covering in January (Wilcoxon MCP, $P = 0.0019$) (Fig. 2.11). However, insect diversity in July, collected after the fertilization amendment and second covering, was significantly lower than both January and April (Wilcoxon MCP, $P < 0.0001$ for both pairwise comparisons) (Fig. 2.11). Insect abundance was the greatest after the first covering in April, and lowest after the fertilizer amendment and second covering in July. The average abundance in July was also significantly lower than both January and April (Wilcoxon MCP, $P < 0.0001$ for each pair-wise comparison)

(Fig. 2.11). The arthropods that dominated abundance counts for each pitfall trap collection were from *Amphipoda, Isopoda,* and *Diplopoda* (Table 2.8).

Turfgrass and weed community

All 64 plots received near uniform turfgrass and weed ratings on January 19, before the first covering. Before the first cover, turfgrass coverage was uniform (95-100%), with little to no weed pressure. Less than 5% weed coverage was found in any given plot, and included species such as *Kylinga* spp. and *Oxalis* spp. After four weeks of cover, visual observations of low and medium density weed mat plots showed only a small difference in turf dieback and color. However, the high density (density 4) did appear to be slightly more effective in terms of old turf elimination. Grass appeared more dry and withered. The medium density (density 2) plot from Block 3 retained more moisture than the other densities. The glyphosate controls appeared to have weed growth around the perimeter of the plots and along the exposed space of the nylon clip base fixtures. They also appeared to be more dried out than the other plots. The low density (density 1) cover in block 4 had similar sideline growth and weed presence. Visual observations of all plots after six weeks showed no difference in turf color or weed presence, indicating that six weeks was sufficient time for all densities to effectively eliminate old turf.

Fertilizer treatments were randomized and assigned to the 64 plots (Table 2.9). Weed diversity and coverage percent were analyzed using the data that was collected in July, one month after fertilization. There were no significant differences in weed diversity due to treatment ($\chi^2 = 3.21$, DF = 4, $P = 0.5232$) (Fig. 2.12). There were also no significant effects of different cover types from the first covering ($\chi^2 = 2.24$, DF = 3, $P = 0.5232$). The same phenomena were observed for coverage percent, with no significant differences due to fertilizer treatment ($\chi^2 = 2.21$, DF = 4, $P = 0.697$), or cover types from the first covering ($\chi^2 = 2.87$, DF = 3, $P = 0.4114$)

(Fig. 2.12). The second covering completely eliminated all newly germinated weeds. The plots were visually similar, with decaying weed matter and muddy bare ground. All but one plot had zero Shannon-Weiner diversity and coverage percent.

The collection of weed diversity and coverage percent data resumed after the new Bermudagrass seed was planted. Ratings were made on a weekly or bi-weekly basis from July 19, 2016 to August 29, 2016, approximately one-week after new Bermudagrass seeds were planted. Ratings from all weeks were combined in order to analyze changes in weed diversity and percent coverage due to cover type and fertilizer treatment. The data were assessed using a Kruskal-Wallis test. If significance was found, a Wilcoxon multiple comparison procedure (MCP) was used to assess pairwise differences. There was a significant difference in weed diversity between fertilizer treatments ($\chi^2 = 98.63$, DF = 4, $P < 0.0001$) and weed mat density ($\chi^2 = 95.5$, DF = 3, $P < 0.0001$) (Fig. 2.13). All plots that received fertilizer amendments had significantly less weed diversity when compared to control plots (Wilcoxon MCP, $P < 0.0001$ for all comparisons to control), but there were no significant differences between treatments (Fig. 2.13). Additionally, all cover types had significantly less weed diversity when compared to control plots (Wilcoxon MCP, $P < 0.0001$ for all comparisons to control), but there were no significant differences between cover types (Fig. 2.13). A similar trend occurred for weed coverage percent after the Bermudagrass was planted. Control plots had significantly greater weed coverage than all cover types ($\chi^2 = 72.5$, DF = 3, $P < 0.0001$) and fertilizer treatments ($\chi^2 = 73.9$, DF = 4, $P < 0.0001$). However, neither had significant pairwise differences between cover types or treatments (Fig. 2.13).

Changes between weekly ratings in terms of weed diversity and percent coverage were also analyzed (Fig. 2.14). A one-way ANOVA was used to calculate differences between ratings.

Weed diversity had a clear increasing trend over the course of the ratings, and was significantly different due to the date of rating (F = 25.44, DF = 5, $P < 0.0001$). Three weeks after the second covering, weed diversity had only established an average Shannon-Weiner Diversity index rating of 0.19. By late August (8/29), weeds had established an average index rating of 0.86. Weed diversity from August ratings (8/09, 8/23, and 8/29) were significantly greater than the July ratings (7/19 and 7/26) for all pairwise comparisons (Tukey HSD $P < 0.05$) (Fig. 2.14). Additionally, weed diversity from the late August ratings (8/29 and 8/23) were significantly greater than weed diversity of from the early August ratings (8/03 and 8/09) for all pairwise comparisons (Tukey HSD, $P < 0.05$). However, the rating from 8/03 was not significantly different from the July ratings in terms of weed diversity (Fig. 2.14).

There was also a significant difference in percent weed coverage over time (F = 39.78, DF = 5, $P < 0.0001$), with a similar trend to the weed diversity data (Fig. 2.14). Weed coverage percent from the 3 August ratings (8/09, 8/23, 8/29) were significantly greater than the two July ratings (7/19 and 7/26) for all pairwise comparisons (Tukey HSD, $P < 0.05$). Coverage percent of the two late August (8/23 and 8/29) ratings were significantly greater than the early August (8/03 and 8/09) ratings for all pairwise comparisons (Fig. 2.14). Overall, average weed coverage percent had a maximum of 32.9% across all plots, regardless of treatment or density. Some treatments and densities had visible and statistical differences.

Biomass

There was a significant difference for dry weight of weeds based on treatment (χ^2 = 12.74, DF = 4, P = 0.0126) and weed mat density (χ^2 = 12.69, DF = 3, $P = 0.0053$). The low rate of potassium nitrate had lower average weed biomass when compared to controls ($P = 0.002$) (Fig. 2.15). The low and high rate of ammonium sulfate also had lower average weed biomass

when compared to controls (Wilcoxon MCP $P < 0.05$) All weed mat densities had significantly lower weed biomass than controls. High density weed mats had the lowest weed biomass (Wilcoxon MCP, $P < 0.05$) (Fig. 2.15). The low and medium densities had similar score mean differences, and also had lower weed biomass than the controls (Wilcoxon MCP, $P < 0.05$). Grass biomass was also significantly different for treatment ($\chi^2 = 16.1$, DF = 4, $P = 0.0029$) and weed mat density ($\chi^2 = 17.99$, DF = 3, $P = 0.0004$) (Fig. 2.15). Additionally, pairwise comparisons showed that all treatments had greater grass biomass when compared to controls. The low rate of potassium nitrate had the largest grass biomass, followed by the high rate of ammonium sulfate (Wilcoxon MCP, $P < 0.05$). The high rate of potassium nitrate and the low rate of ammonium sulfate had the next largest grass biomass when compared to controls (Wilcoxon MCP, $P < 0.05$). However, treatments were not statistically different from one another for any pairwise comparison (Fig. 2.15).

All density types had significantly greater grass biomass than controls (Fig. 2.15). The high density cover had the largest grass biomass when compared to controls (Wilcoxon MCP, $P = 0.0004$), and when compared to the medium density (Wilcoxon MCP, $P = 0.0194$). The low density also had a significant difference between densities with the next largest difference in score mean when compared to controls (Wilcoxon MCP, $P = 0.0023$) (Fig. 2.15). The medium density also had larger biomass when compared to control, but it had the smallest difference in score mean when compared to the other differences (Wilcoxon MCP, $P = 0.0316$) (Fig. 2.15). This biomass information suggests that low rates of potassium nitrate, and high rates of ammonium sulfate both preformed well as fertilizer amendments to stimulate weed seed germination and flush the weed seed bank before the Bermudagrass was planted.

Nematode community

All parameters concerning the nematode community from before the first cover were also analyzed using Kruskal-Wallis tests. Before the first covering, there were significant differences for Maturity Index between cover types ($\chi^2 = 8.39$, DF = 3, $P = 0.0385$), Genera between treatments ($\chi^2 = 10.27$, DF = 4, $P = 0.0360$), and Enrichment Index between both fertilizer treatment ($\chi^2 = 18.06$, DF = 4, $P = 0.0012$) and density ($\chi^2 = 15.52$, DF = 3, $P = 0.0014$). Plots that would receive high density (4x) and low density (1x) covers had larger maturity indices than medium density (Wilcoxon MCP, $P = 0.0302$ and 0.0215, respectively). Plots that would receive the high rate of ammonium sulfate tended to have a larger number of genera than those receiving the low rate of ammonium sulfate or high rate of potassium nitrate (Wilcoxon MCP, $P = 0.0126$ and 0.0034, respectively). Control plots had a lower average enrichment index than plots that would eventually be treated with the low and high rate of potassium nitrate, and the low rate of ammonium sulfate (Wilcoxon MCP, $P = 0.0114$, 0.0003, and 0.039, for each respective comparison to control). Additionally, plots that would receive the low rate of potassium nitrate had lower enrichment when compared to plots that would be treated with the low or high rate of ammonium sulfate (Wilcoxon MCP, $P = 0.021$ and 0.014, respectively). Plots that would receive covers with density 1 and 4 had lower enrichment than controls (Wilcoxon MCP, $P = 0.0007$ and 0.0024, respectively).

After the first covering, there were no significant differences due to cover type for the Maturity Index ($\chi^2 = 3.78$, DF = 3, $P = 0.2862$), the Plant-Parasitic Index ($\chi^2 = 2.31$, DF = 3, $P = 0.5097$), or the Combined Maturity Index ($P = 0.8144$) (Fig. 2.16). However, there were significant differences in numbers of plant-parasitic nematodes ($\chi^2 = 8.14$, DF = 3, $P = 0.043$) and free-living nematodes ($\chi^2 = 10.22$, DF = 3, 0.0167) (Fig. 2.17). Plots with the low density weed mat had lower numbers of plant-parasitic nematodes when compared to control plots

(Wilcoxon MCP, $P = 0.0314$) and medium density weed mats (Wilcoxon MCP, $P = 0.0346$) (Fig. 2.17). There were no significant differences due to cover type for the ratio of free-living to plant-parasitic nematodes ($\chi^2 = 2.98$, DF = 3, $P = 0.3938$), or nematode richness (number of genera) ($\chi^2 = 0.35$, DF = 3, $P = 0.20$) (Fig. 2.17). There were no differences in the enrichment index due to cover type ($\chi^2 = 1.36$, DF = 3, $P = 0.715$) or the structure index ($\chi^2 = 2.93$, DF = 3, $P = 0.402$) (Fig. 2.18).

After the fertilization amendment and the second covering, nematode maturity (MI, PPI, and CMI) did not have any significant differences due to fertilizer treatment or weed mat density (Fig. 2.19). However, significant differences were observed with abundance of free-living nematodes due to cover type ($\chi^2 = 12.96$, DF = 3, $P = 0.0047$). Plots with high density weed mats had significantly lower average absolute abundance when compared to medium density plots and the controls (Wilcoxon MCP, $P = 0.0052$ and 0.0043, respectively) (Fig. 2.20). There was no significant difference amongst abundance of plant-parasitic nematodes due to fertilizer treatment, but there were differences with respect to cover type ($\chi^2 = 11.14$, DF = 3, $P = 0.0110$). The low density cover (1x) had significantly lower numbers of plant- parasitic nematodes when compared to control plots (Wilcoxon MCP, $P = 0.0259$) and plots that received medium density covers (Wilcoxon MCP, $P = 0.0025$). There were no differences in ratios of free-living to plant-parasitic nematodes for treatment ($\chi^2 = 4.38$, DF = 4, $P = 0.357$) or cover type ($\chi^2 = 4.11$, DF = 3, $P = 0.2493$) (Fig. 2.20). There were no differences in nematode richness (number of genera) due to fertilizer treatment ($\chi^2 = 4.72$, DF = 4, $P = 0.3169$), but did exhibit differences due to cover type ($\chi^2 = 14.27$, DF = 3, $P = 0.0026$) (Fig. 2.20). Medium density cover types had the highest number of genera when compared to both low density (Wilcoxon MCP, $P = 0.0018$) and high density covers (Wilcoxon MCP, $P = 0.0067$). Plots with low density

cover types also had significantly lower numbers of genera when compared to control plots (Wilcoxon MCP, $P = 0.0126$). Interestingly, the overall enrichment and structure indices remained intact regardless of treatment or cover type (Kruskal-Wallis, $P > 0.5$) (Fig. 2.21).

Nematode observations from before covering in January and after covering in April were compared to observations from after fertilization and second covering in July in terms of time. Significant differences were found for Maturity Index due to covering ($\chi^2 = 55.6$, DF = 2, $P < 0.0001$) (Fig. 2.22). MI was significantly lower after the first covering (Wilcoxon MCP, $P < 0.0001$ for pairwise comparison to January). The maturity index was also lower after the second covering (Wilcoxon MCP, $P < 0.0001$ for the pairwise comparison to January) (Fig. 2.22). Changes in the plant-parasitic index were not statistically different ($\chi^2 = 4.256$, DF = 2, $P = 0.119$), but did show a marginally significant increase after the first cover (Wilcoxon MCP, $P = 0.0476$) (Fig. 2.22). The Combined Maturity Index (CMI) yielded a highly significant difference in means over time ($\chi^2 = 38.7$, DF = 2, $P < 0.0001$). All pairwise differences were significant (Fig. 2.22). CMI from April after the first cover and July after the second cover had significantly lower values than CMI from January before covering (Wilcoxon MCP, $P = 0.0002$ and 0.0001, respectively). CMI was also significantly different when values from after the second covering were compared with values from after the first covering (Wilcoxon MCP, $P = 0.0162$) (Fig. 2.22).

Significant differences were also found for the number of plant-parasitic nematodes over time ($\chi^2 = 15.85$, DF = 2, $P = 0.0004$) (Fig. 2.23). The number of plant-parasitic nematodes was greater in April after the first covering when compared to January before the covering, but this difference was not significant (Wilcoxon MCP, $P = 0.5448$). After the second covering in July, PPN abundance decreased significantly when compared to the abundance in April (Wilcoxon

MCP, $P = 0.0009$) (Fig. 2.23). The abundance numbers from July were also significantly lower than from January (Wilcoxon MCP, $P = 0.0005$). There were also significant differences for the abundance of free-living nematodes over time. The number of free-living nematodes were significantly lower after both April and July when compared to January (Wilcoxon MCP, $P = 0.0002$ and 0.0001, respectively) (Fig. 2.23). The ratio of free-living to plant-parasitic nematodes thus changed as well ($P = 0.0001$). This ratio decreased significantly in April after the first covering (Wilcoxon MCP, $P < 0.0001$), and then increased in July after the second covering ($P = 0.0265$) (Fig. 2.23). However, there was no significant difference in the ratio when comparing observations from January to July (Wilcoxon MCP, $P = 0.0701$) (Fig. 2.23). There were significant differences in number of genera over time ($\chi^2 = 28.54$, DF = 2, $P < 0.0001$). January had a larger number of genera than both April (Wilcoxon MCP, $P = 0.0237$) and July (Wilcoxon MCP, $P < 0.0001$). April also had a larger number of genera than July (Wilcoxon MCP, $P = 0.0011$) (Fig 2.23). Some of the most abundant genera in year 2 were *Diplogaster, Plectus, Dorylaimus, Eudorylaimus, Eucephalobus, Tylenchus,* and *Filenchus* (Table 2.10).

Interestingly, there were no significant differences in enrichment index between sampling times ($\chi^2 = 5.52$, DF = 2, $P = 0.06$), but non-parametric comparisons showed a difference when comparing pre-cover conditions in January to conditions in July (Wilcoxon MCP, $P = 0.03$). There was a clear difference in structure between sampling times ($\chi^2 = 47.53$, DF = 2, $P < 0.0001$). Structure for both April and July decreased when compared to pre-cover conditions (Wilcoxon MCP, $P < 0.0001$) (Fig. 2.24).

Light meter

The meter revealed that the low, medium, and high density weed mats allowed near equivalent amount of light penetration, even though the fabric types were all unique. This may

explain the lack of visual differences over time. For example, the low, medium, and high density mats recorded average measurements of 2.8, 3.5, and 4.5 umol.$m^{-2}s^{1}$ respectively (Table 2.12). Readings were repeated several times to ensure proper instrument calibration and technique. This indicated that while the high density may have maintained a higher weight (5.8 oz/yd^2), it let in more light in than the lower densities. This evidence confounded the original assumption that the mechanism of increased light exclusion would increase effectiveness of high density cover types. Analysis of the product information also revealed little difference between cover types in terms of permittivity, and indicated that handling, storage and shipping may change properties of the mats.

Discussion

Year 2 results provided more information on how Lights Out mulching affects the plant, insect, and nematode community. The Berlese funnel data did not yield enough microarthropods to conduct make a conclusive summary of the effects of Lights Out mulching on soil arthropods. A rough approximation that 1 out of 10 funnel samples had an abundance of 1-2 arthropods, usually from the family Formicidae, indicated that no statistical conclusion could be made from the Berlese funnel protocol (Table 2.7). Collembola abundance was also very low in the soil (Table 2.7), which was unexpected because they play an important role in soil food web diversity. The second (July) and third (August) soil samples had even fewer collembola. An average of one specimen would be found every 20-30 samples. Collembola are sensitive to soil compaction, and often decrease in abundance after May until August (Schrader and Lingnau 1997). The soils from year 2 were very moist, compacted, and remained that way once they were stored in the refrigerator in the lab. It is possible that soil compaction and collection date played a role in the lack of soil microarthropod abundance. Future protocol may include drying the soil

before refrigeration, agitation to break up soil clumps, and using more than 50 grams of soil per funnel.

Insect diversity decreased after the first covering, with significant differences due to cover type (Fig. 2.9). The low density (1x) cover type had lower insect diversity when compared to the controls (Fig. 2.9). It was determined that average temperatures underneath the low density mat were nearly identical those under the high density mat, and were significantly greater than both medium density and controls. The manufacturer label indicates that storage and transport can affect the thermal, optical, and tensile properties of the weed mats, which offers a plausible explanation for the decrease in insect diversity based on the low density cover type. Although this plastic mulch excludes light, the reduction in insect diversity with thinner materials echoes results from soil solarization studies that have found thinner mulches to be more effective at trapping heat and reducing pathogens (McGovern and McSorley 1997, Gill 2010). Several caveats should be made when making this assumption about decreased insect diversity with lower density covers: 1) the "thinner" mulches used in the reference studies were mostly transparent polyethylene plastic that was measured in microns. This indicates unique thermal properties that may not apply to opaque, polypropylene plastic that was measured by weight in grams. 2) The insects that were reduced were not considered pathogenic. For example, studies have demonstrated that insect response to soil solarization can be dependent on the actual color of the plastic (Csizinsky 1995, Necibi et al. 1992, Orzolek 1993). The insects used in these studies were mostly aphids, thrips and whitefly. These are not common turfgrass pests, but may provide some insight into why covering prompts reduction in insect diversity. Only one color was used in this experiment because black mulch was the best choice in order to accomplish the study objectives, but it is possible that because black plastic mulch was the only color mulch

tested, that the arthropod species that were reduced (Table 2.8) were more sensitive to the color. Interestingly, there were no significant differences in insect abundance due to cover type after the first covering (Fig. 2.9). This may support the observation that some arthropods can persist through the soil solarization process (Gill 2010). These results are unique from year 1, because insect abundance increased after the first covering. In order to further assess a larger number of arthropod and insect species and their absolute abundances, sticky traps could be used in addition to the pitfall traps to observe any adult turfgrass pests that visit the site (i.e. *Herpetogramma* spp.) After the second covering and fertilization amendment, insect diversity was the greatest in plots treated with the low rate of ammonium sulfate when compared to both control plots and plots treated with high rates of potassium sulfate (Fig. 2.10). This result is unique from year 1 reports on insect diversity, where fertilizer treatment had no significant effect. There were no other significant interactions for insect diversity or abundance due to treatment or cover type (Fig. 2.10).

Insect diversity and abundance also changed significantly over time (Fig. 2.11). The largest index values for diversity were seen after the first covering in April, and the lowest values were seen after the fertilization and second covering in July (Fig. 2.11). This trend was also observed with insect abundance. The largest abundance occurred after the first covering, and the smallest after the fertilization and second covering (Fig. 2.11). The increase in both diversity and abundance after the first covering is unique when compared to year 1, where there was a decrease in insect diversity and an increase in abundance. However, the year 2 results are in agreement with other studies in several ways. The results from after the first covering were taken in June, which has been shown as a month where higher insect abundance can be found (Ying-Hua et al. 2013). Additionally, the data collected after the fertilizer amendment from year 2 does

support results from studies that have found decreased insect abundance due to fertilization (Hancock et al. 2012), and that response to fertilization is species-specific (Garrat et al.). This could mean that isopods and amphipods were disproportionately and negatively affected by fertilization. They do not benefit from fertilization in terms of increased nutrient availability and tissue health in the plant, so it is a possible explanation for the result. The limitation on the two-way interaction between time and treatment due to the split block approach limit our understanding of which fertilizer contributed to the changes in diversity and abundance. This provides additional information from year 1, where changes in insect abundance due to fertilization were not significant.

Additional insect research as it relates to Lights Out mulching may include effects of different mulch types on common turfgrass arthropods such as isopods and amphipods. Transparent plastic mulch of different thickness could be compared to the black geotextile weed mats that were used in the study to understand if black opaque plastic is more harmful to isopods, amphipods, or any of the other identified species from the experiment. Future study areas could also be surveyed for insect diversity before the experiment in order to identify areas with high insect abundance, and whether these insects are beneficials or pests. That way, managers will have more conclusive information on how Lights Out mulching could affect any insects or pests associated with the recreational or home turfgrass they would like to treat. More refined statistical analysis could also be used in order to determine which specific species are being reduced and by how much, compared to others.

Overall, differences in fertilizer rate or type did not seem to affect weed seed germination one month after fertilization (Fig 2.12). This was not consistent with the initial findings during the class demonstration, because visual observations of ammonium sulfate plots appeared to

stimulate weed seed germination more effectively than others (Cheng and DeFrank, 2014). However, it does support year 1 findings that there was no significant difference in weed seed germination due to fertilizer treatment. There were no statistically significant pairwise comparisons, indicating that fertilizer rate and type were not significant factors in determining weed diversity and coverage. However, the weekly ratings demonstrated that treated plots have lower weed diversity and coverage when compared to control plots (Fig. 2.13). The biomass results also supported this observation, with no pairwise significance between fertilizer treatments, but all treatments faring better (higher grass dry weight and lower weed dry weight) than control plots (Fig. 2.15). Managers should take note that leaving newly renovated turf to grow with no additional weed management will encourage increased weed diversity and coverage over time (Fig. 2.14). Nonetheless, this provides managers with excellent insight about how to choose fertilizers to renovate turf. It indicates that so long as managers use correct application practices (i.e. following label protocols, watering, etc.), fertilizer selection is not the most critical factor for lights out mulching.

Additionally, results of reduced nematode numbers are consistent with initial projections made during protocol development that prolonged temperatures would eliminate surface arthropods and nematodes (Cheng and DeFrank, 2014). These results are also consistent with other studies that report reductions in plant-parasitic and free-living nematodes due to covering (Coates-Beckford et al. 1998, Chellemi et al., McGovern and McSorley 1997, McGovern et al. 2002, Stapleton and Heald 1991, Domen and Wang, 2016**).** However, the significant decrease in free-living and plant-parasitic abundance (Fig. 2.17) may be a result of the order in which samples were counted. A larger percentage of control plot samples were analyzed toward the end of the counting and identification period. These samples remained preserved in a refrigerator for

approximately 3 months, which may have interfered with the integrity of the samples by making individual specimens from control plots more difficult to count and identify. The structure and enrichment indices of the community also decreased over time. An additional soil sample would have been beneficial to see nematode communities recovered with the newly established Bermudagrass. These results are in agreement with year 1, where free-living and plant-parasitic nematode abundance were also reduced over time, indicating that Lights Out mulching alters the nematode community. This is not an inherently negative outcome, especially if turf managers are struggling with plant-parasitic nematodes that act as pests of turfgrass.

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Table 2.7. Absolute abundance of arthropods found in the first 10 samples from the April 2016 collection. An average of 1-2 arthropods would be found every 10 plots.

Plot Number	Collembola	Formicidae
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	2	2
10	0	0

Table 2.8. List of arthropods identified in year 2 of the Lights Out mulching study. Arthropods were collected from pitfall traps and identified to family, sub-family, or genus and species depending on the quality of the specimen.

Class	Sub-class/Order	Family	Sub-family/Genus spp.	January 2016 Total	April 2016 Total	July 2016 Total
Arachnida	Araneae	Aranidae		4		1
Chilopoda	Scolopendridae	Scolopendridae			1	
Diplopoda	Julida	Blaniulidae		3	4	1
	Polydesmida	Paradoxosomatidae		1		
	Polydesmida	Paradoxosomatidae		1	4	
Entognatha	Collembola				16	
	Protura				1	
Insecta	Blattodea	Blattidae			1	
	Coleoptera	Carabidae		4	1	4
		Curculionidae		1		
		Staphylinidae				
	Dermaptera	Forficulidae		1		1
	Diptera	Culicidae				
		Fannidae		1		
		Muscidae				
	Hemiptera	Aleyrodidae				
		Miridae		5		
		Reduviidae		3		
	Hympenoptera	Formicidae				
		Formicidae				
		Formicidae				
		Pompillidae		1		
		Xylocompinae		1		
	Lepidoptera	Pyralidae				
	Orthoptera	Gryllotalpidae				
Malacostraca	Amphipoda			33	496	4
	Isopoda	Porcellionidae		356	618	177
Total				415	1142	188

Table 2.9. Fertilizer treatments used during year 2 of the Lights Out mulching experiment.

Treatment Code	Type and Rate[a]	Weight (g)	Bag Color[b]
1	KNO_3-22.5	28.5 g/plot	Green
2	KNO_3-90	113.8 g/plot	Yellow
3	$(NH_4)_2SO_4$-22.5	18.6 g/plot	Blue
4	$(NH_4)_2SO_4$-90	74.4 g/plot	Red

[a] Rate calculations are in kg N/ha

[b] Fertilizer treatments were color-coded in order to identify them in the field.

Table 2.10. Common and scientific names of weeds identified during year 2 of the Lights Out mulching experiment.

Common Name	Scientific Name
Ageratum	*Ageratum conyzoides*
Alsike Clover	*Trifolium hybridum*
Amaranth, Slender	*Amaranthus viridis*
Amaranth Weed	*Amaranthus* spp.
Cinderella Weed	*Asclepias* spp.
Creeping Indigo	*Indigofera* spp.
Filarees	*Erodium* spp.
Garden Spurge	*Euphorbia hirta*
Goosegrass	*Eleusine indica*
Graceful Spurge	*Euphorbia glomerifera*
Hairy Crabgrass	*Digitaria sanguinalis*
Hairy Indigo	*Indigofera hirsuta*
Haole Koa	*Leucaena leucocephala*
Honohono	*Commelina diffusa*
Kyllinga	*Kyllinga brevifolia*
Love Grass	*Eragrostis tenella*
McCoy Grass	*Cyperus gracilis*
Morning Glory	*Ipomoea obscura*
Oxalis Weed	*Oxalis corniculata*
Panama Paspalum	*Paspalum fimbriatum*
Pitted Beardgrass	*Andropogon pertusus*
Prostrate Spurge	*Euphorbia prostrata*
Purple Nutsedge	*Cyperus rotundus*
Radiate Finger grass	*Chloris radiata*
Sensitive Plant	*Mimosa pudica*
Sida	*Sida cordifolia*
Sprawling Horseweed	*Calyptocarpus vialis*
Unidentified Grass	*Gramineae*

Table 2.11. Year 2 index of nematode genera, feeding types and respective colonizer-persister (CP) values, and absolute abundance collected of the the Lights Out mulching experiment.

Genus	Feeding Group	January 2016 Total	April 2016 Total	July 2016 Total
Acrobeles	**BF2**	21	4	6
Acrobeloides	**BF2**	66	40	18
Aglenchus	**PF2**	0	0	0
Alaimus	**OM4**	4	0	0
Aphelenchoides	**FF2**	128	39	32
Aphelenchus	**FF2**	83	56	148
Aporcelaimellus	**OM5**	0	0	0
Cephalobus	**BF2**	78	18	118
Cervidellus	**BF3**	4	0	2
Chiloplacus	**BF2**	1	0	0
Criconemoides	**PF3**	0	0	0
Diplogaster	**BF1**	32	27	49
Discolaimus	**OM5**	0	0	0
Dorylaimus	**OM4**	77	30	88
Eucephalobus	**BF2**	176	36	72
Eudorylaimus	**OM4**	79	21	60
Filenchus	**PF2**	113	55	89
Helicotylenchus	**PF3**	76	44	64
Heterodera	**PF3**	3	0	0
Hoplolaimus	**PF3**	0	0	0
Longidorus	**PF5**	0	0	0
Malenchus	**PF2**	6	0	0
MesoCriconemoides	**PF3**	0	0	0
Mesodorylaimus	**OM4**	0	0	0
Monhystera	**BF1**	61	10	21
Mononchus	**PR4**	0	0	5
Nygellus	**OM4**	0	0	0
Panagrolaimus	**BF1**	0	0	0
Paratylenchus	**PF2**	0	6	30
Pelodera	**BF1**	1	0	0
Plectus	**BF2**	3	6	7
Pratylenchus	**PF3**	60	77	20
Psilenchus	**PF2**	2	0	0
Pungentus	**OM4**	2	0	13
Rhabditis	**BF1**	102	65	13
Rotylenchulus	**PF1**	96	13	2
Rotylenchus	**PF3**	0	1	0
Telotylenchus	**PF2**	0	0	0
Turbatrix	**BF1**	0	0	0
Tylenchorynchus	**PF3**	0	0	2
Tylenchus	**PF2**	133	47	83
Unidentified		550	0	82
Wilsonema	**BF2**	0	0	0
Xiphinema	**PF5**	0	0	4

Table 2.12. Light meter results. Measurements from May 24, 2016 are from an overcast day. Measurements from May 25, 2016 are from a sunny day. Readers were taken with a PAR Quantum Light Meter (Spectrum Systems, INC). Units are umol.$m^{-2}s^{-1}$.

Date	Meter Location[a]	Low weed mat density	Medium weed mat density	High weed mat density
May 24, 2016				
	Under cover	1	1	1
		0	1	1
		0	0	1
	Outside	301	291	288
		294	340	295
		283	350	293
May 25, 2016				
	Under cover	5	6	7
		6	6	8
		5	7	9
	Outside	1779	1996	1766
		1630	1773	1821
		1730	1694	1706

[a] Light meter was held underneath each weed mat density and above it to compare conditions of plots that were covered with controls.

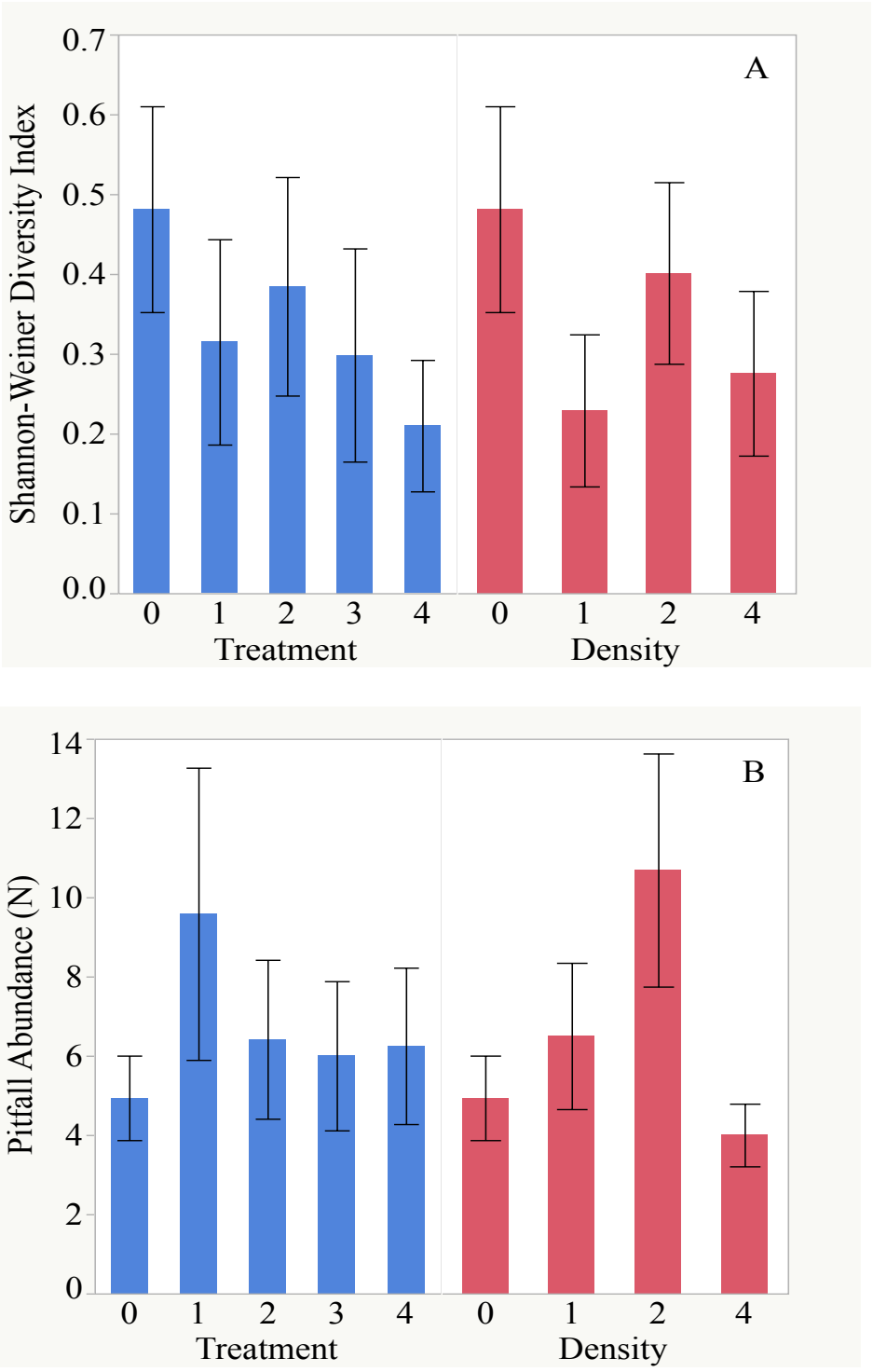


Fig. 2.8. Year 2 arthropod diversity (A) and abundance (B) before the first cover based on treatment (left) and cover type (right) randomization. Low and high rates for treatments 1-2 are KNO_3 and 3-4 are $(NH_4)_2SO_4$. Low (1), medium (2), and high (4) density cover types. Error bars represent one standard error from the mean. Bars with different letters indicate a significant difference.

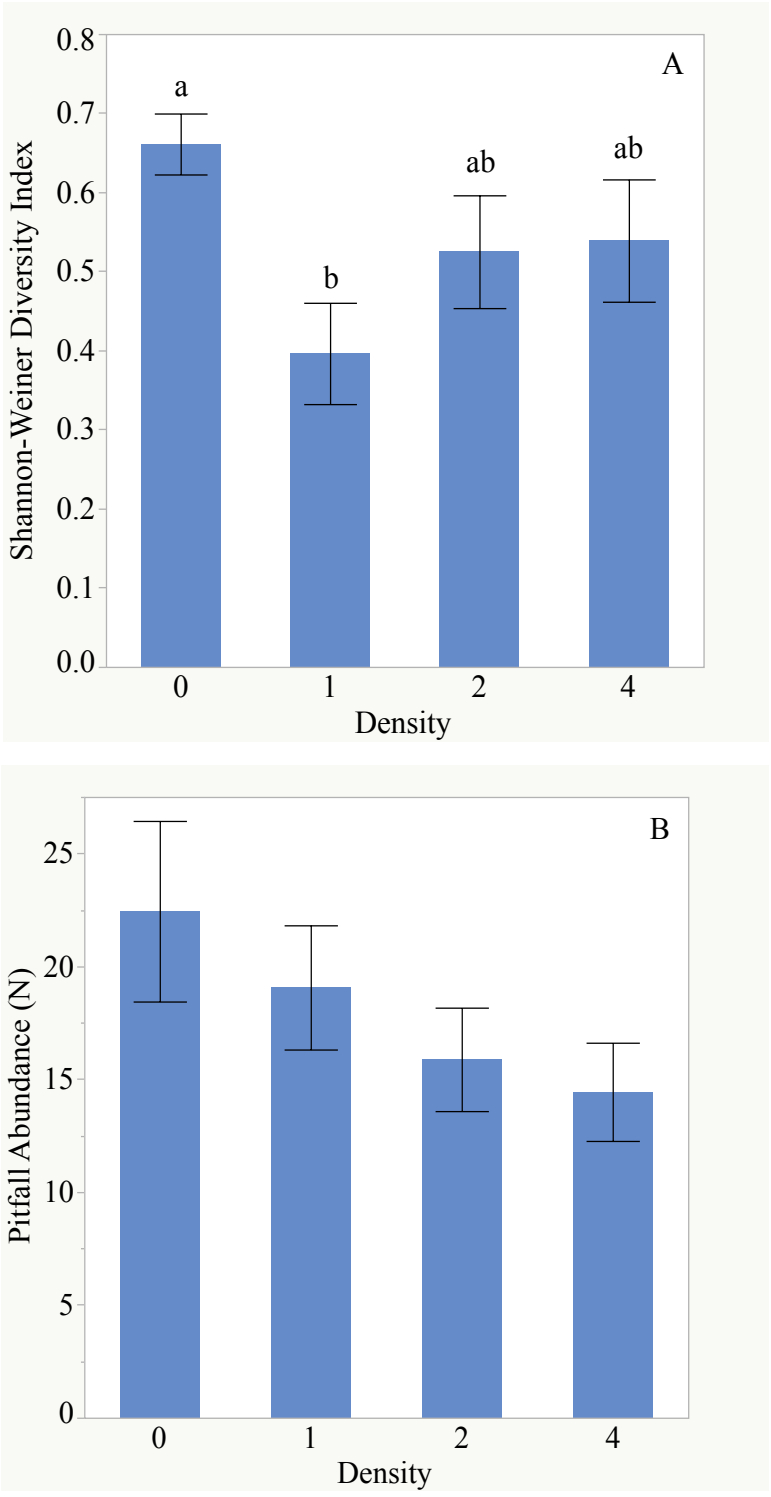


Fig. 2.9. Year 2 arthropod diversity (A) and abundance (B) after the first cover based on density. Low (1), medium (2), and high (4) density cover types. Error bars represent 1 standard error from the mean. Bars with different letters indicate a significant difference.

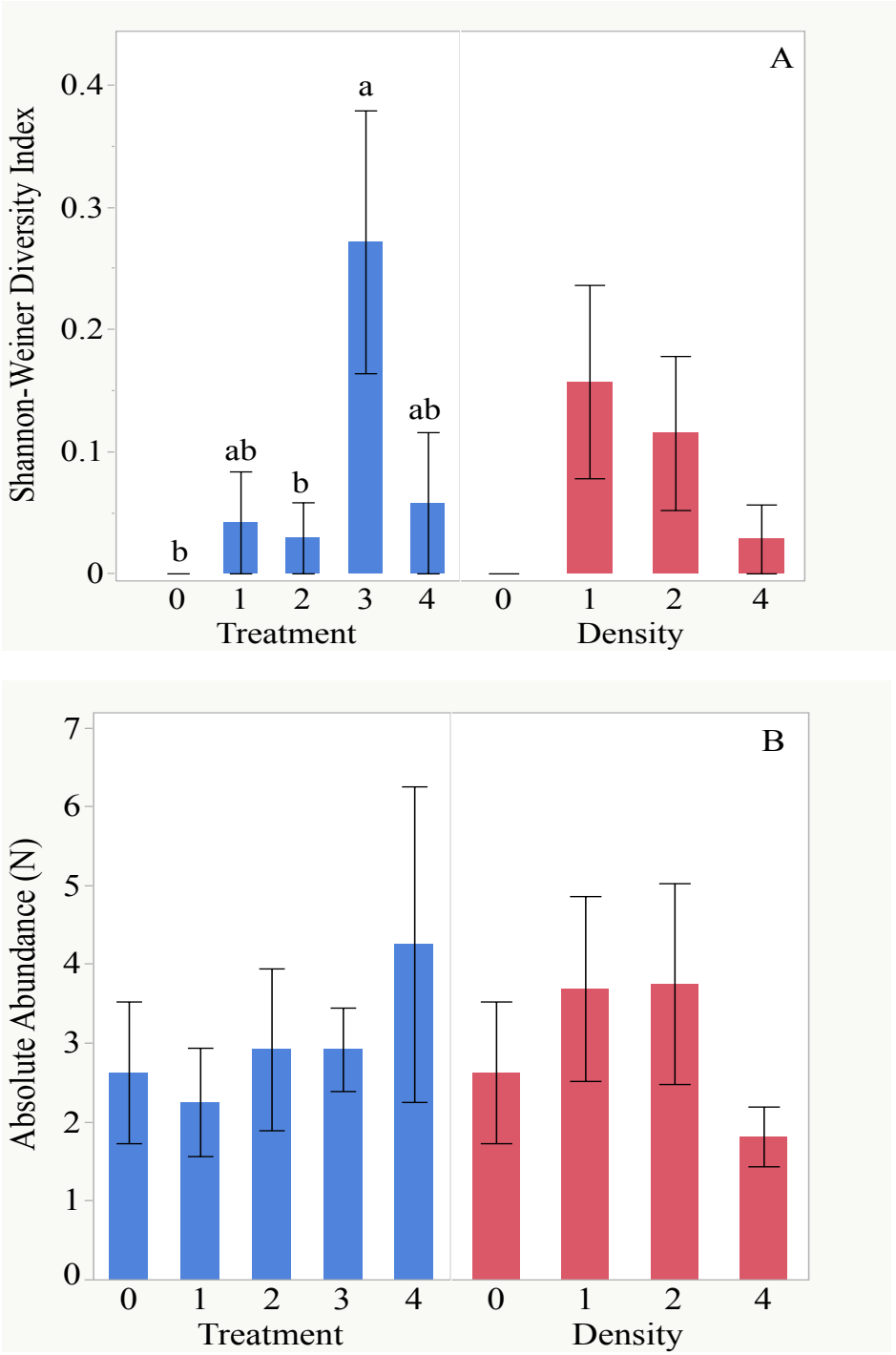


Fig. 2.10. Year 2 arthropod diversity (A) and abundance (B) based on treatment (left) and cover type (right) after fertilization and the second covering. Low and high rates for treatments 1-2 are KNO_3 and 3-4 are $(NH_4)_2SO_4$. Low (1), medium (2), and high (4) density cover types. Error bars represent one standard error from the mean. Bars with different letters indicate a significant difference.

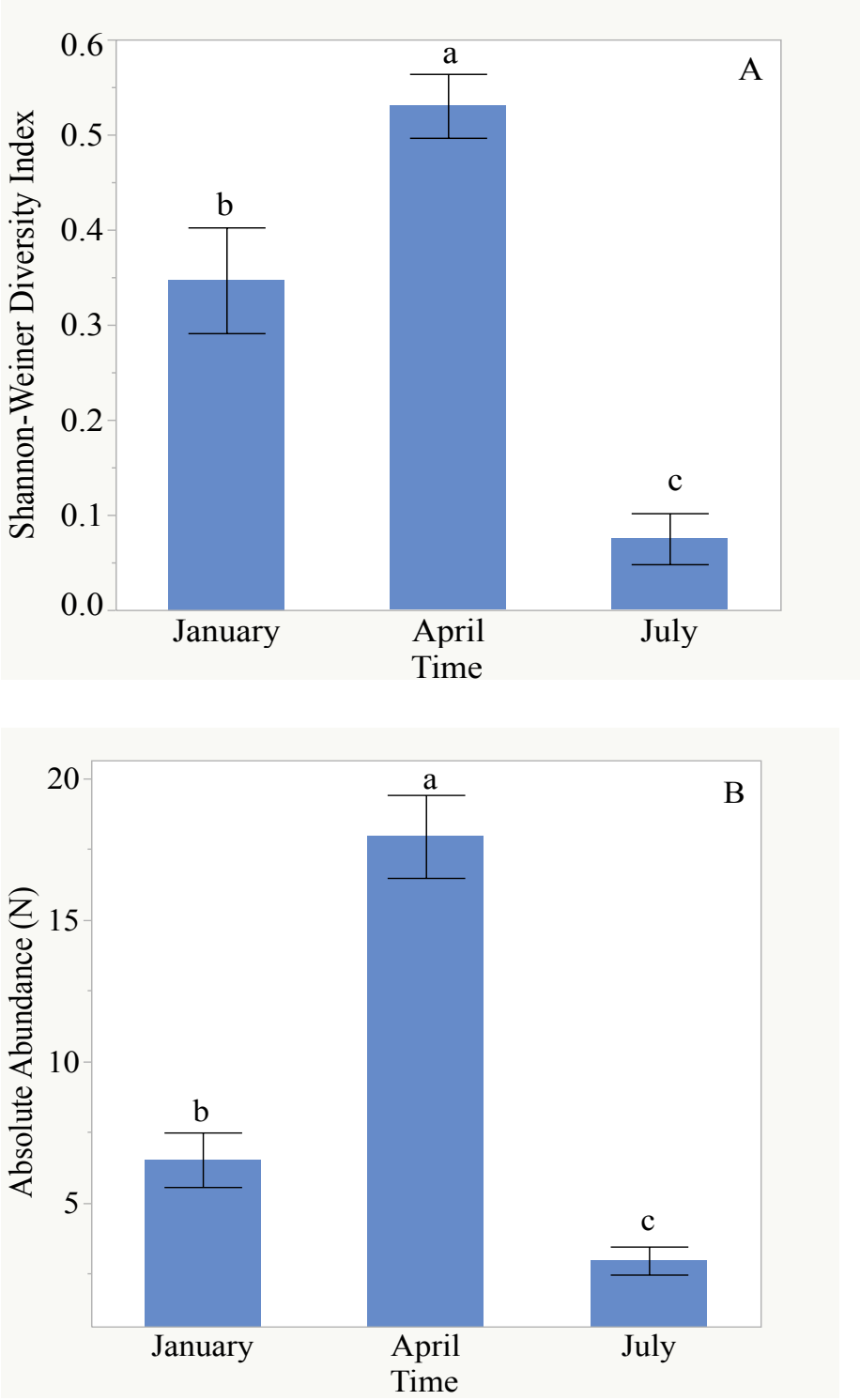


Fig. 2.11. Year 2 change in arthropod diversity (A) and abundance (B). January is before the first cover. April is after the first cover. July is after fertilization and second cover. Error bars indicate one standard error from the mean. Bars with different letters indicate a significant difference.

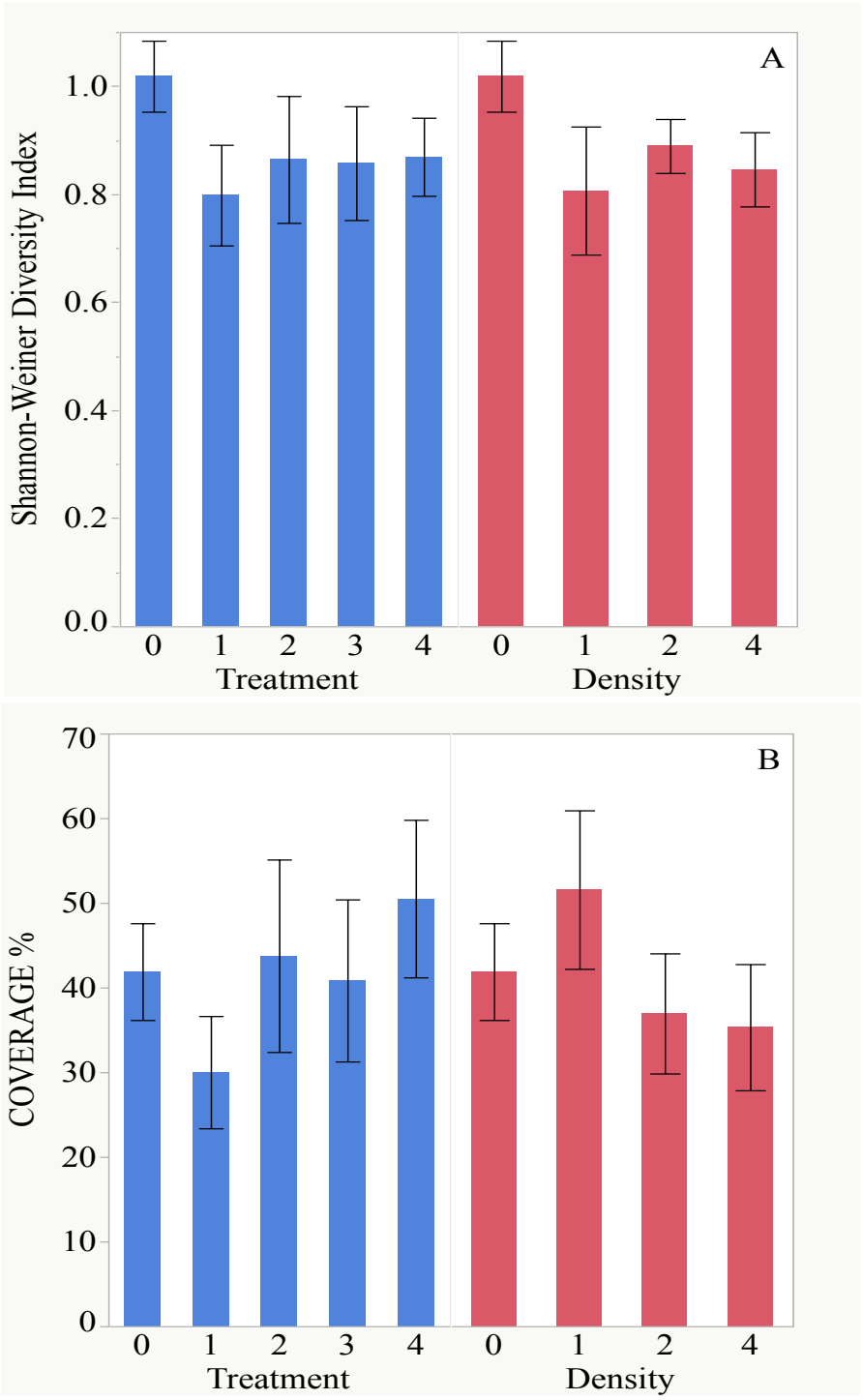


Fig. 2.12. Year 2 changes in weed A) diversity and B) coverage percent due to treatment (left) and density (right) one-month post fertilization. Low and high rates for treatments 1-2 are KNO_3 and 3-4 are $(NH_4)_2SO_4$. Low (1), medium (2), and high (4) density cover types. Error bars represent 1 standard error from the mean.

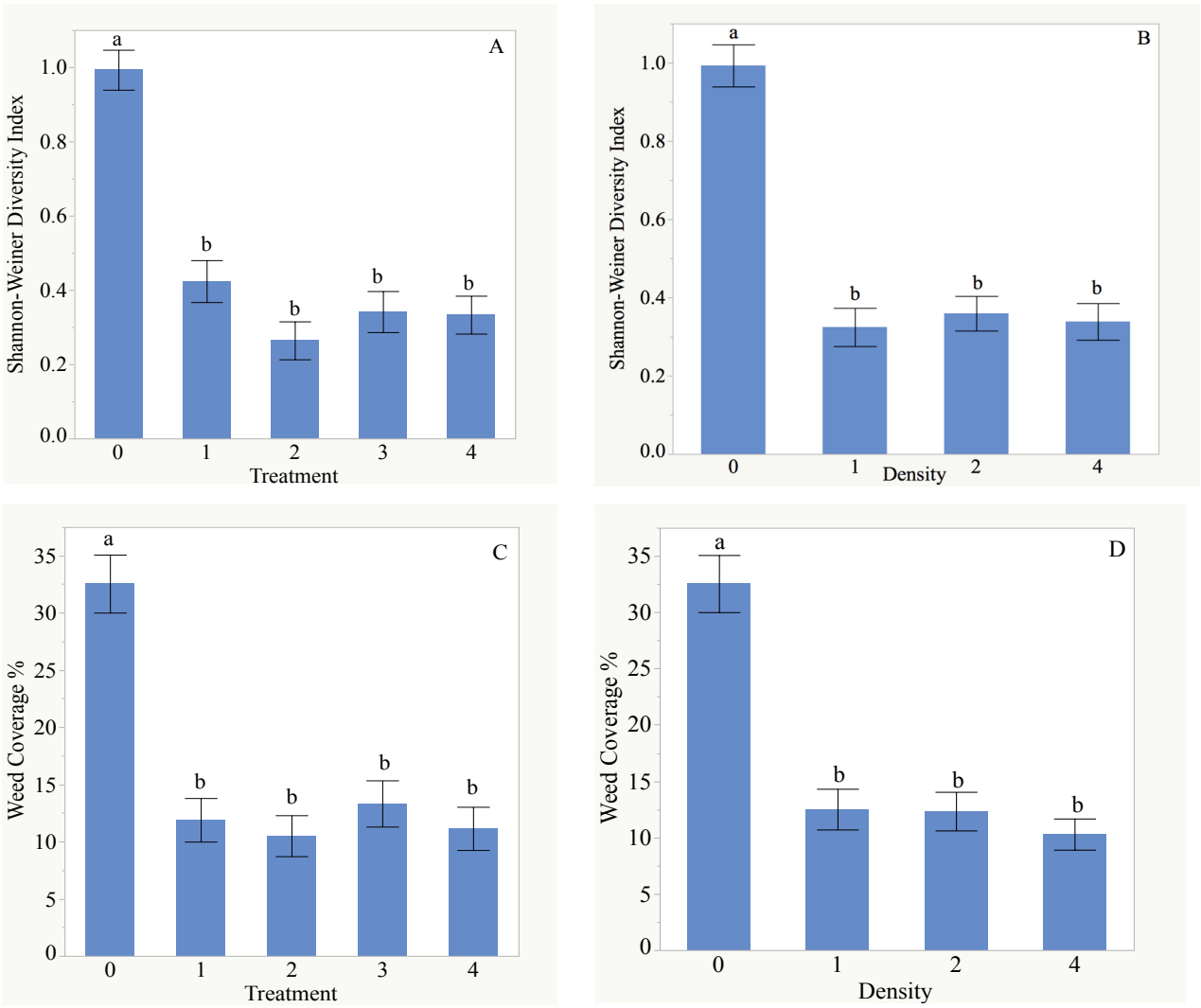


Fig. 2.13. Combination of weekly weed ratings from July 19, 2016 to August 29, 2016. Differences in weed diversity due to A) treatment and B) cover type as well as changes in weed coverage percent due to C) treatment and D) cover type. Low (1), medium (2), and high (4) density cover types. Error bars represent one standard error from the mean. Bars with different letters indicate a significant difference.

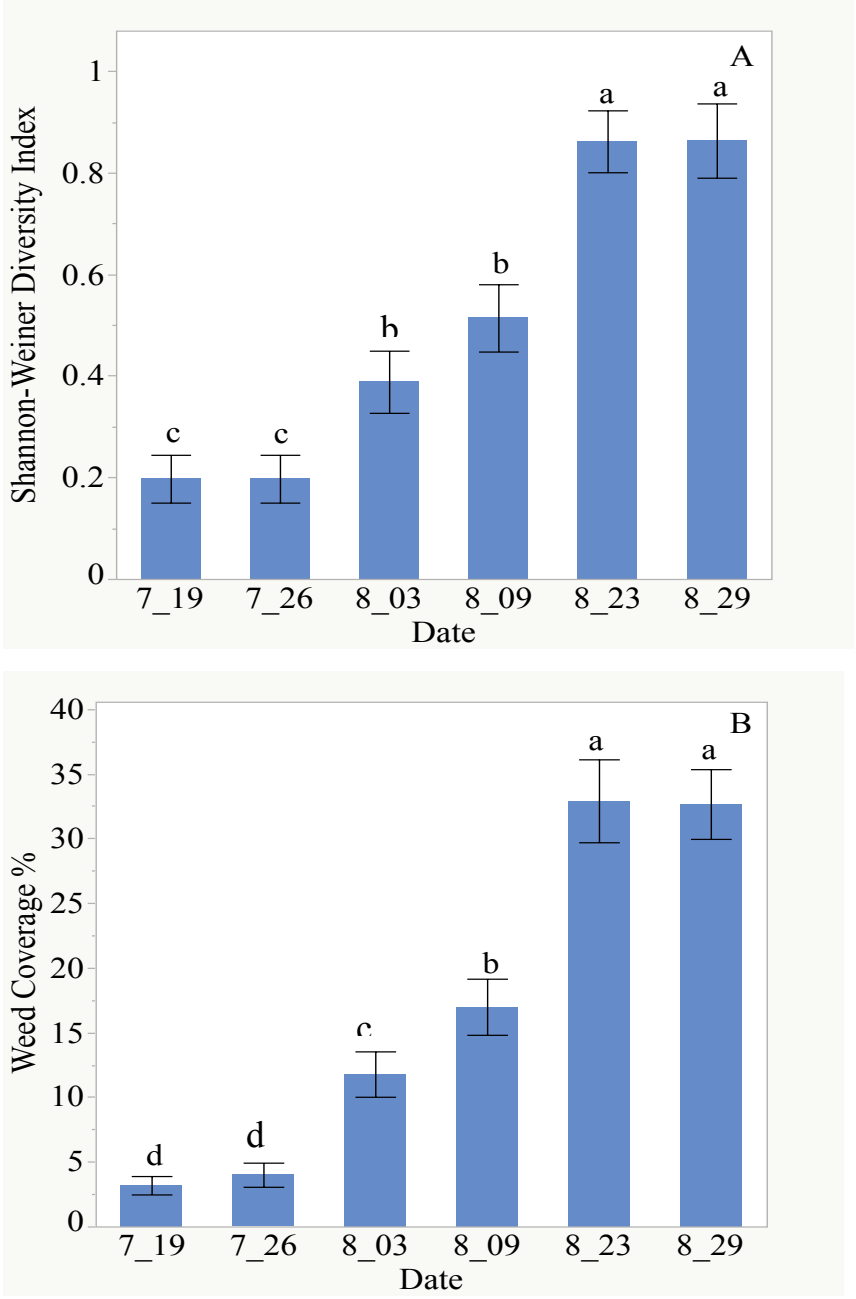
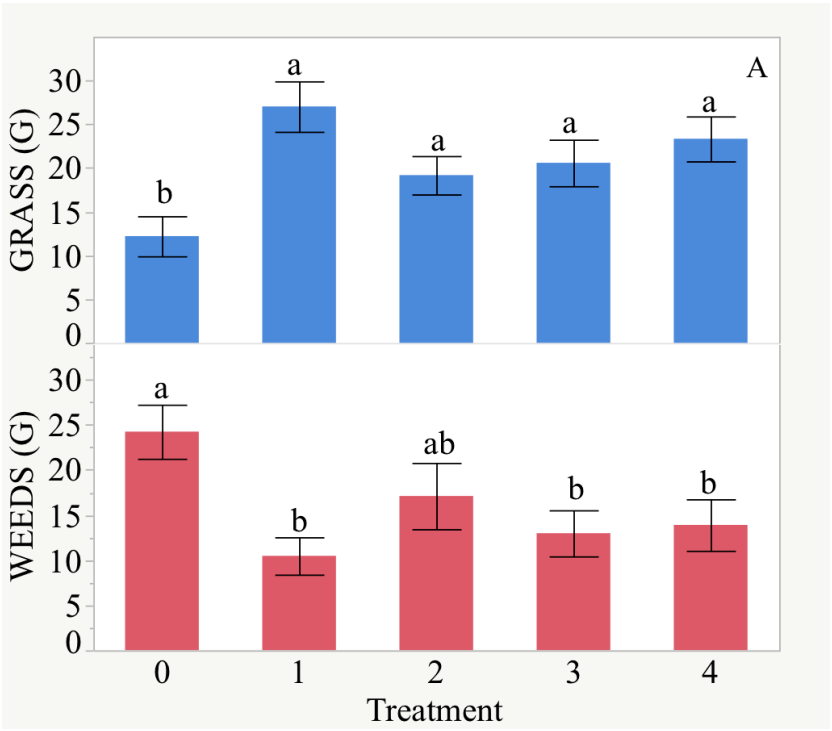


Fig. 2.14. Year 2 change in A) weed diversity and B) coverage percent over time. Error bars indicate one standard error from the mean. Different letters indicate a significant difference. Analyzed using a Kruskal-Wallis and Wilcoxon multiple comparison procedure.



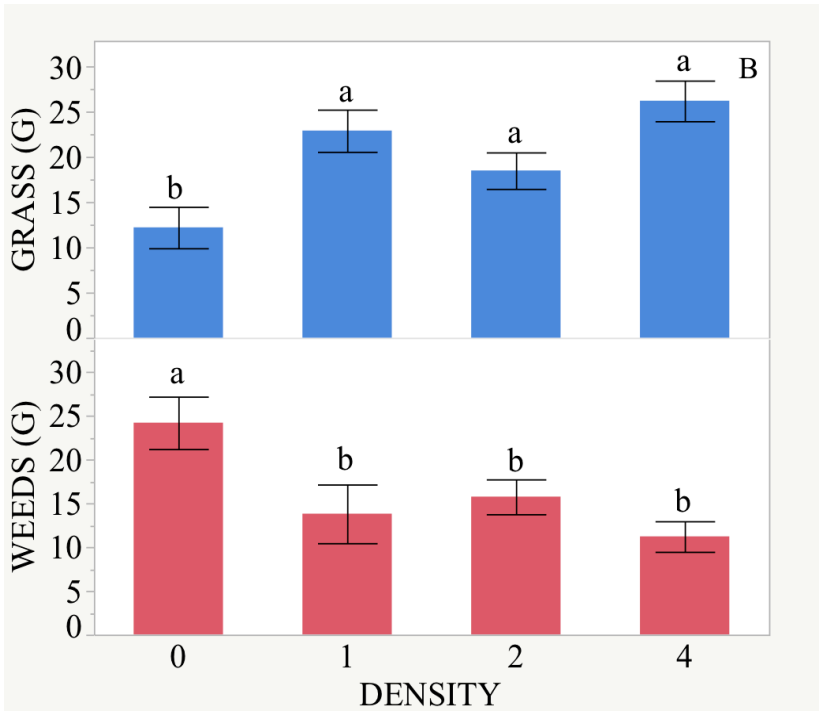


Fig. 2.15. Plant biomass for year 2 based on A) treatment and B) density. Blue indicates dry weight of grass and red indicates dry weight of weeds. Treatment and density for both weeds and grass were statistically significant when compared to controls ($P < 0.05$), no other pairwise comparisons were found. Analyzed using Kruskal-Wallis and Wilcoxon tests.

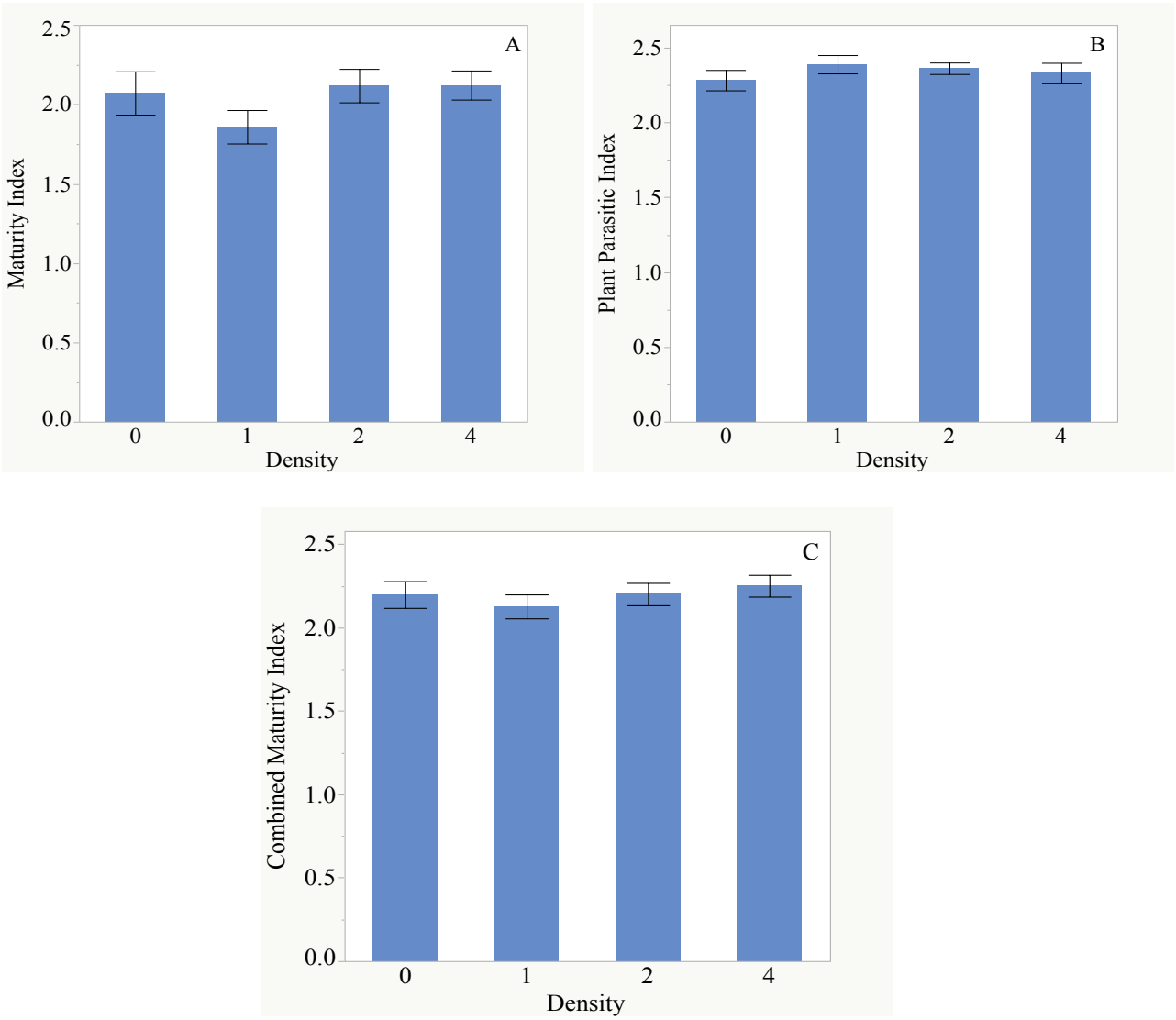


Fig. 2.16. Year 2 differences in nematode A) Maturity Index B) Plant-Parasitic Index and C) Combined Maturity Index after the first covering. Error bars indicate one standard error from the mean. Analyzed using a Kruskal-Wallis test.

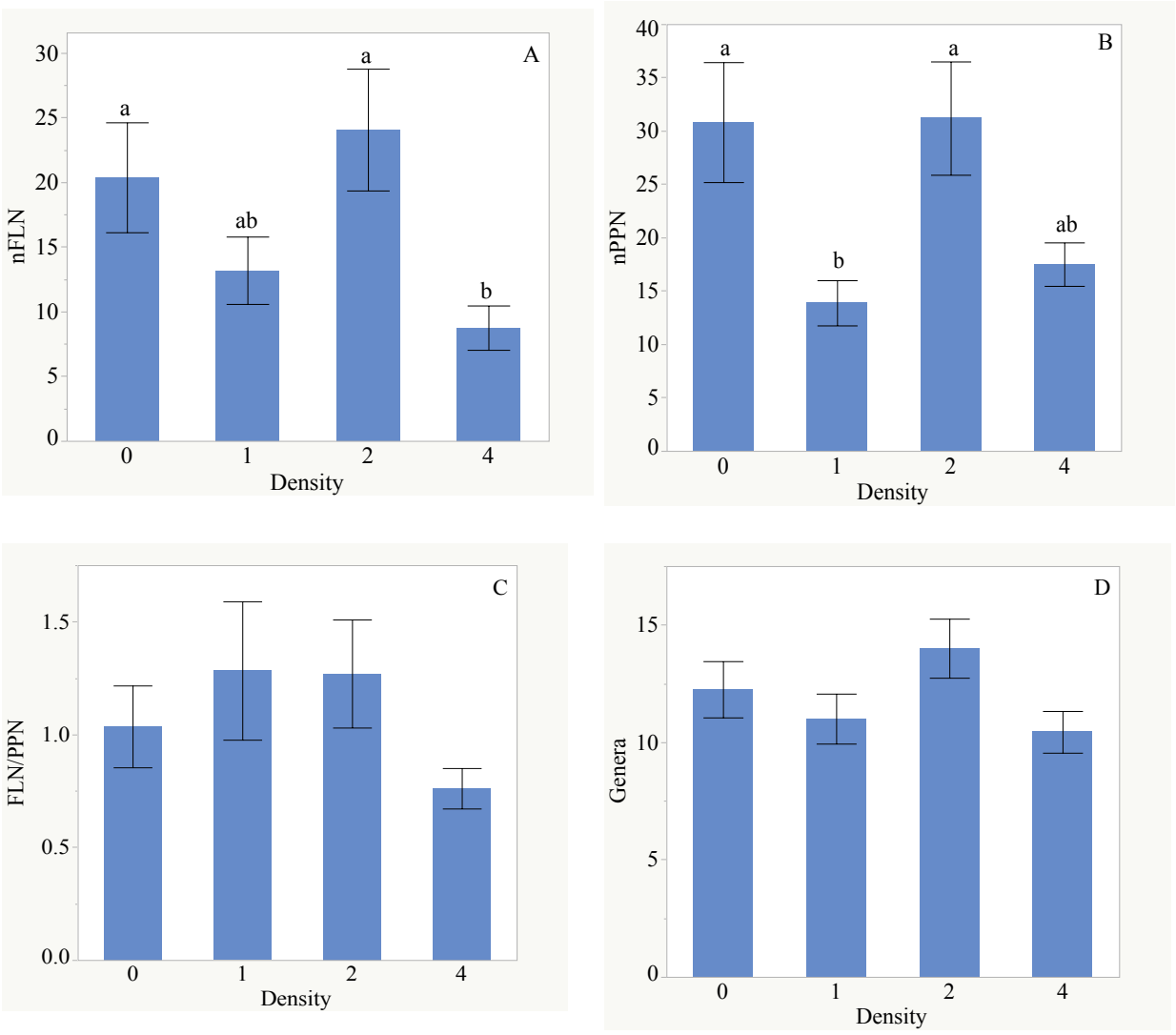


Fig. 2.17. Year 2 differences in numbers of A) free-living and B) plant-parasitic nematodes, the C) ratio of free-living to plant-parasitic nematodes, and D) number of genera due to cover type. Low (1), medium (2), and high (4) density cover types. Error bars indicated one standard error from the mean. Bars with different letters indicate significant differences.

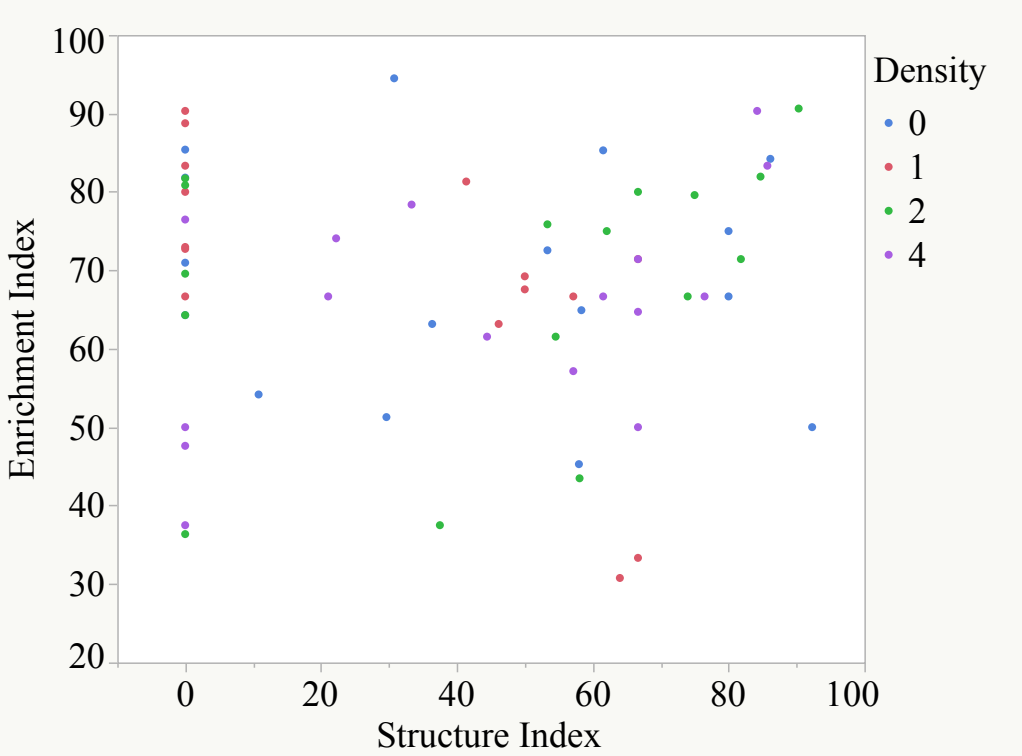


Fig. 2.18. Year 2 changes in nematode enrichment and structure after the first cover. Low (1), medium (2), and high (4) density cover types. Analyzed using a Kruskal-Wallis test and a Wilcoxon multiple comparison procedure.

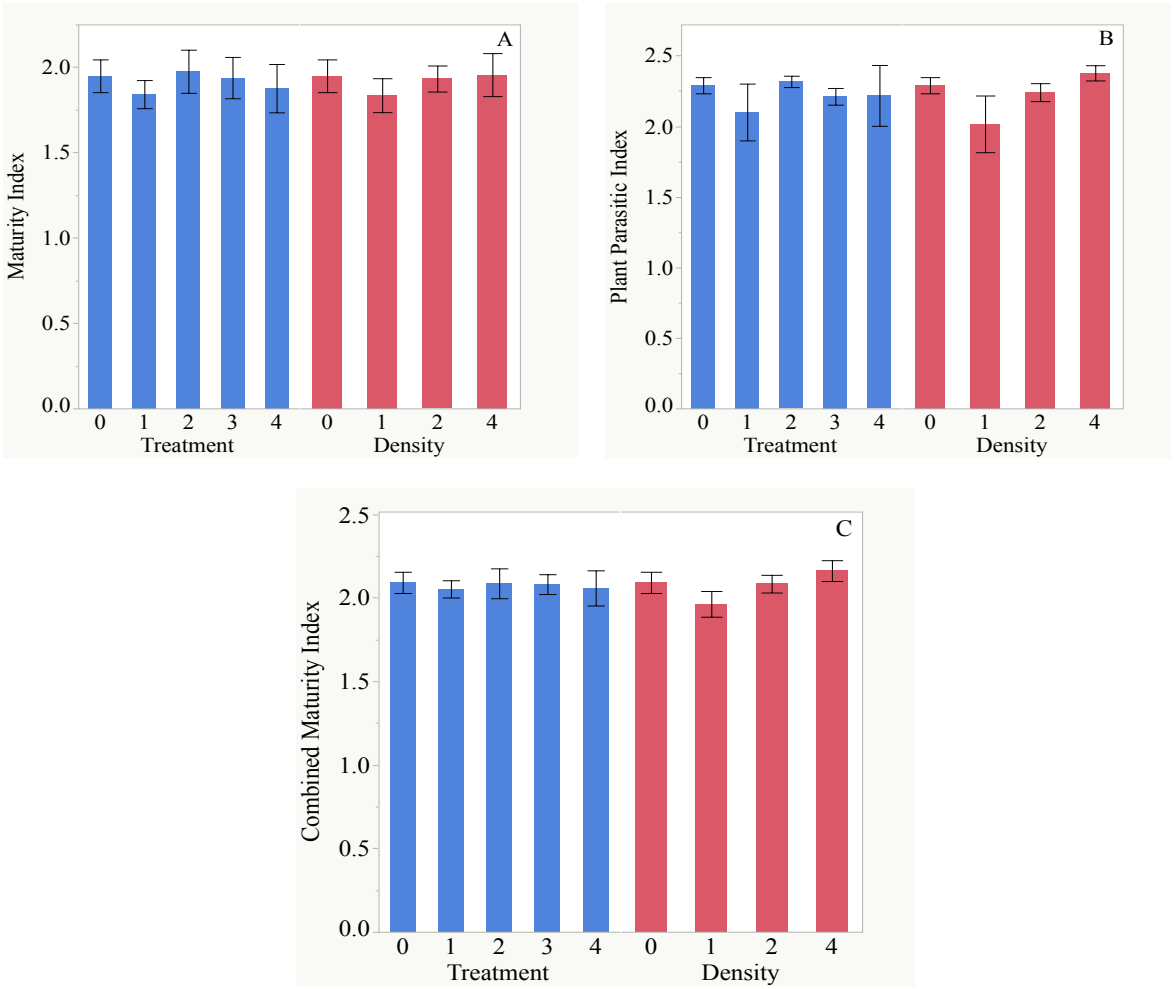


Fig. 2.19. Year 2 differences in nematode A) Maturity Index B) Plant-Parasitic Index and C) Combined Maturity Index based on treatment (left) and density (right) after fertilization and the second cover. Low and high rates for treatments 1-2 are KNO_3 and 3-4 are $(NH_4)_2SO_4$. Low (1), medium (2), and high (4) density cover types. Error bars represent one standard error from the mean. Bars with different letters indicate a significant difference.

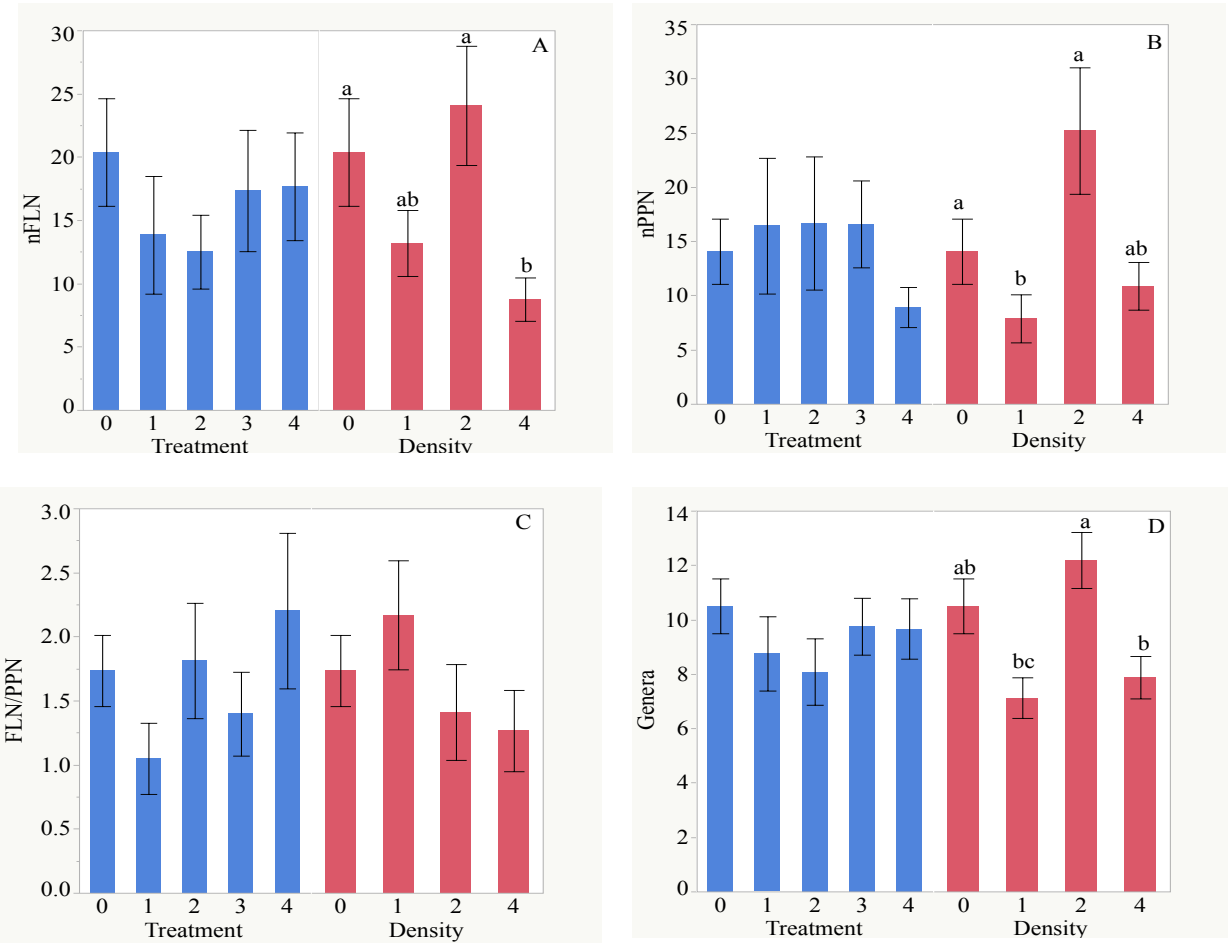


Fig. 2.20. Year 2 differences in numbers of A) free-living and B) plant-parasitic nematodes, the C) ratio of free-living to plant-parasitic nematodes, and D) number of genera due to cover type. August sample from after fertilization and the second cover. Treatments 1-2 are low and high rates of KNO_3 and 3-4 are $(NH_4)_2SO_4$. Low (1), medium (2), and high (4) density cover types. Error bars indicated one standard error from the mean. Bars with different letters indicate significant differences.

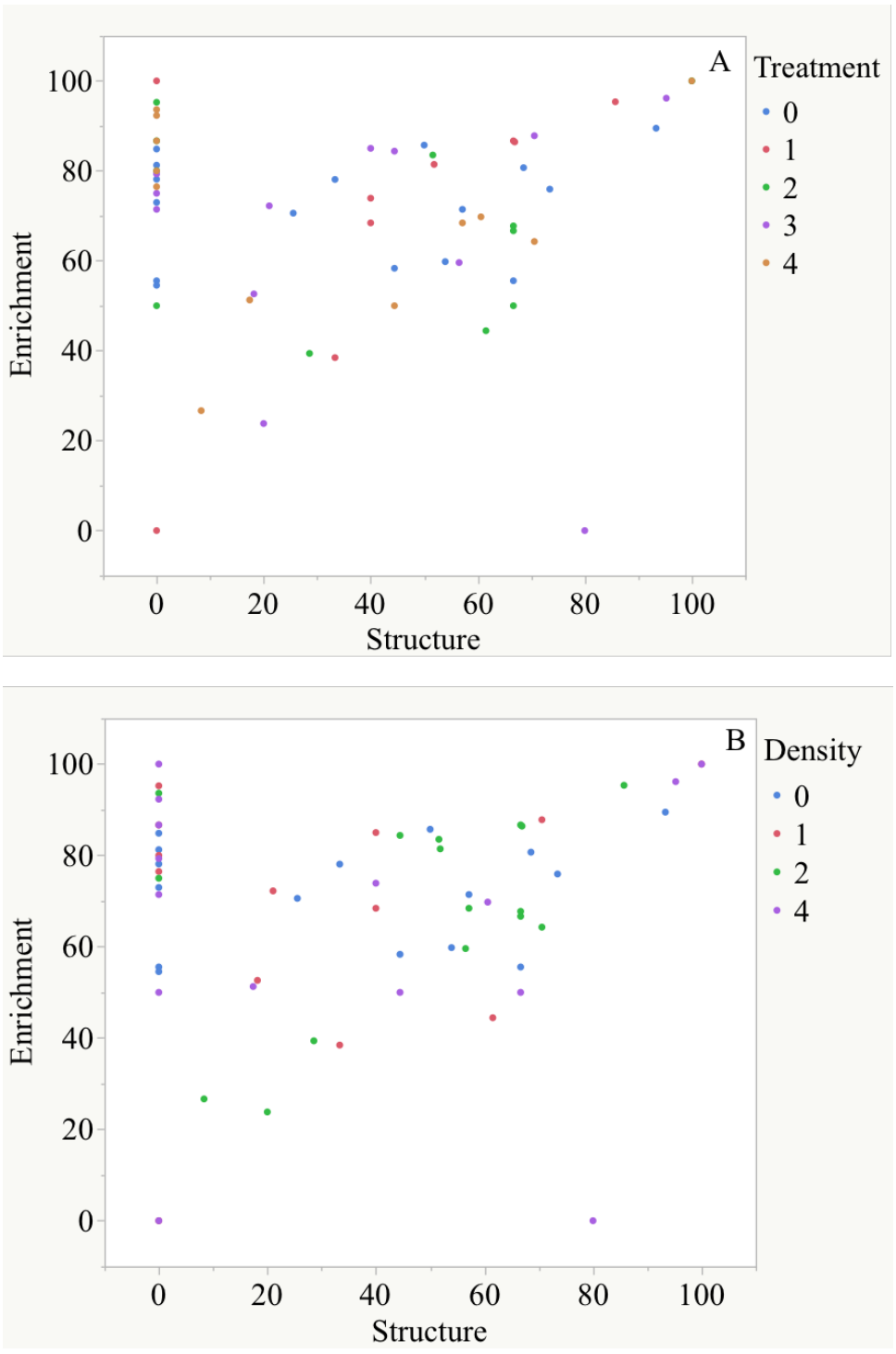


Fig. 2.21. Year 2 changes in nematode enrichment and structure after the fertilization and second cover due to A) treatment and B) cover type. Low and high rates for treatments 1-2 are KNO_3 and 3-4 are $(NH_4)_2SO_4$. Low (1), medium (2), and high (4) density cover types. Analyzed using a Kruskal-Wallis test and a Wilcoxon multiple comparison procedure.

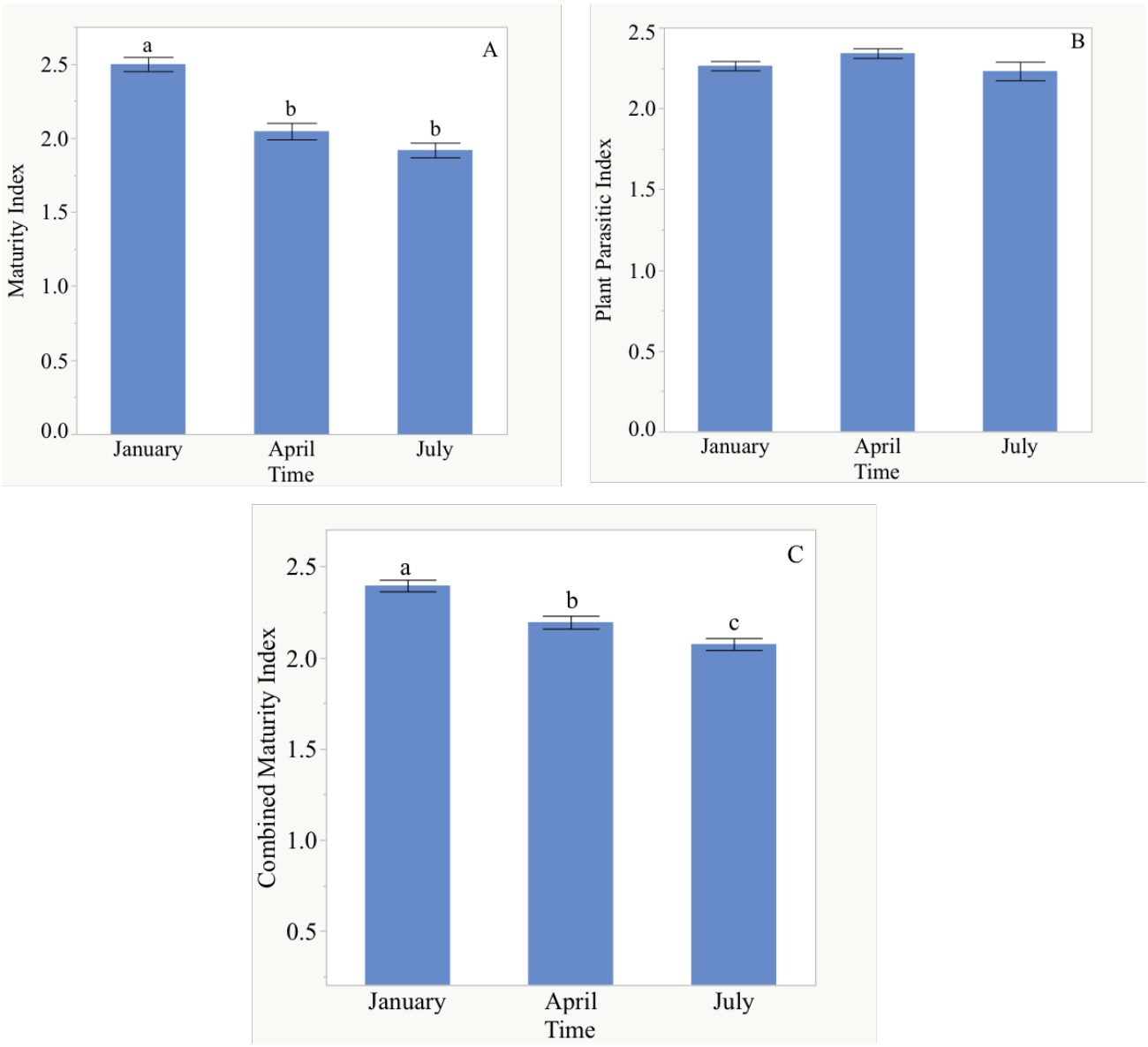


Fig. 2.22. Year 2 differences in nematode A) Maturity Index B) Plant-Parasitic Index and C) Combined Maturity Index over time. January is before the first cover. April is after the first cover. July is after fertilization and second cover. Error bars represent one standard error from the mean. Bars with different letters indicate a significant difference.

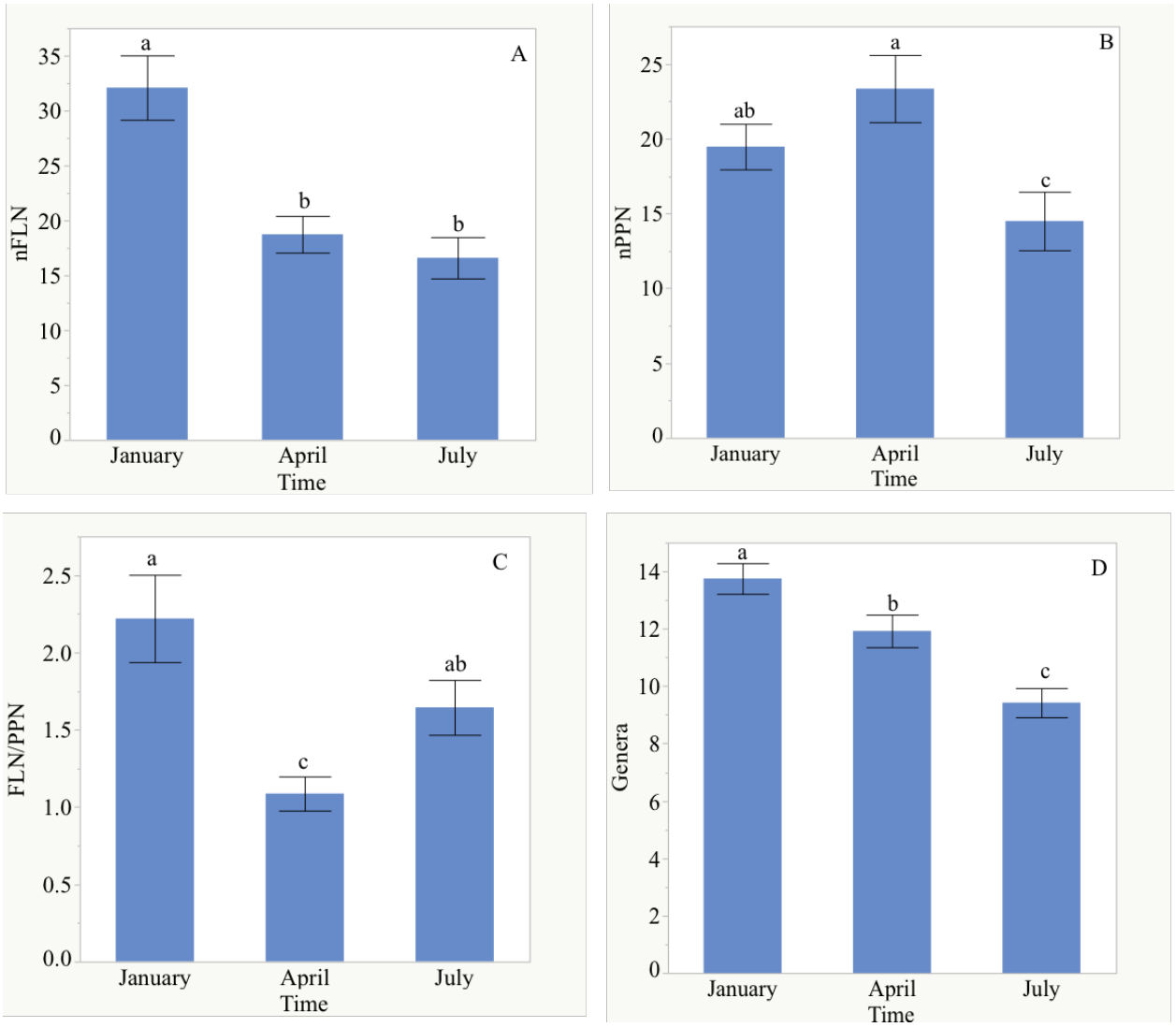


Fig. 2.23. Year 2 differences in numbers of A) free-living and B) plant-parasitic nematodes, the C) ratio of free-living to plant-parasitic nematodes, and D) number of genera over time. January is before the first cover. April is after the first cover. July is after fertilization and second cover. Error bars represent one standard error from the mean. Bars with different letters indicate a significant difference.

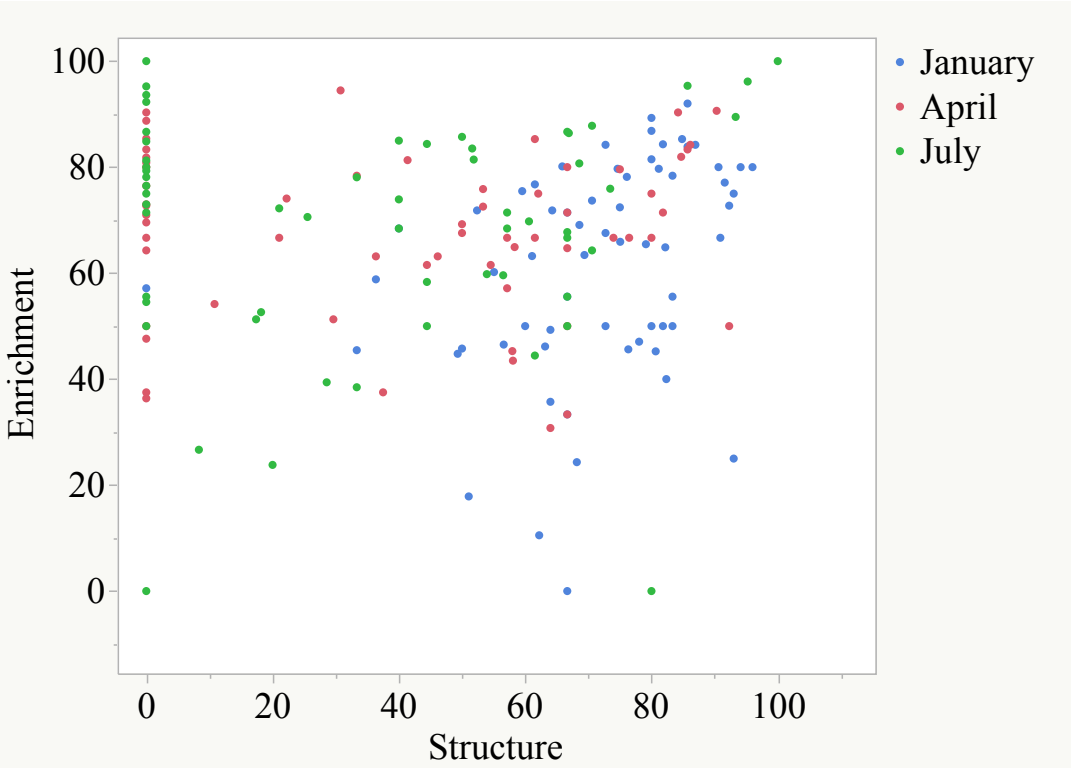


Fig. 2.24. Year 2 change in nematode enrichment and structure over time. January is before the first cover. April is after the first cover. July is after fertilization and second cover.

Chapter 3. Conclusions and Considerations

Conclusions

Based on the identified objectives from year 1, it can be concluded that: 1) a double layer weed mat (Belton 1859 style, Belton industries) effectively reduced weeds after series of three coverings, 2) the high rate of ammonium sulfate did not stimulate weed seed germination with greater efficacy than other fertilizer treatments (however, significant increases in weed diversity after fertilization indicates fertilization is a crucial step in flushing the weed seed bank), and 3) arthropod diversity was reduced due to covering, but seemed to tolerate the fertilizer amendment well. The nematode Plant-Parasitic Index was reduced, which is beneficial for turf managers who have existing problems with nematodes that attack grass roots (Domen and Wang, 2016). Although total nematode abundance was also reduced after each covering, the overall structure and enrichment of the nematode community remained in-tact, indicating no disturbance to the soil food web structure.

Based on the results from year 2, it can be concluded that 1) all weed mat densities (cover types) were more effective in reducing weed pressure (measured in weed diversity and coverage percent) over time when compared to the traditional glyphosate method of control, but no differences were detected among weed mat densities tested, 2) no differences in fertilizer rate or type were detected, but after the final covering and planting Bermudagrass, all had less weed diversity and coverage when compared to the traditional glyphosate method of control, 3) the low density weed mat was associated with a decreased in arthropod diversity, the nematode Plant-Parasitic Index, and plant-parasitic abundance. The low density weed mat was shown to maintain similar average temperatures to the high density weed mat, but had significantly higher average temperatures compared to the control. Belton industries indicates that handling storage

and shipping can change the thermal, optical, and tensile properties of the weed mats. Managers should take this into consideration, and select a weed mat that will maximize light reduction, retain temperatures as high as 60° C. Additionally, arthropod and nematode diversity and abundance were determined to be reduced as a result of the weed mat covering. This is not an inherently negative outcome especially if turf managers are struggling with soil-borne insect pests or destructive nematodes. Although changes in the soil food web are likely to occur when using Lights Out mulching, the effect is temporal but recoverable as indicated by the lack of differences in total abundance of FLN and MI compared to the control. For long-term turfgrass sustainability, managers should pay close attention to the overall health of the soil system that will lead to lower maintenance of the system over time.

Considerations

The Hawaiian Islands have many different types of turfgrass systems. Whether it is turf that has been renovated for use in a small recreational park in Waikiki, or a PGA golf course on Maui, turf plays an important role in the economy and culture of Hawaii. It is critically important for turf managers to be stewards of the landscape that they manage and enjoy. Managers must stay informed on new technological innovations that will help improve their turf in efficient and sustainable ways. While chemical control can be a fast and effective method, the Lights Out mulching method allow managers to incorporate additional non-chemical practices into their management protocol to better maintain the turfgrass landscape.

The most critical approach to turfgrass management is the proactive approach. Turf managers should work diligently to ensure that their first line of defense is a robust, healthy turf. Healthy turf provides aesthetically pleasing recreational areas, decreases runoff and soil erosion, and adds economic value to the landscape (Stier, 2000). Proper irrigation and mowing are two common strategies managers can use to improve the overall health of the turf, and reduce the

likelihood that major renovation will be needed. Irrigation should be performed early in the morning. The water should not be siphoned from contaminated sources, because this can increase the likelihood of bringing weed seeds to the turf (Stier, 2000). Irrigation schedules are left up to the discretion of the management team, and often depend on climate, grass type, and budget. Turf should be watered enough to keep the grass and soil moist. Mowing is also a critical practice for healthy turf. Blades should always be sharpened and cleaned on a regular basis, and managers should follow protocol with mow heights in order to prevent scalping and grass damage. Grass that is mowed at the proper height is well equipped to combat encroaching weeds (McCarty and Murphy, 2004). If any pressing weed problems remain, managers should carefully consider which herbicide to use to control the problem and which season it should be applied. They should also follow application instructions on the label closely, to ensure that weeds are controlled but grass remains stable.

On occasion, weed pressure surmounts these proactive strategies to manage turf, and a partial or complete renovation is required. Traditionally, herbicides such as glyphosate have been used to accomplish this (Stier, 2000). After two years of study, the Lights Out method was determined to be a viable alternative to chemical renovation of turfgrass systems. Some overarching strategies managers can incorporate when implementing Lights Out mulching that have become apparent over the course of the study include:

1. Have an informed, efficient team that will assist throughout the renovation.
2. Prepare mulching materials with team in advance to reduce renovation time
3. Incorporate additional cultural and chemical strategies if needed

Working with a team will greatly reduce the amount of time it takes to renovate the surface. The weed mats can be very large, and the coverings can be difficult for a single person to do alone. Having approximately 5 able-bodied individuals will put at least one person on each side of the weed mat, where people can detach the mat from a clip base (if one is used), or remove any weights that are holding the mat down. Teamwork is essential to the Lights Out process, and managers that understand the principles of team work will undoubtedly benefit from this renovation technique. Once a viable team is established, make sure that materials are prepared in advance so they can be incorporated and removed as quickly as possible. This includes, measuring and cutting weed mats at the desired size, installing irrigation around the renovation site, calibrating any and all equipment used for the renovation, and identifying any tools (i.e. shovels, wrenches, etc.) that will be needed to keep the system functioning. While Lights Out has been shown to effectively renovate old, weedy turf, it is important for managers to still assess the need for additional cultural and chemical control. It is also important to routinely check for drought and pest damage, to address problems before they become severe.

Some other observations that are key for managers to recognize if they are considering lights out mulching include budgeting time and finance. Although the length of renovation using the Lights Out technique took anywhere from 6-10 months depending on the scale, a team of committed managers and staff could effectively renovate a surface in about six months, including new turfgrass establishment time. Some managers may be able to spend this amount of time with part of their course or recreational closed, others may not. Another observation is that abundance of arthropod and nematode communities did change as a result of the mulching. This result can be beneficial if turfgrass managers desire to eliminate insects and nematodes that are pests. However, managers should understand that beneficial or non-target insects may also be affected.

It will benefit managers to do soil evaluations before and after the Lights Out renovation in order to assess the biotic communities that are present. Soil evaluations are affordable measurement tools that give managers a better understanding of how both traditional and alternative methods of control affect the surrounding biotic community (Murphy, 2004).

Grass selection is also an important choice during the renovation process. Managers should decide before implementing the first cover what type of grass variety is best for the surface they are renovating, and how it should be established (i.e. seed, sprig, plug, sod). For the Lights Out study, maximum rates of Bermudagrass seed were used with a hydro-cap mulch to minimize seed predation or wind removal. In some circumstances the desired grass variety may establish best with sprigs or plugs. Some managers may even determine that laying sod is the best approach to renovation. In this case, managers should work with sod farms to grow the desired variety, and have the order ready for transport to the renovation site once the second cover has been removed and soil has been prepared. Depending on the grass type, fertilizer selection and implementation plays an important role in aiding turf establishment. In the Lights Out mulching process, fertilization is also used to maximize weed seed germination between coverings and flush the weed seed bank. A different type and rate of fertilizer may be needed on stripped ground compared to germinating/newly planted turf. High rates of ammonium sulfate can be used to stimulate weed seed germination, but a different rate or type may be needed for the establishment of young turf. Nitrogen fertilizer has been shown to be effective with young grass, and is an option turf managers use often to help establish new seed and sod (Kawate et al., 2015).

Lights Out mulching has the potential to be used on recreational surfaces, home lawns, and possibly golf courses. It addresses a large variety of turf problems including insect, weed,

and nematode infestations, all with one simple technique. Geotextile weed mats are durable, and can be washed and reused for future renovations. Using these weed mats may reduce the total amount of pesticide applied to the system, relieving public concerns about the use of pesticides and saving managers the cost of investing in an expensive chemical that may only be able to be used once. The techniques from Lights Out mulching can even be applied to problems that arise in agricultural systems (Domen and Wang, 2016). It is a widely applicable and effective method that encourages the longevity of the natural and man-made turfgrass systems of Hawaii.

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