

## ABSTRACT

ADAMS III, PAUL RICHARD. Above and Below Ground Interactions in Corn (*Zea mays* L.) and Soybean *Glycine max* (L.) Merrill Under Various Production Systems. (Under the direction of Dr. Yasmin J Cardoza).

The objective of Chapter One of this thesis was to measure the effects of system level management programs common to the Southeastern United States on soil and foliar arthropod abundance and diversity in both corn (*Zea mays* L.) and soybeans (*Glycine max* (L.) Merr.). Studies on soil and foliar arthropod populations have shown that they are sensitive to environmental disturbance and degradation and which impacts functional biodiversity in agroecosystems that can maintain key ecosystem services such as pollination, nutrient cycling, and natural pest control. Our field experiment was a completely randomized block design with three replicates for each of five farming systems which include: Conventional clean till (CCT), conventional long rotation (CLR), conventional no till (CNT), organic clean till (OCT), and organic reduced till (ORT). Sampling for soil arthropods was accomplished by pitfall trapping while foliar arthropod sampling was accomplished by scouting and sweep netting for corn and soybeans respectively. Pitfall trapping resulted in 39,994 and 37,119 specimens in corn and soybeans while foliar scouting in corn produced 2,375 specimens and sweep netting in soybeans resulted in 18,207 specimens. Soil and foliar arthropod abundance were significantly impacted by cropping systems and sampling dates. This suggests that the sum of management practices, i.e. systems, impact soil and foliar arthropod abundance and diversity and that the effects of these systems are dynamic over time. Furthermore, our results suggest that agricultural systems that are lower in management intensity, especially those employing reduced or no tillage, foster greater arthropod abundance and diversity.

The objective of Chapter Two of this thesis was to investigate the effects of organic (poultry litter) and synthetic nitrogen source on mycorrhization of corn roots. Moreover, to assess the potential effects on performance by two important corn insect pests: the generalist

corn earworm, *H. zea* and the specialist corn leafhopper, *D. maidis*. Effects of nitrogen source, mycorrhization and insect infestation on below- and above-ground plant biomass production were also assessed. The benefits of root mycorrhizal colonization to host plants is several fold and includes increased nutritional status, abiotic stress mediations, and defense priming against herbivory however, these effects are dependent on feeding habit, feeding specialization, and mycorrhizal species. One approach to better our understanding of these interactions is to assess the impacts of macronutrient nutrition i.e. phosphorus or nitrogen, on the AMF-plant-insect system. Our experiment was a randomized complete block design with factorial treatment arrangements for 3 factors which include: mycorrhization (non-mycorrhizal control vs. 3 spp. live inoculum blend), nitrogen source (no nitrogen, organic i.e. poultry litter, and synthetic nitrogen), and insect (no insect, *Helicoverpa zea*, *Dalbulua maidis*). Organic nitrogen increased pupal survival and mean pupal mass of *H. zea* but tended to decrease *D. maidis* nymph production and significantly decreased mycorrhizal colonization. Mycorrhization tended to reduce pupal survival and mean pupal mass but overall did not significantly impact our insects. Mycorrhization did however reduce root and shoot biomass. Altogether our results show the nuances of these ecological interactions and the importance of understanding their fluctuations in response to production management.

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Above and Below Ground Interactions in Corn (*Zea mays* L.) and Soybean *Glycine max* (L.)  
Merrill Under Various Production Systems.

by  
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A thesis submitted to the Graduate Faculty of  
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## **DEDICATION**

To my parents for their unwavering support, love, and belief in my ability to overcome my circumstances, whatever they may be.

To my friends for providing laughter, encouragement, and the occasional open ear.

To the art form that is music, for its ability to soothe the soul and motivate the mind.

## **BIOGRAPHY**

Paul Adams is a North Carolinian through and through. He was raised in the small coastal town of Beaufort N. C. along the crystal coast. He thought he wanted to be an engineer and so fate led him to North Carolina State University where he got his B. S. in Zoology and a Minor in Entomology. As luck would have it, he decided that he should go to graduate school and it turns out the professor whose entomology lab he worked in, had an opening. Because of these happy circumstances and NCSU's high ranking among Entomology programs, he gladly accepted. He will graduate sometime in 2015 and plans to stay in the Raleigh area.

During his time here the majority of his work has been on determining how agricultural management practices impact foliar and soil arthropod abundance and diversity in NC corn and soybean systems. He has also dabbled in work with entomopathogenic nematodes, pollinator responses to vermicompost amendments in cucurbits, and how mycorrhization and nitrogen nutrition and source impact chewing and sucking pest performance in corn.

In his spare time he likes to make loud noises on his electric guitars, collect vinyls, and go see shows. When not contributing to his own inevitable hearing loss he also enjoys spending time in the great outdoors whether that be fishing, hunting, running (only for health & wellness), or playing disc golf, among other things. He also fancies grilling, craft beer, a good book, and tattoos.

## ACKNOWLEDGEMENTS

Since the inception of my project there has been but one person who has given me constant insight, encouragement, and put in her own hard hours to accomplish this research. Of course I mean my major advisor, Dr. Yasmin J. Cardoza. Certainly it can be said that without her I would not be here nor would I have made it this far. Even after her difficult decision to pursue her own career goals beyond the university she was constantly available to provide wise counsel via phone, email, or face-to-face meetings. Her work ethic, clearly visible in her help with my own research, is something to be admired and I'd like to thank her for all her wisdom and the opportunities that she has given me.

Similarly, I must share my gratitude with Dr. D. Wes Watson. He is an exemplary man, one who stepped up to the plate and accepted responsibility as our own department head. But beyond that he is a great teacher and a friend. When obstacles arose within my project he was constantly available to talk and lend advice. Wes has been a source of encouragement and an example of what can happen with hard work and the willingness to seize opportunity when it arises.

Though the last of my advisors, certainly not the least in any respect. Dr. Michelle Schroeder-Moreno has stood by me despite the immense work load she seems to find herself under. Between advising her other students, teaching, and heading up many other projects she too was always available should I need her. And at times it was for sure that I did. She too offered many words of encouragement and scholarly advice. I must thank her specifically for all the knowledge she and her technician imparted to me about mycorrhizal fungi and research techniques in general. For all these things, as well as putting up with me, I thank her.

Beyond my advisors there is quite a list of people that I need to thank for all of their hard work. Perhaps, the length of the list is a reflection of the difficulties of working with me. Because this may be true, I owe all of the following people a debt of gratitude. Emily, Ana, Ashely, Jennifer, Sofia, Axel, Amanda, and anybody I may have forgotten I couldn't have done this without all of your help with my colonies, my field work, and all of the countless hours we spent identifying specimens. I know that I was at times intolerable and that the job you performed was often thankless and interesting only at the best of times. Regardless, I made it and it was because of your efforts. I'd like to thank Dolly Watson and Tomas Moreno specifically for all of their help. Dolly, your patience and willingness to teach and discuss my work with mycorrhizae was invaluable and you provided so many resource. Tomas, your knowledge of the FSRU and its happening was worth its weight in gold and for that you deserve many thanks. I'd also like to thank all the other people involved in the Greenhouse Gas project and all of the farm hands at CEFS for doing a bang up job despite all of the issues and dealing with a bunch of crazy scientists.

Lastly, I'd like to thank the USDA-NIFA for funding my research and SARE for helping fund some of my assistants. Outside of the fact that my project wouldn't exist without their funding they allowed me to collect two years' worth of data and helped give me the resources and time to excel as an academic and meet all of the demands of my research.

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## **CHAPTER 1 - Soil and foliar arthropod abundance and diversity in five cropping systems in the Southeastern United States.**

### **Introduction**

Approximately 40% of the land globally is dedicated to agriculture (Asner et al., 2004) and it is increasingly recognized that the expansion of intensive agriculture has led to reductions in global diversity of both, flora and fauna (Foley et al., 2005; Pimm and Raven, 2000). The demand on agricultural lands has increased dramatically, with global population increase necessitating greater production of food, feed and fiber, with ensuing negative impacts on biodiversity, and the ecosystem services provided therein (Altieri, 1999; Norris, 2008; Tscharntke et al., 2012). Preserving functional biodiversity in agroecosystems helps maintain key ecosystem services such as pollination, nutrient cycling, and natural pest control (Altieri, 1999; Cardinale et al., 2012; Flynn et al., 2009; Zhang et al., 2007). Thus, future increases in agricultural production will necessitate systems that balance net productivity with biodiversity conservation and ecosystem functioning.

Soil arthropod populations are sensitive to environmental disturbance and degradation and can serve as bio-indicators of management intensity within agricultural production systems (Burgio et al., 2015; Jerez-Valle et al., 2014). The response of arthropod diversity and abundance to agricultural production practices has been documented, and varies widely depending on the agricultural management practices (e.g. tillage, rotational scheme, organic vs. conventional) implemented. For instance, organic crop production increases species richness and evenness for ground beetles, ants, pollinators and natural enemies compared to conventional crop production, especially when pesticides are applied (Drinkwater et al., 1995; Dritschilo and Wanner, 1980; Krauss et al., 2011; Mone et al., 2014). Organic crop production also increases the density of

spiders in wheat over conventional fields (Schmidt et al., 2005). Furthermore, for some crops such as tomatoes, pest prevalence and damage are not different in organic systems compared to conventionally managed systems, yet organic systems maintain greater overall diversity (Letourneau and Goldstein, 2001). Similarly, organic and conventional maize and soybean systems produce statistically similar yields (Pimentel et al, 2005).

Not surprising, tillage has been demonstrated to reduce soil arthropod diversity (House and Alzugaray, 1989; Rodriguez et al., 2006) but also soil arthropod predation (Brust et al., 1986). Conventional tillage, generally accomplished by mold board plowing, has been shown to negatively impact arthropod populations while conservation tillage, i.e. tillage reduced in frequency, intensity, or field area, has elicited the opposite response. For instance, conventional tillage reduces generalist predators in the coleopteran families Carabidae and Staphylinidae as well as ground dwelling spiders in soybean and cereal fields (House, 1985; Thorbek and Bilde, 2004) corn, hairy vetch, crimson clover, and wheat grown in reduced- or no-tillage systems support higher numbers and diversity of soil arthropods, increased predation rates by natural enemies, and increased presence of natural enemies (Brust et al., 1986; House and Alzugaray, 1989; Rodriguez et al., 2006). No tillage systems have been proven to reduce numbers of western and northern corn rootworm larvae as well as reduce damage from rootworms over conventionally plowed systems (Gray and Tollefson, 1987; Stinner et al., 1986). However, increases in specific pest species have also been noted of reduced and no till systems when compared to conventional tillage (Tonhasca and Stinner, 1991; Willson and Eisley, 1992).

All the above studies illustrate that arthropod populations are responding to tillage however they are generally addressing a few targeted species or groups and are often within the context of factorial treatments as opposed to systems as a whole. Drinkwater (2002) discusses

how multiple factor experiments are complimentary to research addressing the effects of system level agricultural management (e.g. the sum of all agricultural management practices in a given agroecosystem) with each methodology having its own limitations and strengths. However, systems level research on both foliar and epigeic arthropod diversity and abundance is limited and warrants greater scientific attention. This is especially true in production systems for corn-soybean rotations commonly used in the Southeastern United States. The few studies published on the subject have until now focused on geographic regions other than the southeastern U.S. and on crops other than corn and soybeans. Even so, these studies indicate that organic and reduced tillage systems are more beneficial for soil and foliar arthropod biodiversity (Gkissakis et al., 2015; Henneron et al., 2015; Perez-Bote and Romero, 2012).

The overarching objective of this study then was to measure the effects of system level management programs common to the Southeastern United States on soil arthropod abundance and diversity in both corn (*Zea mays* L.) and soybeans (*Glycine max* (L.) Merr.). The five cropping systems studied: conventional clean till, conventional long rotation, conventional no till, organic clean till and organic reduced till. These systems vary in being organic vs. conventional management, tillage, herbicide use, the use of transgenic hybrids, and other factors which have been independently shown to impact soil and foliar arthropods. As such, we also measured the effects of these same cropping systems on beneficial and pest foliar arthropods on the same two crops. We hypothesize that organic vs. conventional management will be more predictive of management intensity on arthropod abundance and diversity than other factors (Figure 1).

## Materials and Methods

Research was conducted at the Center for Environmental Farming Systems, Goldsboro, NC (CEFS, <http://www.cefs.ncsu.edu/>), which was established in 1998 by a group of scientists at North Carolina State University (NCSU), North Carolina Agriculture and Technical State University (NCA&T), and North Carolina Department of Agriculture (NCDA) with major inputs of farmers. The Farming Systems Research Unit, FSRU, at CEFS comprises more than 200 acres and 5 different farming systems (Mueller et al. 2006). These systems include: a) Conventional clean Till (CCT) b) Conventional long rotation (CLR) c) Conventional no till (CNT), d) Organic clean till (OCT) and e) Organic reduced till (ORT). The field experiment is a completely randomized block design with three replicates for each of five farming systems. Each plot (i.e., field replicate) ranges from 1.2 to 3.8 ha (Mueller et al., 2002). The organic farming system finished its transition in 2002 and is certified with International Certification Services, Inc (Medina, North Dakota, USA). A total of 15 plots, representing 3 replicates of each of the aforementioned systems were sampled as part of this project.

The rotational schemes in place dictated planting to specific crops. As such, to prevent crop from being a confounding factor, plots not intended for corn or soybeans, in 2013 and 2014 respectively, received an 8 row micro-plot which ran the entire length of the field. All full plots or micro-plots were planted to corn on April 16<sup>th</sup>, 2013 during the first year of the study, and to soybeans on June 2<sup>nd</sup>, 2014 during the second year of the study. Organic plots were planted to ‘Augusta’ (Verona, VA) A7664 corn and ‘Hutcheson’ soybean varieties while conventional plots were planted to ‘Mycogen’ (Indianapolis, IN) 2T784 corn and Pioneer (Johnston, IA) ‘96M’ soybean varieties. The ‘Mycogen’ corn is a genetically modified (GM) cultivar marketed as “SmartStax” which contains 5 *Bacillus thuringiensis* producing genes 3 of which are active

against Lepidoptera and 2 active against Coleoptera. The Pioneer '96M' soybean cultivar is also a GM cultivar and is resistant to the herbicide glyphosate, also known as "Round Up" ready. All other agronomic practices executed within cropping systems i.e. cover crop kill, tillage, fertilization, for both soybeans and corn were derived from the North Carolina Corn Producers Guide (2000), Crop Profile for Soybeans in North Carolina (2005), and the North Carolina Organic Grain Production Guide (2013) for conventional and organic systems, respectively.

**Soil arthropod Sampling.** Soil arthropod population abundance and diversity were assessed by means of 3 pitfall traps per plot for 72hrs. Pitfall traps consisted of a plastic container measuring approximately 10.2 cm tall, 8.1 cm in diameter, and volume of approximately 500 ml whose design was modified from Pearce et al. (2005). Each trap was filled with 250ml of 50% ethanol solution (KOPTEC, Deacon Labs Inc., King of Prussia, PA) to catch and preserve the specimens during the 72h trap deployment period. Each trap was protected from rain and debris by a shelter made of corrugated plastic sheeting approximately 15 cm<sup>2</sup> with galvanized nails to hold the shelter 2.54 centimeters above the trap rim. The shelter was to prevent rain water overflowing the trap and washing specimens away.

A baseline sample for soil arthropod abundance and diversity was taken by deploying three pitfall traps/plot two days after planting for 72 hrs. in both corn and soybean. Four additional samplings were obtained throughout the growing season 1, 3, 6, and 12 weeks after corn planting and at 4, 8, 12, and 16 weeks after soybean planting. On each sampling date, three pitfall traps were set in each of fifteen plots approximately 1m apart from each other within the same row of corn or soybeans, depending on year. Sampled rows were haphazardly selected each sampling date making sure to avoid the two border rows at either side of the field or micro-plot, and being careful to select sites not sampled during the previous sampling. During each

collection date, the contents of all three pitfall traps from each plot were sieved through a fine organza fabric mesh to remove all liquid, then transferred to labeled Mason jars and covered with 70% ethanol. This resulted in one composite sample per plot per sampling period, for a total of 15 samples per sampling period.

**Sample processing and specimen identification.** Large specimens and organic debris were removed by decanting samples through a No. 16 and No. 80 standard testing sieves (1.19mm & 177 microns, W. S. Tyler Inc. U.S.A) and rinsing samples with tap water. Larger specimens were transferred out of the coarse sieve into a mason jar with 70% ethanol. The smaller organisms collected by the No. 80 sieve were separated from soil sediment using a sucrose flotation method adapted after Pask and Costa (1971). Supernatant containing small arthropods was decanted through a fine organza mesh (Bridal Inspirations White Organza, Jo-Ann's Fabrics) filter for collection. The sugar floatation process was repeated three times followed by gently rinsing the collected micro-arthropods with tap water to remove sucrose residues. Clean specimens were then rinsed into the Mason jar containing the larger specimens with 70% ethanol. The sample cleaning process was repeated for all jars collected throughout each field season.

Contents from each sample were observed under a dissecting microscope for specimen classification and abundance determination. Arthropods were identified to family level using identification keys in Borror and DeLong's Introduction to the Study of Insects (Triplehorn and Johnson, 2005), and BugGuide ([www.bugguide.net](http://www.bugguide.net)), as necessary. Specimens proving difficult to identify through these means were sent out for identification by experts at the NCSU Plant Disease and Insects Clinic (Raleigh, NC). Sample date, cropping system, replicate, arthropod order and family, and number of individuals per family were recorded.

**Foliar arthropod sampling.** To assess for the impact of these 5 cropping systems on foliar arthropod abundance and diversity, sampling was carried out for corn through direct visual inspection of the plants while sampling in soybeans was done using sweep netting only. A comprehensive visual scouting of corn was performed in all 5 crop production systems over 4 sampling times through the season at 8, 12, 14, and 16 weeks after planting and both foliar pests and beneficials were identified at least to order and family and counted. In soybeans, sweep net sampling was conducted 7 times throughout the season at 3, 5, 8, 9, 10, 12, and 14 weeks after planting in 2014 for all 5 crop production systems. Aerial sampling in corn and soybeans was performed in 3 randomly selected transects within each whole or micro-plot with 25 individual sweeps for soybeans or inspection of 10 corn plants per transect and one transect per third of total crop area. After the last sweep of each transect was taken in soybeans, the contents of the net were emptied into pre-labeled 3.78 L Ziploc bags, closed tightly, and placed in a cooler for transport to the lab. Samples were frozen in a -20 °C freezer for storage until processing. Frozen arthropod samples were emptied into plastic serving trays (NCT 1418 Carlisle Foodservice Products, Oklahoma City, OK) where specimens could be manually separated from organic debris and classified to family, genus, or species level, if possible, using identification keys in Borror and DeLong's Introduction to the Study of Insects (Triplehorn and Johnson, 2005), and BugGuide ([www.bugguide.net](http://www.bugguide.net)), as necessary.

**Statistical Analysis** Effects of cropping system (treatment), on log transformed overall soil arthropod abundance (summed across orders and sampling dates) was tested using a Poisson distribution in PROC GLIMMIX (SAS 9.4, Cary NC, 2013). Mean separations tests were performed on significant effects using the Tukey-Kramer adjustment for post-hoc test ( $P \leq 0.05$ ). Each sampling year was analyzed separately due to different crop species. Similarly, effects of

cropping system (treatment), sampling date, and their interactions on total (summed of all arthropods within a sampling date) soil arthropod abundance was tested using a Poisson distribution in PROC GLIMMIX (SAS 9.4, Cary NC, 2013). Mean comparisons were performed on significant effects using the Tukey-Kramer adjustment for post-hoc comparisons ( $P \leq 0.05$ ). The 2013 corn and 2014 soybean foliar samplings were analyzed in the same manner as the soil arthropod abundance.

## Results

**Soil Arthropod Diversity.** A total of 39,994 specimens were collected over the 2013 sampling season in corn. The specimens were classified into 15 different taxa including 10 orders of Insecta, 3 orders of Acari, 13 families of Araneae and 3 classes of Myriapoda. Cropping system (treatment) did significantly affect overall soil arthropod abundance in corn ( $F=1043.86$ ;  $df=4, 1$ ;  $P=.0232$ ) (Figure 2a). Conventional no till (CNT) had significantly greater overall abundance of soil arthropods compared to all other treatments except CLR (Figure 2a). Conventional long rotation (CLR) cropping systems had overall soil arthropod abundance equivalent to ORT, but significantly greater than CCT and OCT (Figure 2a). However, total (sum of all arthropods within a sampling date) soil arthropod abundance was not significantly affected by cropping system (treatment) but was significantly affected by sampling date ( $F=531.17$ ;  $df=4,51$ ;  $P<.0001$ ) and the interaction of cropping system and sampling date (not pictured) ( $F=344.33$ ;  $df=16,51$ ;  $P<.0001$ ) (Figure 2b). Total soil arthropod abundance was significantly higher 12 weeks after planting compared to all other sampling dates (Figure 2b). Interestingly, weeks 3 and 6 after planting showed the next highest soil arthropod abundance, which were both significantly higher

than those of weeks 6 and 1 (Figure 2b). Total soil arthropod abundance on week 6 was significantly higher than that of week 1 (Figure 2b).

The most predominant orders across systems sampled in corn were Acari (Figure 3a), Collembola (Figure 3b), Hymenoptera (Figure 3c), Coleoptera (Figure 3d), Diptera (Figure 3e), and Hemiptera (Figure 3f) making up 32.4, 26.6, 12.9, 11.1, 7.6, and 3.7% of the specimens collected respectively. Acari were especially dominant in CLR and CNT systems exceeding all other systems 3-5 fold, and especially low in the ORT system (Table 1). Spiders (Araneae) were  $\sim 2 \times$  more abundant in the CLR and ORT systems compared to all others. Similar to Acari, Collembola were dominant in CLR and CNT plots as well but unlike Acari were extremely abundant in the ORT system (Table 1). Interestingly, Coleoptera abundance was similar for all systems but CCT, which yielded the lowest abundance for this group (Table 1). Abundance of Hemiptera was equivalent across systems, with the exception of ORT, which yielded  $\sim 20 \times$  higher abundance for this group (Table 1), due to a cereal aphid outbreak early in the season. Generally, Diptera was not extremely abundant but the OCT system had more than  $10 \times$  the abundance of most other systems (Table 1). Like the Acari. Hymenoptera were most prevalent in the CLR and CNT systems and lowest in the ORT system (Table 1). Orthoptera and Thysanoptera were also found across cropping systems and showed a slightly higher abundance in the ORT system for Orthoptera, and in the OCT and CNT systems for Thysanoptera (Table 1).

The 2014 soybean soil arthropod sampling season yielded 37,119 total specimens. Total and overall soil arthropod abundances were not significantly affected by treatment, sampling date or their interaction (Figure 4a, 4b). However, total soil arthropod abundance did exhibit a trend in which less intensively managed systems i.e. conventional long rotation and organic reduced till systems, exhibited highest total soil arthropod abundance (Figure 4a). Conversely,

more intensively managed systems i.e. CCT, exhibited lowest total soil arthropod abundance (Figure 4a) in soybean fields. Conventional no tillage systems (CNT) exhibited an intermediate level of total soil arthropod abundance (Figure 4a). Overall abundance exhibited trends similar to that of total soil arthropod abundance means across production systems and were also not statistically different (Figure 4b).

The most predominant orders across soybean cropping systems sampled were Acari (Figure 5a), Collembola (Figure 5b), Hymenoptera (Figure 5c), Coleoptera (Figure 5d), Orthoptera (Figure 5e), and Diptera (Figure 5f) making up 30.7, 25.4, 22, 9.5, 3.4, and 2.7% of the specimens collected respectively (Figure 5). In soybeans, Acari were similarly high in CNT and ORT systems, however; the CLR system exhibited the highest Acari abundance compared to all systems (Table 2). Spiders (Araneae) yielded the highest abundance in the ORT system and showed equivalent abundance in all other cropping systems (Table 2). Coleoptera abundance was highest in the ORT system, followed by OCT, CLR, CCT and CNT (Table 2). Collembola abundance was far greater in the ORT, followed by CLR, with intermediate abundances for CNT and OCT and lowest abundance in the CCT system (Table 2). Interestingly, Diptera abundance was 1-2× higher for organic systems (OCT and ORT) compared to all other systems (Table 2). Hemiptera showed highest abundance in the ORT system, intermediate abundance in the CNT and CLR and lowest in the CCT and OCT (Table 2). Hymenoptera in soybeans were most prevalent in the CNT system, showing intermediate abundances for CCT, CLR, and ORT, and having the lowest abundance in the OCT system (Table 2). Orthoptera being in greater abundance, exhibited their highest abundances in ORT and CLR systems, and yielded similarly lower abundances across all other cropping systems (Table 2). Abundance of Thysanoptera was

highest in the ORT system, followed by CNT, OCT and CLR and yielding the lowest abundance in the CCT system (Table 2).

**Foliar Arthropod Diversity.** Foliar scouting for the 2013 corn season yielded 2,375 arthropod specimens of which 2,070 were classified as beneficial and 305 as pests based on feeding habits i.e. on plants or on other arthropods. The three most abundant pest taxa were *Rhopalosiphum padi* (Figure 6a) *Euschistus servus* (Figure 6b) and *Helicoverpa zea* (Figure 6c), making up 64.6 and 26.2 of all pest population respectively. The three most abundant beneficial taxa were Arachnida (Figure 6d), *Chrysoperla carnea* (Figure 6e), and *Orius insidiosus* (Figure 6f), making up 63.3, 13.5, and 10.3% of beneficial taxa, respectively. *Euschistus servus* abundance was relatively low regardless of cropping system being examined (Table 3). *Helicoverpa zea* abundance was greatest in OCT, being 3 × greater than the second highest system, CCT (Table 3). Abundance of *R. padi* was relatively low in all cropping systems except for the CLR, in which *R. padi* abundance exceeded the second highest system by more than 5 × (Table 3). Among the beneficial taxa, Arachnida abundance sequence was OCT > CLR > CNT > CCT > ORT (Table 3). On the other hand, *C. carnea* abundance was similarly high in abundance for CCT, CLR and CNT, intermediate abundance in OCT and the lowest abundance in ORT (Table 3). Abundance of *Coleomegilla maculata* was highest in CNT, followed by CCT and OCT and lowest in CLR and ORT (Table 3). *Hippodamia convergens* showed equivalently low abundance across cropping systems (Table 3). *Orius insidiosus* abundance values were similar across cropping systems (Table 3). Both pest and beneficial foliar arthropod abundance were highest in the CLR system followed by the OCT system and much lower in the ORT system in corn (Figure 7a).

Total foliar arthropod abundance in the 2013 corn growing season was significantly impacted by cropping system ( $F=6.34$ ;  $df=4, 8$ ;  $P=.0124$ ) but not by sampling date or its interactions with cropping system (Figure 8a). Conventional long rotation and organic clean till cropping systems exhibited significantly higher total foliar arthropod abundance than the ORT cropping system (Figure 8a). Conventional no till and conventional clean till cropping systems exhibited intermediate total foliar arthropod abundance (Figure 8a). Overall foliar arthropod abundance was not significantly impacted by cropping systems (Figure 8b). However, while overall abundance was equivalent across most cropping systems, there was a strong tendency for lower overall foliar abundance in the ORT system (Figure 8b).

The most predominant orders in soybean across cropping systems sampled were Diptera, Hemiptera, Orthoptera, Coleoptera, and Araneae making up 42.7, 32.9, 7.8, 7.6, and 4.4% of the total specimen count respectively. Araneae was highest in the CLR system, followed by the ORT, CNT systems with CCT and OCT exhibiting lowest Araneae abundance (Table 4). Coleoptera abundance was highest in the OCT system and lowest in the CLR system but similar for all other systems, which exhibited intermediate abundance (Table 4). Diptera abundance was greatest in the CLR system, followed by the ORT and CNT and lowest in the both clean tilled systems, CCT and OCT (Table 4). Hemiptera abundance sequence was  $ORT > CCT = OCT > CNT > CLR$  (Table 4). Hymenoptera abundance was slightly higher in the CLR, CNT and ORT systems compared to the other two systems (Table 4). Lepidoptera abundance ranged from 16-31 specimens across systems (Table 4). Interestingly, Orthopteran abundance was over 2× as high in the ORT system than all other systems which exhibited similar abundances to each other (Table 4). Thysanoptera exhibited low abundances across cropping systems but were highest in CNT and OCT compared to all other systems (Table 4). Pest

abundance in soybean was highest in the ORT, followed by CLR which was equivalent to CNT, CCT and OCT (Figure 7b). Beneficial arthropod abundance in soybean yielded similar abundance across cropping systems (Figure 7b). Total abundance of foliar arthropods in soybeans was significantly impacted by cropping system (treatment) ( $F=22.92$ ;  $df=4,10$ ;  $P<.0001$ ), sampling date ( $F=213.57$ ;  $df=6,71$ ;  $P<.0001$ ), and their interaction ( $F=50.26$ ;  $df=24,71$ ;  $P<.0001$ ) (Figures 9a, 9b). The organic reduced till (ORT) system yielded the highest total foliar arthropod abundance and was significantly higher than for all other systems (Figure 9a). Conventional long rotation (CLR) showed the second highest total foliar arthropod abundance which was significantly lower than ORT, and significantly greater than OCT and CCT systems, but not significantly different from CNT (Figure 9a). Total foliar arthropod abundance was significantly higher 14 weeks after planting than all previous dates (Figure 9b). Samples obtained 3 and 8 weeks after planting yielded significantly lower total foliar arthropod abundance than all other sampling dates but were not different from each other (Figure 9b). Samplings at 5 and 9 weeks after planting exhibited the next highest total foliar arthropod abundance and were not different from each other (Figure 9b). Sampling dates 10 and 12 weeks after planting exhibited two intermediate but statistically different levels of total foliar arthropod abundance (Figure 9b). Unlike in corn, soybean overall foliar arthropod abundance was also impacted by cropping system ( $F=25.8$ ;  $df=4,9.91$ ;  $P<.0001$ ) and mean separations followed the trends of total foliar arthropod abundance (Figure 10).

### **Discussion**

Results from our study show significant impacts of cropping system, sampling date and their interaction, on soil and foliar arthropod abundance, which are dependent on the cash crop being sampled. Total soil arthropod abundance in corn was significantly impacted by sampling

date and the interaction between date and cropping system while overall soil arthropod abundance was affected by cropping system. In contrast, soil arthropod abundance was not significantly impacted by cropping system or sampling date in soybeans.

We expected that abundance and diversity of various soil arthropods would be lower in tilled conventionally managed systems than in reduced or no-till systems (House and Alzugaray 1989; Rodriguez et al. 2006). Greater overall soil arthropod abundance was observed in corn in the CLR and CNT systems compared to CCT, OCT, ORT systems. This observed greater overall soil arthropod abundance exhibited by the CLR and CNT systems in corn could be a product of reduced cultivation events (i.e., tillage) and increased organic residue accumulation on the soil surface in both systems. Tillage and the fate of crop residues have been documented to impact soil arthropod abundance with conservation i.e. reduced or no tillage, generally increasing diversity, though some taxon specific disparate responses have been reported (House, 1985; Stinner and House, 1990; Tonhasca and Stinner, 1991). The high soil arthropod overall abundance of the CLR and CNT systems in corn resulted from increased numbers of Acari, Collembola, and Hymenoptera. Positive responses in Collembola and Acari populations have been reported in no tillage sorghum systems, in which increased abundance of both groups were found in no tillage compared to conventional tillage production systems (House and Parmelee, 1985). Complete surface shading by the crop, in addition to the use of herbicides in the CCT system, kept rows mostly devoid of weeds which may explain why this system exhibited lower arthropod abundance. As agricultural landscapes move further from natural habitat to more monotypic landscapes, species diversity is reduced (Attwood et al. 2008). This is a fair description of our CCT systems, which had no cover crop residues and little to no plant diversity due to herbicide use. Further supporting the negative impacts of tillage events on soil arthropods,

the timing of the cultivations in OCT and CCT systems during our study precedes the observed depressions in arthropod abundance, which suggests the effects of sampling date on total soil arthropod abundance in corn may have indeed resulted from management practices. In the ORT system, during the 2013 corn season, the roller crimping of the leguminous cover crops was unsuccessful in killing the cover crop and resulted in the need for two previously unscheduled cultivation events during the growing season. These extra cultivations of cover crop may be the driving factor in the reduced arthropod abundance observed for this system in corn. Certain groups of soil arthropods are more responsive and negatively affected by tillage disturbance. For example, abundance of Acari in ORT corn systems was  $7.5 \times$  lower than that of the reduced and no till systems. In our study OCT and CCT systems yielded lower order abundances of Acari, Collembolans, and Hymenoptera as well as Araneae, Orthoptera, and Hemiptera compared to CLR and CNT systems, although we saw no significance. Given the propensity of Acari abundance and diversity to be lowered in response to tillage or other cultivations (Rodriguez et al., 2006; Sapkota et al., 2012), it is possible the effects of unscheduled cultivation on Acari and other sensitive groups heavily influenced total arthropod abundance in the ORT systems.

Similar trends to those in corn in total soil arthropod abundance were observed in soybeans across production systems, except for the ORT systems. Compared to corn, the opposite trend was observed in the ORT system during the 2014 growing soybean season. That is, this production system exhibited the highest total soil arthropod abundance in soybean. We attribute this difference to the fact that the roller crimping of cover crops was fully successful in killing the cover crop in 2014 and thus, no additional unscheduled cultivation events were necessary during the soybean growing season, which resulted in the ORT system having higher total soil arthropod abundance compared to other systems. The main contributors to the trend of

high abundance of soil arthropods in ORT soybean systems in soybeans were Acari and Collembola, with contributions from other taxa, including Araneae, Orthopterans, and Thysanoptera, as well.

In addition to the direct effects of tillage on soil arthropods, Stinner et al. (1986) showed that tillage also has indirect influences on foliar arthropod pest and predator abundance, though in a taxon specific manner. Taxon specific increases in foliar arthropod abundance have been shown for pests and predators of maize agroecosystems (Brust and King, 1994) as well as of conservation tillage soybean systems (Hammond and Stinner, 1987). Similar to the soil arthropod results, foliar arthropod abundance in corn was responsive to cropping system. The OCT system yielded the highest total foliar arthropod abundance in corn. This was largely due to the high incidence of Araneae (spiders, beneficial) and larval *Helicoverpa zea* (corn earworm, pests). Given that conventional systems were planted with a *Bt* cultivar it was expected for OCT and ORT systems to exhibit greater incidence of Lepidoptera pests, such as *H. zea*, compared to all conventional systems in corn. Surprisingly, higher incidence of *H. zea* was only recorded in the OCT systems and not in the ORT systems for corn. These disparate results could be attributed to poor overall corn crop performance in the ORT systems, due to roller crimper failure to kill the cover crop, which created competition between the cover crop and the cash crop. Reduction in *H. zea* populations is the major contributor to the lower abundance in foliar arthropods in the ORT systems. The CLR corn system had comparable total foliar arthropod abundance to the OCT system but as a result of large numbers of aphids (*Rhopalosiphum padi*), green lacewings (*Chrysoperla carnea*) and minute pirate bugs (*Orius* spp). The presence of these three taxa is interrelated as it has been shown in other crops, such as cotton, that numbers of cotton aphid predators, which included both *Chrysoperla* and *Orius* spp., spiked following

increases in aphid population abundance (Shrestha and Parajulee, 2013). The ORT system, having the lowest abundance of foliar arthropods, exhibited significantly fewer *Chrysoperla* spp., *Orius* spp., and Araneae. These aforementioned arthropod groups, with the addition of *Coleomegilla maculata*, were also responsible for the large increase in foliar abundance 12 weeks after planting. Thorbek and Bilke (2004) showed that reductions in arthropod abundance continue for several weeks following crop management practices. Given that tillage generally reduces foliar arthropod abundance and that the execution of in-field residue and weed management coincides with reduction in foliar arthropod abundance it would seem likely that tillage practices are responsible for variations in total foliar arthropod abundance within the systems over time.

Foliar dwelling arthropods in soybeans were also impacted by date and cropping system but, unlike in corn, closely resembled the results of soil dwelling arthropods in soybeans. That is to say, the least intensively managed systems, like ORT and CLR, exhibited the highest foliar arthropod abundance while more intensively managed systems like the OCT and CCT systems exhibited the lowest total foliar arthropod abundance, with CNT systems being intermediate. Similarly, peaks and depressions in foliar abundance seemed to vary in response to tillage events in each of the systems. Lundgren et al. (2013) showed that systems with cover crops or reduced chemical inputs exhibited increased activity density and abundance of predators and soybean aphids compared to high chemical inputs and clean tillage which generally showed the inverse.

In addition to variations in total foliar arthropod abundance, pest and beneficial populations were also dynamic within cropping systems, especially for corn. Conventional clean till and CNT systems exhibited much lower pest abundance compared to beneficial abundance while CLR, ORT, and OCT systems had similar pest and beneficial abundance. Pest and

beneficial populations within the soybean systems were all very similar across cropping systems save for both organic systems which exhibited trends for higher pest abundance in relation to their respective beneficial arthropod abundance. In a meta-analysis by Garratt et al. (2011), both pests and natural enemies show significant positive responses to organic and low intensity agricultural systems. From our data we cannot confirm that organic systems positively impact both pest and beneficial populations given that foliar arthropod abundance generally was not consistent within years. Our data does confirm however, that systems incurring lower management intensity exhibit higher pest and beneficial arthropods and by extension total foliar arthropod abundances, which is in accordance with the findings of Garratt et al., 2011.

Our results suggest that both foliar and soil arthropod abundance are affected by management practices on a production system scale, at least in the coastal plains region of North Carolina. It is also evident that arthropod populations also exhibit temporal variation. Generally, our data suggests that systems that employ reduced disturbance (i.e. tillage) and are less intensively managed, tend to sustain increased abundance of soil and foliar arthropods. Therefore, it appears conserving arthropod diversity and arthropod dependent ecosystem services can be accomplished by implementing production systems that include alternative management practices, such as no tillage and long rotational schemes.

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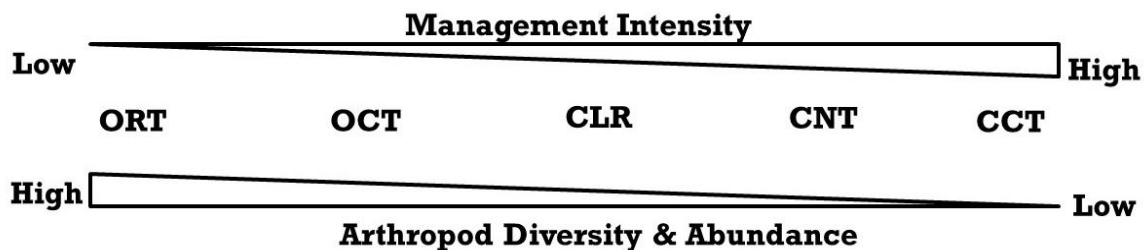
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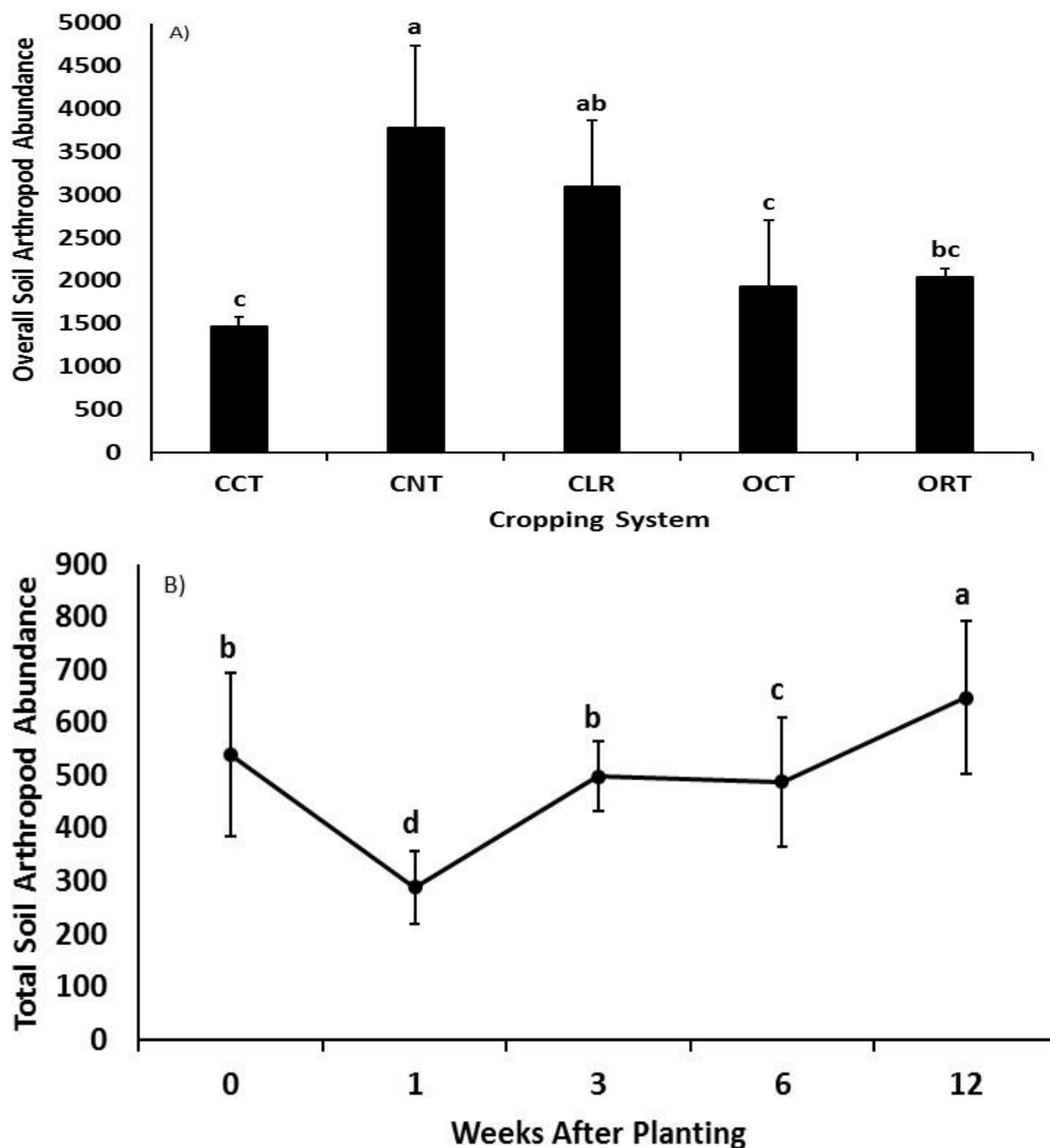
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## FIGURES



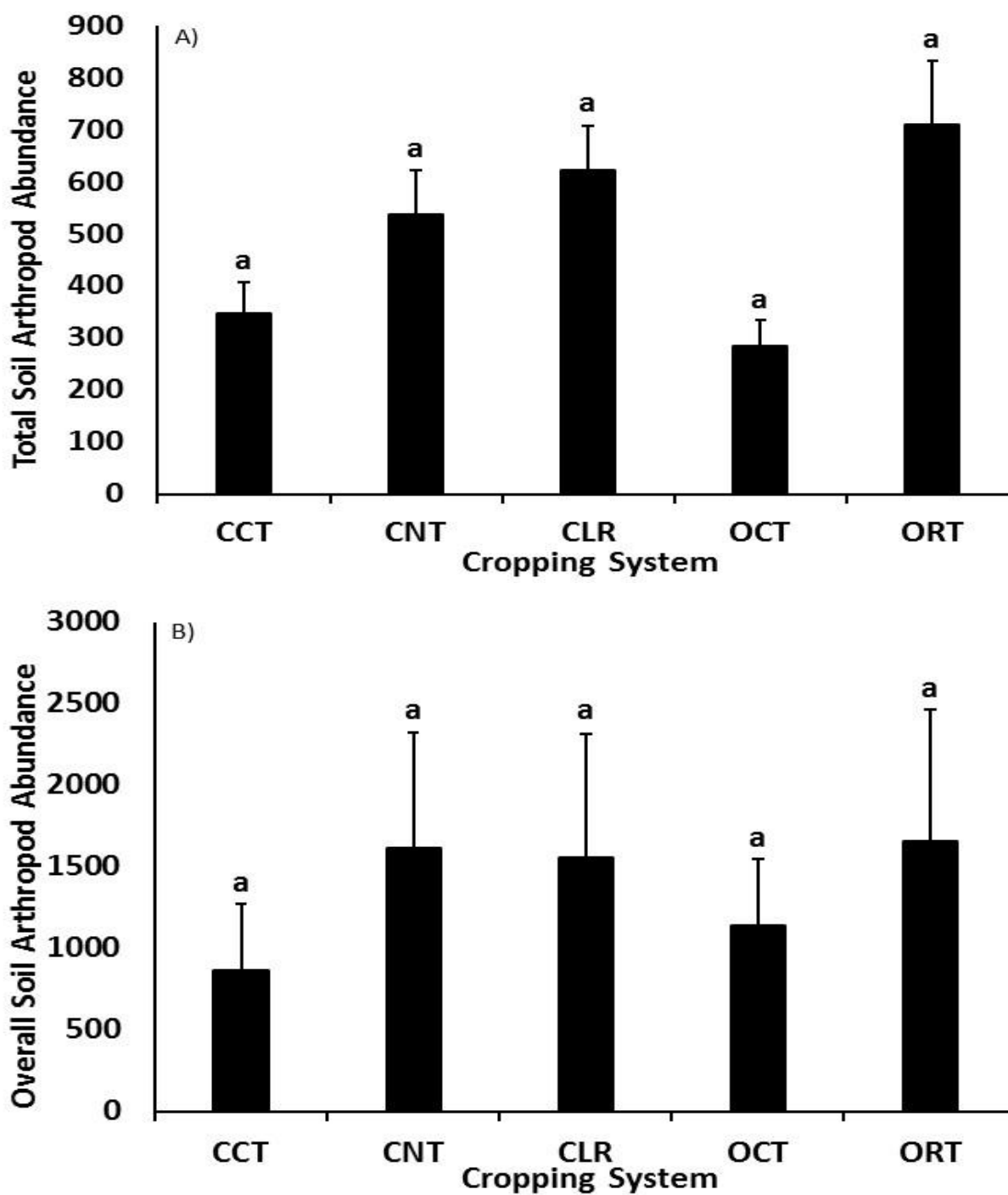
**Figure 1.1.** Hypothesized responses of arthropod abundance and diversity with changing management intensity across the 5 cropping systems. Investigated systems appear in the expected order of abundance and diversity in the center of the diagram as Organic reduced till (ORT), organic conventional till (OCT), conventional long rotation (CLR), conventional no till (CNT) and conventional clean till (CCT)



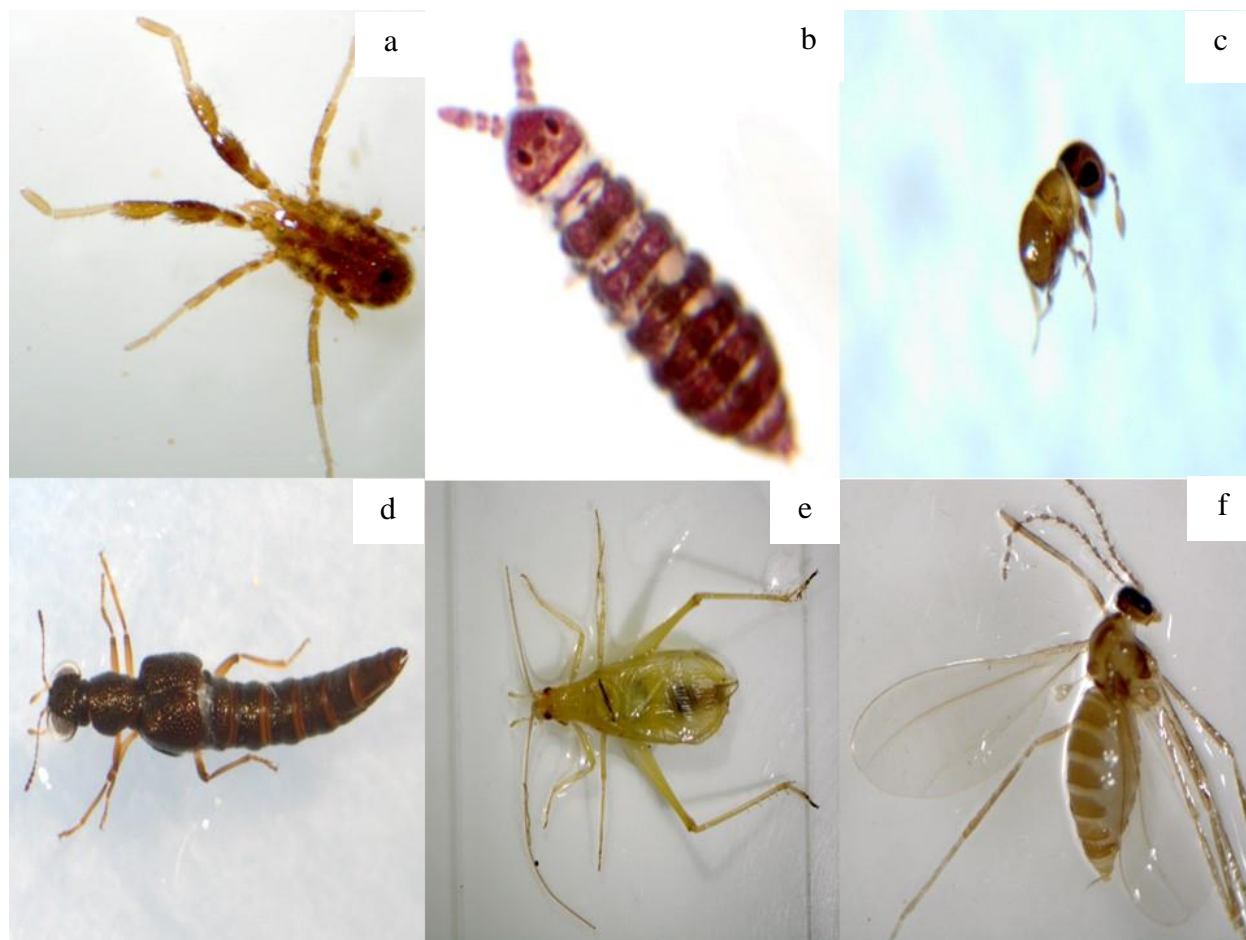
**Figure 1.2.** Effects of cropping system on **A)** overall soil arthropod abundance and effect of sampling date on **B)** total soil arthropod abundance for the 2013 corn growing season. Bars represent means with error bars representing standard error. Bars headed by the same letter are not significantly different, Tukey-Kramer mean separation test ( $\alpha = 0.05$ )

**Figure 3**

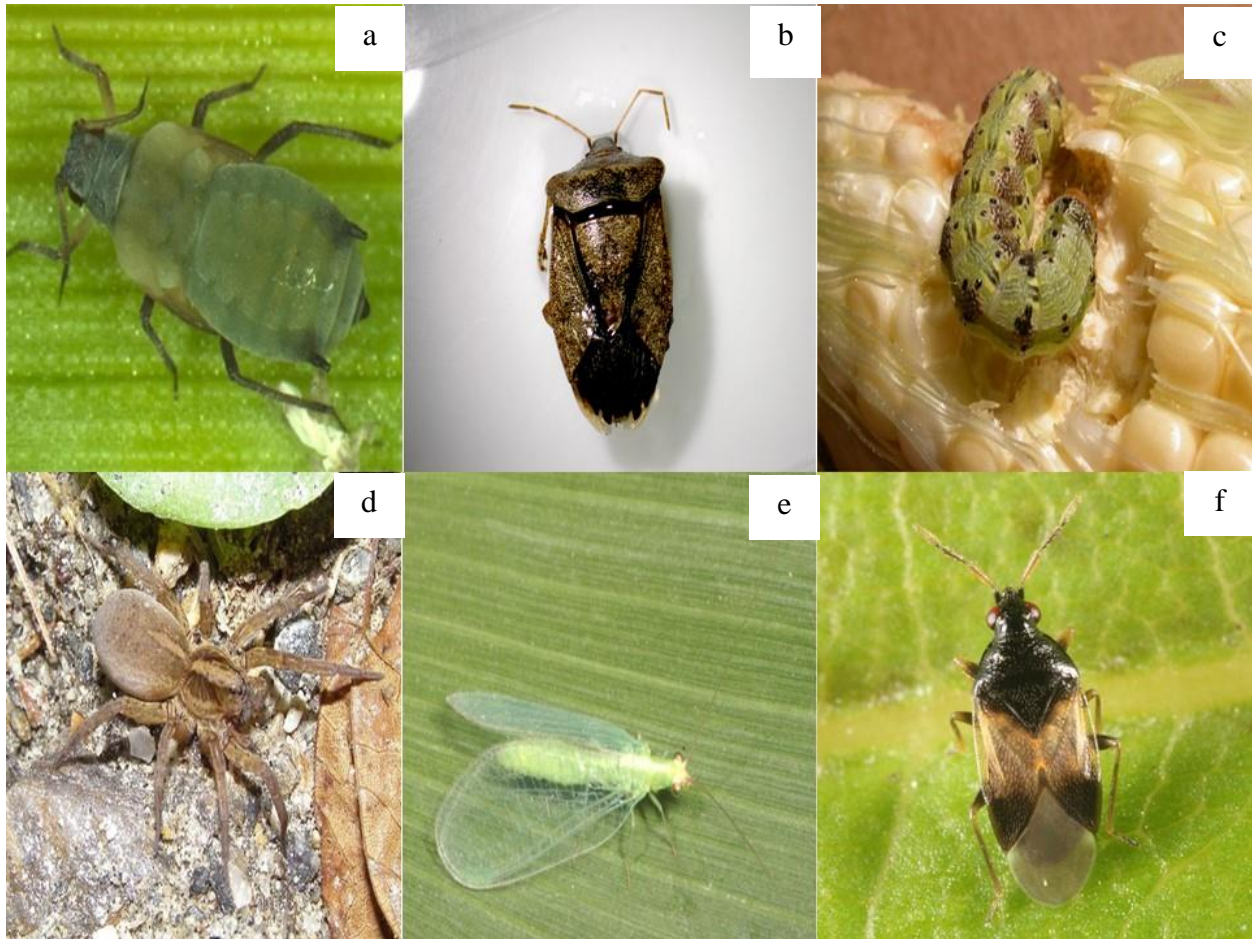
**Figure 1.3.** Representative specimens of the six most prevalent soil arthropod orders found in pitfall traps during the 2013 corn growing season: a) Acari, b) Collembola, c) Hymenoptera, d) Coleoptera, e) Diptera, and f) Orthoptera



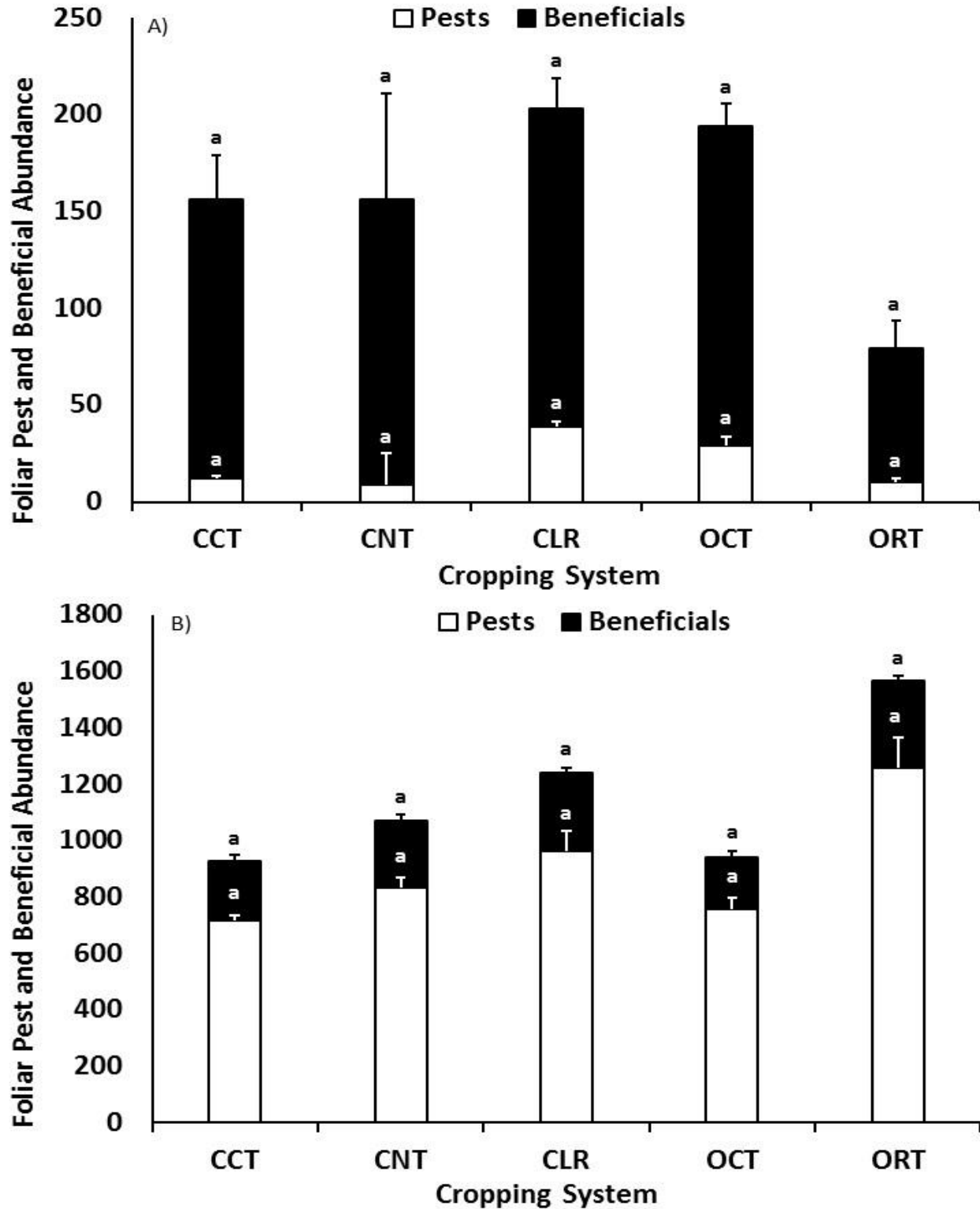
**Figure 1.4.** Means and standard errors for trends observed in A) total and B) overall soil arthropod abundance for the 2014 season in soybeans. No significant effects of were detected for these two variables at the ( $\alpha = 0.05$ ) level with Tukey-Kramer mean separation test.



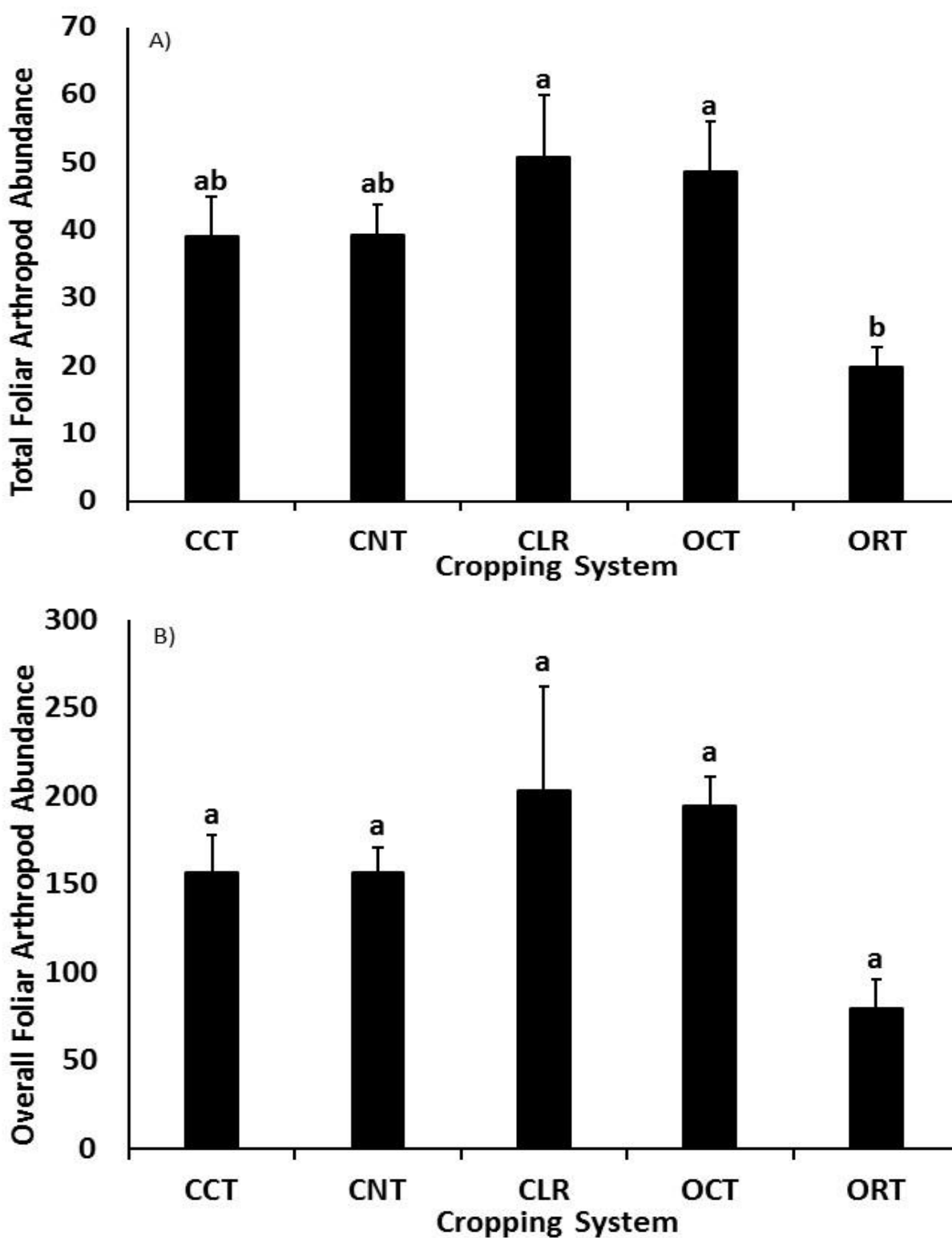
**Figure 1.5.** Representative specimens of the six most abundant soil arthropod orders found in pitfall traps during the 2014 soybean growing season: a) Acari, b) Collembola, c) Hymenoptera, d) Coleoptera, e) Orthoptera, and f) Diptera.



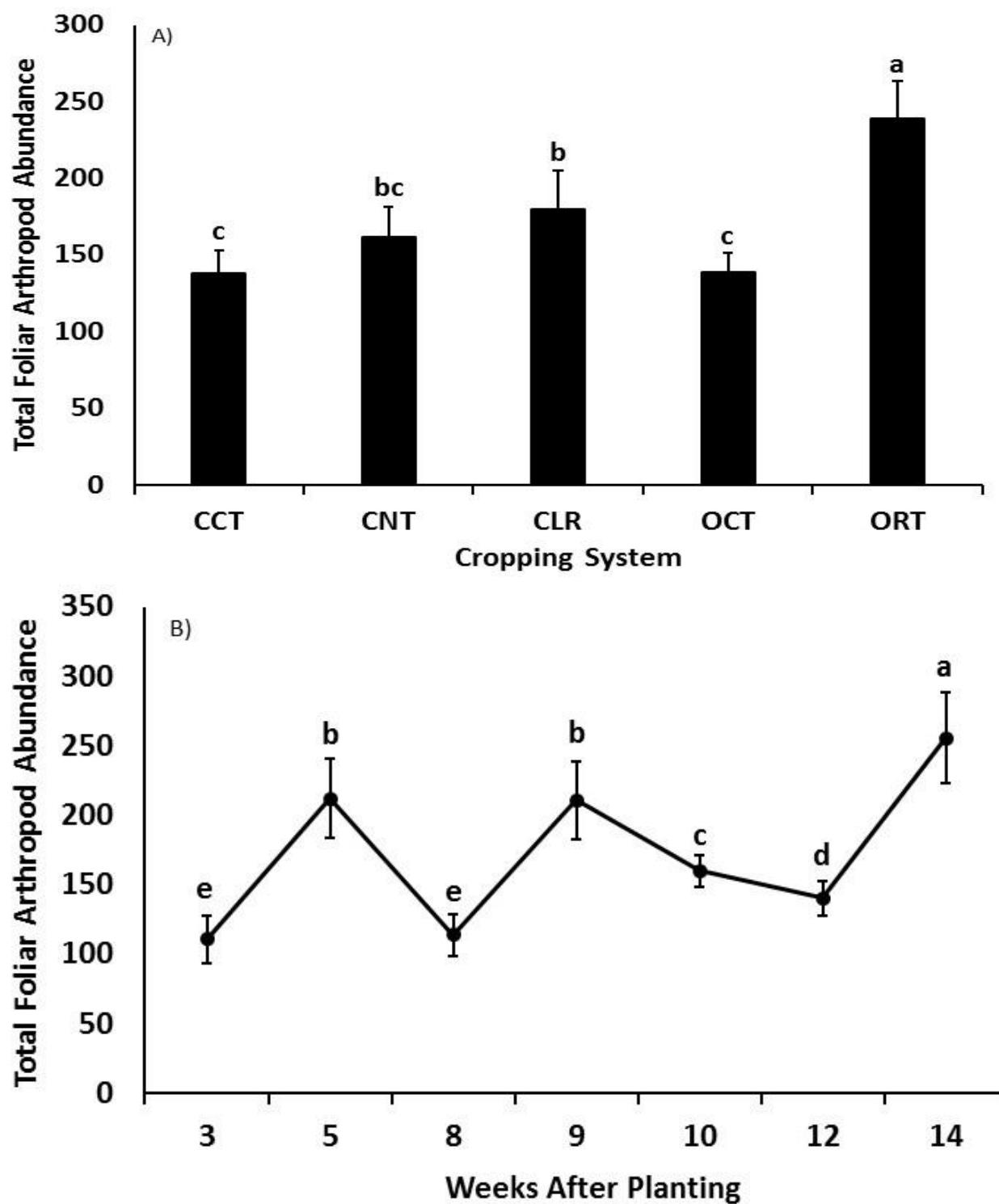
**Figure 1.6.** Representative specimens for the three most abundant foliar pest and beneficial arthropod taxa found during the 2013 corn growing season: Top row left to right show most abundant pest species: a) *Rhopalosiphum padi* (Hemiptera: Aphididae) (L.), b) *Euschistus servus* (Hemiptera: Pentatomidae) (Say) and c) *Helicoverpa zea* (Lepidoptera: Noctuidae) (Boddie). Bottom row shows representatives of the most abundant beneficial organisms: d) Arachnida (Aranea), e) *Chrysoperla carnea* (Neuroptera: Chrysopidae) (Stephens), and f) *Orius insidiosus* (Hemiptera: Anthicoridae) (Say).



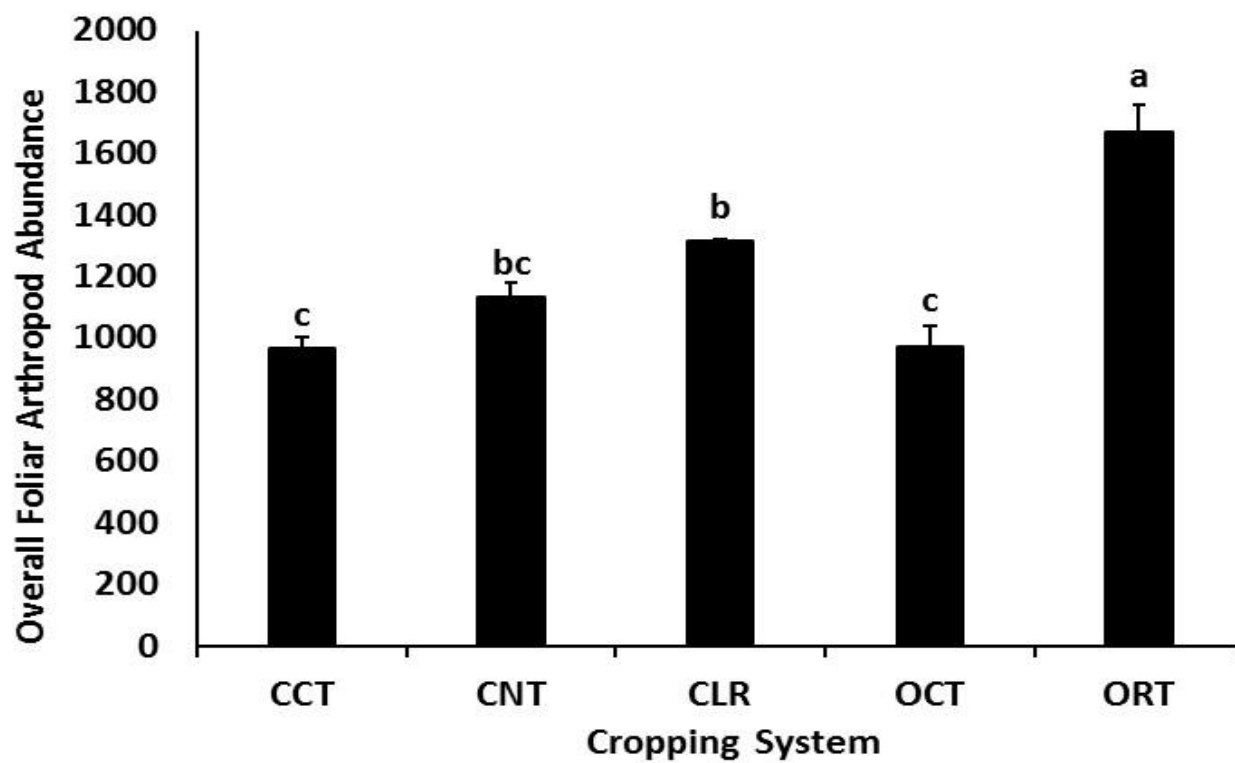
**Figure 1.7.** Effects of cropping system on **A)** corn foliar pest and beneficial arthropods and **B)** soybean foliar pest and beneficial arthropods. Bars represent means with error bars representing standard error.



**Figure 1.8.** Effects of cropping system on **A)** total foliar arthropod abundance and **B)** means and standard errors for overall foliar arthropod abundance in corn. Bars headed by the same letter are not significantly different, Tukey-Kramer mean separation test ( $\alpha = 0.05$ ).



**Figure 1.9.** Effects of **A)** cropping system and **B)** sampling date on total foliar arthropod abundance in soybean during the 2014 season. Bars headed by the same letter are not significantly different according to Tukey-Kramer mean separation test ( $\alpha = 0.05$ ).



**Figure 1.10.** Effects of cropping system on overall foliar arthropod abundance in soybeans. Bars headed by the same letter are not significantly different according to Tukey-Kramer mean separation test ( $\alpha = 0.05$ ).

## TABLES

**Table 1.1.** Overall soil arthropod order abundance (Means±SE) across cropping systems for the 2013 growing season in corn.

<b>Order</b>	<b>CCT</b>	<b>CNT</b>	<b>CLR</b>	<b>OCT</b>	<b>ORT</b>
Acari	403.0±151.28 a	1793.7±941.19 a	1293.0±664.60 a	261.0±93.03 a	239.0±16.50 a
Araneae	42.3±9.74 a	53.7±6.69 a	100.7±10.17 a	62.0±16.17 a	92.3±5.04 a
Coleoptera	381.0±70.50 a	235.0±21.07 a	203.0±42.23 a	302.7±76.79 a	249.0±23.30 a
Collembola	273.7±89.25 a	1202.3±493.12 a	654.3±166.86 a	388.3±97.20 a	767.0±126.75 a
Diptera	66.7±8.41 a	43.3±2.60 a	50.7±15.03 a	664.3±552.34 a	109.3±19.46 a
Hemiptera	24.7±9.28 a	16.3±1.20 a	18.0±7.21 a	14.7±4.41 a	385.0±167.18 a
Hymenoptera	235.3±29.90 a	333.3±55.73 a	728.7±198.95 a	163.0±14.47 a	125.7±23.55 a
Orthoptera	5.3±0.67 a	17.3±6.94 a	5.0±0.58 a	9.3±7.36 a	27.7±13.57 a
Thysanoptera	35.0±8.96 a	73.3±31.47 a	40.7±5.67 a	61.7±24.31 a	48.0±2.65 a

**Table 1.2.** Overall soil arthropod order abundance (means  $\pm$  SE) across cropping systems for the 2013 growing season in soybean.

<b>Order</b>	<b>CCT</b>	<b>CNT</b>	<b>CLR</b>	<b>OCT</b>	<b>ORT</b>
Acari	552.0 $\pm$ 142.08 a	772.3 $\pm$ 285.21 a	1316.0 $\pm$ 357.53 a	457.7 $\pm$ 182.25 a	703.0 $\pm$ 111.08 a
Araneae	35.7 $\pm$ 7.36 a	54.0 $\pm$ 6.35 a	62.3 $\pm$ 4.48 a	54.7 $\pm$ 21.26 a	105.0 $\pm$ 8.89 a
Coleoptera	216.0 $\pm$ 18.01 a	139.3 $\pm$ 10.33 a	215.3 $\pm$ 63.06 a	253.7 $\pm$ 15.21 a	355.0 $\pm$ 37.65 a
Collembola	248.3 $\pm$ 43.11 a	470.3 $\pm$ 55.25 a	774.0 $\pm$ 16.37 a	419.0 $\pm$ 145.81 a	1228.3 $\pm$ 33.72 a
Diptera	70.3 $\pm$ 4.98 a	44.7 $\pm$ 3.18 a	51.3 $\pm$ 8.09 a	83.7 $\pm$ 9.82 a	82.0 $\pm$ 9.61 a
Hemiptera	4.3 $\pm$ 1.20 a	35.0 $\pm$ 28.51 a	23.7 $\pm$ 3.38 a	9.3 $\pm$ 1.20 a	59.3 $\pm$ 11.33 a
Hymenoptera	548.0 $\pm$ 367.66 a	1064.0 $\pm$ 356.27 a	506.3 $\pm$ 224.69 a	142.0 $\pm$ 38.48 a	467.0 $\pm$ 161.98 a
Orthoptera	26.0 $\pm$ 10.12 a	44.3 $\pm$ 16.70 a	109.7 $\pm$ 35.00 a	38.7 $\pm$ 13.02 a	207.7 $\pm$ 62.09 a
Thysanoptera	18.7 $\pm$ 2.67 a	60.7 $\pm$ 24.50 a	44.0 $\pm$ 3.06 a	56.7 $\pm$ 25.20 a	108.0 $\pm$ 19.08 a

**Table 1.3.** Foliar arthropod species abundance (means  $\pm$  SE) across cropping systems for the 2013 growing season in corn.

Type	Insect	CCT	CNT	CLR	OCT	ORT
Pest	<i>E. servus</i>	3.7 $\pm$ 0.67 a	4.7 $\pm$ 1.76 a	4.3 $\pm$ 0.67 a	6.0 $\pm$ 2.52 a	5.7 $\pm$ 2.2 a
	<i>H. zea</i>	5.0 $\pm$ 0.58 a	2.3 $\pm$ 2.33 a	2.3 $\pm$ 0.88 a	14.3 $\pm$ 2.60 a	2.7 $\pm$ 0.3 a
	<i>R. padi</i>	2.0 $\pm$ 0.58 a	1.3 $\pm$ 0.33 a	32.0 $\pm$ 16.29 a	5.7 $\pm$ 5.17 a	0.3 $\pm$ 0.3 a
Beneficial	Araneae	81.3 $\pm$ 17.07 a	90.3 $\pm$ 10.93 a	106.3 $\pm$ 55.05 a	119.0 $\pm$ 11.53 a	40.0 $\pm$ 9.29 a
	<i>C. carnea</i>	26.7 $\pm$ 6.17 a	22.3 $\pm$ 4.26 a	28.3 $\pm$ 4.81 a	13.7 $\pm$ 3.84 a	2.0 $\pm$ 0.00 a
	<i>C. maculata</i>	11.7 $\pm$ 2.73 a	17.3 $\pm$ 5.24 a	6.0 $\pm$ 4.04 a	11.3 $\pm$ 2.67 a	7.3 $\pm$ 1.86 a
	<i>H. convergens</i>	3.7 $\pm$ 1.67 a	2.0 $\pm$ 1.53 a	1.0 $\pm$ 1.00 a	1.7 $\pm$ 0.88 a	2.0 $\pm$ 0.58 a
	<i>O. insidiosus</i>	16.0 $\pm$ 1.73 a	12.3 $\pm$ 1.88 a	19.0 $\pm$ 4.93a	14.0 $\pm$ 1.73 a	10.0 $\pm$ 5.51 a

**Table 1.4.** Foliar arthropod order abundance (Means  $\pm$  SE) across cropping systems for the 2014 growing season in soybeans.

<b>Type</b>	<b>Insect</b>	<b>CCT</b>	<b>CNT</b>	<b>CLR</b>	<b>OCT</b>	<b>ORT</b>
Pest	Acrididae	51.0 $\pm$ 34.51 a	65.7 $\pm$ 22.45 a	50.3 $\pm$ 9.17 a	29.7 $\pm$ 12.44 a	83.7 $\pm$ 30.46 a
	<i>S. festinus</i>	32.3 $\pm$ 5.90 a	61.0 $\pm$ 21.78 a	46.0 $\pm$ 24.34 a	26.0 $\pm$ 1.15 a	29.3 $\pm$ 7.17 a
	<i>C. trifurcata</i>	47.3 $\pm$ 11.78 a	31.7 $\pm$ 8.09 a	16.7 $\pm$ 3.48 a	94.0 $\pm$ 21.17 a	11.0 $\pm$ 2.52 a
	Miridae	42.0 $\pm$ 4.93 a	17.7 $\pm$ 5.84 a	23.0 $\pm$ 11.79 a	63.7 $\pm$ 12.02 a	62.3 $\pm$ 30.01 a
Beneficial	Dolichopodidae	92.0 $\pm$ 14.15 a	115.3 $\pm$ 6.33 a	105.7 $\pm$ 22.18 a	64.3 $\pm$ 17.70 a	83.0 $\pm$ 13.11 a
	<i>Geocoris spp.</i>	38.7 $\pm$ 8.11 a	8.0 $\pm$ 2.08 a	5.3 $\pm$ 2.03 a	20.7 $\pm$ 9.84 a	49.0 $\pm$ 8.89 a
	Oxyopidae	7.7 $\pm$ 2.19 a	12.3 $\pm$ 1.67 a	26.3 $\pm$ 3.93 a	4.7 $\pm$ 0.33 a	16.7 $\pm$ 2.03 a
	Salitricidae	9.0 $\pm$ 1.53 a	16.0 $\pm$ 4.04 a	13.7 $\pm$ 1.45 a	8.0 $\pm$ 2.00 a	13.0 $\pm$ 2.00 a

## CHAPTER 2 - Effects of nitrogen fertilization and root mycorrhization on the performance of *Helicoverpa zea* and *Dalbulus maidis* in corn (*Zea mays*, L.)

### Introduction

Corn, *Zea mays* L. is inarguably one of the most important field crops grown globally, primarily for animal feed, but also for human consumption and the production of numerous products (USDA ERS 2013, NCGA 2013). The United States produced almost 14 billion bushels of corn worth nearly 62 billion dollars in 2013 and over 14 billion bushels worth over 52 billion dollars in 2014, contributing the largest percentage of corn grown globally (USDA ERS 2014, USDA NASS 2014). However, worldwide corn productivity is severely limited by lepidopteran pests including the corn earworm (CEW), *Helicoverpa zea*, Boddie (Lepidoptera: Noctuidae) and by corn diseases vectored by phloem sucking insects such as the corn leafhopper (CLH), *Dalbulus maidis*, DeLong & Walcott (Hemiptera: Cicadellidae) (Ortega, 1987).

The corn earworm is a generalist chewing pest that damages corn by direct consumption of corn plant tissues while the corn leafhopper is a phloem feeding pest, generally accepted as a corn specialist, which is capable of vectoring several known diseases of corn such as corn stunt spiroplasma and maize bushy stunt spiroplasma, that result in stunting and yield loss (Boddie, 1850; Ebbert and Nault, 2001; Nault et al., 1980). Conventional methods for controlling these pests include insecticidal spraying and transgenic corn hybrids. However, insecticides impart large selection pressures which result in evolutions of resistance in the target pests. On the other hand, transgenic corn hybrids carrying *Bacillus thuringiensis* (*Bt*) traits, though effective on lepidopterans such as the CEW, have not been shown to be effective against CLH (Siebert et al., 2012). Furthermore, Bt technology is only a viable option for conventional farmers and there exists the potential of resistance development.

The inherent lack of a singular solution to controlling these and other pests and the consequences of using insecticides under such a mentality spurred the integrated pest management (IPM) movement of the 1970's. The tenants of this movement recognized the need to acknowledge the dynamics of the system as a whole and to only intervene with insecticides and other management tactics when naturally derived services proved to be inadequate (Lewis et al., 1997). Addressing intensive, conventional agriculture's negative impacts on biodiversity, ecosystems, and the services provided therein, remains a focus of interest (Norris, 2008; Tscharrntke et al., 2012). To reduce the negative impacts of intensive agricultural on biodiversity and ecosystem services, Altieri et al. (1999) and Krauss et al. (2011) have suggested adoption of less intensive agricultural practices, such as organic farming, reduced pesticide use, decreased mechanical perturbations, and biologically based practices which conform to the philosophy of IPM. An example being the use of plant growth promoting organisms like bacteria or mycorrhizas to help aid crop plants in gaining nutrition and fighting disease.

Arbuscular mycorrhizal fungi (AMF), class Glomeromycetes, are ubiquitous in soils and associate with approximately 80% of plant roots examined worldwide, including many agricultural crops (Wang and Qiu, 2006), making them important for biologically based agricultural systems, yet understudied,. Benefits of AMF root colonization to host plants include increased nutritional status, abiotic stress mediations, and defense priming against various pests (Smith and Read, 1997). More specifically, AMF has been shown to increase phosphorus (P) (Moss et al., 1973) and micronutrient uptake (Clark and Zeto, 2000), while alleviating salt (Poss et al., 1985), heavy metal (Weissenhorn et al., 1995), and drought stresses (Ruiz-Lozano et al., 2001).

Mycorrhizae root colonization is also reported to impact insect herbivore performance in a AMF species specific manners for various plant species (Gange et al., 1999; Rabin and Pacovsky, 1985). However, studies on mycorrhizal colonization's influence on herbivorous insects is the least studied compared with AMF's impact on plant nutrition with several theories regarding the mechanism by which the symbiosis impacts the herbivore (Jung et al., 2012). Mycorrhizae's impact on plant nutrition and growth impacts herbivore and plant performance through tolerance but mycorrhizal influence on phytohormones, such as jasmonic and salicylic acid, also seem to play an integral role in mycorrhizal induced defense response to herbivory (Jung et al., 2012). Of these studies, only a selected few address corn pests, specifically Western corn rootworm and European cornborer (Dematheis et al., 2013, Murrell et al., 2015). Western corn rootworm larval development was negatively affected by AMF (Dematheis et al., 2013) while European corn borer oviposition was either increased or decreased depending on the interaction of AMF and soil treatment i.e. fertilization combined and crop rotation (Murrell et al., 2015). The meta-analysis of 34 studies conducted by Koricheva et al. (2009) found that AMF influence insect herbivore performance but these effects are dependent on insect feeding habit and feeding specialization, as well as mycorrhizal species. Variation amongst herbivore performance across feeding habits suggests that nutrient content, or possibly secondary metabolites, vary within various tissues consumed by herbivores may exhibit variation in AMF colonized plants. Further, it suggests that addressing pest problems through proper nutrition, AMF, and their effects on secondary plant compounds would be different depending on the pest complex, such as chewing or sucking pest problems.

There are ample studies that have examined the effects of P fertilization on AMF benefits to hosts but fewer in regards to similar nitrogen (N) effects on AMF impacts on herbivores.

Nitrogen nutrition alone can significantly impact insect development, feeding, and oviposition (Awmack and Leather, 2002; Mattson, 1980; Scriber and Slansky Jr., 1981). Moreover, fertilizer source (organic vs. conventional) can increase insect performance through faster larval development as has been shown for European corn borer in corn (Murrell and Cullen, 2014). Similarly, beet armyworm preferentially fed and performed better i.e. increased growth rate and survival, on high N supplemented corn than on low N supplemented corn (Ren et al., 2013). This response was due in part to increased nutritional quality of corn tissue, but also due to reduced corn plant phenolics (Ren et al., 2013). Mycorrhizae also affect plant phenolic levels (Jung et al., 2012) and given the growing evidence for AMF uptake of N (Ames et al., 1983; Miransari, 2011) it seems likely that the interaction of these two factors can significantly alter plant defensive compounds and nutritional status, which can lead to concomitant impacts on plant-insect interactions, especially at the second trophic level.

Given the potential of N and mycorrhizae to impact plant-herbivore interactions, we investigated the potential effects of N source and mycorrhizal colonization on the performance of two corn pests: the generalist corn earworm, *H. zea* and the specialist corn leafhopper, *D. maidis*. In addition to having a different feeding range (generalist vs specialist), these insect species also differ in their feeding habits as a chewer (*H. zea*) and as a sucking insect (*D. maidis*). We also investigated the effects of N source, AMF inoculation and insect infestation on below- and above-ground corn biomass production.

## Materials and Methods

This study was conducted in a NCSU greenhouse facility kept on a 14:10 L:D schedule and maintained at approximately  $27 \pm 5$  °C. The experiment was set up in a randomized

complete block design with factorial treatments consisting of three N treatments (no N, synthetic, or organic) × two AMF inocula (sterile vs. live) × three insect infestations (none, *H. zea* or *D. maidis*). For the N amended treatments, the control treatment received no N addition, but was provided mineral potash (K<sub>2</sub>O, 0-0-60) and Phosphorous (P<sub>2</sub>O<sub>5</sub>, 0-46-0), both produced by the Espoma Co. (Millville, NJ, 08332). The synthetic N treatment consisted of nitrate of soda (15-0-0, Bonide Products Inc., Oriskany, NY, 13424) in addition to phosphorus and potassium equivalent to the control treatment. The organic N was provided by MicroSTART60 pelletized poultry litter (3-2-3 N-P-K) (Purdue Agrirecycle LLC., Seaford, DE) which in addition to N, P, and K contained small amounts of Ca, Mg, Cu, Fe, Mn, and Zn. Experimental fertilizer rates for synthetic and organic N treatments were derived from in-field pre-planting fertilization rates used in the Farming Systems Research Unit (FSRU) at the Center for Environmental Farming Systems, Goldsboro, NC (CEFS, <http://www.cefs.ncsu.edu/>) which were based on recommendations from the North Carolina Corn Production Guide (Heiniger et al., 2000) and the North Carolina Organic Grain Production Guide (Hamilton et al., 2013) for conventional and organic systems, respectively. All treatments began with a 1:1 sterile soil sterile sand mixture with negligible starting nutrient content. The low N control treatment was given a mixture of synthetic phosphate and potassium at a rate of 96.64 and 123.4 mg per plant, respectively. The synthetic N treatment received the same equivalents of P and K with the addition of 223.4 mg of N calculated from the 75 lbs. per acre at 30% pre-planting. Litter was applied to the fields at 10,000 lbs per acre with 30,600 plants per acre resulting in 148.22 grams of litter per plant. Each of our litter treatment pots received 988 mg of litter, to reflect the field application rates.

**Rearing of *Helicoverpa zea* and *Dalbulus maidis*.** Corn earworm, *Helicoverpa zea* (Boddie) larvae, used for this study were from an established colony maintained by the Cardoza lab at the Biological Resource Facility at NCSU. Adult *H. zea* were kept in 1 gallon plastic buckets (Leticia Corp., Rochester, MI, USA) with lids centers cut out to allow access to light and aeration. A 25.4 cm<sup>2</sup> piece of bleached, Grade 90 cheesecloth (Fisher Scientific, Marietta OH, USA) was used to cover the holes in the bucket lids and to serve as oviposition substrate for the moths. Each bucket was provided with a 125 ml Erlenmeyer flask filled with 10% sucrose solution as sustenance for the moths and eggs were collected three times weekly by exchanging the cheese cloth pieces. Eggs were removed from the cheese cloth by immersing in a 1 L beaker filled with ~300 ml 1% NaClO for 4 minutes. Eggs were allowed to settle to the bottom of the beaker and then transferred to a 20 cm diameter basket style paper coffee filter (HOME360) to remove eggs from the water. Eggs were then surface sterilized and removed from cheese cloth by rinsing with water to remove excess bleach. Eggs were allowed to air-dry and were then stored in placed in a covered plastic dish (Dart Container Corp. Mason, MI, USA) and placed in a rearing room at 25° C ± 2° 55 ± 5% humidity and 14:10 L:D cycle to allow embryo development and larval hatching which took approximately 48 hrs. After hatching, neonates were transferred in groups of three into 30 ml cups (Dixie Consumer Products, Atlanta, GA, USA) filled with ~9 ml of artificial diet (Southland Products Inc. Lake Village, AR, USA). Insects were allowed to feed on the artificial diet until pupation, for colony maintenance, or until needed for experiments. Pupae were collected and surface sterilized by rinsing with 1% NaCl for one minute followed by 2-3 rinses with de-ionized water (Waldbauer et al., 1984). After air drying, pupae were transferred to adult buckets, described above. All *H. zea* stages were maintained at 25° C ± 2° and 55% humidity ±

5% respectively. Lights within the rearing room were automatically set to 14:10 h L:D. Third instar larvae were used in this experiment.

*Dalbulus maidis*, otherwise known as the corn leafhopper, adults were obtained from a lab colony at Texas A & M maintained in Dr. Julio Bernal's lab in the Department of Entomology. Upon arrival the adults were placed on young Augusta A7664 (Verona, VA) corn plants, approximately 10 days old, inside of a 0.33 m<sup>3</sup> PVC cage covered with a sleeve made of white organza fabric to contain *D. maidis* and exclude other pests from the corn plants. Both corn plants were grown and colony cages were maintained in a greenhouse kept under natural light conditions during summer approximately 14:10 L:D and on a 14:10 L:D schedule during winter with the use of artificial lighting, at approximately  $27 \pm 5$  °C. Adults were allowed access to the plants for feeding and oviposition for 3-5 days and then plants were removed and placed in separate cage for the eggs to hatch. Adults were provided with 14 day old plants for feeding and oviposition and plants were watered as needed. Cages receiving corn plants post oviposition, containing eggs laid within the plant stems, were watered as necessary until nymphs emerged. As nymphs progressed through instars and corn plant health deteriorated the old plants were severed and left in the cage which was provisioned with new corn plants. After adult emergence began the plants were changed again to provide healthy oviposition substrates and in this manner colonies of known ages were maintained.

**Arbuscular mycorrhizae inoculum production.** Initial AMF inocula were provided by Dr. Michelle Schroeder-Moreno's lab (NCSU, Crop Science). These originated from AMF single species isolates obtained via trap cultures from soil samples collected from the Farming Systems Research Unit (FSRU) at the Center for Environmental Farming Systems (CEFS, Goldsboro,

NC) in December of 2012. The three species chosen for this project were *Gigaspora margarita*, *Glomus mosseae*, and *Glomus intraradices* because they are ubiquitous at CEFS and they are known to colonize corn as well as other plants readily and previously documented to impact Western corn rootworm and European cornborer in corn (Dematheis et al., 2013; Koricheva et al., 2009, Murrell et al., 2015).

Each individual species within the three species AMF inocula were produced separately by initially using one corn plant receiving 50 g by weight of whole inoculum which grew for approximately 1 month before pots were over-seeded with *Sorghum bicolor* x *S. bicolor* var. *sudanense* for approximately 5 months. The sudangrass-sorghum hybrid was used as host plants because it is easier to maintain for long periods in a greenhouse setting. Spore production was checked by taking 10g soil samples from single species pot cultures and collecting spores via a wet-sieving methodology adapted from An et al. (1990). All pots of any one species were combined and macerated using a rubber mallet to separate the bulk of root material from the soil and to make storing the whole inoculum easier post 1 week of room temperature drying. The bulk of root material was discarded and the soil and small root fragments remaining were kept. Single species AMF inoculum was stored in a -4 C refrigerator for approximately 1 month, to break spore dormancy before being used for the experiments. Spore density in the bulk inoculum of each AMF species was assessed from a 10 gram sub-sample again by an adaptation of the wet-sieving procedure in An et al. (1990). Spores collected were placed in a gridded petri dish and counted via a stereomicroscope. In this manner spore density was determined to be 48.2, 10, and 0 spores/gram for *Rhizophagus intraradices*, *Gigaspora margarita*, and *Funneliformis mosseae*, respectively. Since our *G. mosseae* inoculum production failed to produce enough spores for our needs, this species was obtained commercially from the International Culture

Collection of (Vesicular) Arbuscular Mycorrhizae (INVAM, 1090 Agricultural Sciences Building, Morgantown, WV). The spores received from INVAM were a mix of two *G. mosseae* strains with a combined spore density of 35 spores per gram.

Mycorrhizal, inocula consisted of a 1:1:1 spore count homogenized blend, containing 50 spores of each of the three fungal species either live spores (AMF, mycorrhizal treatment) or autoclave-sterilized inoculum (control). The sterile inoculum was produced in a similar manner but was subsequently autoclaved at 250° F for 60 minutes on a gravity cycle, allowed to sit for 24 hours, and then autoclaved once more under the same settings. Prior to planting, a microbial filtrate was produced, the live inoculum measured and mixed, the sterile inoculum measured and sterilized, and the fertilizer blends pre measured. Filtrate production was modified from van Kessel et al. (1985) where in filtration by Whatman #1 filter paper was done three times.

**Experimental corn planting.** Terracotta pots (15-cm diameter) were filled with the 1:1 sterile sand and soil mixture amended with the appropriate fertilizer type and rate, 20 ml of microbial filtrate, and inoculum for each treatment. Each pot received approximately 250 ml of tap water, and 3 ‘Augusta’ (Verona, VA) A7664 corn seeds were distributed evenly at a depth of approximately 1.27 cm. Post germination, each plant was thinned to two seedlings and the two healthiest seedlings were allowed to continue growing throughout the experiment. Plants were watered with tap water as needed and grown for approximately 2 weeks, when plants were at the 3<sup>rd</sup> -true leaf stage. Insect infestation levels included a no insect control, two 3<sup>rd</sup> instar *H. zea*, or ten (five male and five female) adult *D. maidis*. The number of *H. zea* used is indicative of 1 larvae per ear for corn plants producing two ears while the number of *D. maidis* was arbitrary since the damage they cause is as a vector a corn stunting diseases and is not directly comparable

to that of CEW. Each replicate block was housed within a PVC cage approximately 1 m<sup>3</sup> enclosed in an organza sleeve to prevent accidental infestation by opportunistic pests. On the day of infestation, each plant was confined within an organza sleeve held at the base of the pot by a large rubber band and at the top by a binder clip attached to overhead rope for support. All plants were covered with organza sleeves regardless of insect infestation to ensure homogeneity of microclimatic conditions across treatments. This experiment was carried out in duplicates over time to produce a total of 10 replicates for each nitrogen/mycorrhiza/insect treatment combination. Plants infested with *H. zea* were checked daily for insect feeding activity and when larvae reached the wandering stage, and no more feeding activity was observed, they were removed from the plants and number of surviving larvae per treatment was recorded. Surviving larvae were placed individually in 30 ml plastic cups labeled with their respective treatment and replicate and transferred to the laboratory. Time from infestation to pupation and pupal weight and gender were recorded. Treatments containing *D. maidis* were allowed 14 days for feeding and reproduction. After this time, plant stems were severed at the soil surface and confined with insects within the organza sleeve. The sleeves containing the plant and insect material were frozen in a -20°C freezer (97055-078 VWR International LLC., Radnor, PA) for at least 24 h to kill the adults and nymphs. After freezing, nymphs were counted and total insect mass per plant was recorded for each of the treatments.

To determine treatment impact on plant productivity, at the conclusions of the 2 week infestation period, all plants were severed at the soil level with pruning shears to separate stems and leaves from root mass. Stems were placed in brown paper bags immediately after separation from roots. Roots were removed from pots, the majority of soil shaken loose and the remaining soil washed away under running tap water. Roots were patted dry with paper towels and placed

in brown paper bags. Bagged root and stem material was placed in a drying oven (Model 6530, Thermo Fischer Scientific, Marietta, OH, USA) at 70 °C for 72 h and then weighed on a digital scale (Model MS105DU, Mettler Toledo, Columbus, OH, USA) to obtain dry biomass. AMF colonization was assessed after root dry weights were recorded; the roots of plants were rehydrated for 30 min., cut into 1-2 cm pieces, homogenized, and stained via an adaptation of Phillips and Hayman's (1970) protocol so that mycorrhizal colonization could be quantified. This was accomplished via the root line intersect method described in Giovannetti and Mosse (1980).

**Statistical analyses.** Data were  $\log(x + 1)$  transformed to fit assumptions of normality. Effects of mycorrhizal colonization and N source on *H. zea* survival to pupation, days to pupation, mean pupal mass, as well as on *D. maidis* total nymph mass, and mean mass per nymph were tested using an analysis of variance (ANOVA) using PROC GLM (SAS 9.4 Cary, NC 2013). Data were also subjected to  $\log(x + 1)$  transformation to fit assumptions of normality. Effects of mycorrhizal status, N source, and insect treatment and their interactions on root and shoot dry weights as well as percent mycorrhizal colonization were tested using an analysis of variance (ANOVA) using PROC GLM (SAS 9.4 Cary, NC 2013). Mean comparisons, for significant effects, were performed on significant effects using the Tukey-Kramer adjustment for post-hoc comparisons ( $P \leq 0.05$ ).

## Results

Nitrogen source impacted AMF colonization ( $F=13.66$ ;  $df=2,174$ ;  $P<.0001$ ; Table 2), *H. zea* pupal success ( $F=15.56$ ;  $df=2,56$ ;  $P<.0001$ ) and mean pupal mass ( $F=6.54$ ;  $df=2,29$ ;  $P=.0045$ ) as well as both shoot and root biomass ( $F=21.25$ ;  $df=2,174$ ;  $P<.0001$ ). Organic N treatments had significantly lower AMF colonization compared to the other N treatments

( $F=13.66$ ;  $df=2,174$ ;  $P<.0001$ ; Table 2). Of the 120 larvae infested on all *H. zea* treatments, only 43 survived to pupation. Sixty four percent larvae died, likely as a result of cannibalism by their plant mate. Pupal *H. zea* survival was significantly greater in organic N treatments ( $F=15.56$ ;  $df=2,56$ ;  $P<.0001$ ), in the order of  $3 \times$  higher than either the no N or synthetic N treatments which were not different from each other (Figure 1a). Mean pupal mass of *H. zea* was similarly significantly higher in the organic N treatment ( $F=6.54$ ;  $df=2,29$ ;  $P=.0045$ ) than compared to the no N treatment (Figure 1b). Mean number of nymphs by *D. maidis* tended to be higher for plants receiving the synthetic N treatment followed by those on plants receiving no N and finally by those on plants receiving the organic N source (Table 1). The organic N treatment yielded significantly ( $F=21.25$ ;  $df=2,174$ ;  $P<.0001$ ) more shoot biomass than the no N controls and the synthetic N treatment, which were equivalent to one another (Figure 3b). The organic N treatments had significantly more root biomass than either synthetic or no N treatments which were not significantly different from each other ( $F=7.30$ ;  $df=2, 174$ ;  $P=.0009$ ) (Figure 3b).

Results did not reveal any significant impacts of AMF or its interaction with N source on pupal survival ( $P=.5593$ ,  $P=.4321$ ) or mean pupal mass ( $P=.1482$ ,  $P=.6700$ ), respectively. However, both pupal survival (Figure 2a) and mean pupal mass (Figure 2b) exhibited negative trends in response to mycorrhizal treatment when compared to non-mycorrhizal controls. *Dalbulus maidis* response variables were not significantly affected by AMF, N source, nor their interaction. Mean and total nymph mass were not significantly impacted by treatment effects (Table 1). Non-mycorrhizal control plants had negligible colonization in comparison to mycorrhizal plants ( $F=512.28$ ;  $df=1,174$ ;  $P<.0001$ ) (Table 2). Percent colonization was highest in corn roots from plants infested with *D. maidis* which was equivalent to colonization of roots from control (uninfested) plants, but significantly higher than AMF colonization of roots from

plants infested *H. zea* ( $F=5.9$ ;  $df=2,174$ ;  $P=.0033$ ) (Table 2). Shoot biomass showed significant ( $F=5.45$ ;  $df=1,165$ ;  $P=.00207$ ) reductions in biomass for mycorrhizal plants relative to non-mycorrhizal controls (Figure 3a). Mycorrhizal colonization caused a significant reduction in root biomass compared to non-mycorrhizal controls  $F=18.73$ ;  $df=1, 174$ ;  $P<.0001$ ) (Figure 3a).

Shoot biomass was significantly affected by insect treatment ( $F=10.18$ ;  $df=2,174$ ;  $P<.0001$ ) (Figure 3c). Feeding by *H. zea* resulted in significantly ( $F=10.18$ ;  $df=2,174$ ;  $P<.0001$ ) lower shoot biomass compared to *D. maidis* and the no insect control, which were not significantly different from each other (Figure 3c). Root biomass was also significantly impacted by insect treatment ( $F=17.06$ ;  $df=2, 174$ ;  $P<.0001$ ) (Figure 3c). Insect treatment resulted in significantly lower root biomass for the *H. zea* compared to the control (no insect) and the *D. maidis* treatments, which were not significantly different from each other ( $F=17.06$ ;  $df=2, 174$ ;  $P<.0001$ ) (Figure 3c).

## Discussion

In this study nitrogen fertilization impacted AMF colonization, insect performance, and plant biomass. Our study found that organic N source significantly impacted AMF colonization of host roots, resulting in lower colonization than either control or synthetic N treatments. These results were in accord with previous field data showing lower mycorrhizal colonization in corn roots grown under organic production systems (MSM personal observation). Similarly, Ellis et al. (1992) found that manure amended soybeans and sorghum exhibited reduced colonization compared to unfertilized and nitrogen fertilized treatments. The response of AMF to organic N that we found as well as reported by Ellis et al. may have resulted from nutrient overabundance or potentially from competition of soil microbes with AMF. For example, *Aspergillus niger* (Van

tiegh), *Streptomyces griseoviridis*, and *Trichoderma harzianum* reduce colonization of *Glomus mosseae* in corn and soybeans (McAllister et al., 1995; Wyss et al., 1992). Furthermore, high phosphorous content, in general, as well as organic fertilizers and manures can lead to reduction in mycorrhizal root colonization (Joner, 2000; Linderman and Davis, 2004; Tarkalson et al. 1998).

Nitrogen source differentially impacted the performance of our two corn pests, *H. zea* and *D. maidis*. *H. zea* larvae that fed on plants supplemented with an Organic N source were 3 × more likely to survive to pupation and exhibited increased pupal mass over no N controls with synthetic N supplemented plants being intermediate in regards to pupal mass only. Host plant nitrogen nutrition has been shown to vary due to nitrogen availability, nitrogen source, and to impact secondary metabolites. Moreover, the nitrogen content of insect host plants (food sources) has been shown to impact insect herbivore performance and fecundity (Awmack and Leather, 2002; Mattson, 1980). Ren et al. (2013) showed that increased N availability increased *Spodoptera exigua* (Lepidoptera: Noctuidae) (Hubner) performance (growth rate) over lower N availability treatments. This supports our work assuming that our organic N treatments increased N availability. In field trials, Schomberg et al. (2011) showed that N mineralization and corn biomass were greater with poultry litter fertilization compared to commercial fertilizers. Similarly, Ma et al. (1999) showed that dairy manures exhibited increased N use efficiency, N uptake, and biomass over ammonium nitrate fertilization. The response of *H. zea* coupled with the increased biomass of our own study amongst organic treatments supports the idea that nitrogen availability was better for our organic treatments. However, *D. maidis* performance based on nymph production was decreased in organic N supplemented corn. Aqeel and Leather (2011) showed that as N supply to several wheat cultivars increased so did tissue nitrogen content and subsequent performance of

aphid species such as *Rhopalosiphum padi* (Hemiptera: Aphididae) (L.). However, this does not explain why organic treatments were so reduced in nymphs produced in comparison to no N treatments. In contrast to our findings, Staley et al. (2010) showed that presence of *Brevicoryne brassicae* was greatest on organic manure fertilized *Brassicas* than synthetic treatments but likely as a result of secondary metabolites i.e. glucosinolates, than plant nutrient content. The organic litter product which contained Ca, Mg, Cu, Fe, Mn, and Zn likely increased the availability of these micronutrients in our organic N treatments which may have also influenced *D. maidis* performance. Furthermore, plant available nitrogen is highest early in corn growth for conventional (synthetic) fertilized fields, decreasing overtime, while in organic (poultry litter) fertilized corn fields mineralized nitrogen increases over time (Schomberg et al., 2011). Decreased available plant nitrogen in the organically fertilized plants could limit nitrogen available for insect nutrition and development, especially in nitrogen deficient vascular tissues such as phloem.

Organic N treatments produced significantly more plant biomass than either synthetic or no N treatments. Just as plant available nitrogen could increase insect performance it is well documented to impact plant growth. Plant available nitrogen is highest early in corn growth for conventional (synthetic) fertilized fields, decreasing overtime, while in organic (poultry litter) fertilized corn fields mineralized nitrogen increases over time (Schomberg et al., 2011). As such, the continued vigor and growth of organic N treatments may be in response to increasing mineralized nitrogen. Micronutrients can also be limiting to corn growth if scarce or absent making it plausible that the increased availability of micronutrients, specifically Ca, Mg, Cu, Fe, Mn, and Zn supplied by the Organic N treatments could have been responsible for this disparity between N treatments. Sistani et al. 2008 found that poultry litter applications to corn fields increased yield and soil levels of P, Zn, and Cu. Given our Organic N treatment also increased yield and

presumably Zn and Cu levels it is plausible that micronutrients, notably Zn, could have played a role in the observed results.

In our study mycorrhizal colonization had no significant impacts on either insect species but a tendency for reduced *H. zea* pupal survival and pupal mass was observed. This response to mycorrhizal colonization was similar to reports by Koricheva et al. (2009) whose meta-analysis showed chewing insects tended to perform poorly on mycorrhizal host plants compared to non-mycorrhizal hosts. Similarly, Rabin and Pacovsky (1985) showed that mycorrhizal soybeans increased mortality, reduced pupal weights, and increased time to pupation for *H. zea* compared to non-mycorrhizal controls. Rabin and Pacovsky posit nutritional differences and induction of secondary metabolites or defenses as potential causes for the observed differences. As expected, the lack of impact of mycorrhizal colonization on *D. maidis* fecundity observed in our study agrees with previous studies that demonstrate that phloem feeder specialists tend to be either neutrally or positively affected when feeding on AMF-colonized plants (Jung et al. 2012; Koricheva et al. 2009).

Effects of AMF can vary from providing benefits, no responses, to negative responses on host plants and the outcome of the interaction is a product of the specific host species, AMF species, and soil environment, especially as related to nutrient availability (Bolan, 1991; Miransari, 2011). AMF frequently benefit host plants when nutrients are deficient, especially P, but carbon costs of AMF can outweigh the benefits when nutrient availability is abundant relative to host demand (Bolan, 1991; Miransari, 2011). However, evidence exists to support both phosphorus and N uptake increase by AMF root colonization (Ames et al. 1983; Stribley et al. 1980). Generally, this nutrient recruitment leads to increases in plant growth which is well correlated with P and N uptake (Mensah et al., 2015), especially when soils are deficient in these macronutrients, the most

documented of which is phosphorus. In our study, root and shoot dry weights of corn plant biomass was differentially impacted by AMF root colonization. Mycorrhizal inoculation decreased all biomass measures, as did infestation by *H. zea*. The tradeoffs between AMF and its host plant involve the exchange of photosynthetic products for macronutrients. As such, our AMF positive treatments may have acted as a carbohydrate sink thereby reducing plant available carbohydrates for use in growth. Kothari et al. (1990) demonstrated corn infected with *Glomus mosseae* had up to 16% reductions in root dry weight compared to non-mycorrhizal controls. Similarly, Simpson and Daft (1990) showed that some *Glomus* and *Acaulospora* species reduced overall plant growth in both corn and sorghum, which they posited to be a result of high carbohydrate usage by the AMF fungus.

Insect species had disparate effects on root colonization by AMF, where *D. maidis* had no measurable impacts on colonization while *H. zea* reduced AMF root colonization. Similarly, the effects of each insect species on root and shoot biomass were also variable. The reduction of colonization by *H. zea* defoliation is supported by the work of Daft and El-Giahmi (1978) which showed mechanical defoliation of corn reduced mycorrhizal infection by *Glomus mosseae* and *Glomus macrocarpus* var. *geosporus*. Specifically, defoliation reduces plant production of photosynthates that are available to support plant-AMF relations. Corn earworm infestation significantly reduced both root and shoot biomass while *D. maidis* infestation exhibited no observable effects on root or shoot biomass. Corn earworm feeds via the direct consumption of plant tissues including: leaves, stem, silks, and kernels. As such, reductions in shoot biomass resulted from tissue consumption which, in turn, likely interrupted photosynthate availability to root tissues causing a subsequent loss in biomass. The corn leafhopper on the other hand, much like aphids, have to be in extremely high numbers during early corn development to cause direct

damage. Given the low infestation level in our treatments it is likely that numbers were too low to cause feeding related reductions in shoot biomass.

Altogether our results show the nuances of bottom-up, top-down ecological interactions and the importance of understanding their fluctuations in response to production management. The greater implication of this research being that agricultural systems and management must strive to balance sustainability while considering the interrelatedness of above and below ground systems.

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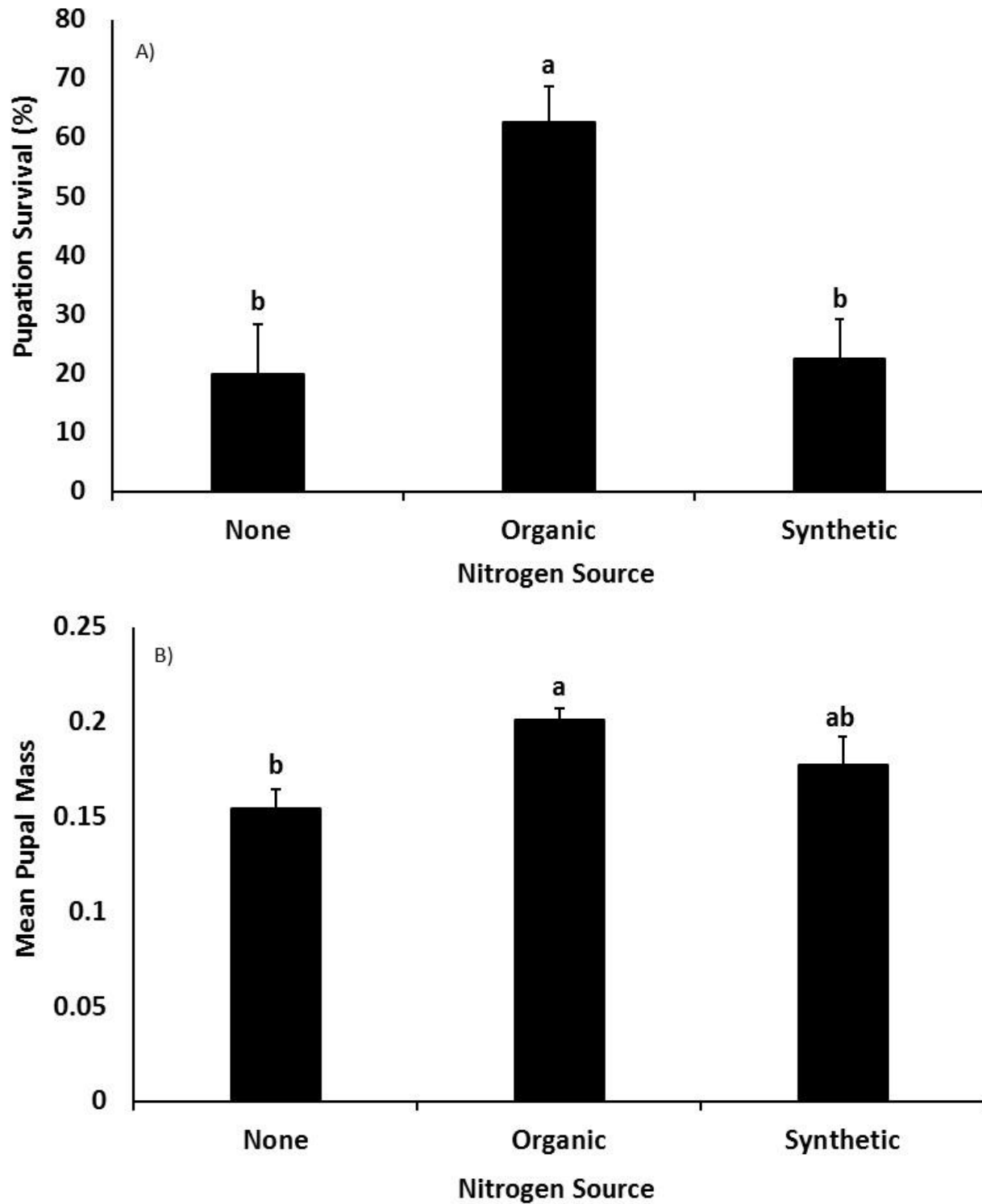
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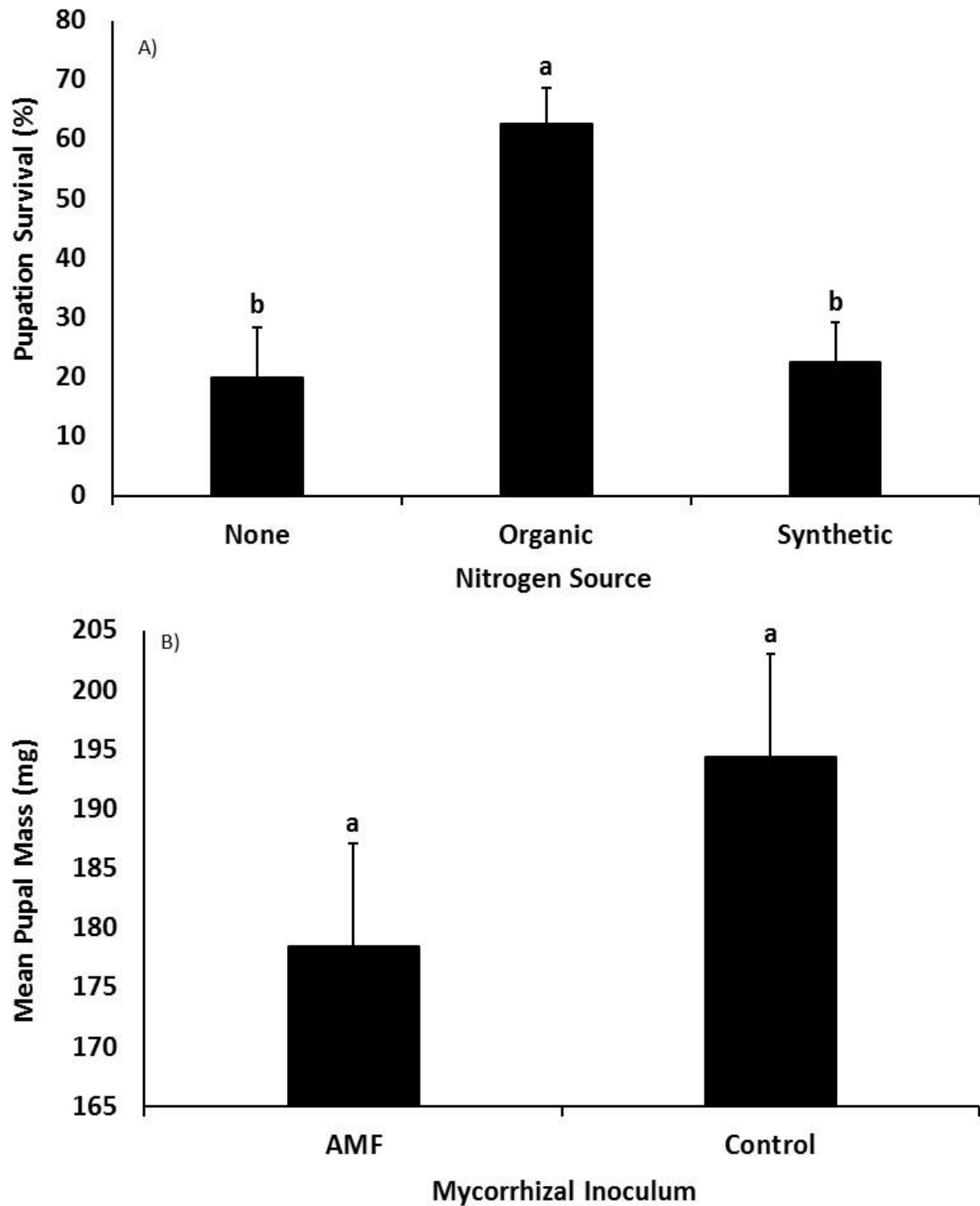
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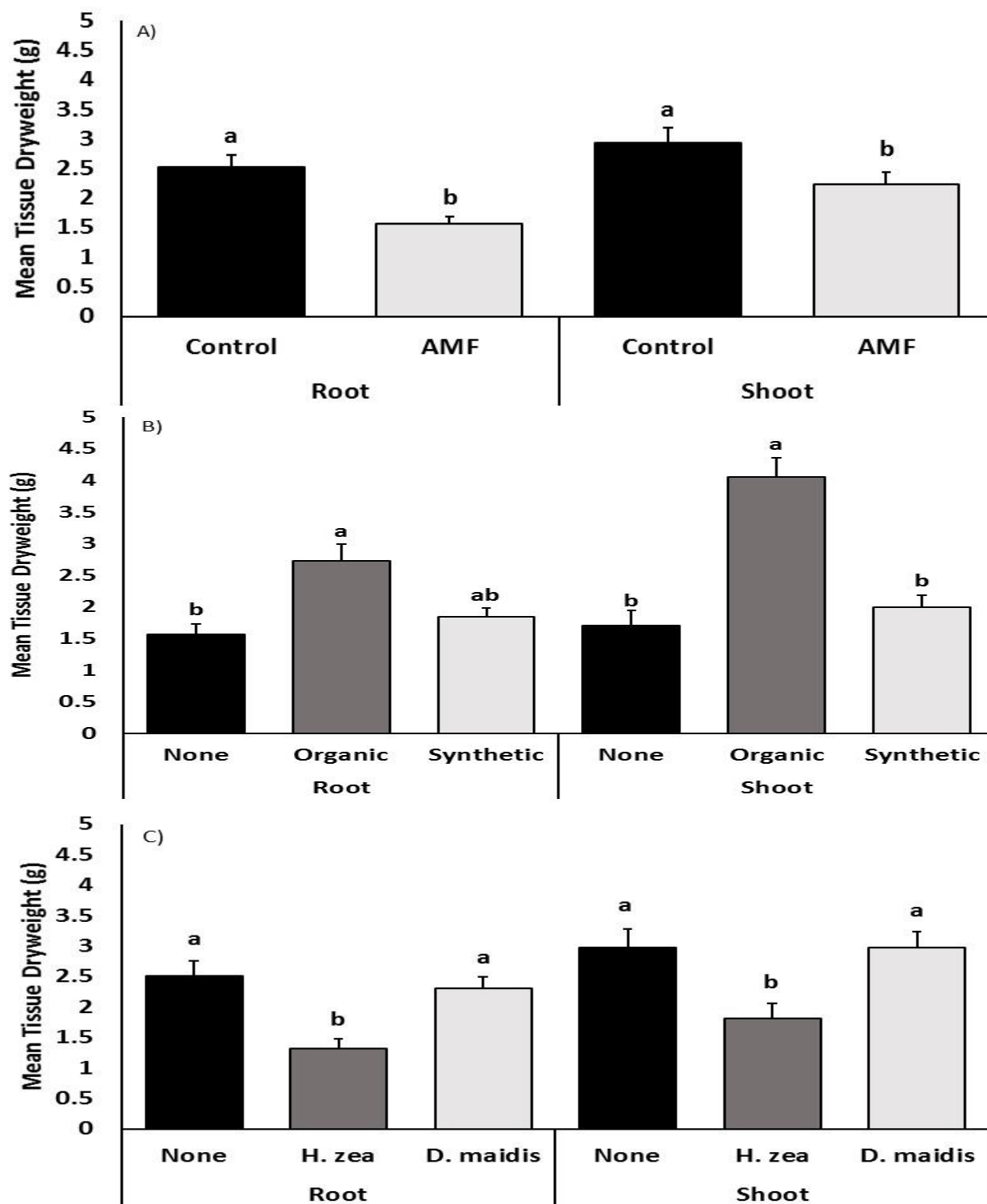
## FIGURES



**Figure 2.1.** Effect of nitrogen source on *H. zea* pupal survival **A)** and pupal weight **B)**. Values represent Means  $\pm$ SE for 10 replicates. Bars headed by the same letter are not significant according to Tukey-Kramer mean separation test ( $\alpha=0.05$ ).



**Figure 2.2.** Effect of mycorrhizal root colonization on *H. zea* pupal survival **A)** and pupal weight **B)** Values represent Means  $\pm$  SE for 10 replicates. Bars within mycorrhizal treatment headed by the same letter are not significant according to Tukey-Kramer mean separation test ( $\alpha=.05$ ).



**Figure 2.3.** Effects of **A)** mycorrhizal inoculum, **B)** nitrogen source and **C)** insect treatment on root and shoot dry weights. Values represent Means $\pm$ SE for 10 replicates. Bars within treatment headed by the same letter are not significant according to Tukey-Kramer mean separation test ( $\alpha=.05$ ).

## TABLES

**Table 2.1.** Trends exhibited by number and total mass of *D. maidis* in response to treatment effects. Values represent Means $\pm$ SE for 10 replicates. Values within treatments headed with the same letter are not significantly different, Tukey-Kramer mean separation test ( $\alpha=0.05$ )

Treatment	Level	No. nymphs per plant	Mean nymph mass ( $\mu\text{g}$ )	Total nymph mass ( $\mu\text{g}$ ) per plant
Nitrogen source	None	44.8 $\pm$ 11.34a	20.0 $\pm$ 3.00a	1300.0 $\pm$ 400.00a
	Organic	29.5 $\pm$ 6.29a	20.0 $\pm$ 2.00a	600.0 $\pm$ 200.00a
	Synthetic	51.2 $\pm$ 12.26a	20.0 $\pm$ 2.00a	1200.0 $\pm$ 300.00a
AMF	No	42 $\pm$ 8.9a	20.0 $\pm$ 2.00a	950.0 $\pm$ 200.00a
	Yes	41 $\pm$ 8.0a	20.0 $\pm$ 2.00a	1100.0 $\pm$ 300.00a

**Table 2.2.** Effect of nitrogen source and insect infestation on percent mycorrhizal colonization (Mean  $\pm$  SE) of experimental corn plant roots. Values within treatments headed with the same letter are not significantly different, Tukey-Kramer mean separation test ( $\alpha=0.05$ ).

Treatment	Level	Colonization (%)
Nitrogen source	None	44.0 $\pm$ 2.71a
	Organic	24.6 $\pm$ 3.49b
	Synthetic	41.3 $\pm$ 3.51a
Insect	None	37.3 $\pm$ 3.25a
	<i>D. maidis</i>	38.4 $\pm$ 3.14a
	<i>H. zea</i>	34.1 $\pm$ 4.33b