# EXPLORING EFFECTS OF WEED MANAGEMENT IN AGROECOSYSTEMS: ARTHROPODS, SOIL PROPERTIES AND SOYBEAN PRODUCTION

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EXPLORING THE EFFECTS OF WEED MANAGEMENT IN AGROECOSYSTEMS: ARTHROPODS, SOIL PROPERTIES AND SOYBEAN PRODUCTION

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**MASTER OF SCIENCE**

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## ABSTRACT

Soil-dwelling arthropods contribute to agroecosystems, but it's unclear how specific taxa respond to weed management practices. Objectives were to explore 1) response of Collembola to weed management in a glyphosate-tolerant soybean system and 2) impacts of weed management and soil arthropod reduction (via insecticide) on soil and plant parameters. Weed management had variable effects on Collembola, whereas location had a consistent effect on diversity and density. Increased weed pressure decreased soil nitrate and reduced soybean yield. Reduction of soil arthropods didn't impact soil nitrate or yield, but increased the number of soybean root nodules. This could be due to decreased root herbivores, or overcompensation of the plant. Previous research emphasized effects of plant communities on soil arthropods, but our study suggests soil properties strongly influence arthropod communities. Although this study does not show obvious benefits of soil arthropods, long term insecticide application may be detrimental to crop production.

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# CHAPTER 1. INTRODUCTION

Over seventy million acres of soybean were planted in the United States in 2013, second only to corn (NASS 2014). Soybeans are processed primarily as a protein source in livestock feed and vegetable oil for human consumption. Insect pests and diseases threaten soybean yield, but the biggest concern for soybean producers is weed control (Vivian et al. 2013).

The main weed control used in soybean is the application of herbicides, commonly glyphosate (Young 2006). Glyphosate is a broad spectrum herbicide used not only in soybean, but also in several other crops as well as urban areas (Baylis 2000, Helander et al. 2012). Eighty-five percent of soybean acres were treated with glyphosate in 2012 (NASS 2014). With the intensification of agriculture it is important to understand how glyphosate and other weed management practices are impacting the agroecosystem.

One important but often overlooked aspect of agricultural ecosystems is the soil arthropod community. Although some soil arthropods can be pests, many are beneficial. They perform essential services including aerating the soil, fragmenting organic matter and cycling nutrients as well as consuming other insect pests and weed seeds. Since many soil arthropods are small and cannot move to avoid unfavorable conditions, they are susceptible to small changes in their environment including those resulting from weed management.

This research was designed to answer two main questions. First, how do soil arthropods, specifically Collembola, respond to common weed management practices (Chapter 1)? And how do soil arthropods and weed management affect soil and soybean parameters (Chapter 2)?

## CHAPTER 2. EFFECTS OF WEED MANAGEMENT OF DENSITIES OF COLLEMBOLA IN GLYPHOSATE-TOLERANT SOYBEAN

### Introduction

Weed management is an essential aspect of agricultural production. Weeds compete with crops for space, water, sunlight, and soil nutrients required for growth (Staniforth and Weber 1956, Lindquist et al. 2010, Green-Tracewicz et al. 2012). Reducing weed populations can enhance crop growth and yield, ultimately increasing grower revenue (Acker et al. 1993, Dieleman et al. 1995). While profitability is a primary concern for producers, it is also imperative to ensure that common agricultural practices are not detrimental to ecosystem function and sustainability. Herbicides, specifically glyphosate, are the primary means of weed control in many agricultural systems (Cerdeira and Duke 2006).

Glyphosate is a broad-spectrum, non-selective herbicide used in both agricultural and urban settings. One common use on farms is for the control of weeds in glyphosate resistant crops such as corn, cotton, canola, and soybean (Duke and Powles 2008). Glyphosate kills weeds by inhibiting the 5-enylpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, which disrupts synthesis of aromatic amino acids (Dill 2005). Since only plants and bacteria have this enzyme, glyphosate is considered to have low toxicity to humans and other animals and is generally considered environmentally safe, especially compared other herbicides, such as atrazine (Cerdeira and Duke 2006, Dill et al. 2008, Green 2012). Glyphosate is also beneficial in that it is compatible with conservation tillage (Givens et al. 2009). It is extremely effective and cost efficient, which has resulted in rapid adoption from growers (Green 2012). In 2012, over 80% of soybean acres in the United States were planted with glyphosate resistant varieties (NASS 2014). Since glyphosate is so effective and commonly used, some concerns have been voiced about potential negative impacts on biodiversity of farmland wildlife due to decreased weed density in agricultural fields (Heard et al. 2003). Other concerns include possible long term retention in the soil and toxicity to microbes and soil arthropods (Helander et al. 2012).

Soil arthropods are important to soil and plant health in many ways, including nutrient cycling and pest management (Moore et al. 1988), but their ecological impacts are often overlooked in managed agroecosystems. Soil arthropods can be either euedaphic (belowground), spending all of their life below the soil surface, or epedaphic (aboveground or epigeal), dwelling on the soil surface. Depending on where the arthropods live, they may be more or less exposed to changes in their environments. This difference in habitat usually results in morphological differences, such as size and presence or absence of hairs, eyes or other sensory organs (Eisenbeis and Wichard 1987). As a result, most aboveground species do not actually enter the soil and vice versa (Eisenbeis and Wichard 1987). Nevertheless, there is some overlap of species between these two environments (Wallwork 1970). For example, the collembolan taxa Entomobryomorpha and Sminthuridae are more common above the soil surface, while Onychiuridae are typically found below the soil surface and Isotomidae are both euedaphic and epedaphic (Eisenbeis and Wichard 1987). All soil arthropods, euedaphic or epedaphic, rely on the soil for protection and regulation of the microclimate, but conditions can still be unfavorable at times (Villani and Wright 1990).

Collembola (springtails) are one example of a soil arthropod that is abundant in agroecosystems. Collembola are cosmopolitan small soft-bodied arthropods that primarily feed on microbes, fungi, and dead plant matter, although some species occasionally consume living plant matter (Curl et al. 1988). Collembola are vital to the decomposition process because they break down large pieces of organic matter, making it easier for microorganisms to access and further decompose the material, thus returning nutrients to the soil (Moore et al. 1988). They are also a sustaining food source for generalist predators when pest populations are low or absent (Agusti et al. 2003, Warner et al. 2003, Oelbermann et al. 2008). Because Collembola are an integral part of the soil ecosystem, they are often used as model organisms to investigate the toxicity of some chemicals (Hopkin 1997). There is contrasting evidence regarding effects of glyphosate on Collembola. Some studies found no effect of glyphosate on densities of belowground Collembola (Gomez and Sagardoy 1982, Lins et al. 2007), whereas Bitzer et al. (2002) found that densities of aboveground Collembola were negatively impacted by this herbicide, although effects were attributed to differences in weed cover rather than chemical toxicity.

Although weeds compete with crops for nutrients, water and sunlight, they also increase the plant biodiversity of agricultural fields, which is generally beneficial to soil arthropods (Altieri and Whitcomb 1979, Hadjicharalambous et al. 2001, Marshall et al. 2003). Many arthropods benefit from the food sources, protection from enemies, and habitat modification weeds provide (Andow 1991). Regulation of extreme temperatures, reduction of wind speed, and increases in humidity are some of the ways weeds can modify environmental conditions near the soil surface (Norris and Kogan 2005). Different types of weeds offer different resources to arthropods as a result of their differences in size, shape and chemicals they produce (Norris and Kogan 2005). These more favorable living conditions often result in higher densities of some arthropods (Altieri et al. 1985, House 1989) while others appear to be unaffected (Andow 1991, Norris and Kogan 2005). Weed management in agricultural systems can result in changes that are unfavorable for some soil arthropods depending on whether they are euedaphic or epedaphic (Villani and Wright 1990). Most of the relevant research was gathered from studies involving foliar arthropods. However, relatively little work has been done exploring how soil arthropods respond to decreases in weed diversity and density in agricultural fields. It is predicted that many soil arthropods will not easily adjust to habitat changes because of their limited dispersal abilities (Hedlund et al. 2003), especially euedaphic species, whose movement is generally related to the amount of pore space in the soil (Larsen et al. 2004).

Adequate soil pore space is essential for movement of soil arthropods, but it is also crucial for root growth and water movement (Vreeken-Buijs et al. 1998). Porosity is impacted by several factors, including compaction and soil texture. Soil texture in turn can influence soil arthropods. Higher densities of soil arthropods are often found in sandy soils (Vreeken-Buijs et al. 1998, Larsen et al. 2004). Texture also influences water movement and retention in soil, availability of plant nutrients, and adsorption of pesticides that come in contact with the soil surface (Sprankle et al. 1975, Cryer and Laskowski 1998, Brady and Weil 2009). Water flows more readily through the large pores in sandy soils compared to fine-textured soils, and plant nutrients as well as pesticides can be washed away with the water. When pesticides absorb to soil particles they are less likely to leach or runoff. Adsorption is highest in clay soils and soils high in organic matter (Sprankle 1975).

The purpose of this study was to determine the effect of glyphosate application on densities of soil arthropods, specifically Collembola, in a glyphosate-resistant soybean production system. The study was designed to allow us to differentiate direct effects of chemicals on soil arthropods from indirect effects related to the presence or absence of weeds. Based on literature discussed above, we expected that densities of Collembola would be highest in plots where weeds were present, and that direct effects of glyphosate would be minimal. We also expected to see greater densities of Collembola and other soil arthropods in sandier soil. In addition, we hypothesized that aboveground soil arthropods would be affected by weed management practices to a greater degree than belowground arthropods.

## Materials and Methods

### Experimental Design

Site Details

The experiment was conducted in 2012 and 2013 at two field sites over the course of the growing season (May-Sept). Both fields were located in Cass County, ND; one field was located two miles north of Leonard (sandy field, GPS coordinates: 46°39'58.3560", -097°14'32.9640"). The other field was located two miles east and two and a half miles north of Mapleton (clay field, GPS coordinates: 46°55'42.1680", -097°01'03.1800"). The two sites were chosen based on their soil type, specifically soil texture.

*Soil Texture*

Soil texture was determined by collecting samples from three different areas of each field. These particular areas were chosen after examining the Web Soil Survey (http://websoilsurvey.sc.egov.usda.gov) to see if soil type differed within plots. Soil samples were collected in the same method described below. After samples were collected, they were taken back to the lab for particle size analysis using the hydrometer method (Kroetsch and Wang 2008). Fifty grams of soil mixed with 100 mL of 5% HMP and allowed to disperse overnight. The following day, water was added to the mixture for a total of 1000 mL, mixed with a plunger and allowed to settle for eight hours before using a hydrometer to measure the amount of clay in the suspension. The soil was rinsed through a 53 µm sieve to collect sand particles which were then dried in an oven at 105C for 24 hours. The Leonard site had a relatively sandy soil (fine sandy loam) and will be referred to as the sandy field. All three samples from Leonard had the same soil texture (averages: 64% sand, 24% silt, 12% clay). The Mapleton location had relatively high clay content (silty clay to silty clay loam) and will be referred to as the clayey field. Two of the samples were a silty clay loam (averages: 6% sand, 56% silt, 38% clay). The other sample was a silty clay with 5% sand, 53% silt and 42% clay.

Soil texture was important to consider in this experiment for several reasons. Texture not only affects physical and chemical attributes of soil, but can also directly and indirectly impact plant growth, biology of subterranean invertebrates, and fate of chemicals. The size of soil particles and the space between them can have a large impact on soil functions. Sand particles are large and have a few large pores between the particles. Clay particles on the other hand are very small and have many small pores. Clay soils also attach to organic matter, so while the organic matter content of clay soils is higher, decomposition is slower because it is not as available to soil organisms (Brady and Weil 2009).

Soils with large pores, like uncompacted soils and sandy soils, are easier for plants shoots and roots to penetrate, but sands also offer less stability for the plant (Tanner and Hume 1978). These large pores also allow water to move through the soil easier, which can be good when there is too much water, but detrimental to plant health when soil moisture is already low (Brady and Weil 2009). In addition to water, other nutrients and organic matter can also leach out of sandy soil. The charges and large surface area in clay soils on the other hand, can prevent nutrients and organic matter from leaching (Brady and Weil 2009).

Soil texture not only impacts plant growth, but also soil invertebrates, including arthropods. The larger pores in sandy soils allow more space for movement (Vreeken-Buijs et al. 1998, Larsen et al. 2004), but some of the course particles in these soils can be abrasive to soft bodied arthropods (Choudhuri 1961). Proper humidity is also important for soil arthropods, and the amount of moisture in the soil varies depending on texture (Ferguson and Joly 2002, Chikoski et al. 2006).

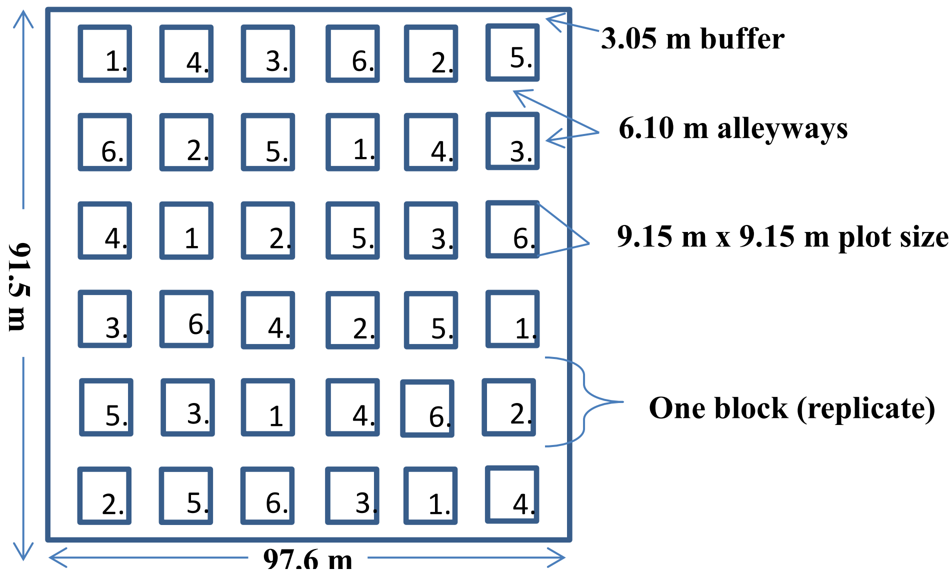


Figure 1. Field design at both sites for 2012 and 2013. Numbers represent treatments: 1=Glyphosate, +Insecticide 2=Hand-weeded, +Insecticide 3=Weedy, +Insecticide 4=Glyphosate, no Insecticide 5=Hand-weeded, no Insecticide 6=Weedy, no Insecticide.

Soil texture can also influence the fate of pesticides once they encounter the soil surface. Adsorption of chemicals is highest in clayey soils with greater amounts of organic matter (Sprankle et al. 1975) while sandy soils can result in chemical runoff and leaching.

Field Design and Land Preparation

The field experiment was set up as a randomized complete block design with six replicates in a split plot arrangement. Location was the whole-plot factor, weed management and insecticide treatment were subplot factors, and block (i.e., replicate) was considered a random factor. A total of 36 experimental units (plots) were present at each location. Each plot was 9.15

m x 9.15 m with 6.10 m between each plot (alleyways) and a 3.05 m border around the entire field (buffer) (Figure 1). In 2012 weeds in buffers and alleyways were controlled by cultivation with a John Deere wide field cultivator (spring tooth harrow, 2.29 m wide) when the weeds reached a height of six inches. In 2013, weed control in buffers and alleyways was achieved by periodic (approximately July 2 and July 30) application of glyphosate at 2.34 L + 187.08 L of water per hectare at 275.80 kPa (sprayer details are discussed below).

In both years and at both locations, the land was prepared the same way and planted at similar times. Fields were cultivated on May 16 both years using the cultivator described above to prepare soil for planting. Soybean variety Roughrider Genetics 607 Roundup Ready® (Monsanto Company; St. Louis, MO) was planted using a John Deere 71 flex planter with 76.20 cm between rows and twelve rows per plot at approximately 370,658 seeds per hectare resulting in a within row spacing of approximately 3 cm. In 2012 the sites were planted on May 22, whereas in 2013 the sites were planted on May 24. Field location, location of individual experimental plots, and assignment of treatments to each plot remained the same for both years in order to elucidate potential long term effects of the treatments.

<u>Experimental Treatments</u>

The two main factors in this experiment, weed management and insecticide, were established using a factorial arrangement with three levels of the former and two levels of the latter (Figure 2). The weed management treatments were: *glyphosate*: +glyphosate, no weeds; *hand-weeded*: no glyphosate, no weeds; and *weedy*: no glyphosate, +weeds. This arrangement was designed to separate effects of the herbicide (glyphosate) from effects related to the presence or absence of weeds. Comparing glyphosate plots with hand weeded plots should show effects of

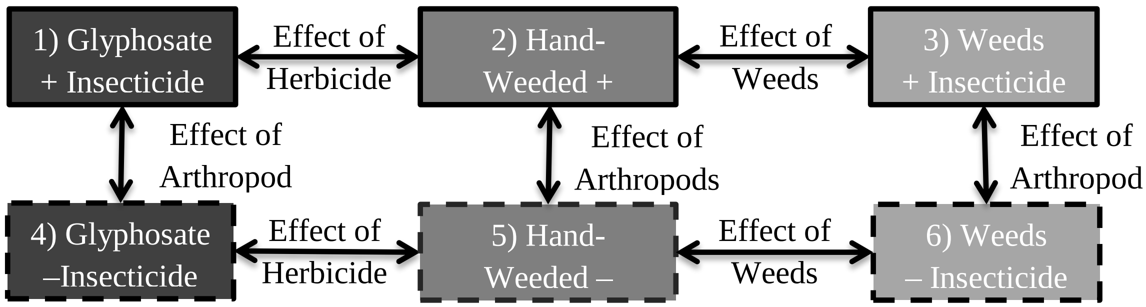


Figure 2. Diagram of treatments with arrows showing the designed effect difference between each of the treatments.

the herbicide, whereas comparing hand-weeded plots with weedy plots should demonstrate effects of the weeds. The insecticide treatment had two levels: with or without a soil insecticide (chlorpyirfos; details given below). Using a soil insecticide was intended to reduce densities of soil arthropods within half of the plots, thus allowing us to determine what effects they may be having on overall soil and soybean health (Figure 2).

**Establishment of Treatments**

Weed Management

*Glyphosate Plots*

Weeds in the +glyphosate plots were removed using glyphosate (Buccaneer Plus®; Tenkoz, Inc; Alpharetta, GA), which is an herbicide commonly used in soybean fields for control of broadleaf weeds and grasses. In our experimental plots, glyphosate was applied twice each summer at the recommended label rate of 2.34 L + 187.08 L of water per hectare at 275.80 kPa. The herbicide was applied using a tractor mounted boom sprayer (3.048 m long) elevated 45.72 cm off the ground with 9 - 8015 nozzles (80 degrees, 0.15 gallons/minute at 40 psi) spaced 40.64 cm apart. In 2012, glyphosate was applied on June 15 and July 13 and in 2013, it was applied on

June 18 and July 16. We chose to apply glyphosate twice to control the weeds in the +glyphosate plots and to reflect common farming practices in the area.

*Hand-weeded Plots*

Weeds in the hand-weeded plots were manually removed. The plots were intended to be weed free. However, due to the high levels of weed pressure, especially in late June – early July, 2012 at the sandy site, we were unable to keep these plots completely weed free for the duration of the season. In 2012, weeds were pulled by hand within the rows and flat edged garden hoes were used to remove weeds between the rows while taking care to minimize disturbances to the soil. Plots were weeded on June 12-13, 25-26 and July 5-6 at the sandy site and June 13-15 and July 2 at the clay site. In 2013, in addition to previous methods, we used mini cultivators (MC 43, Earthquake, Cumberland, WI) in order to improve the degree of weed reduction. Cultivator tines were set at the highest position (resulting in a tillage depth of about 4 cm) to reduce soil disturbance. Hand-weeded plots were weeded on June 14, 19, July 9, and 24 at the sandy site and June 13, 18, July 11, and 24 at the clay site.

*Weedy Plots*

Weeds from the naturally existing seed bank were allowed to grow in plots assigned to the weedy treatment. In 2012, the weed population was so robust that weeds had to be managed in order for the soybeans to survive. Therefore, weeds between the rows were cut with a grass trimmer (FS 45 C, Stihl®, Waiblingen, Baden-Württemberg, Germany) to a height of approximately 10 cm once in each of the weedy plots during the last week of June (June 25-29). In 2013, at the land owner's request and to allow the soybeans to grow, weeds between the rows were cut periodically with a hedge trimmer (HS 45, Stihl®, Waiblingen, Baden-Württemberg, Germany) rather than a grass trimmer because the hedge trimmer was more effective. Weed management began on July 12 and was done as needed to prevent weeds from going to seed.

Insecticide

Chlorpyrifos (Lorsban® 15G Dow AgroSciences; Indianapolis, IN) is a broad spectrum granular insecticide often used for control of soil dwelling pests. This insecticide was chosen because the active ingredient (*chlorpyrifos*) is known to be toxic to Collembola (Wiles and Frampton 1996) and many other important soil dwelling insects (as referenced in Frampton, 1999). The insecticide was distributed by hand across the +insecticide plots at a rate of 266.49 g per 304.80 m of row. Soybeans and weeds were gently shaken with rakes to knock the insecticide granules to the soil and granules were raked into the soil to a depth of about 4 cm. This was done pre-planting (sandy site: May 21, 2012 and May 23, 2013; clayey site: May 22, 2012 and May 23, 2013) and again mid-season (July 12, 2012 & July 17, 2013) to keep the soil arthropod densities low throughout the growing season.

**Weed Pressure**

Weed pressure was quantified mid-season in order to assess the establishment and potential effectiveness of our weed management treatments. Three different options were considered to determine overall weed pressure. One option was the use of a ceptometer to measure leaf area index (LAI). This would have been a useful non-destructive method to quantify weed pressure, but would not have been able to take multiple weed species into account, and the soybean leaves could interfere with the LAI readings. A second option was to calculate the cylindrical plant volume using height and width of the weeds (Bussler et al. 1995), but this method was rejected because it would have been overly time consuming. Instead weeds were sampled destructively and biomass was quantified. Biomass is known to be a good indicator of the amount of stress a plant is able to exert (Wilson 1991, Guo and Rundel 1997) and the information can be gathered quickly.  The downside to this method is that taking destructive samples limits the number of times weed pressure can be quantified without significantly altering weed populations within a plot.

Weeds were destructively sampled at each site from three 0.25 $m^2$ quadrats in each plot on July 31 and August 1, 2012 and July 31 and August 1, 2013. A tape measure was stretched diagonally across the square plots and quadrats were placed between the rows of soybeans at 3.35 m, 6.71 m and 10.06 m. All of the weeds within a quadrat were identified using a weed identification field guide (Iowa State University Extension, 2010), the density of each weed species quantified, weeds clipped at ground level, and placed in paper bags by species. Bags were placed in ovens (71°C) after returning to the lab.  Weeds were dried to a constant weight (timing varied from 48 hours to 1 week depending on weed size) and weighed on a scale (Sartorius, Brinkmann Instruments, Co., Westburg, NY) immediately after being removed from the drier.

**Arthropod Sampling**

We identified and counted all arthropods collected that we expected to find on or in the soil surface. We focused on how treatments affected Collembola. Collembola are important to look at because they play a vital role in the decomposition process (Moore et al. 1988) and the soil food web (Agusti et al. 2003). They are often used as model organisms to investigate the toxicity of some chemicals (Hopkin 1997).

Subsurface (Belowground) Arthropods

Soil samples were taken periodically throughout the growing season to assess how experimental treatments affected the identity and density of the belowground arthropods. One sample was taken from each plot, with four subsamples taken between soybean rows near each plot corner (approximately 1.5m from edge). Samples were taken using a golf cup cutter (11 cm in diameter; Par Aide Products Co., Lino Lakes, MN) by inserting it approximately 15 cm into the soil. When the soil was dry and difficult to penetrate, oil-based cooking spray was used to lubricate the cup cutter. In the weedy plots, the soil was shaken off the roots and plant matter was discarded. Soil from each subsample was combined in a 19 L plastic bucket and mixed by hand. Approximately 3.8 L of the mixed soil was placed in a two-gallon freezer safe bag (Ziploc®, S. C. Johnson & Son Inc; Racine, WI). The soil was transported back to the lab and stored in a walk in refrigerator (10°C) until the soil was processed. After breaking apart soil aggregates and removing large pieces of debris or plant material by hand, 3,240 mL of soil from each individual plot was placed in a Berlese funnel. A specific volume was used rather than weight because it allowed us to standardize the amount of soil processed regardless of differences in weight due to texture or moisture. Remaining soil was returned to the fridge and saved for soil parameter testing.

The soil samples were stored in the cooler until they could be processed due to the large volume of samples collected in 2012. However, this may have impacted the number of arthropods extracted from soil samples. Increasing storage time in a 6°C refrigerator decreased the number of some arthropod taxa extracted from organic soils (Lakly and Crossley 2000). Effects were seen even after 48 hours of storage, and extraction efficiency decreased linearly to approximately fifty percent after 192 hours of storage. Decreases were most extreme for immature soft-bodied mites. The authors suggested this may have been caused by the decreased temperature in the soil resulting in decreased mobility of the smaller soft-bodied organisms.

Sampling dates were chosen based on application of insecticide and glyphosate (Table 1). In both years, the first sample was taken in mid-May to determine arthropod densities prior to any experimental modifications. Subsequent samples were taken less than 72 hours after glyphosate application (to investigate short-term effects) and after approximately 2 weeks (to investigate longer-term effects) (Table 1). The last samples were taken towards the end of the

Table 1. Timing of soil samples for Berlese extraction in relation to treatment applications and duration of cooler storage and extraction time.

| Sample # | Sample Date | Reason for Sampling | Days in Fridge | Days in Berlese Funnel |
|---|---|---|---|---|
| 2012 Field Season | | | | |
| 1 | Sandy field: 5-18<br>Clay field: 5-17 | Sample prior to any chemical application or planting | 12-13 d | 9 d |
| 2 | 6-7 | After chlorpyrifos application and planting (before glyphosate) | 4 d | 9 d |
| 3 | 6-18 | < than 72 h after glyphosate application | 3 d | 10 d |
| 4 | 6-27 | 12 d after 1$^{nd}$ glyphosate application | 11 d | 9 d |
| 5 | 7-16 | 4 d after 2$^{nd}$ insecticide and less than 72 h after 2$^{nd}$ glyphosate application | 8 d | 10 d |
| 6 | 7-27 | 14 d after 2$^{nd}$ glyphosate | 34 d | 10 d |
| 7 | 8-10 | Continued monitoring | 35 d | 10 d |
| 8 | 8-24 | Continued monitoring | 33 d | 9 d |
| 2013 Field Season | | | | |
| 1 | 5-16 | Sample prior to any chemical application or planting | 0 d | 11 d |
| 2 | Sandy field: 6-22<br>Clay field: 7-1 | 96 h after glyphosate application<br>12 d after glyphosate application | 0 d | Sandy field: 9 d<br>Clay field: 10 d |
| 3 | 7-16 | 48 h after glyphosate application | 0 d | 9 d |
| 4 | 8-12 | Continued monitoring | 0 d | 9 d |

growing season (when soybeans were at the R4-R5 growth stage), to document potential cumulative effects. In 2012, a total of eight samples were collected while in 2013 sampling was reduced to four dates, and the timing of samples focused on short-term and cumulative season-long effects.

*Berlese Funnel*

Berlese funnels were used to process soil samples. Berlese funnels use a light source that gradually increases soil temperature and decreases soil moisture, thus causing arthropods in the soil to migrate to the bottom of the apparatus where they are captured in a jar. This method allows for collection of active arthropods that are able to move through the soil structure in response to heat and/or light. It is considered the best option for extraction of soil arthropods that are not surface active (Sakchoowong et al. 2007, Sabu and Shiju 2010). It is also an attractive option because it allows many samples to be processed at once (Edwards 1991).

Berlese funnels were assembled in the lab from steel tractor funnels (1 gallon lock on, Behren's Manufacturing LLC, Winona, MN) measuring 25 cm in diameter across the top of the funnel. Two layers of wire mesh (with 0.50 $cm^2$ and 0.30 $cm^2$ holes respectively, 13.5 cm in diameter), were overlaid 19 cm from the top of each funnel and a steel heat lamp (27 cm in diameter; fitted with a 25W incandescent light bulb was placed on top of the funnel. Glass jars (half pint; Kerr®, TMs Kerr Group Inc., Lancaster, PA) were affixed to the bottom of the funnel (3 cm diameter) by inserting the funnel tip through a modified jar lid (rubber matting was glued to the band with a hole 3 cm in diameter cut into the center which fit perfectly over the bottom of the funnel) and wire was used to secure it in place. This design prevented soil arthropods from escaping and arthropods from the surrounding environment from entering (Figure 3). The jars were covered in duct tape (providing a dark, cool area) to help entice arthropods to move down into the jar) and filled with approximately 75 mL of propylene glycol in order to preserve arthropods until they could be processed.

The extraction time for Berlese funnels varies depending on the amount of soil used and how moist the soil is. Extraction time ranges from 5 days (Sabu and Shiju 2010) to 7 days (Lakly and Crossley 2000, Sakchoowong et al. 2007). After one week of extraction time, our samples were checked and the soil was still moist. After another four days a majority of the soil was dry except the soil along the edges of the funnel, which was probably due to condensation.

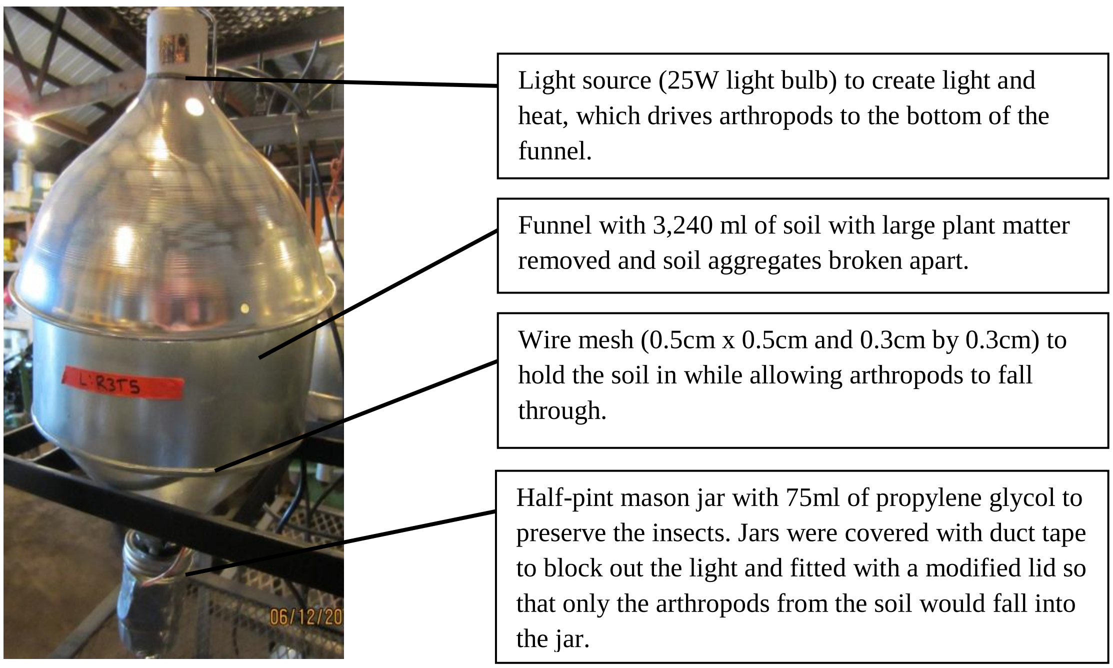


Figure 3. Photo of Berlese funnel set up used to extract soil arthropods and description of modifications used.

Epigeal (Aboveground) Arthropods

Berlese funnels capture mostly small belowground dwelling arthropods, but some species of soil arthropods are more abundant at or on the soil surface rather than deeper within the soil (i.e., epigeal arthropods) (Jagers Op Akkerhuis et al. 1988). Certain families of Collembola are found aboveground, others live belowground, while some families move up and down the soil profile. Experimental treatments may have different impacts on soil arthropods depending on whether they live above or below the soil surface (Verhoef and Brussaard 1990). Aboveground arthropods are more likely to be directly exposed to chemicals and experience more extreme environmental conditions, such as temperature, precipitation, wind, and shading from weeds. Belowground arthropods are more likely to come in contact with chemicals after they have traveled through the soil or plant roots (i.e., during the degradation process). Belowground arthropods are also more sheltered from extreme changes in abiotic factors, but are more influenced by weed roots. To more fully understand how weed management treatments were impacting focal soil arthropod taxa, we also quantified densities of epigeal soil arthropods.

Pitfall traps are the primary method used to assess surface active arthropods (Sabu and Shiju 2010). Pitfalls are an attractive option because they are relatively cheap and easy to use. Unfortunately, they only measure population size based on activity density (Thomas et al. 2006). Activity can be influenced by many factors, including the particular species (Thomas et al. 2006), temperature (Honek 1997), precipitation (Thomas et al. 1998), time of year (Thomas et al. 1998) and vegetation density (Thomas et al, 2006). Even though pitfall traps have their drawbacks, they were the most attractive option in our study to examine the aboveground soil arthropod community.

*Pitfall Trap Design and Placement*

Each trap consisted of a 473 ml plastic cup (9 cm in diameter x12 cm deep; Dart Container Corporation, Mason, MI) filled with approximately 150 ml of propylene glycol. Each cup was inserted into a hole in the ground (11cm in diameter x 15 cm deep) made by using a golf cup cutter, and gaps were filled with soil so that the cup's rim was level with the soil surface. In the weedy plots, attempts were made to disturb the weeds as little as possible except the weeds that were removed when creating the hole for the cup. Cups were covered with a 13.5 $cm^2$, 1.5 cm thick piece of plywood to prevent rain and plant debris from falling into the trap. Wire mesh with 1 $cm^2$ holes was stapled around the outside of the plywood and pushed gently into the soil to hold the plywood off the ground (6.5 cm) and prevent mice, frogs or other small animals from entering the trap.

In 2012, two pitfall traps were placed in each of the 72 experimental plots on August 10 and recovered on August 24. These traps were placed between the soybean rows approximately 2.5 meters from the north and south edge and in the center (east/west) of the plot.

In 2013, we changed our methods for both scientific and logistical reasons. Scientifically, we wanted to be able to compare how the above and belowground soil arthropods were responding to the treatments. To accomplish this, four traps were placed approximately 1.5 m x 1.5 m from each corner of the plot (approximately 80 cm from where the Berlese samples were collected). This was only done in the 36 experimental plots that did not receive an insecticide application. Traps were set out on June 24 (sandy site) July 1 (clay site), July 22 (both sites) and August 16 (both sites). The first samples from each location (June 24 and July 1) were discarded due to heavy rains that damaged the samples. Pitfall traps were left in the field for one week before they were collected. We chose to shorten the trapping duration from two weeks in 2012 to one week in 2013 to shorten exposure of samples to the environment. The +insecticide plots were only sampled on August 16 and only two pitfall traps were set per plot due to logistical and time constraints. These traps were placed the same distance from the edges as the traps in the no insecticide plots, but traps were only in two of the four corners.

## Processing of Berlese and Pitfall Samples

In order to separate arthropods from the preservation liquid for sorting and identification, each sample was transferred to a Büchner funnel lined with a #2 filter paper (1002 110 mm; Whatman™, Maidstone, United Kingdom). The funnel was set atop an Erlenmeyer flask attached to a vacuum hose and suction was used to drain away the liquid, thus leaving the arthropods behind on the filter paper. Although most arthropods were collected and identified (to order and/or family), the primary focus of this study was on Collembola. Identification of Collembola (Christiansen and Bellinger 1998), Coleoptera (Arnett et al. 2002) and other taxa (Dindal 1990, Triplehorn and Johnson 2005) was performed using the relevant dichotomous keys and a dissecting microscope (Zeiss Stemi 2000-C; Carl Zeiss Microscopy, Jena, Germany). Due to difficulty with identification, the collembolan families Isotomidae, Hypogasturidae and Onychuridae were lumped together for analysis.

## Data Analysis

Data from each year were analyzed separately. Only plots without insecticide application were examined for this chapter (except weed management). The insecticide treatment was included to determine what effect soil arthropods had on soil and plant parameters, which are discussed in Chapter 2. Plots with and without insecticide treatment were included for weed pressure because this information was be used in Chapter 2. Graphical analysis of residuals by prediction plots were used to determine if data needed to be transformed prior to analysis using parametric statistics. In cases where data was transformed, details can be found below. LSMeans contrasts were used to compare treatments within a site when weed management was significant to see if the same thing was happening at both sites (Quinn and Keough 2002). Data on each dependent variable were analyzed separately using JMP (SAS Institute, 2013).

Weed Pressure

For both weed density and weight, data from each transect within a plot were averaged prior to analysis. Total weed density and total weed biomass data (all weed species combined) were log X+1 transformed and analyzed using factorial ANOVA with site, block, weed management, insecticide and weed management*insecticide as the independent variables. The last four factors were nested within site and block was considered a random variable.

Collembola Diversity

We were interested in determining if weed management treatments altered the community composition of Collembola taxa. Therefore, we analyzed the relative proportion of each group (Entomobryidae, Sminthuridae, IOH), which were expressed as a percentage of total Collembola collected, prior to and after weed management treatments were applied.

For 2012, Berlese data from May 17 and June 7 were summed for the pre-weed management analysis (i.e., data taken prior to glyphosate application). For post-weed management analysis, data from June 18 through August 24 were combined. In 2013, pre-weed management data from May 16 were analyzed. Data from the three sampling dates after glyphosate application (i.e., June 22 through August 14) were used for the post-weed management analysis.

Percentage of IOH and Sminthuridae were analyzed using MANOVA with site, block and weed management as the independent variables. The last two factors were nested within site. Since the variables were proportions of a total, each row added up to one. In order for the analysis to work properly, one group had to be left out so that each response variable was an independent measurement (Cisneros and Rosenheim 1998). Entomobryidae was left out of the analysis because it was the least dominant. For the pitfall samples, proportions of Collembola collected from each group were analyzed using MANOVA with site, block and weed management as independent variables. The last two factors were nested within site. This time Sminthuridae was left out of the analysis because it had the least percentage of total Collembola. In 2013, data from both sampling were combined for analysis.

Collembola Density

All data were analyzed using factorial ANOVA with site, block and weed management as independent variables. The last two factors were nested within site and block was considered a random variable.

Short Term Impacts of Glyphosate

Weed management practices may have had both immediate (short term) and cumulative (long term) impacts on arthropod populations, In order to assess potential short term effects of glyphosate on belowground Collembola (i.e., 3-12 d post application), we examined Collembola densities (all species combined) prior to glyphosate application (May 17, 2012, June 7, 2012, and May 16, 2013) and the change in density after glyphosate application.  For the latter analysis, Collembola densities from the first sampling date 3 d post glyphosate application (June 18, 2012 and June 22, 2013) were subtracted from pre-glyphosate application densities (on June 7, 2012 and May 16, 2013, respectively). In addition, in 2012, we also examined the change in Collembola densities 12 d post glyphosate application (i.e., densities from the second sampling date, June 27, subtracted from pre-weed management, June 7 densities). The absolute value of the difference was log (X+1) transformed and negative values were reassigned prior to analysis.

Long Term Impacts of Weed Management

To address potential long term effects of weed management treatments on Collembola densities, for each year we analyzed Collembola densities summed across the season, excluding dates prior to glyphosate application and hand-weeding (i.e., June 18 through August 24, 2012 and June 22 through August 14, 2013).Cumulative density data were log (x+1) transformed for ANOVA.

Weed Management Effects on Aboveground Collembola

A similar method was used to analyze epigeal cumulative densities. In 2012, Collembola were only sampled on one date and average density per pitfall trap per plot was calculated. The two sampling dates (July, 22 and August 16) from 2013 were added together and average cumulative density per pitfall trap per plot was determined. The cumulative density was log (x+1) transformed for ANOVA.

## Results

### Weed pressure

We measured weed pressure to have a quantifiable means of determining whether our weed management treatments worked the way we designed them to and to determine the difference in weed communities between the two sites. We not only measured total weed density and biomass, but also individual species. Each species or group of plants can impact the soil arthropods differently which could have implications for our results.

Weed Diversity

Both sites had similar weed community compositions, but the weeds were present in different proportions at each site as well as in different weed management treatments. Weed diversity was highest in the weedy plots and lowest in the glyphosate plots. This was especially evident at the sandy site where only two weed species were found in the glyphosate plots compared to seven weed species in the weedy plots.

In 2012, at the beginning of the season, the sandy site had a dense carpet of *Chenopodium album* (L.). Throughout the season, it was difficult to keep up with the weeding at this site, as demonstrated by the higher biomass of weeds in the hand-weeded plots (43.79 ± 10.31 g $m^{-2}$) versus the glyphosate plots (15.88 ± 5.00 g $m^{-2}$; Table 2). Weed density was higher in the glyphosate plots (31.58 ± 8.14 weeds $m^{-2}$) compared to the hand-weeded plots (18.17 ±3.14 weeds $m^{-2}$). The glyphosate plots had many small *C. album* seedlings that likely emerged after the last glyphosate application causing this trend. *Chenopodium album* was the dominant weed at the sandy site in 2012 in all three weed management treatments.

Many more weeds were present in the weedy plots at the sandy site (312.22 ± 40.95 weeds $m^{-2}$) than at the clayey site (47.89 ± 19.16 weeds $m^{-2}$), but the biomass was similar between the two sites (sandy: 311.13 ± 32.18 g $m^{-2}$; clayey: 311.84 ± 76.25 g $m^{-2}$). In general, at the sandy site there were many small weeds, while at the clayey site there were fewer weeds, but they were much larger. This was especially true in the weedy plots where *Amaranthus retroflexus* (L.) made up most of the biomass (76.34%) but only 28.54% of the density. The densest weeds in the weedy plots as well as the hand-weeded and glyphosate plots were *Ulmus pumila* (L.) seedlings. Although they never got very large, they were difficult to kill with physical or chemical control. Once again, the biomass in the hand-weeded plots (11.98 ± 6.25 g $m^{-2}$) was higher than in the glyphosate plots ($0.48 \pm 0.21$ g m$^{-2}$), while glyphosate plots had the higher density (glyphosate: $11.56 \pm 4.26$ weeds m$^{-2}$; hand-weeded: $6.11 \pm 1.78$ weeds m$^{-2}$). This was mostly a result of the small elm trees that were not killed by the chemical.

In 2013, the weed species were similar as in 2012. At the sandy site, *C. album* still dominated in terms of density (50.28%) but grasses (33%) and *A. retroflexus* (31.68%) made up most of the biomass. *Chenopodium album* made up most of the density and biomass in the hand-weeded plots. The glyphosate plots were dominated by *U. pumila,* but *C. album* again made up most of the biomass. At the clayey site, *C. album* and *A. retroflexus* were again the most dominant weeds in the weedy plots. The hand-weeded plots had mostly *A retroflexus* while the glyphosate plots were dominated by *U. pumila* seedlings. In 2013, three weeds were present in the plots that were not there the previous year: *A. tuberculatus* ((Moq.) Sauer), *Conyza canadensis* (L), and *Cirsium arvense* (L.).

<u>Weed Density and Biomass</u>

As a result of the differences discussed above, site had a significant effect on both total weed density ($P = 0.0025$; Table 3) and weed biomass ($P = 0.0043$) in 2012. Weed management had a significant effect on both total weed density ($P < 0.0001$) and total weed biomass ($P < 0.0001$). Density was significantly higher in the weedy plots ($186.06 \pm 35.33$ weeds m$^{-2}$; Table 3; Figure 4), but not significantly different between the hand-weeded ($12.14 \pm 2.67$ weeds m$^{-2}$) and glyphosate plots ($21.64 \pm 4.69$ weeds m$^{-2}$). Weed biomass on the other hand, was significantly different between all three weed management treatments, likely due mostly to the high biomass in the hand-weeded plots at the sandy site. Ideally the hand-weeded and glyphosate plots would have had the same amount of weed pressure, but we were unable to achieve that in

2012. This may have implications when interpreting the results of collembolan densities. A mini cultivator was used in 2013 to allow for better weed control.

Even though the weeds in the weedy plot were trimmed periodically in 2013, weed management still had a significant effect on both total weed density ($P < 0.0001$) and total biomass ($P < 0.001$). At both sites, weed density was significantly higher in the weedy (614.40 ± 67.20 weeds $m^{-2}$; Table 4) plots than the hand-weeded (10.81 ± 2.05 weeds $m^{-2}$) and glyphosate (42.35 ± 18.63 weeds $m^{-2}$) plots. Weed biomass was also significantly higher in the weedy plots (281.61 ± 36.36 g $m^{-2}$). The mini cultivator allowed us to better control the weed populations in the hand-weeded plots resulting in no significant difference between the hand-weeded and glyphosate plots.

Insecticide did not significantly impact weed density or biomass in either year. There was a small weed management*insecticide treatment effect on weed density in 2012, but this was only marginal ($P = 0.0707$).

Table 2. Weed pressure across weed treatments expressed as mean ± SEM density and percentage of total weed density (D, number m-2) as well as mean ± SEM biomass and percentages of total biomass (B, g m-2) collected, approximately 18 d, and 14 d after glyphosate application and 22 d and 20 d after hand weeding in 2012 and 2013 respectively.

| | **Weedy** | | | | **Hand-weeded** | | | | **Glyphosate** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | %D | D | %B | B | %D | D | %B | B | %D | D | %B | B |
| **Sandy 2012** | | | | | | | | | | | | |
| *Amaranthus retroflexus* | 4.41 | 13.78±4.60 | 13.58 | 42.25±25.99 | 2.75 | 0.50±0.22 | 14.89 | 6.52±3.91 | 3.07 | 0.97±0.41 | 4.41 | 0.70±0.37 |
| *Taraxacum spp.* | 0.68 | 2.11±1.40 | 2.00 | 6.23±5.78 | 3.69 | 0.67±0.45 | 0.78 | 0.34±0.26 | 2.31 | 0.73±0.73 | 0.76 | 0.12±0.12 |
| *Chenopodium album* | 90.54 | 282.67±43.23 | 82.37 | 256.27±25.24 | 80.74 | 14.67±3.04 | 72.94 | 31.94±8.92 | 85.59 | 27.03±7.54 | 93.45 | 14.84±4.75 |
| Poaceae | 2.49 | 7.78±3.16 | 1.60 | 4.98±1.94 | 7.93 | 1.44±0.81 | 9.43 | 4.13±2.45 | 8.07 | 2.55±2.28 | 1.39 | 0.22±0.16 |
| *Solanum nigrum* | 1.74 | 5.44±1.50 | 0.36 | 1.11±0.37 | 4.29 | 0.78±0.31 | 1.96 | 0.86±0.55 | 0.57 | 0.18±0.18 | 0 | 0 |
| *Ulmus pumila* | 0.11 | 0.33±0.33 | 0.08 | 0.25±0.23 | 0.61 | 0.11±0.11 | <0.01 | <0.01 | 0.38 | 0.12±0.12 | <0.01 | <0.01 |
| unknown weeds | 0.04 | 0.11±0.11 | 0.02 | 0.06±0.05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 100 | 312.22±40.95 | 100 | 311.13±32.18 | 100 | 18.17±3.14 | 100 | 43.79±10.31 | 100 | 31.58±8.14 | 100 | 15.88±5.00 |
| **Clayey 2012** | | | | | | | | | | | | |
| *Amaranthus retroflexus* | 28.54 | 13.67±6.69 | 76.34 | 238.05±67.25 | 32.73 | 2.00±0.89 | 92.99 | 11.14±6.21 | 0.95 | 0.11±0.11 | <0.01 | <0.01 |
| *Chenopodium album* | 1.17 | 0.56±0.31 | 15.50 | 48.35±32.08 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Hibiscus trionum* | 0.69 | 0.33±0.33 | 0.20 | 0.63±0.63 | 1.80 | 0.11±0.11 | 1.59 | 0.19±0.19 | 0 | 0 | 0 | 0 |
| *Zea mays* | 0.46 | 0.22±0.15 | 3.86 | 12.04±8.24 | 1.80 | 0.11±0.11 | 0.67 | 0.08±0.08 | 0 | 0 | 0 | 0 |
| Other Poacea | 2.78 | 1.33±0.96 | 1.28 | 3.98±3.42 | 3.60 | 0.22±0.15 | 0.83 | 0.10±0.08 | 0 | 0 | 0 | 0 |
| *Portulaca oleracea* | 0.23 | 0.11±0.11 | <0.01 | 0.02±0.02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ulmus pumila* | 66.13 | 31.67±15.44 | 2.82 | 8.78±5.64 | 60.07 | 3.67±1.47 | 4.00 | 0.48±0.23 | 98.96 | 11.44±4.26 | 100 | 0.48±0.21 |
| Total | 100 | 47.89±19.16 | 100 | 311.84±76.25 | 100 | 6.11±1.78 | 100 | 11.98±6.25 | 100 | 11.56±4.26 | 100 | 0.48±0.21 |
| **Sandy 2013** | | | | | | | | | | | | |
| *Amaranthus retroflexus* | 25.27 | 114.33±34.58 | 31.68 | 48.04±10.30 | 24.26 | 2.55±1.02 | 41.10 | 0.60±0.27 | 5.52 | 0.56±0.31 | 6.25 | 0.03±0.02 |
| *A. tuberculatus* | 12.05 | 54.53±31.43 | 10.57 | 16.03±5.79 | 0 | 0 | 0 | 0 | 0.99 | 0.10±0.10 | 12.50 | 0.06±0.06 |
| *Conyza canadensis* | 0.02 | 0.10±0.10 | <0.01 | 0.01±0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Taraxacum spp.* | 0.19 | 0.87±0.58 | 2.03 | 3.08±2.82 | 0 | 0 | 0 | 0 | 0.99 | 0.10±0.10 | 2.08 | <0.01 |
| *Chenopodium album* | 50.28 | 227.44±54.19 | 18.49 | 28.04±5.62 | 44.34 | 4.66±1.50 | 42.47 | 0.62±0.30 | 38.36 | 3.89±1.39 | 41.67 | 0.20±0.09 |
| Poaceae | 7.92 | 35.82±7.76 | 33.00 | 50.04±9.87 | 0.95 | 0.10±0.10 | 0.68 | 0.01±0.01 | 0 | 0 | 0 | 0 |
| *Solanum nigrum* | 3.30 | 14.93±4.51 | 4.14 | 6.28±2.33 | 0.95 | 0.10±0.10 | 8.22 | 0.12±0.12 | 0 | 0 | 0 | 0 |
| *Ulmus pumila* | 0.89 | 4.00±3.34 | 0.04 | 0.06±0.06 | 29.50 | 3.10±1.86 | 7.53 | 0.11±0.07 | 53.20 | 5.40±2.71 | 27.08 | 0.13±0.05 |
| unknown weeds | 0.07 | 0.33±0.25 | 0.03 | 0.04±0.04 | 0 | 0 | 0 | 0 | 0.99 | 0.10±0.10 | 10.42 | 0.05±0.05 |
| Total | 100 | 452.36±47.38 | 100 | 151.63±14.52 | 100 | 10.51±2.53 | 100 | 1.46±0.38 | 100 | 10.14±3.77 | 100 | 0.48±0.17 |
| **Clayey 2013** | | | | | | | | | | | | |
| *Amaranthus retroflexus* | 46.18 | 358.56±64.94 | 45.71 | 188.15±31.77 | 52.03 | 5.78±2.44 | 39.66 | 1.15±0.53 | 0 | 0 | 0 | 0 |
| *A. tuberculatus* | 14.35 | 111.44±24.76 | 19.09 | 78.59±14.82 | 0.99 | 0.11±0.11 | 26.55 | 0.77±0.77 | 1.05 | 0.78±0.53 | 11.19 | 0.33±0.22 |
| *Cirsium arvense* | 0.10 | 0.78±0.78 | 0.20 | 0.82±0.82 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Chenopodium album* | 36.83 | 286.00±83.73 | 31.65 | 130.28±36.34 | 1.98 | 0.22±0.22 | <0.01 | <0.01 | 1.05 | 0.78±0.56 | 6.44 | 0.19±0.15 |
| *Hibiscus trionum* | 0.10 | 0.78±0.45 | 0.18 | 0.74±0.51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Poaceae | 1.32 | 10.22±6.21 | 2.61 | 10.74±6.95 | 5.04 | 0.56±0.56 | <0.01 | <0.01 | 0 | 0 | 0 | 0 |
| *Ulmus pumila* | 0.64 | 5.00±1.70 | 0.27 | 1.11±0.53 | 33.03 | 3.67±1.56 | 30.00 | 0.87±0.71 | 96.87 | 72.22±35.56 | 76.91 | 2.26±1.03 |
| unknown weeds | 0.47 | 3.67±2.51 | 0.28 | 1.16±0.73 | 6.93 | 0.77±0.48 | 4.14 | 0.12±0.09 | 1.05 | 0.78±0.38 | 6.10 | 0.18±0.09 |
| Total | 100 | 776.45±108.93 | 100 | 411.60±47.49 | 100 | 11.11±3.35 | 100 | 2.90±1.92 | 100 | 74.55±35.32 | 100 | 2.95±1.00 |

Scientific name followed by common name organized alphabetically by family. Amaranthaceae: *A. retroflexux* = redroot pigweed, *A. tuberculatus* = water hemp; Asteraceae: *C. arvense* = Canada thistle, *C.* Canadensis = horseweed, Taraxacum *spp.* = dandelion species; Chenopodiaceae: *C. album* = common lambsquarter; Malvaceae: *H. trionum* = veinice mallow; Poacea: *Z. mays* = volunteer corn, other grasses; Portulacaceae: *P. oleracea* = common purselane; Solanaceae: *S. nigraum* = eastern black nightshade; Ulmaceae: *U. pumila* = Siberian elm

Table 3. F-values, degrees of freedom and P-values from ANOVA test for total weed density and biomass using log(x+1) transformed data with data averaged across all three transects. Least significant mean contrast used to determine difference between weed management treatments.

| **Factors** | **2012 P-Values** | | **2013 P-Values** | |
|---|---|---|---|---|
| | **Density** | **Biomass** | **Density** | **Biomass** |
| Site | F = 15.9533<br>df = 1, 10<br>**P = 0.0025** | F = 13.5317<br>df = 1, 9.982<br>**P =0.0043** | F = 4.7193<br>df = 1, 10<br>**P = 0.0549** | F = 31.7763<br>df = 1, 10<br>**P = 0.0002** |
| Weed Management (Site) | F = 29.2855<br>df = 4, 50<br>**P < 0.0001** | F = 46.6378<br>df = 4, 48.21<br>**P < 0.0001** | F = 55.4381<br>df = 4, 50<br>**P < 0.0001** | F = 213.2328<br>df = 4, 50<br>**P < 0.0001** |
| Insecticide Treatment (Site) | F = 1.9948<br>df = 2, 50<br>P = 0.1467 | F = 0.2772<br>df = 2, 48.22<br>P = 0.7591 | F = 0.8838<br>df = 2, 50<br>P = 0.4196 | F = 0.7111<br>df = 2, 50<br>P = 0.4960 |
| Weed Management*Insecticide Treatment (Site) | F = 2.3065<br>df = 4, 50<br>P = 0.0707 | F = 1.7255<br>df = 4, 48.21<br>P = 0.1597 | F = 2.0262<br>df = 4, 50<br>P = 0.1049 | F = 1.1043<br>df = 4, 50<br>P = 0.3649 |
| Glyphosate vs. Hand-weeded | F = 1.3330<br>df = 1, 50<br>P = 0.2538 | F = 14.0688<br>df =1, 48.32<br>**P = 0.0005** | F = 1.0102<br>df = 1, 50<br>P = 0.3197 | F = 0.2336<br>df = 1, 50<br>P = 0.6309 |
| Glyphosate vs. Weedy | F = 69.3115<br>df = 1, 50<br>**P < 0.0001** | F = 167.7389<br>df = 1, 48.32<br>**P < 0.0001** | F = 150.0095<br>df = 1, 50<br>**P < 0.0001** | F = 647.3403<br>df = 1, 50<br>**P < 0.0001** |
| Hand-weeded vs. Weedy | F = 89.8689<br>df = 1, 50<br>**P < 0.0001** | F = 89.5520<br>df = 1, 48<br>**P < 0.0001** | F = 175.6400<br>df = 1, 50<br>**P < 0.0001** | F = 622.9772<br>df = 1, 50<br>**P < 0.0001** |

Table 4. Mean ± SEM density (number of weeds per square meter) and mean ± SEM biomass (grams per square meter) of weeds for each year with data summed across sites and insecticide treatment.

| Weed Management | **Mean ± SEM** | | | |
|---|---|---|---|---|
| | **2012 Density** | **2012 Biomass** | **2013 Density** | **2013 Biomass** |
| Glyphosate | 21.64 ± 4.69 | 8.48 ± 2.96 | 42.35 ± 18.63 | 1.72 ± 0.56 |
| Hand-weeded | 12.14 ± 2.67 | 27.89 ± 6.77 | 10.81 ± 2.05 | 2.18 ± 0.97 |
| Weedy | 180.06 ± 35.33 | 311.49 ± 40.47 | 614.40 ± 67.20 | 281.61 ± 36.36 |

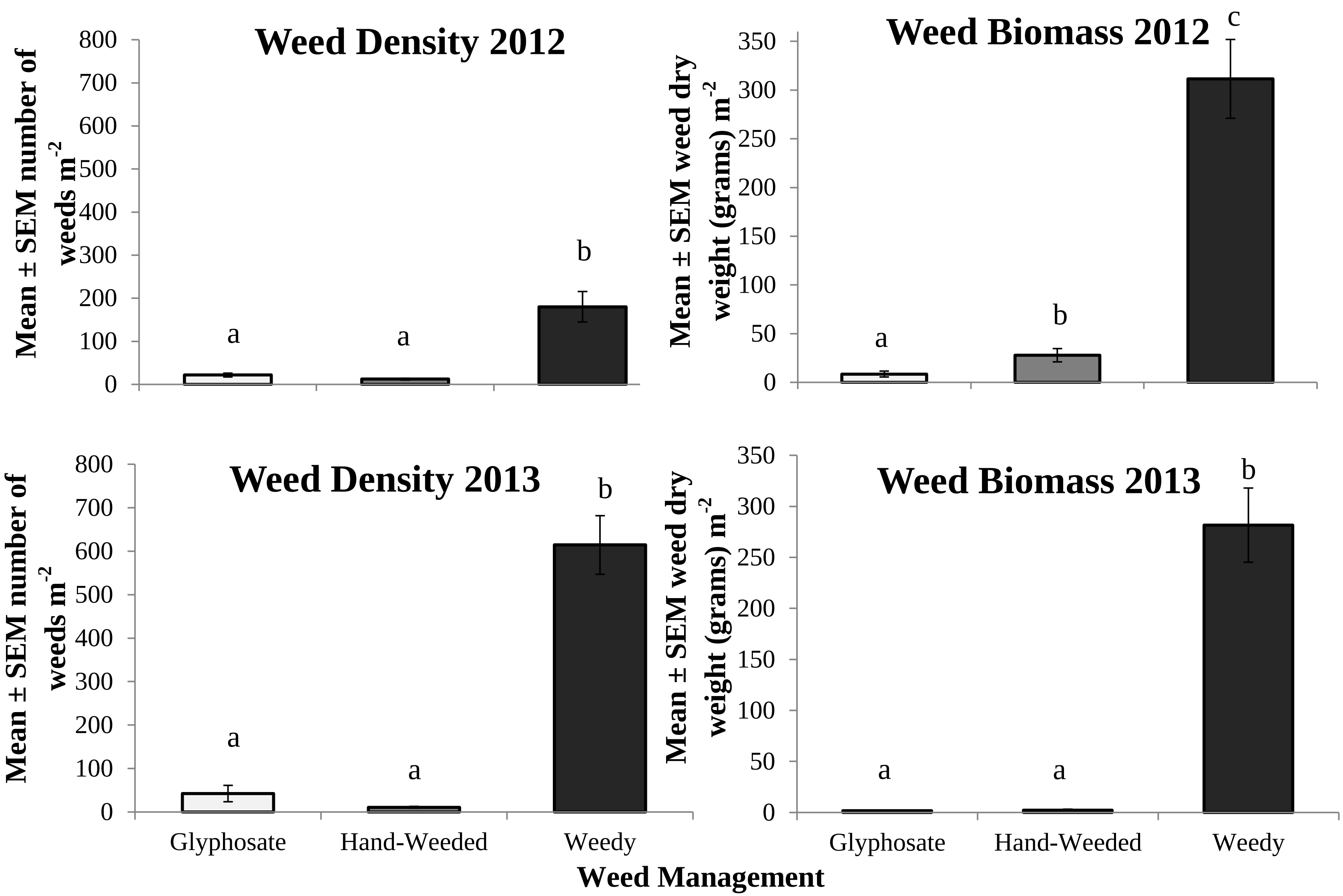


Figure 4. Mean ± SEM density (number of weeds per square meter) and mean ± SEM biomass (grams per square meter) of weeds for each year with data summed across sites and insecticide treatment. Different letters indicate significant differences at $P \leq 0.05$.

**Total Taxa Collected**

Several different groups of arthropods were collected throughout the experiment (Table 5). Larvae of any groups were not counted due to difficulty with identification. Collembola were the most numerous taxa. The most arthropods were collected at the sandy site in both 2012 and 2013. The most abundant group collected in the Berlese funnels differed depending on location. At the sandy site, Collembola were the most abundant. At the clayey site, Acari (mites) were the most abundant. In the pitfall traps, Collembola was the dominant group (mites were not counted in the pitfall traps. Total numbers were lower in 2013 than 2012. This was partially due to fewer collection dates, but also to lower densities in general which will be shown later on. We chose to focus the remainder of this chapter on Collembola due to their abundance as well as their importance in the soil food web and in nutrient cycling.

Table 5. Total numbers of relevant taxa collected over the course of the experiment.

| | | | | 2012 | | 2013 | |
|---|---|---|---|---|---|---|---|
| **Trap** | **Class** | **Order** | **Family** | **Sandy** | **Clayey** | **Sandy** | **Clayey** |
| **Berlese** | Arachnida | Acari | | 7886 | 16285 | 4486 | 10598 |
| | Symphyla | | | 176 | 11 | 117 | 5 |
| | Hexapoda | Collembola | | 24515 | 9323 | 7602 | 1892 |
| | | Diplura | Japygidae | 111 | 14 | 30 | 10 |
| | | Coleoptera | Carabidae | 12 | 57 | 16 | 51 |
| | | | Staphylinidae | 81 | 20 | 23 | 7 |
| Berlese Total | | | | 32781 | 25710 | 12274 | 12536 |
| **Pitfall** | Arachnida | Araneae | | 97 | 473 | 360 | 326 |
| | | Opiliones | | 34 | 1125 | 471 | 209 |
| | Malacostraca | Isopoda | | 10 | 165 | 22 | 59 |
| | Chilopoda | | | 51 | 351 | 57 | 85 |
| | Hexapoda | Collembola | | 4284 | 3208 | 1898 | 1844 |
| | | Coleoptera | Carabadae | 294 | 680 | 951 | 932 |
| | | | Staphylinidae | 478 | 376 | 149 | 61 |
| | | | Scarabaeidae | 6 | 25 | 6 | 12 |
| | | | Elateridae | 6 | 47 | 9 | 36 |
| | | | Nitidulidae | 46 | 71 | 446 | 56 |
| | | | Phalacridae | 1041 | 975 | 651 | 233 |
| | | | Latridiidae | 59 | 55 | 211 | 110 |
| | | | Anthicidae | 63 | 162 | 92 | 157 |
| Pitfall Total | | | | 6469 | 7713 | 5133 | 4120 |
| **Total Arthropods Collected** | | | | 39250 | 33432 | 17404 | 16656 |

Note: number of traps were not equal between years.

## Collembola Diversity

<u>Euedaphic (Belowground) Collembola</u>

To determine if collembolan families were affected differently by the weed management treatments, we examined the diversity of Collembola before and after weed management treatments were applied. The belowground Collembola were mostly composed of three different families Isotomidae, Onychiuridae and Hypogasturidae that have been lumped into one group (i.e., IOH). This was the most dominant group in all three weed management treatments both before (Figure 5) and after (Figure 6) treatments were applied (Table 6). Weed management was not significant in either year (Table 7), but site was significant. The sandy site had few Collembola other than from the IOH group. Although IOH was dominant at the clayey site, the percentages of the other families were higher at the clayey site than the sandy site.

Table 6. Percentage of collembolan families based on average densities collected from belowground before and after weed management.

| | | **Sandy** | | | **Clayey** | |
|---|---|---|---|---|---|---|
| | **Ento** | **IOH** | **Sminth** | **Ento** | **IOH** | **Sminth** |
| **2012 Pre Weed Management** | | | | | | |
| Glyphosate | 0.04% | 99.17% | 0.78% | 0.16% | 90.30% | 9.54% |
| Hand-weeded | 0.04% | 98.77% | 1.19% | 0.44% | 85.86% | 13.70% |
| Weedy | 0.00% | 99.87% | 0.13% | 0.59% | 90.65% | 8.76% |
| **2012 Post Weed Management** | | | | | | |
| Glyphosate | 0.00% | 97.54% | 2.46% | 0.25% | 82.54% | 17.21% |
| Hand-weeded | 0.48% | 94.29% | 5.23% | 0.45% | 92.05% | 7.50% |
| Weedy | 0.03% | 99.80% | 0.18% | 0.08% | 87.68% | 12.24% |
| **2013 Pre Weed Management** | | | | | | |
| Glyphosate | 0.00% | 100.00% | 0.00% | 0.00% | 91.83% | 8.17% |
| Hand-weeded | 0.00% | 100.00% | 0.00% | 0.00% | 98.33% | 1.67% |
| Weedy | 0.00% | 100.00% | 0.00% | 0.00% | 93.94% | 6.06% |
| **2013 Post Weed Management** | | | | | | |
| Glyphosate | 0.00% | 99.83% | 0.17% | 2.98% | 95.32% | 1.70% |
| Hand-weeded | 0.00% | 99.90% | 0.10% | 2.87% | 91.87% | 5.26% |
| Weedy | 0.00% | 92.92% | 7.08% | 9.73% | 89.36% | 0.91% |

Ento – Entomobryidae; IOH – combination of three families (Isotomidae, Onychiuridae and Hypgasturidae); Sminth - Sminthuridae

Table 7. F-values, degrees of freedom and P-values from MANOVA test using proportions of collembolan groups collected. No insecticide plots only, Wilks' Lambda values reported.

| | 2012 | | | 2013 | | |
|---|---|---|---|---|---|---|
| | **F-Value** | **df** | **P-Value** | **F-Value** | **df** | **P-Value** |
| **Pre Weed Management** | | | | | | |
| Site | 23.5501 | 2, 19 | **<0.0001** | 3.9340 | 2, 18 | **0.0382** |
| Weed Management(Site) | 0.6531 | 8, 38 | 0.7284 | 1.382 | 8, 36 | 0.2434 |
| **Post Weed Management** | | | | | | |
| Site | 4.5119 | 2, 19 | **0.0249** | 6.8090 | 2, 19 | **0.0059** |
| Weed Management(Site) | 1.3375 | 8, 38 | 0.2553 | 1.4289 | 8, 38 | 0.2163 |

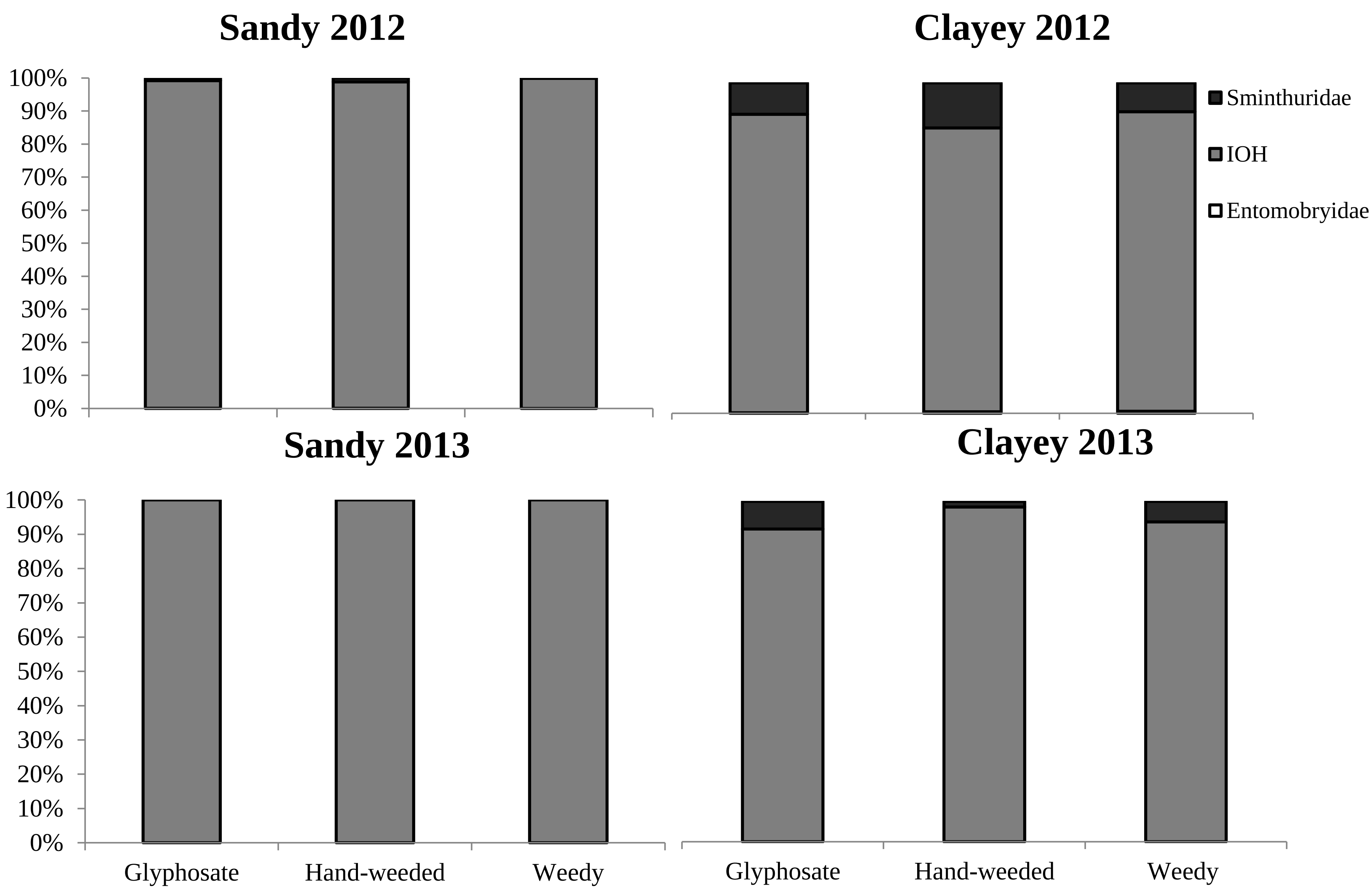


Figure 5. Subsurface collembolan families collected prior to weed management expressed as a percentage of total Collembola. IOH is a combination of three families (Isotomidae, Onychiuridae and Hypogasturidae).

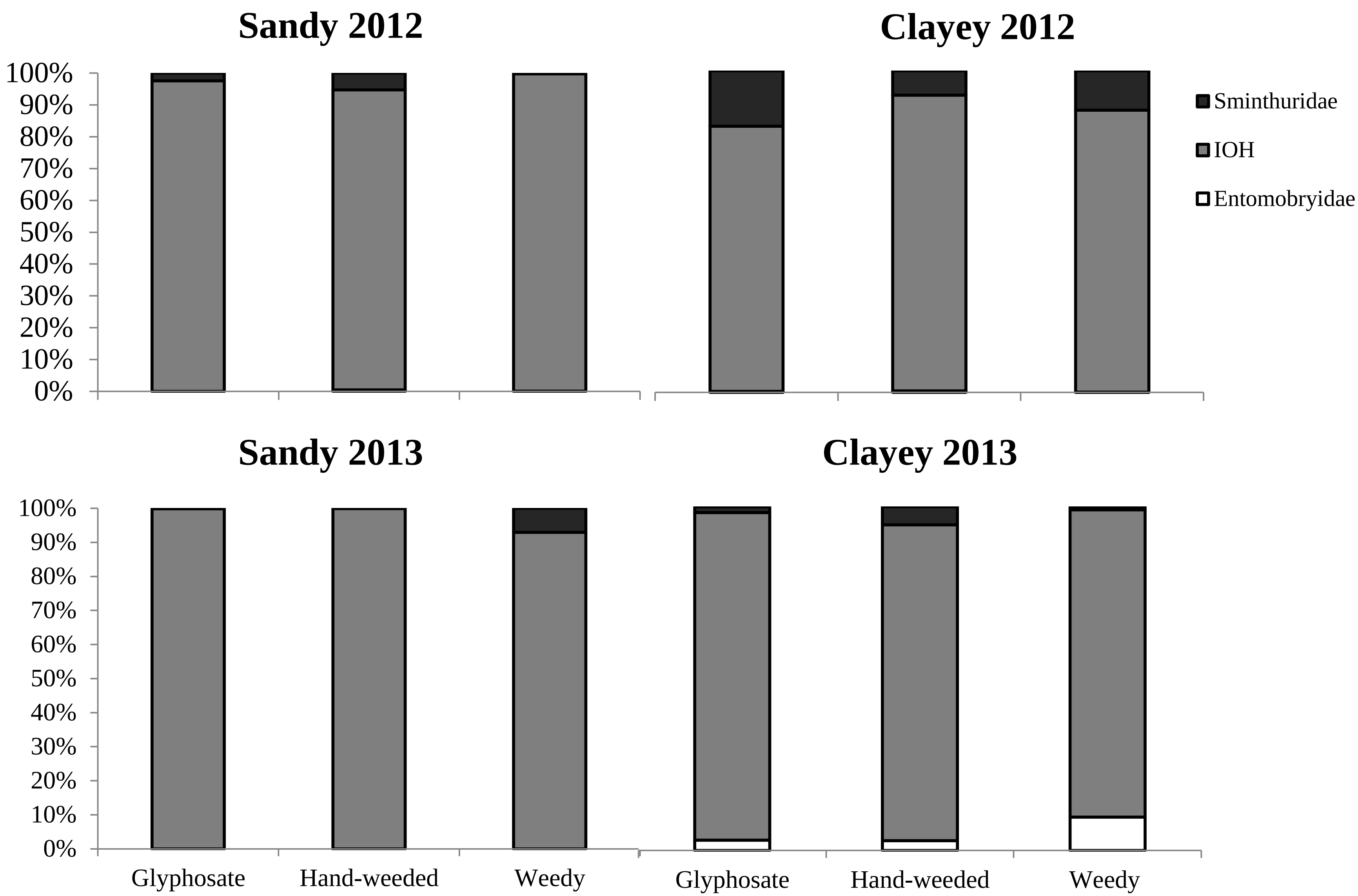


Figure 6. Subsurface collembolan families collected after weed management expressed as percentages of total Collembola. IOH is a combination of three families (Isotomidae, Onychiuridae and Hypogasturidae).

Epedaphic (Aboveground) Collembola

The percentages of aboveground Collembola families collected were more evenly distributed than the belowground Collembola. Samples were not collected prior to applying weed management treatments, so we only have diversity information from after weed management. At the sandy site in 2012, the most dominant group in the hand-weeded and weedy plots was the IOH group (47.48% and 61.19% respectively; Table 8; Figure 7). Sminthuridae was the dominant family in the glyphosate plots (54.12%), but this difference in dominant groups was not strong enough to result in a significant weed management effect (P = 0.1061; Table 9). Entomobryidae was the dominant group at the clayey site in all three weed management treatments, followed closely by IOH. In 2013, weed management caused significant differences in Collembola diversity (P = 0.0257), but only at the sandy site. At the sandy site, the dominant group in the glyphosate plots was IOH (61.10%). In the other two weed management treatments, all three groups had similar proportions. The proportions of collembolan taxa in glyphosate plots was significantly different than both the hand-weeded (P = 0.0023) and the weedy (P = 0.0034) plots because the glyphosate plots had a significantly higher proportion of IOH.

Table. 8. Percentage of collembolan families based on average densities collected aboveground after weed management.

| | **Sandy** | | | **Clayey** | | |
|---|---|---|---|---|---|---|
| | **Ento** | **IOH** | **Sminth** | **Ento** | **IOH** | **Sminth** |
| **2012** | | | | | | |
| Glyphosate | 11.27% | 34.61% | 54.12% | 54.19% | 45.33% | 0.48% |
| Hand-weeded | 19.99% | 47.48% | 32.53% | 57.45% | 41.61% | 0.93% |
| Weedy | 13.73% | 61.19% | 25.08% | 54.81% | 44.91% | 0.28% |
| **2013** | | | | | | |
| Glyphosate | 36.49% | 61.10% | 2.41% | 79.31% | 14.99% | 5.70% |
| Hand-weeded | 40.20% | 26.07% | 33.73% | 80.02% | 18.19% | 1.80% |
| Weedy | 38.80% | 25.62% | 35.58% | 94.31% | 5.35% | 0.34% |

Note: Ento = Entomobryidae; IOH = Isotomidae, Onychuridae, Hypogastruridae; Sminth = Sminthruidae

Table 9. F-values, degrees of freedom and P-values from MANOVA test using proportions of collembolan groups collected from pitfall samples. No insecticide plots only, Wilks' Lambda values reported.

| | | 2012 | | | 2013 | | |
|---|---|---|---|---|---|---|---|
| | | **F-Value** | **df** | **P-Value** | **F-Value** | **df** | **P-Value** |
| **Post Weed Management** | | | | | | | |
| Site | | 27.7153 | 2, 17 | **<0.0001** | 9.4309 | 2, 19 | **0.0014** |
| Weed Management(Site) | | 1.8265 | 8, 34 | 0.1061 | 2.5346 | 8, 38 | **0.0257** |
| Glyphosate vs. Hand-weeded | Sandy | N/A | N/A | N/A | 8.4675 | 2, 19 | **0.0023** |
| | Clayey | N/A | N/A | N/A | 0.1102 | 2, 19 | 0.8962 |
| Glyphosate vs. Weedy | Sandy | N/A | N/A | N/A | 7.7643 | 2, 19 | **0.0034** |
| | Clayey | N/A | N/A | N/A | 0.6374 | 2, 19 | 0.5396 |
| Hand-weeded vs. Weedy | Sandy | N/A | N/A | N/A | 0.0547 | 2, 19 | 0.9470 |
| | Clayey | N/A | N/A | N/A | 0.6975 | 2, 19 | 0.5101 |

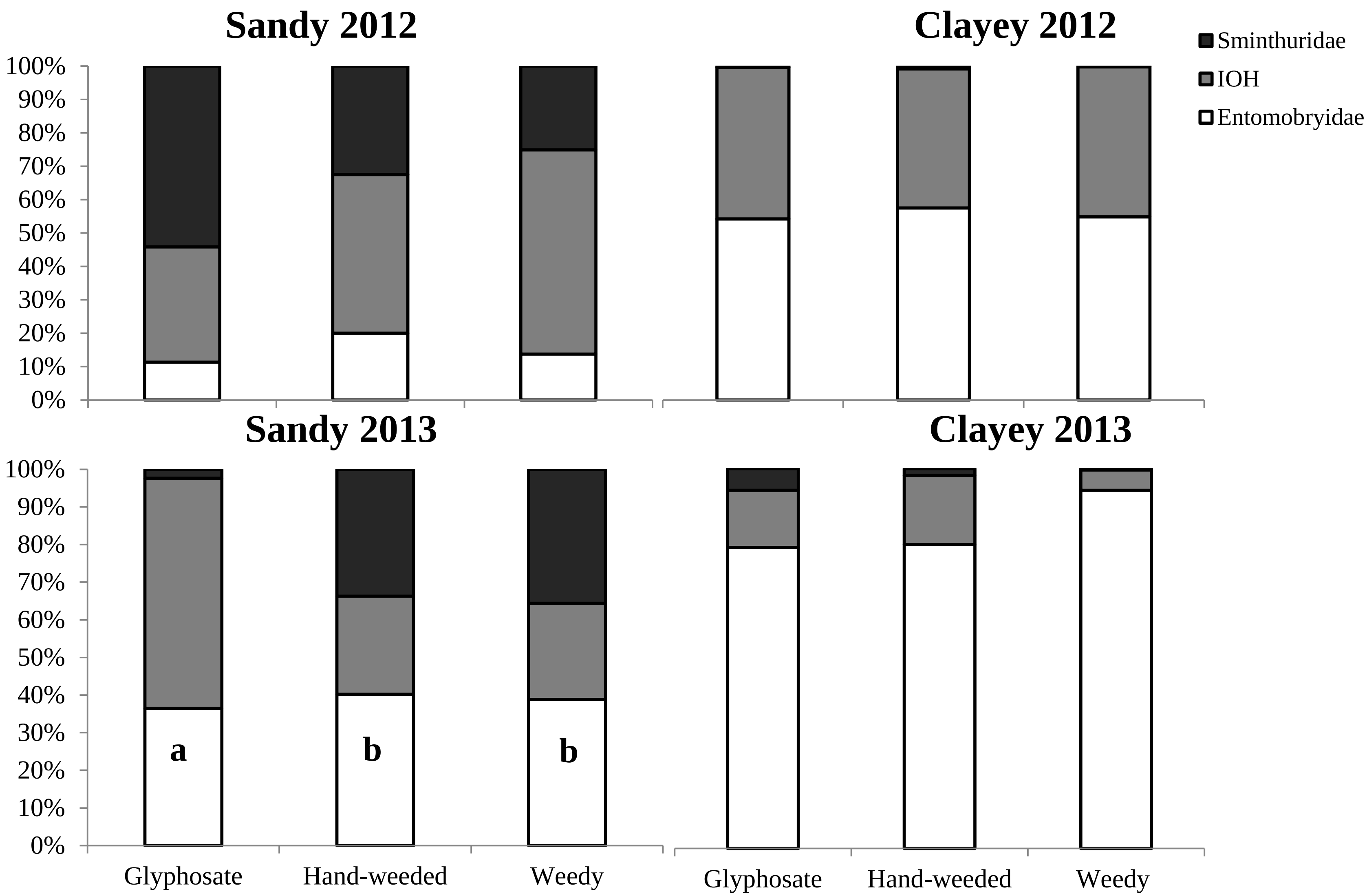


Figure 7. Percentages of average collembolan families collected per pitfall trap in the no insecticide plots. One sampling date for 2012, summed across 2 sampling dates for 2013. Where weed management was significant, different letters indicate significant differences between weed management treatments.

## Seasonal Variation

Euedaphic (Belowground) Collembola

The densities of belowground Collembola changed over the course of the season (Figure 8, Figure 9). In 2012 at the sandy site, the highest densities were collected on June 7 (398.8 ± 125.0; Table 10; Figure 8), densities were the lowest on July 16 (18.2 ± 3.6). Collembolan densities were lower at the clayey site. Densities ranged from 60.6 ± 10.4 on June 18 to only 20.9 ± 4.5 on July 16. There was less variation in 2013 due to overall low collembolan densities. At the sandy site the highest densities were collected on May 16 (87.9 ± 17.6) and the lowest were on August 14 (17.8 ± 3.8). At the clayey site collembolan densities did not change much (from 17.0 ± 4.5 on July 18 to 10.3 ± 2.6 on June 7). This tells us that regardless of weed management treatment, collembolan densities naturally vary over the course of this season as a result of various biotic and abiotic factors, except when densities are low. Also, these variations were more dramatic at the sandy site where there were large peaks in populations at certain times in the season.

Table 10. Mean ± SEM collembolan density extracted from the Berlese soil samples collected on each date over the course of the season (averaged across weed management treatments).

| 2012 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Date Collected** | **5/17** | **6/7** | **6/18** | **6/27** | **7/16** | **7/27** | **8/10** | **8/24** |
| **Sandy** | 164.4 ± 28 | 398.8 ± 125 | 110.1 ±25 | 31.9 ± 7 | 18.2 ± 4 | 151.6 ± 34 | 79.0 ± 23 | 32.6 ± 12 |
| **Clayey** | 59.4 ± 7 | 51.7 ± 10 | 60.6 ± 10 | 21.4 ± 4 | 20.9 ± 5 | 55.7 ± 20 | 30.7 ± 8 | 26.4 ± 5 |

| 2013 | | | | |
|---|---|---|---|---|
| **Date Collected** | **5/16** | **6/22** | **7/18** | **8/14** |
| **Sandy** | 87.9 ± 17.6 | 77.6 ± 10.9 | 21.3 ± 6.0 | 17.8 ± 3.8 |
| **Clayey** | 11.1 ± 3.3 | 10.3 ± 2.6 | 17.0 ± 4.5 | 15.7 ± 3.4 |

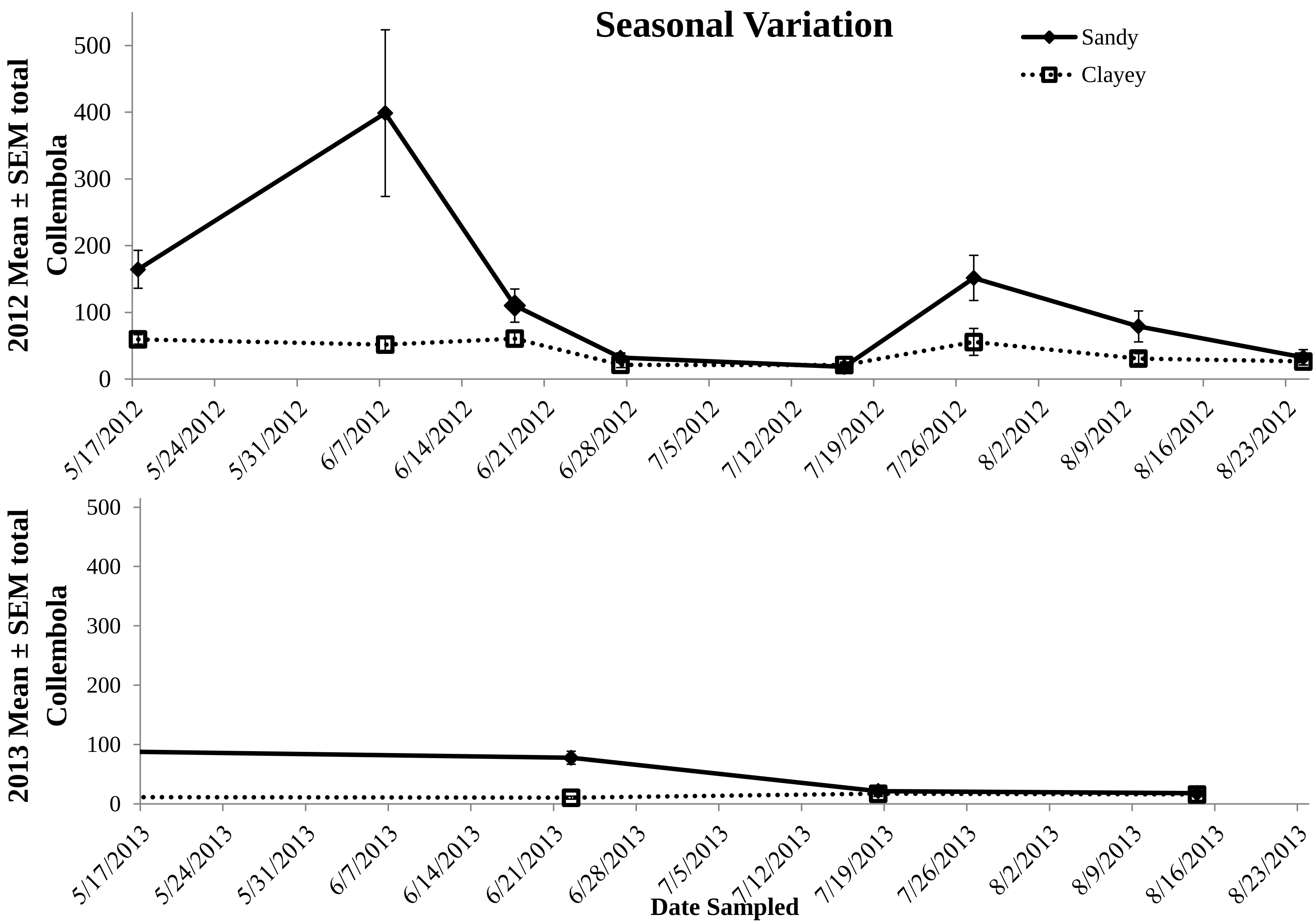


Figure 8. Mean ± SEM of seasonal variation of Collembola (all families combined), averaged across weed management treatment.

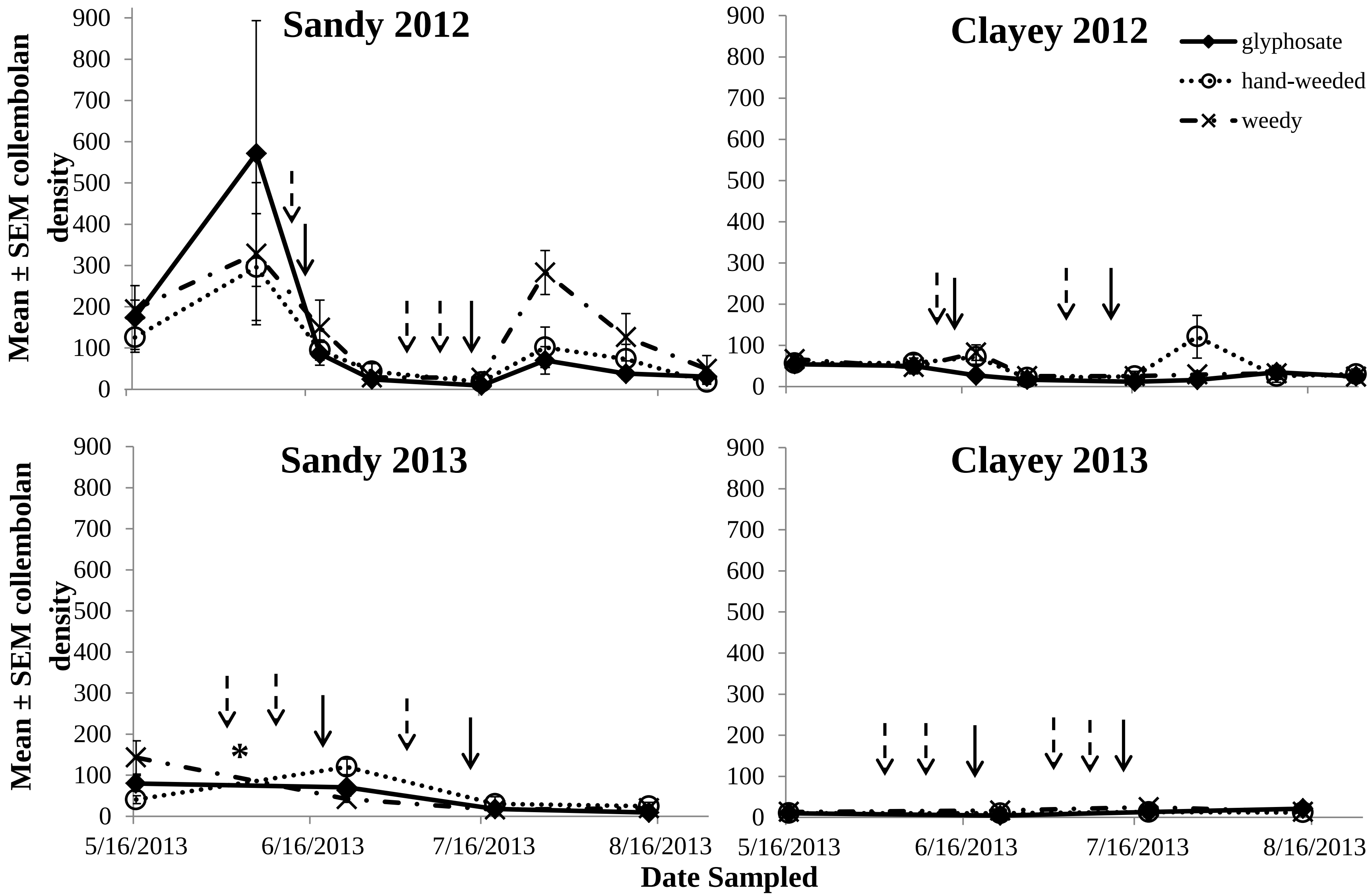


Figure 9. Changes in mean Collembola density over the season separated by weed management. Solid arrows indicate dates of glyphosate application, dashed arrows indicate hand-weeding. Stars indicate where the change in density from one sampling date to the next was significantly (P < 0.05) or marginally significantly (P < 0.10) affected by weed management.

## Changes in Collembolan Densities

<u>Euedaphic (Belowground) Collembola</u>

In 2012, belowground collembolan densities at the beginning of the season (May 17; Table 11) were not statistically different between treatments (Table 12) at either site. On June 7, densities in the glyphosate plots (prior to glyphosate application or hand weeding) were 571.3 ± 322.2 (Table 11; Figure 9) compared to only 296.2 ±129.4 in hand-weeded plots and 328.8 ±172.2 in weedy plots, but it was not significantly different (Table 12). The large variation in the glyphosate plots was due to a few outliers. Densities in all three weed management treatments decreased from the pre-weed management sampling date (June 7) to the post 1 sampling date (June 18), but this was not significantly different between treatments (Table 11). Densities continued to decrease in the post 2 sampling date (June 18), but again, there was no significant difference between weed management treatments.

In 2013, collembolan densities were not different at the beginning of the season (May 16). When the difference between post 1 (June 22) and pre (May 16) sampling dates were analyzed, a significant weed management effect was discovered ($P = 0.0022$), which was driven by differences at the sandy site (Table 12). At that site, densities in the glyphosate treatment remained about the same between sampling dates (79.8 ± 19.3 to 70.7 ± 12.4). Densities in the hand-weeded plots increased from 40.7 ± 9.7 to 120.3 ± 18.9. Densities in the weedy plots on the other hand, decreased from 143.2 ± 40.2 to 41.8 ± 7.8. All three weed management treatments were significantly different from each other (Figure 10). These changes in density could be a result of reproduction or movement between plots.

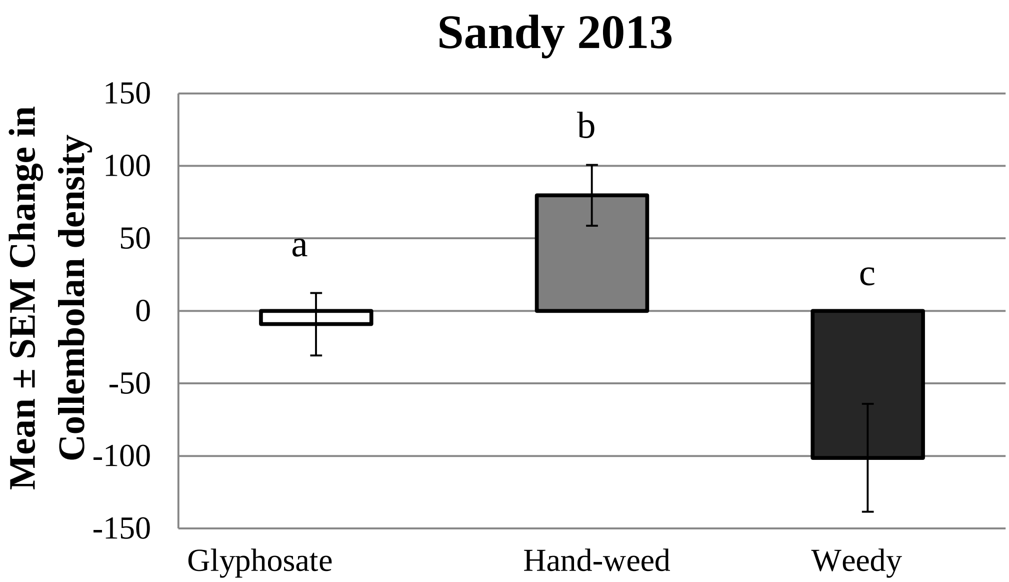


Figure 10. Mean ± SEM change in collembolan densities after first glyphosate application. (6/22 (post 1) – 5/16 (pre)).

Table 11. Mean ± SEM of collembolan densities collected on each date separated by year, location, and weed treatment.

| **2012** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **5/17** | **6/7** | **6/18** | **6/27** | **7/16** | **7/27** | **8/10** | **8/24** |
| **Sandy 2012** | | | | | | | | |
| Glyphosate | 174.0±77 | 571.3 ± 322 | 86.0 ± 28 | 24.0 ± 8 | 9.2 ± 4 | 70.0 ± 33 | 37.7 ± 13 | 30.8 ± 15 |
| Hand-weeded | 125.5 ±36 | 296.2 ± 129 | 95.0 ± 24 | 43.2 ± 19 | 17.3 ± 5 | 101.5 ± 50 | 73.2 ± 36 | 17.5 ± 6 |
| Weedy | 193.7 ± 23 | 328.8 ± 172 | 149.3 ± 67 | 28.7 ± 10 | 28.0 ± 8 | 283.2 ± 53 | 126.2 ± 57 | 49.3 ± 32 |
| **Clayey 2012** | | | | | | | | |
| Glyphosate | 54.8 ± 18 | 50.0 ± 19 | 27.3 ± 10 | 17.2 ± 7 | 12.2 ± 2 | 16.3 ± 2 | 34.7 ±17 | 25.0 ± 6 |
| Hand-weeded | 56.7 ± 11 | 57.7 ± 19 | 72.0 ±17 | 21.5 ± 9 | 25.2 ±12 | 121.3 ±52 | 25.3 ± 15 | 30.2 ± 13 |
| Weedy | 66.8 ± 8 | 47.3 ± 13 | 82.5 ± 19 | 25.5 ± 6 | 25.5 ± 6 | 29.3 ± 9 | 32.2 ± 7 | 24.2 ± 8 |

| **2013** | | | | |
|---|---|---|---|---|
| | **5/16** | **6/22** | **7/18** | **8/14** |
| **Sandy 2013** | | | | |
| Glyphosate | 79.8 ± 19 | 70.7 ± 12 | 17.7 ± 5 | 9.2 ± 2.6 |
| Hand-weeded | 40.7 ± 10 | 120.3 ± 19 | 30.0 ± 17 | 24.7 ± 9.0 |
| Weedy | 143.2 ± 40 | 41.8 ± 8 | 16.3 ± 5 | 19.5 ± 5.9 |
| **Clayey 2013** | | | | |
| Glyphosate | 10.2 ± 4 | 4.5 ± 2 | 13.3 ± 9 | 21.3 ± 8.4 |
| Hand-weeded | 10.2 ± 4 | 9.8 ± 3 | 13.0 ± 6 | 12.0 ± 5.1 |
| Weedy | 13.2 ± 10 | 16.5 ± 7 | 24.7 ± 9 | 13.7 ± 3.7 |

Table 12. F-Value, degrees of freedom and P-values from factorial ANOVA of collembolan densities on 5/17/12 and 6/7/12 prior to any weed management and density changes 72 hours (6/18/12) and 2 weeks (6/27/12) following glyphosate application in 2012 as well as on 5/16/13 prior to weed management and 72 hours (6/22/13) after glyphosate application in 2013.

| | | **5/17/12** | | | **6/7/12** | | | **6/18/12 – 6/7/12** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F-Value | df | P-value | F-Value | df | P-Value | F-Value | df | P-Value |
| Site | | 20.3861 | 1, 10 | **0.0011** | 6.3472 | 1, 10 | **0.0304** | 3.0099 | 1, 9.412 | 0.1153 |
| Weed Management | | 0.8471 | 4, 20 | 0.5113 | 0.5211 | 4, 20 | 0.7213 | 1.3097 | 4, 18.57 | 0.3028 |
| | | **6/27/12-6/7/12** | | | **5/16/13** | | | **6/22/13-5/16/13** | | |
| | | F-Value | df | P-Value | F-Value | df | P-Value | F-Value | df | P-Value |
| Site | | 0.6076 | 1, 10 | 0.4537 | 70.5287 | 1, 9.79 | <**0.0001** | 0.0204 | 1, 10.21 | 0.8892 |
| Weed Management | | 0.6580 | 4, 20 | 0.6282 | 1.2378 | 4, 19.71 | 0.3272 | 6.1237 | 4, 20.12 | **0.0022** |
| Glyphosate vs. Hand-weeded | Sandy | N/A | N/A | N/A | N/A | N/A | N/A | 6.5788 | 1, 19.7 | **0.0186** |
| | Clayey | N/A | N/A | N/A | N/A | N/A | N/A | 0.5079 | 1, 19.7 | 0.4844 |
| Glyphosate vs. Weedy | Sandy | N/A | N/A | N/A | N/A | N/A | N/A | 5.0169 | 1, 19.7 | **0.0368** |
| | Clayey | N/A | N/A | N/A | N/A | N/A | N/A | 1.3435 | 1, 21.05 | 0.2594 |
| Hand-weeded vs. Weedy | Sandy | N/A | N/A | N/A | N/A | N/A | N/A | 23.0856 | 1, 19.7 | **0.0001** |
| | Clayey | N/A | N/A | N/A | N/A | N/A | N/A | 0.2270 | 1, 21.05 | 0.6386 |

## Cumulative Densities

Euedaphic (Belowground) Collembola

Cumulative collembolan densities were calculated to determine if there was a season long weed management effect. In 2012, cumulative belowground collembolan densities were significantly affected by weed management (P = 0.0080; Table 13). At the sandy site, the collembolan densities were significantly higher in weedy plots (664.7 ± 134.5; Figure 11; Table 14) than hand-weeded plots (347.7 ± 102.5; P = 0.0157) and glyphosate plots (257.7 ± 61.6; P = 0.0026). The hand-weeded and glyphosate plots were not significantly different (P = 0.6392). At the clayey site, glyphosate (132.7 ±24.0) and hand-weeded (295.5 ±86.9) plots were significantly different (P = 0.0447) and densities in weedy (219.2 ± 20.2) plots were marginally significantly higher than glyphosate plots (P = 0.0612). At the sandy site the effect seems to be a result of the presence of weeds while at the clayey site, application of glyphosate appears to have had some kind of negative effect other than absence of weeds. In 2013, cumulative subsurface collembolan densities were significantly affected by site (P = 0.0004; Table 13), but not by weed management.

Table 13. F-values, degrees of freedom and P-values from ANOVA test on effects of site and weed management on belowground cumulative collembolan densities summed after the first glyphosate application.

| | | 2012 | | | 2013 | | |
|---|---|---|---|---|---|---|---|
| | | **F-Ratio** | **df** | **P-Value** | **F-Ratio** | **df** | **P-Value** |
| Site | | 3.9682 | 1, 10 | 0.0744 | 27.3705 | 1, 10 | **0.0004** |
| Weed Management (Site) | | 4.6616 | 4, 20 | **0.0080** | 2.0299 | 4, 20 | 0.1288 |
| Glyphosate vs. Hand-weeded | Sandy | 0.6392 | 1, 20 | 0.6392 | N/A | N/A | N/A |
| | Clayey | 4.5885 | 1, 20 | **0.0447** | N/A | N/A | N/A |
| Glyphosate vs. Weedy | Sandy | 11.8212 | 1, 20 | **0.0026** | N/A | N/A | N/A |
| | Clayey | 3.9331 | 1, 20 | 0.0612 | N/A | N/A | N/A |
| Hand-weeded vs. Weedy | Sandy | 6.9627 | 1, 20 | **0.0157** | N/A | N/A | N/A |
| | Clayey | 0.0252 | 1, 20 | 0.8754 | N/A | N/A | N/A |

Table 14. Mean ± SEM of sum (2012: 6sampling dates; 2013: 3 sampling dates) of belowground Collembola collected after first glyphosate application.

| Weed Management | Mean ± SEM | | | |
|---|---|---|---|---|
| | **2012 Sandy** | **2012 Clayey** | **2013 Sandy** | **2013 Clayey** |
| Glyphosate | 257.7 ± 61.6 | 132.7 ±24.0 | 97.5 ± 11.5 | 39.2 ± 16.9 |
| Hand-weeded | 347.7 ± 102.5 | 295.5 ± 86.9 | 175.0 ± 34.2 | 34.8 ± 9.2 |
| Weedy | 664.7 ± 134.5 | 219.2 ± 20.2 | 77.7 ± 16.4 | 54.8 ±12.4 |

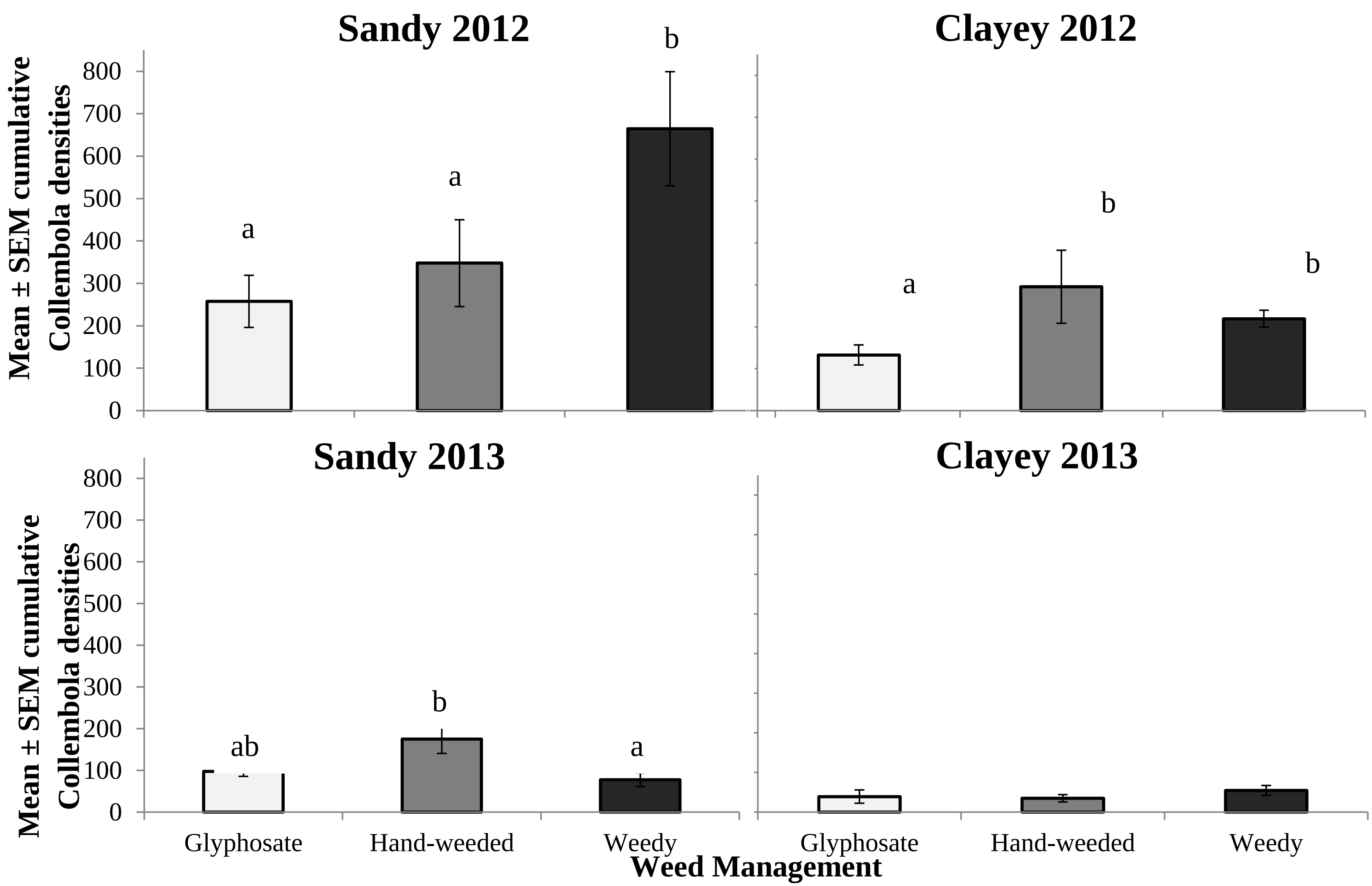


Figure 11. Mean ± SEM of belowground (Berlese) cumulative Collembolan densities collected after the first glyphosate application (six dates for 2012, three dates for 2013). Different letters indicate significant differences at $P \leq 0.05$ between weed management treatments.

Epedaphic (Aboveground) Collembola

In 2012, aboveground collembolan densities were not significantly impacted by site or weed management treatment (Table 15; Figure 12). Samples were only collected once at the end of the season, so we may have missed any short term effects. If collembolan densities were negatively affected by the weed management treatments, they may have been able to rebound by this time. In 2013, weed management significantly affected cumulative aboveground collembolan densities (P = 0.0006; Table 15). At the sandy site, densities in weedy plots (58.4 ± 19.8; Table 16) were significantly higher than in hand-weeded (9.1 ± 2.9; P = 0.0033) and glyphosate (10.4 ± 3.4; P = 0.0038) plots. At the clayey site, densities in the weedy plots (55.5 ± 19.4) were also significantly higher than in both hand-weeded (13.2 ±2.6; P = 0.0157) and glyphosate (6.7 ± 1.7; P = 0.0007) plots. Unlike the results from 2012, the aboveground Collembola appear to have benefited from the presence of weeds at both sites in 2013.

Table 15. F-values, degrees of freedom and P-values from ANOVA test on effects of site and weed management on epigeal cumulative collembolan densities.

| | | **2012** | | | **2013** | | |
|---|---|---|---|---|---|---|---|
| | | **F-Ratio** | **df** | **P-Value** | **F-Ratio** | **df** | **P-Value** |
| Site | | 3.1530 | 1, 10 | 0.1062 | 0.2714 | 1, 10 | 0.6137 |
| Weed Management (Site) | | 0.3552 | 4, 20 | 0.8373 | 7.7783 | 4, 20 | **0.0006** |
| Glyphosate*Hand-weeded | Sandy | N/A | N/A | N/A | 0.0040 | 1, 20 | 0.9501 |
| | Clayey | N/A | N/A | N/A | 1.8366 | 1, 20 | 0.1905 |
| Glyphosate*Weedy | Sandy | N/A | N/A | N/A | 10.7354 | 1, 20 | **0.0038** |
| | Clayey | N/A | N/A | N/A | 15.9663 | 1, 20 | **0.0007** |
| Hand-weeded*Weedy | Sandy | N/A | N/A | N/A | 11.1549 | 1, 20 | **0.0033** |
| | Clayey | N/A | N/A | N/A | 6.9725 | 1, 20 | **0.0157** |

Table 16. Mean ± SEM of sum of epigeal cumulative collembolan densities (2012 = 1 sampling date; 2013 = 2 sampling dates).

| **Weed Management** | **Mean±SEM** | | | |
|---|---|---|---|---|
| | **2012 Sandy** | **2012 Clayey** | **2013 Sandy** | **2013 Clayey** |
| Glyphosate | 96.1 ± 22.4 | 35.7 ± 4.3 | 10.4 ± 3.4 | 6.7 ± 1.7 |
| Hand-weeded | 138.8 ± 48.1 | 26.8 ± 6.9 | 9.1 ± 2.9 | 13.2 ± 2.6 |
| Weedy | 108.7 ± 22.9 | 59.8 ± 22.6 | 58.4 ± 19.8 | 55.5 ± 19.4 |

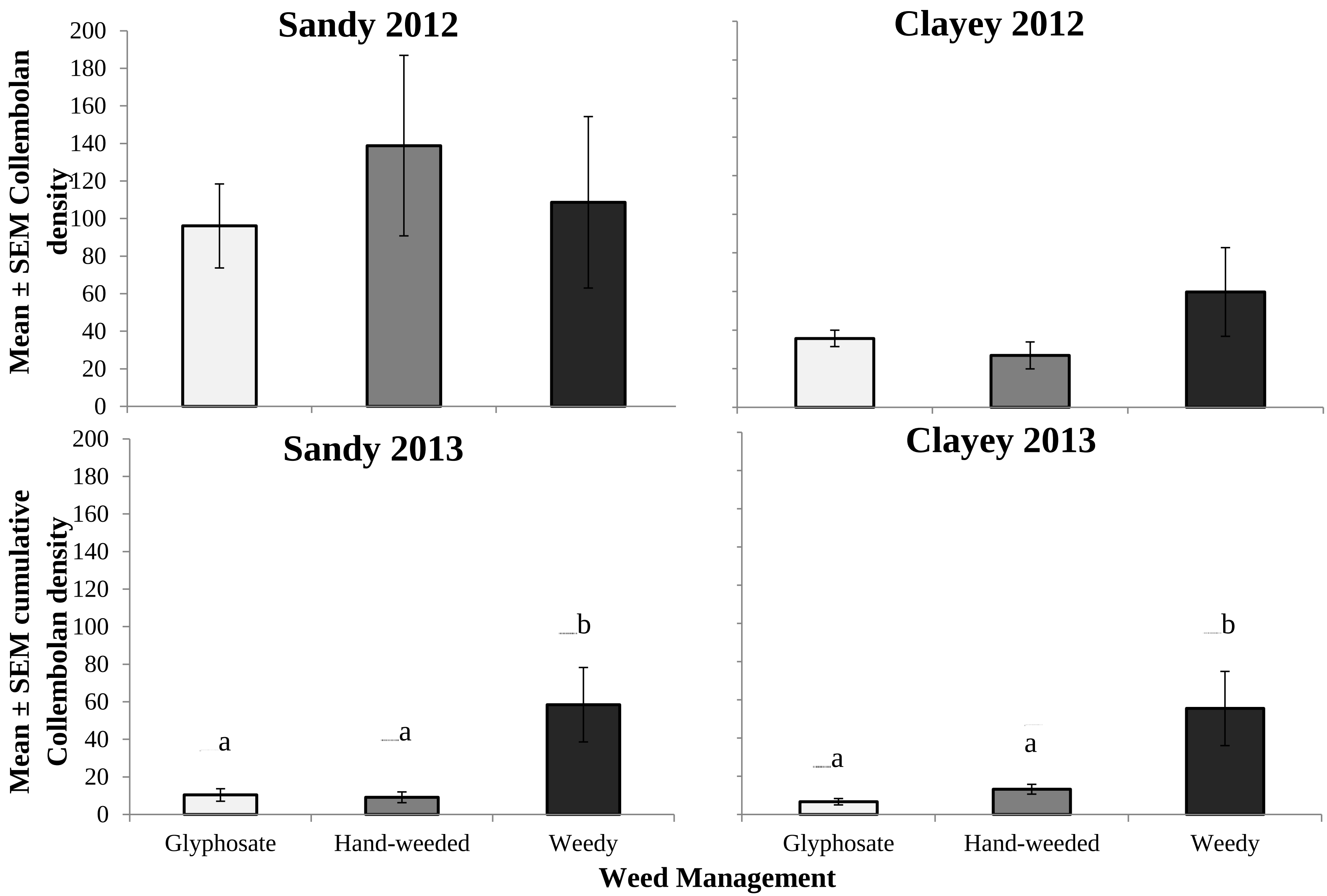


Figure 12. Mean ± SEM of epigeal (pitfall) cumulative Collembolan densities collected after the first glyphosate application (one date for 2012, two dates for 2013). Different letters indicate significant difference at $P \leq 0.05$ between weed management treatments.

# Discussion

The purpose of this experiment was to determine how weed management in a glyphosate-tolerant soybean system impacted Collembola. We hypothesized that both above and belowground collembolan densities would be highest in plots without weed control due to increased resources and favorable habitat modifications. We expected belowground collembolan densities to be less affected as a result of their protection from the soil. This experiment was conducted at two sites with different soil textures and we expected the sandy site to have higher densities as a result of larger pore space.

Several previous studies have examined the effect of weed management in various cropping systems on Collembola and other soil arthropods. Their results vary from no effect of herbicide on collembolan densities (Gomez and Sagardoy 1982) to inconsistent results (Wardle et al. 1993) to higher densities in areas with higher weed densities (Curry and Purvis 1982, Bitzer et al. 2002) or higher densities of Collembola in plots treated with herbicide (Lins et al. 2007). Although several of these studies attribute the differences in density to the presence of weeds, the experimental designs did not distinguish between the presence of chemical or the absence of weeds. This experiment was designed to have two main treatment effects: a weed effect and an herbicide effect.

## Weed Effect

The weed effect can be seen when comparing the hand-weeded and weedy treatments. The weed effect did not alter the diversity of above or belowground collembolan taxa, which remained the same over the two years of the experiment. The presence of weeds did however have an effect on cumulative collembolan densities.

The effect of weeds on collembolan density varied depending on year and site as well as if they were above or belowground. Although it was not always consistent, collembolan densities in the weedy plots tended to be higher than the hand-weeded plots. The belowground collembolan densities were significantly higher in the weedy plots at the sandy site in 2012, but significantly lower in 2013. Although belowground densities were variable, aboveground densities showed an obvious pattern. The aboveground collembolan densities were significantly higher in the weedy plots at both sites in 2013 but not at all in 2012. The effect of weeds on densities may have been clearer in the second year due to an accumulation of food and resources from the previous year. House (1989) also found collembolan densities to be higher in weedy plots. His results were more consistent in that densities were clearly higher in weedy plots than plots with summer cultivation after three years of treatments.

The inconsistent weed effect belowground could have been due to periodically high weed densities in the hand-weeded plots. We were unable to keep the hand-weeded plots completely weed free throughout the entire season. These occasional periods of high weed biomass and density could have resulted in peaks of collembolan densities, although the lack of significant differences between treatment dates suggest that this is not the case. Future tests would be needed to determine if collembolan densities peak prior to hand-weeding and fall after disturbance and weed removal. Our study was two years long, but effect of weeds on belowground densities varied year to year and aboveground densities were only affected in the second year of our study. Perhaps a few more years of repeating the same experiment would give us more consistent results in terms of collembolan density.

## Herbicide Effect

The effect of herbicide can be seen by comparing the hand-weeded and glyphosate plots. A significant herbicide effect was found on both collembolan diversity and density. At the sandy site in 2013, aboveground diversity was different in the glyphosate plots than the hand-weeded plots as a result of two groups: Sminthuridae and IOH. Proportions of Sminthuridae in the glyphosate plots were lower and IOH percentages were higher compared to hand-weeded plots. Sminthuridae were dominant in the glyphosate plot in 2012, but not in 2013. It is difficult to say exactly what may have caused these changes. Collembolan families are quite diverse and without more detailed genus or species information we can only speculate on the causes of these changes (Hopkin 1997). Decreased Sminthuridae proportions could have been a result of sensitivity to the herbicide or movement from the plots to avoid the unfavorable habitat. Proportion of Sminthuridae was smaller because of a decrease in Sminthuridae densities, but also due to an increase in IOH densities. IOH proportions could have been higher because they were able to use resources from glyphosate (actual chemical compounds or dead plants) (Lins et al. 2007) resulting in increased reproduction of that group.

The herbicide effect was only significant on belowground collembolan density at the clayey site in 2012 where collembolan densities were significantly lower in the glyphosate plots. This effect was likely seen at the clayey site and not the sandy site because glyphosate readily binds to clay particles and organic matter (Sprankle et al. 1975) and as a result persists longer in the soil (Veiga et al. 2001).

The apparent herbicide effect could have been a result of direct toxicity. Possible direct toxicity has been shown in some spiders (Evans et al. 2010), but not all species are affected (Haughton et al. 2001). A study by Gomez and Sagardoy (1982) concluded that direct toxicity to

Collembola is not likely, but certain species may be more sensitive to the herbicide (Santos et al. 2010). However, this is difficult to confirm without controlled laboratory experiments where Collembola are exposed to glyphosate via direct spray or by coming in contact with sprayed surface (Haughton et al. 2001).

The herbicide could have also indirectly impacted collembolan densities. The Collembola populations could have moved to a different area or deeper in the soil (Hopkin 1997) to avoid unfavorable conditions. The herbicide can also alter the microbial community (Moorman 1989) which is an important source of food for Collembola in addition to detritus. Previous studies have shown that some predators are sensitive to changes in weed management (Thorbek and Bilde 2004) including glyphosate applications (Haughton et al. 2001). Changes in predator communities could also indirectly impact collembolan densities.

Ideally the only difference between the hand-weeded and glyphosate plots would have been the presence of glyphosate, however, there were other differences. For example, hand-weeding the plots caused soil disturbance, which has been shown to decrease soil arthropod densities (Hendrix et al. 1986). Another difference is that the weed pressure changed over time in both plots. Pressure was highest prior to weed management (glyphosate application or hand-weeding) and lowest after weed management. Periods of high weed pressure were not synchronized between the two treatments. In the glyphosate plots, weeds were present prior to glyphosate application and after application it took several days for the weeds to shrivel up and die. In the hand-weeded plots, the weeds were left on the soil surface to decompose. These periods of high weed pressure could have offered more resources for Collembola at different times throughout the season.

## Weed Management Conclusions

One possible reason for the lack of weed management effect is that the dead weeds from glyphosate application and hand-weeding offered extra food sources for the Collembola, which feed on dead plant matter. This could have off-set the potential benefits we expected to see in the weedy plots. The occasional herbicide effect suggests that further studies should be conducted to determine whether the herbicide alters the soil arthropod community over time.

There appeared to be no immediate or short term effects of weed management on Collembolan densities or diversity. Belowground densities showed no pattern in their response to weed management over the course of two years. Aboveground collembolan densities on the other hand, were affected in the second year of this study suggesting that some longer term effects are likely present. Collembola are not the only soil organisms important in agricultural ecosystems, a more comprehensive study including bacteria, mites and other soil arthropods may provide a clearer picture of how soil organisms respond to weed management.

## Site Effects

Effects of weed management on Collembola densities were mostly inconsistent; effect of site on the other hand, was consistent. As expected, above and belowground Collembolan densities were significantly higher at the sandy site in nearly every case. Others have also found soil texture to significantly affect Collembola and other soil arthropods. Vreeken-Buijs et al. (1998) also found a higher microarthropod biomass in sandy soil compared to loamy soil although Collembola were not significantly affected by soil type in their experiment. Results from Domene et al. (2011) support our results. They discovered that reproduction of certain species of Collembola was lower in clayey soils. Fromm et al. (1993) on the other hand found higher densities of Collembola in clayey soils which is opposite from what we found.

Site not only affected collembolan densities, but collembolan diversity also differed between sites. Diversity belowground was similar between the sites, with the IOH group dominating in both soil types. The aboveground diversity on the other hand, was different between sites. At the clayey site the family Entomobryidae was the most dominant group and Sminthuridae was not often present. The families at the sandy site were more evenly distributed.

## Conclusions

In conclusion, weed management did have an effect on Collembola, but it was not as extreme or consistent as we expected. As we predicted, it appears that the main effect was a result of the absence of weeds (weed effect) rather than the presence of herbicide (herbicide effect). Although glyphosate application did not appear to be directly detrimental to collembolan communities in this study, impacts of weed management on other aspects of the soil community should be considered as well. A lot of emphasis is placed on the effect of plant communities on soil arthropods, but our study suggests that the soil environment can play a stronger role in shaping arthropod communities.

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# CHAPTER 3: EFFECTS OF WEED MANAGEMENT AND SOIL ARTHROPODS ON SOIL AND SOYBEAN PARAMETERS

## Introduction

Soil health is critical for survival of civilizations. Without productive soil, we would not be able to grow enough food to support the population. Cultivation and subsequent exhaustion of soil as a result of overuse has contributed to the demise of many historical civilizations (Montgomery 2007). Soil health refers to the soil's ability to support living organisms, promote environmental quality and sustain plant productivity (Doran and Zeiss 2000). With the intensification of agriculture, it is imperative to understand all the factors that can help keep the soil productive. One of these often overlooked factors is soil arthropods.

Soil arthropods provide two main functions that contribute to soil health: organic matter decomposition and ecosystem engineering (Culliney 2013). As decomposers, soil arthropods fragment and consume macro residues. Digested plant matter is then released as feces and broken down by microbes into plant available nutrients (Seastedt 1984, Culliney 2013). The release of feces also results in robust soil microbial communities (Hanlon and Anderson 1979). Verhoef and Brussaard (1990) concluded that soil fauna contribute to about 30% of soil nitrogen mineralization. As ecosystem engineers, soil arthropods stabilize soil structure allowing for the movement of water, air and nutrients as well as creating space for plant roots (Oades 1993). Actions of soil arthropods moving through soil and releasing fecal pellets result in the mixing of organic matter and contribute to soil aggregation in lower levels of the soil profile (Hole 1981, Lee and Foster 1991).

Soil arthropods can also impact plant performance. These effects can be both direct and indirect and can be either beneficial or detrimental to the plant (Wardle et al. 2004). Some soil arthropods are herbivores and will consume plant roots which in severe cases can result in decreased yield or even plant death (Hunter 2001). In some cases, soil arthropods also compete with the plant for nutrients in the soil, thus indirectly damaging the plant (Wardle et al. 2004). Although some soil arthropods can be harmful to plant production, many are indirectly beneficial (Seastedt and Crossley 1980). Some examples include consumption of plant pests like herbivores (Moore et al. 1988) and root pathogens (Curl et al. 1988). Most importantly, they speed up the nutrient cycling process resulting in more plant available nutrients (Eisenhauer et al. 2010). High densities of Collembola (one type of soil arthropod) can result in increased root and shoot biomass (Lussenhop and BassiriRad 2005) on the other hand, high densities of pests can reduce plant biomass.

Soil arthropods can be especially important in legume crops, like soybean, that depend on symbiotic bacteria to fix nitrogen. Research has specifically been done on how collembolans, small soft bodied arthropods, affect nodulation in soybean. Collembola could potentially impact nodulation in two ways. First, they could increase the amount of bacteria as result of their movement mixing up the soil (Hanlon and Anderson 1979). Second, their movement could transport microbes to or from plant roots (Lussenhop 1993). A lab study showed that high densities of Collembola can result in up to 52% more nodules per plant. However, the same trend was not seen in the field (Lussenhop 1996). Little information is available on how soil arthropods in general impact nodulation, but we predict a few possible outcomes. The first possibility is that higher numbers of soil arthropods would result in increased nutrient cycling and more nitrogen available. When ample nitrogen is available for plant growth, the plant produces about 50% fewer nodules (Hinson 1975). Soil arthropods could also negatively impact nodulation by feeding directly on the nodules or bacteria necessary to form nodules also reducing nodule numbers.

Soil arthropods and microbial activity increase decomposition and nutrient mobilization. Negative effects on their densities from insecticide application could result in decreased nutrients and decreased plant productivity (Eisenhauer et al. 2009). Application of chlorpyrifos resulted in increased microbial activity and biomass either as a result of an increased energy source from the chemical or from a decrease in soil arthropods that consume microbes (Eisenhauer et al. 2009). Sardar and Kole (2005) on the other hand, found nitrogen availability to decrease from 280.9 mg $kg^{-1}$ to 150.9 mg $kg^{-1}$ 90 days after application of chlorpyrifos, possibly due to decreased bacteria numbers.

Reduction of soil arthropods by insecticide in a grassland system resulted a 19% and 60% reduction in productivity depending on the plant species even when pests were present (Eisenhauer et al. 2010). This suggests that although pests were damaging the plants, the presence of beneficial arthropods outweigh the negative effects of pests. We wanted to determine whether the same could be true in an agricultural system. The objective of this study was to determine how soil nitrate as well as soybean plant parameters responded to a decrease in soil arthropods as a result of a soil insecticide application.

## Materials and Methods

### Site Description

The field experiment was conducted during the 2012 and 2013 growing season (May – September) at two field sites. Both fields were located in Cass County, ND; one field was near Leonard (46°39'58.3560", -097°14'32.9640") and will be referred to as the sandy field. The sandy site has a Glyndon soil series, coarse-silty, mixed superactive, frigid Aeric Calciaquoll with a sandy loam soil texture with particle size distributions of 64% sand, 24% silt, and 12% clay. The other field was located near Mapleton (46°55'42.1680", -097°01'03.1800"), and will be referred to as the clay field. The clayey site can be classified as a mixture of both Dovray series, fine, smectitic, frigid Cumulic Vertic Epiaquoll, with silty clay texture (5% sand, 53% silt and 42% clay) and Bearden series, fine-silty, mixed, superactive, frigid Aeric Calciaquoll with particle size distributions of 6% sand, 56% silt and 38% clay. The two sites were chosen based on their soil type, specifically soil texture.

Descriptions of field design, land preparation, experimental treatments, and soil arthropod collection can be found in the previous chapter.

**Soil Nitrate Nitrogen**

Soil nitrate N ($NO_3^-$-N) was determined to find whether weeds were competing with soybean and how presence or absence of soil arthropods via the soil insecticide application influenced soil $NO_3^-$-N levels. One composite soil sample was taken from each plot, with four subsamples taken between soybean rows near each plot corner (approximately 1.5m from edge). Samples were taken using a golf cup cutter (11 cm in diameter; Par Aide Products Co., Lino Lakes, MN) by inserting it approximately 15 cm into the soil. In the weedy plots, the soil was shaken off the roots and plant matter was discarded. Soil from each subsample was combined in a 19 L plastic bucket and mixed by hand. A subsample was submitted to the NDSU testing lab for soil nitrate-N analysis by transnitration of salicylic acid (Vendrell and Zupancic 1990).

## Plant Parameters

Plant sampling was done to determine the soil arthropod effect on plant growth. Five plants were selected from the third row of soybeans from the north edge of each plot. The first plant selected was approximately 1.5 m from the edge and the next four plants were selected. Roots were carefully dug up using a hand trowel. Each individual root was placed in an air tight bag and transported back to the lab where they were placed in a cooler until nodules could be removed by hand (no longer than one week) and counted. In 2012, this sampling was only done once, on July 19 at the sandy site and on July 18 at the clayey site when the beans were in the R1 growth stage. Nodules were averaged per plant for analysis. In 2013, plant samples were taken to correlate with glyphosate applications. At the sandy site, sampling was done on June 24 (V1 growth stage), July 18 (R2), and August 14 (R5). At the clayey site, samples were collected on July 1 (V3), July 19 (R2) and August 15 (R5). Nodule numbers were averaged per plant across all three sampling dates.

## Yield

Soybean yield was examined by harvesting 3.05 m of plants from the center of the middle two rows of each plot. Plants were cut by hand and fed into a thresher. Beans were collected in paper bags and organic matter was removed prior to drying in an oven (71°C) for one week. Beans were weighed (Sartorius, Brinkmann Instruments, Co., Westburg, NY) immediately after removal from the driers.

## Data analysis

Analysis of residuals by prediction plots were used to determine if data needed to be transformed prior to analysis. Average belowground soil arthropod densities collected per sampling date were calculated. Total aboveground soil arthropod densities were averaged per trap from the August sampling dates for each year (see Table 5 in Chapter 1). When necessary, data were log X+1 transformed for analysis using factorial ANOVA with site, block, weed management, insecticide and weed management*insecticide treatment as the independent variables. The last four factors were nested within site and block was considered a random variable. Site factor was taken out of the model for the 2012 yield data because data from the sandy site was not available. When weed management or insecticide factors were found to be significant ($P < 0.05$) or marginally significant ($P < 0.09$), preplanned contrasts were used for mean separation among weed management treatments and insecticide treatments.

# Results

## Soil Arthropod Densities

Belowground Soil Arthropods

Soil arthropod densities did not always respond negatively to insecticide (Figure 13). Belowground soil arthropod densities were significantly affected by insecticide, weed management and site in 2012. Densities were highest in the weedy, no insecticide plots (Table 17) and lowest in the glyphosate +insecticide plots at the sandy site. Soil arthropod densities were significantly higher in the weedy plots than the hand-weeded and glyphosate plots and densities were significantly lower in the +insecticide plots than the no insecticide plots regardless of weed management (Table 18). At the clayey site insecticide treatment was not significant.

However, densities were significantly lower in the glyphosate plots compared to the weedy plots. Densities in the hand-weeded plots were not significantly different from the other two treatments. In 2013, neither insecticide nor weed management impacted soil arthropod densities.

Table 17. Mean ± SEM of total belowground soil arthropod densities collected per date after first glyphosate application (average of 6 dates).

| **Treatment** | **2012** | | | | **2013** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Sandy** | | **Clayey** | | **Sandy** | | **Clayey** | |
| | no ins | +ins | no ins | +ins | no ins | +ins | no ins | +ins |
| Glyphosate | 77.2±15.7 | 19.8±3.5 | 80.9±19.3 | 76.4±17.6 | 70.9±10.2 | 70.6±15.5 | 92.4±32.7 | 85.8±23.8 |
| Hand-weeded | 91.2±25.7 | 30.9±6.0 | 116.7±27.0 | 78.7±14.6 | 135.6±20.8 | 56.8±9.1 | 91.9±20.7 | 119.3±32.6 |
| Weedy | 162.5±34.7 | 42.5±10.0 | 115.7±15.3 | 96.7±10.2 | 97.9±27.0 | 95.4±30.7 | 147.1±55.5 | 57.72±10.3 |

Table 18. F-values, df and P-values for significance of insecticide application on total belowground soil arthropods.

| | | **2012** | | | **2013** | | |
|---|---|---|---|---|---|---|---|
| | | **F-Value** | **df** | **P-value** | **F-Value** | **df** | **P-value** |
| Site | | 8.4560 | 1, 10 | 0.0156 | **0.0179** | 1, 10 | 0.8969 |
| Weed Management | | 4.5225 | 4, 50 | **0.0034** | 0.5152 | 4, 50 | 0.7249 |
| Insecticide | | 25.733 | 2, 50 | **<0.0001** | 1.9237 | 2, 50 | 0.1567 |
| Weed Man*Ins | | 0.1941 | 4, 50 | 0.9404 | 1.7159 | 4, 50 | 0.1612 |
| Glyphosate vs. Hand-weeded | Sandy | 2.2060 | 1, 50 | 0.1438 | | | |
| | Clayey | 1.2241 | 1, 50 | 0.2739 | | | |
| Glyphosate vs. Weedy | Sandy | 13.8579 | 1, 50 | **0.0005** | | | |
| | Clayey | 4.0295 | 1, 50 | **0.0501** | | | |
| Hand-weeded vs. Weedy | Sandy | 5.0058 | 1, 50 | 0.0297 | | | |
| | Clayey | 0.8118 | 1, 50 | 0.3719 | | | |
| Insecticide contrast | Sandy | 50.3240 | 1, 50 | **<0.0001** | | | |
| | Clayey | 1.1485 | 1, 50 | 0.2890 | | | |

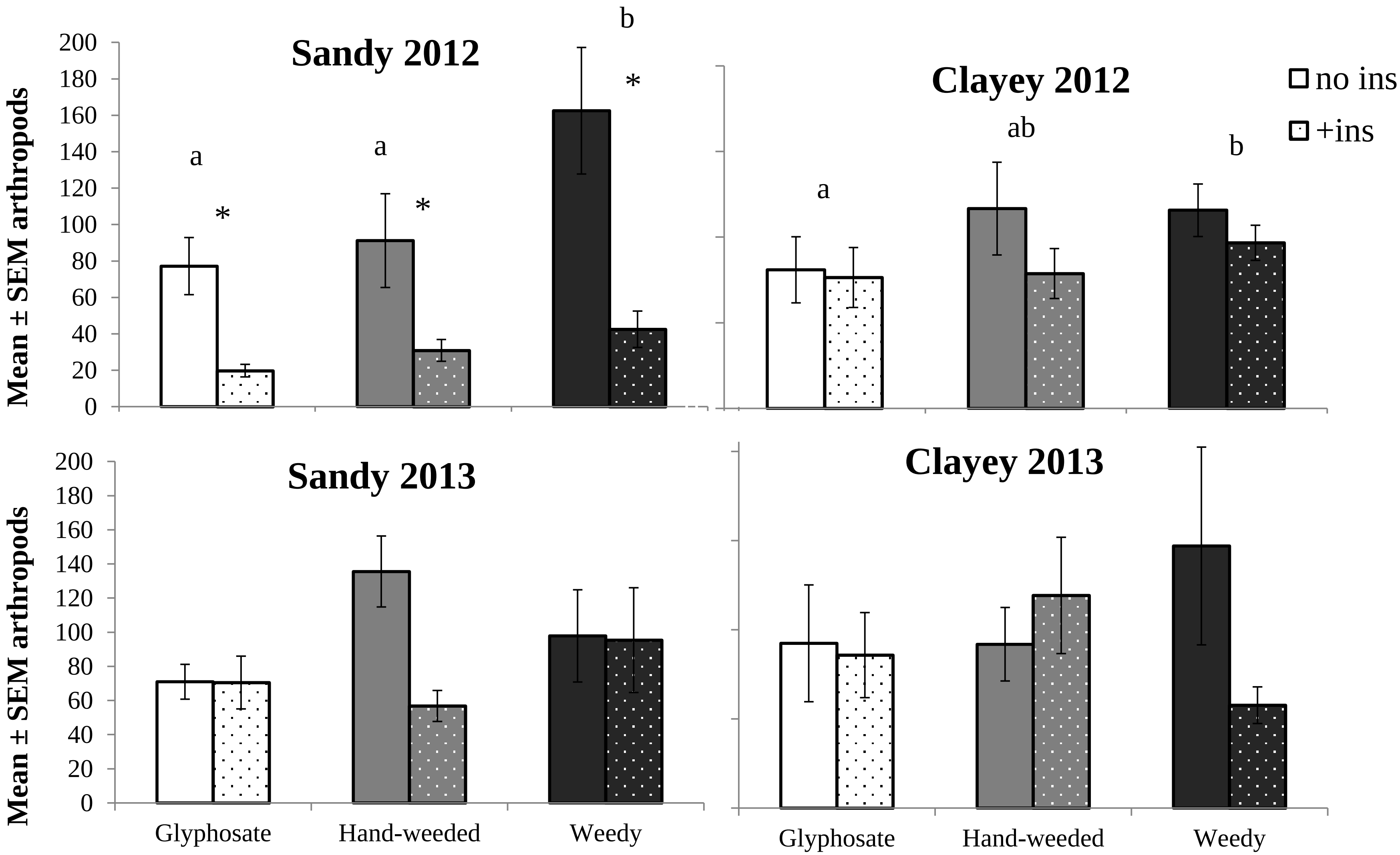


Figure 13. Mean ± SEM total belowground arthropods collected per date starting after the first glyphosate application. * indicate a significant difference (P<0.05) between insecticide treatments. Different letters indicate differences between weed management treatments (P<0.05). Solid bars are no insecticide, spotted bars are +insecticide.

Aboveground Soil Arthropods

In 2012, the aboveground soil arthropod densities were significantly (Table 19) lower in the +insecticide plots compared to the no insecticide plots at both sites (Figure 14; Table 20). Weed management was not significant. In 2013, weed management and insecticide as well as an interaction of the two had a significant effect on aboveground soil arthropod densities. The weedy plots had significantly more soil arthropods than the hand-weeded or glyphosate plots at either site. Insecticide was significant in all three weed management treatments at the sandy site, but only in the weedy treatment at the clayey site.

Table 19. F-values, df and P-values for significance of insecticide application on total aboveground soil arthropods.

| | | 2012 | | | 2013 | | |
|---|---|---|---|---|---|---|---|
| | | **F-Value** | **df** | **P-value** | **F-Value** | **df** | **P-value** |
| Site | | 7.4660 | 1, 10 | 0.0211 | 0.5613 | 1, 10 | 0.4710 |
| Weed Management | | 0.9122 | 4, 50 | 0.4641 | 8.4139 | 4, 50 | **<0.0001** |
| Insecticide | | 81.1645 | 2, 50 | **<0.0001** | 30.8949 | 2, 50 | **<0.0001** |
| Weed Man*Ins | | 0.5766 | 4, 50 | 0.6809 | 3.1051 | 4, 50 | **0.0233** |
| Glyphosate vs. Hand-weeded | Sandy | | | | 0.7194 | 1, 50 | 0.4004 |
| | Clayey | | | | 1.9874 | 1, 50 | 0.1648 |
| Glyphosate vs. Weedy | Sandy | | | | 5.0776 | 1, 50 | **0.0287** |
| | Clayey | | | | 10.8933 | 1, 50 | **0.0018** |
| Hand-weeded vs. Weedy | Sandy | | | | 9.6164 | 1, 50 | **0.0032** |
| | Clayey | | | | 22.1865 | 1, 50 | **<0.0001** |
| Insecticide contrast | Sandy | 113.7580 | 1, 50 | **<0.0001** | 51.3335 | 1, 50 | **<0.0001** |
| | Clayey | 48.5711 | 1, 50 | **<0.0001** | 10.4563 | 1, 50 | **0.0022** |

Table 20. Mean ± SEM of total aboveground soil arthropod densities collected on the August sampling date.

| **Weed Management** | **2012** | | | | **2013** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Sandy** | | **Clayey** | | **Sandy** | | **Clayey** | |
| | no ins | +ins | no ins | +ins | no ins | +ins | no ins | +ins |
| Glyphosate | 133.4±33.2 | 21.8±6.8 | 136.3±19.0 | 51.5±7.3 | 20.9±4.7 | 8.7±1.7 | 13.9±1.7 | 13.5±3.1 |
| Hand-weeded | 187.1±50.8 | 28.4±5.3 | 175.9±39.6 | 57.8±8.8 | 19.0±3.7 | 6.0±0.7 | 13.3±2.4 | 9.9±4.2 |
| Weedy | 166.7±43.8 | 19.4±1.8 | 189.1±34.7 | 38.3±3.3 | 50.6±7.8 | 8.0±1.1 | 46.8±7.0 | 16.2±3.0 |

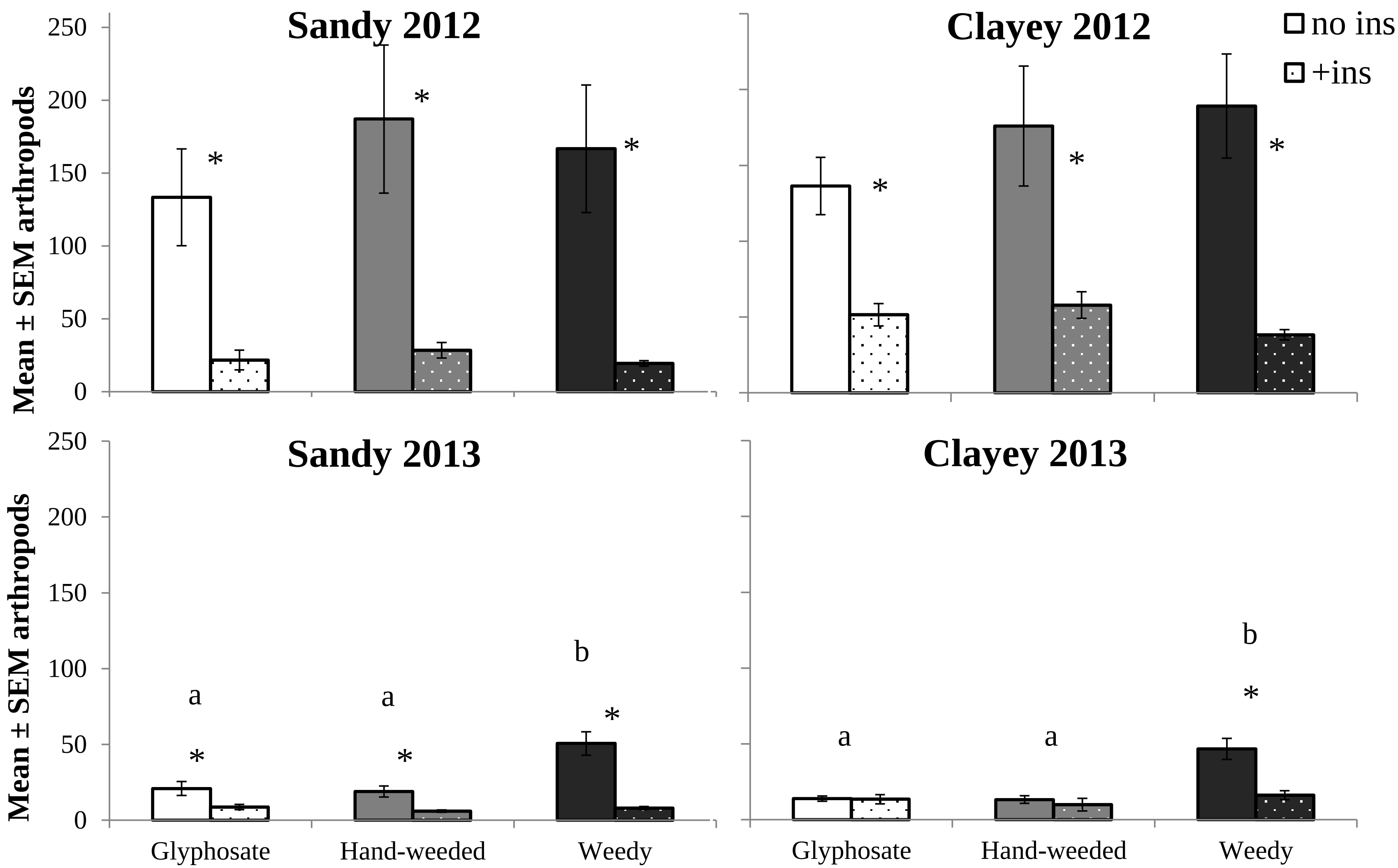


Figure 14. Mean ± SEM total aboveground arthropods collected per date starting after the first glyphosate application. * indicate a significant difference ($P<0.05$) between insecticide treatments. Different letters indicate differences between weed management treatments ($P<0.05$). Solid bars are no insecticide, spotted bars are +insecticide.

## Available Nitrogen

In 2012, available nitrogen was significantly affected by site (Table 21) and marginally affected by weed management. Nitrogen levels at the clayey site in weedy plots (Table 22; Figure 15) were significantly lower than in the glyphosate plots. Insecticide did not significantly affect soil nitrogen.

In 2013, available nitrogen was significantly impacted by weed management. At the clayey site, nitrogen levels were higher in the glyphosate and hand-weeded plots than the weedy plots.

Table 21. F-values, df and P-values for test of significance on available nitrogen.

| | | 2012 | | | 2013 | | |
|---|---|---|---|---|---|---|---|
| | | **F-Value** | **df** | **P-value** | **F-Value** | **df** | **P-value** |
| Site | | 6.2183 | 1, 10.27 | **0.0312** | 2.0846 | 1, 10 | 0.1794 |
| Weed Management | | 2.1547 | 4, 48.24 | 0.0883 | 5.8813 | 4, 50 | **0.0006** |
| Insecticide | | 2.3820 | 2, 48.72 | 0.1030 | 1.188 | 2, 50 | 0.3132 |
| Weed Management*Insecticide | | 0.2983 | 4, 48.24 | 0.8776 | 0.6838 | 4, 50 | 0.6065 |
| Glyphosate vs. Hand-weeded | Sandy | 0.0063 | 1, 49.48 | 0.9372 | 1.3330 | 1, 50 | 0.2538 |
| | Clayey | 1.0444 | 1, 47.63 | 0.3120 | 0.0072 | 1, 50 | 0.9329 |
| Glyphosate vs. Weedy | Sandy | 1.1093 | 1, 47.63 | 0.2975 | 2.0098 | 1, 50 | 0.1625 |
| | Clayey | 6.9075 | 1, 47.63 | **0.0115** | 16.2724 | 1, 50 | **0.0002** |
| Hand-weeded vs. Weedy | Sandy | 1.2802 | 1, 49.48 | 0.2633 | 0.0692 | 1, 50 | 0.7935 |
| | Clayey | 2.5800 | 1, 47.63 | 0.1148 | 15.5964 | 1, 50 | **0.0002** |

Table 22. Mean ± SEM of available nitrogen (kg/hectare).

| **Weed Management** | **2012** | | | | **2013** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Sandy** | | **Clayey** | | **Sandy** | | **Clayey** | |
| | no ins | +ins | no ins | +ins | no ins | +ins | no ins | +ins |
| Glyphosate | 50.0±4.6 | 45.2±5.0 | 47.1±5.2 | 43.3±5.6 | 32.1±4.3 | 36.6±4.7 | 29.0±3.3 | 34.4±3.9 |
| Hand-weeded | 57.9±4.4 | 39.4±3.8 | 43.9±6.0 | 36.2±5.4 | 28.2±5.9 | 35.1±4.2 | 35.3±5.5 | 31.8±5.5 |
| Weedy | 45.5±7.4 | 40.2±7.7 | 32.7±2.4 | 30.6±6.7 | 26.7±2.8 | 28.0±2.9 | 17.9±0.9 | 14.9±0.7 |

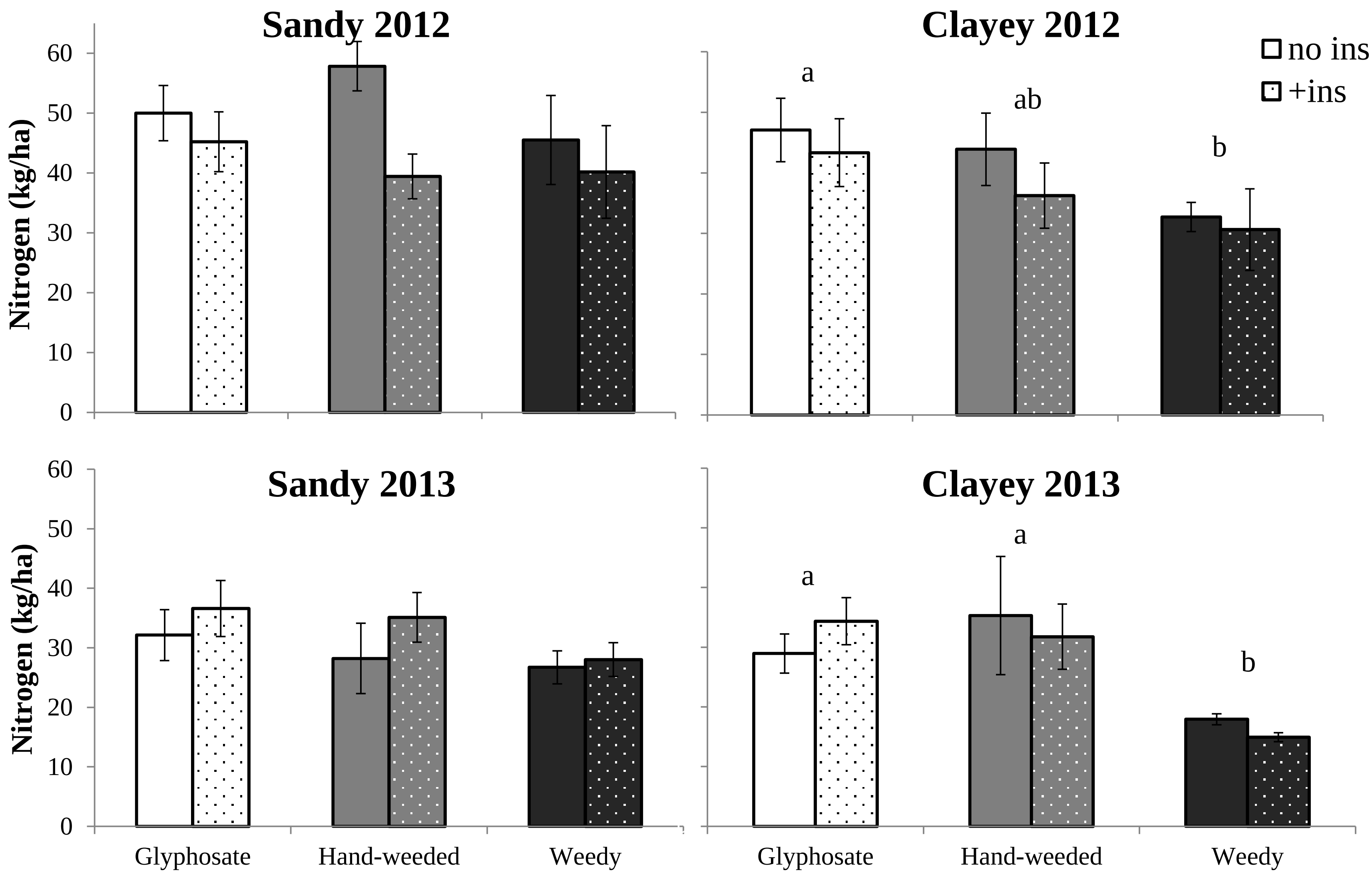


Figure 15. Mean ± SEM nitrogen in kg/ha. Different letters indicate differences between weed management treatments ($P<0.05$). Solid bars are no insecticide, spotted bars are +insecticide.

**Nodule Number**

In 2012, nodule number per plant was significantly affected by weed management (Table 23). This was only true at the sandy site where numbers were higher in the glyphosate plots (Table 24) and hand-weeded plots than in weedy plots. Insecticide treatment was not significant (Figure 16).

Table 23. F-values, df, and P-values on test of significance for nodule number.

| | | 2012 | | | 2013 | | |
|---|---|---|---|---|---|---|---|
| | | **F-Value** | **df** | **P-value** | **F-Value** | **df** | **P-value** |
| Site | | 0.7685 | 1, 10 | 0.4021 | 34.5426 | 1, 10 | **0.0002** |
| Weed Management | | 31.727 | 4, 50 | **0.0212** | 2.7075 | 4, 50 | **0.0405** |
| Insecticide | | 0.5502 | 2, 50 | 0.5803 | 7.9007 | 2, 50 | **0.0010** |
| Weed Management*Ins | | 1.7314 | 4, 50 | 0.1578 | 0.5825 | 4, 50 | 0.6767 |
| Glyphosate vs. Hand-weeded | Sandy | 1.1736 | 1, 50 | 0.2839 | 0.0555 | 1, 50 | 0.8147 |
| | Clayey | 0.8182 | 1, 50 | 0.3700 | 1.6789 | 1, 50 | 0.2010 |
| Glyphosate vs. Weedy | Sandy | 11.3863 | 1, 50 | **0.0014** | 0.0179 | 1, 50 | 0.8942 |
| | Clayey | 0.1922 | 1, 50 | 0.6630 | 10.5466 | 1, 50 | **0.0021** |
| Hand-weeded vs. Weedy | Sandy | 5.2488 | 1, 50 | **0.0262** | 0.1364 | 1, 50 | 0.7135 |
| | Clayey | 0.2173 | 1, 50 | 0.6431 | 3.8096 | 1, 50 | 0.0566 |
| Insecticide | Sandy | N/A | N/A | N/A | 15.7960 | 1, 50 | **0.0002** |
| | Clayey | N/A | N/A | N/A | 0.0053 | 1, 50 | 0.9420 |

Table 24. Mean ± SEM number of nodules per plant in each treatment.

| | 2012 | | | | 2013 | | | |
|---|---|---|---|---|---|---|---|---|
| **Weed Management** | **Sandy** | | **Clayey** | | **Sandy** | | **Clayey** | |
| | no ins | + ins | no ins | + ins | no ins | + ins | no ins | + ins |
| Glyphosate | 23.1±3.0 | 19.7±1.4 | 22.3±2.80 | 18.6±1.5 | 74.9±9.8 | 102.5±11.9 | 68.7±4.1 | 59.6±6.9 |
| Hand-weeded | 16.5±2.7 | 20.3±1.4 | 21.0±3.8 | 17.6±4.3 | 69.9±3.4 | 110.4±8.2 | 55.2±9.2 | 57.9±7.0 |
| Weedy | 11.0±1.7 | 17.0±3.8 | 15.3±1.7 | 23.6±3.1 | 71.7±4.3 | 106.1±18.8 | 41.3±3.6 | 49.3±8.4 |

In 2013, site, weed management and insecticide all significantly affected nodule number (Table 23). At the sandy site, none of the weed management treatments were significantly different, but +ins plots had significantly more nodules (Figure 16). At the clayey site, numbers of nodules were greater in both glyphosate and hand-weeded plots than weedy. Insecticide treatment was not significant at the clayey site

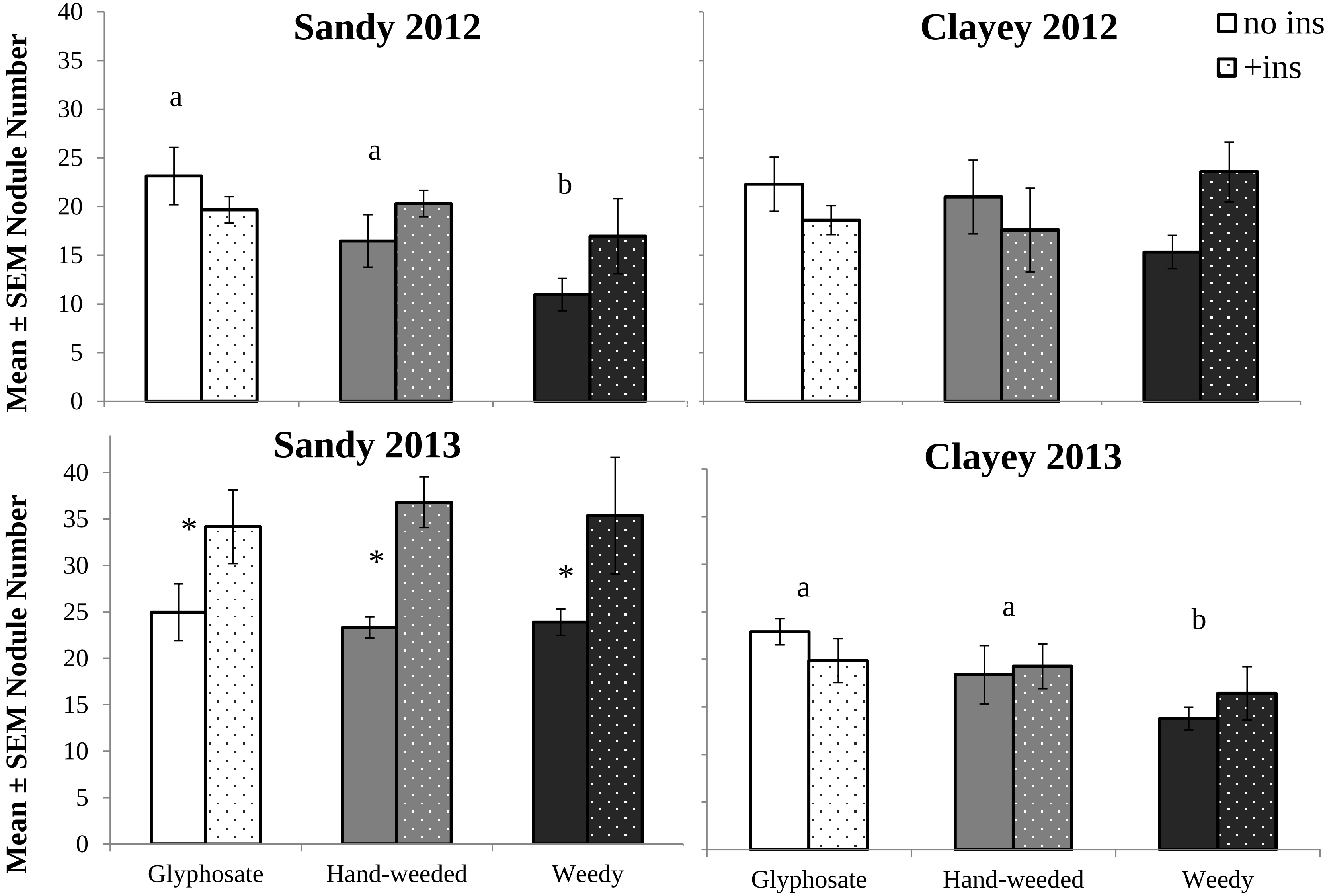


Figure 16. Mean ± SEM nodule number per plant. * indicate a significant difference ($P<0.05$) between insecticide treatments. Different letters indicate differences between weed management treatments ($P<0.05$). Solid bars are no insecticide, spotted bars are +insecticide.

**Yield**

In 2012, only yield data was only available from the clayey site. Yield was significantly affected by weed management (Table 25), but not by insecticide. Yield was significantly lower in weedy plots (Table 26) compared to glyphosate and hand-weeded plots.

In 2013, yield was significantly affected by site and weed management. At the sandy site, yield was higher in glyphosate and hand-weeded than weedy plots. The same trend was seen at the clayey site, with glyphosate and hand-weeded plots having higher yields than weedy plots.

Table 25. F-values, df and P-values on test of significance for yield.

| | | 2012 | | | 2013 | | |
|---|---|---|---|---|---|---|---|
| | | **F-Values** | **df** | **P-Values** | **F-Values** | **df** | **P-Values** |
| Site | | N/A | N/A | N/A | 5.7553 | 1, 10 | **0.0371** |
| Weed Management | | 8.4168 | 2, 25 | **0.0016** | 17.6790 | 4, 50 | **<0.0001** |
| Insecticide Treatment | | 0.5469 | 1, 25 | 0.4665 | 2.1654 | 2, 50 | 0.1253 |
| Weed Management *Insecticide Treatment | | 0.5567 | 2, 25 | 0.5801 | 2.1054 | 4, 50 | 0.0940 |
| Glyphosate vs. Hand-weeded | Sandy | N/A | N/A | N/A | 0.1300 | 1, 50 | 0.7199 |
| | Clayey | 0.0031 | 1, 25 | 0.9560 | 0.0030 | 1, 50 | 0.9564 |
| Glyphosate vs. Weedy | Sandy | N/A | N/A | N/A | 10.6327 | 1, 50 | **0.0020** |
| | Clayey | 12.8216 | 1, 25 | **0.0014** | 43.8109 | 1, 50 | **<0.0001** |
| Hand-weeded vs. Weedy | Sandy | N/A | N/A | N/A | 8.4111 | 1, 50 | **0.0055** |
| | Clayey | 12.4258 | 1, 25 | **0.0017** | 43.0863 | 1, 50 | **<0.0001** |

Table 26. Mean ± SEM of yield (kg $ha^{-1}$).

| **Weed Management** | **2012 Clayey only** | | **2013** | | | |
|---|---|---|---|---|---|---|
| | | | **Sandy** | | **Clayey** | |
| | no ins | +ins | no ins | +ins | no ins | +ins |
| Glyphosate | 4175.0±533.0 | 5001.9±198.8 | 6281.0±484.3 | 6490.0±227.5 | 5468.4±442.9 | 5059.5±441.1 |
| Hand-weeded | 4493.2±260.4 | 4403.3±344.4 | 5169.2±572.6 | 6141.0±572.7 | 5263.9±645.3 | 5364.4±685.2 |
| Weedy | 3086.0±650.0 | 3104.1±609.6 | 1407.2±273.3 | 2630.0±573.9 | 1106.0±498.0 | 1439.5±514.1 |

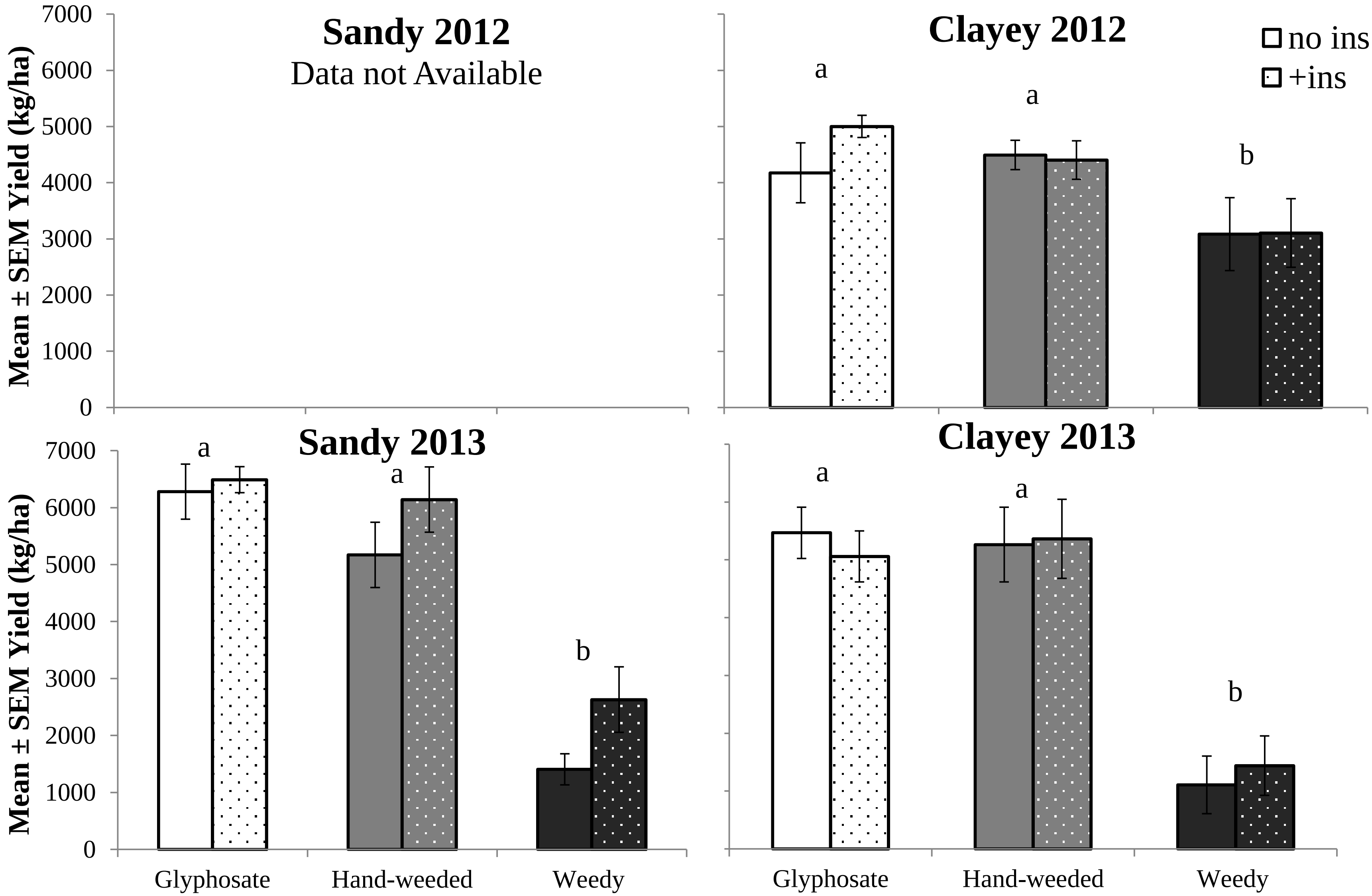


Figure 17. Mean ± SEM yield in kg/ha. * indicate a significant difference ($P<0.05$) between insecticide treatments. Different letters indicate differences between weed management treatments ($P<0.05$). Solid bars are no insecticide, spotted bars are +insecticide.

## Discussion

Weeds compete with soybeans for resources (Staniforth and Weber 1956), so as expected, weed management had a significant impact on many soil and soybean properties. Yield was greatly reduced in the weedy plots compared to the hand-weeded and glyphosate plots (Staniforth and Weber 1956, Vivian et al. 2013). Weeds also lowered the soil nitrogen (Lindquist et al. 2010), but only at the clayey site. Since the weedy plots at the clayey site had lower soil nitrogen, nodule numbers should be higher in the weedy plots (Hinson 1975) at that site. Instead, nodule numbers were significantly lower in the weedy plots at the sandy site in 2012 and the clayey site in 2013. Nodule number in this case did not seem to be correlated with soil nitrate levels.

The insecticide treatment did not exclude total soil arthropod populations as successfully as we had expected it to (Michereff-Filho et al. 2004). Soil arthropod densities were generally not affected belowground except at the sandy site in 2012. Chlorpyrifos residues have been shown to be more toxic in sandy soils (Wiles and Frampton 1996), which explains why this effect was not seen belowground at the clayey site. The general lack of effect on belowground soil arthropods could have been because insecticide was only incorporated into the top 3 cm of soil. The soil arthropods deeper in the soil profile may not have been affected as strongly as the soil arthropods on the soil surface. The aboveground densities on the other hand were significantly reduced by insecticide application (Floate et al. 1989, Epstein et al. 2000) in almost all cases.

We hypothesized that insecticide application would be detrimental to soil nitrate and soybean yield due to a reduction of beneficial soil arthropods. However, our results did not support the hypothesis. Soil nitrate content did not change due to chlorpyrifos application or reduced soil arthropods. Although the lack of effect is not entirely surprising considering belowground soil arthropods were not significantly reduced in the +insecticide plots and both above and belowground arthropods are important for nutrient cycling (Anderson et al. 1983, Seastedt and Crossley, Jr. 1984). Soil arthropods indirectly impact nutrient cycling through litter fragmentation (Culliney 2013). As a result, it may take a few years for detrimental effects of soil arthropod removal to become apparent.

The insecticide application only had a significant impact on soybean nodule number at the sandy site in 2013. The +insecticide plots had more nodules per plant than the plots without insecticide. The insecticide used, chlorpyrifos, is not known to effect nodulation (Revellin et al. 1992). At first glance one might assume that the plant produced fewer nodules because of the higher levels of soil nitrate (Hinson 1975) in the no insecticide plots as a result of soil arthropods decomposing plant matter and cycling nutrients (Verhoef and Brussaard 1990). However, soil nitrate was not higher in those plots so the number of nodules should have been relatively equal unless soil arthropods in the no insecticide plots were feeding on the nodules (Boethel 2004) or indirectly affecting the nodules in some other way.

Legumes are able to compensate for nodule herbivory by producing more nodules. A study on nodule herbivory in alfalfa resulted in more nodules and an increase in existing nodule size after exposure to first instar clover root weevil larvae (Quinn and Hall 1992). However this is not always the case as shown in decreased nodule number as a result of nodule herbivory on white clover (Murray et al. 2002). The higher number of nodules in the +insecticide plot could have been due to a reduction of herbivorous nodule feeding pests rather than the plant overcompensating. It is unknown how soybeans respond to nodule herbivory.

Pest populations have been shown to reestablish quicker than predatory insects following an insecticide application (Ripper 1956, Shepard et al. 1977). The increased numbers of nodules in the + insecticide plots could have been a result of reduced beneficial insects and increased densities of nodule damaging pests (Buschmann and Depew 1990). As a result of nodule herbivory, the plant overcompensated by producing more nodules. The nodule number was only affected in the second year further supporting the case that pest populations may have rebounded faster than predatory arthropods. Pests known to feed on soybean nodules include bean leaf beetle larvae (*Ceromata trifurcata* (Foster))(Kogan and Turnipseed 1987) and the soybean nodule fly (*Rivellia quadrifasciata* (Macquart) (Eastman and Wuensche 1977). In this study, larvae were not quantified, so we are unable to confirm whether these pests were causing the damage.

This study is limited in that we only reported total arthropod densities, and do not give further detail about which soil arthropods were affected by the insecticide. It has been documented that different soil arthropod groups and even certain species of the same family can be more sensitive to insecticide application than others (Stark 1992, Wang et al. 2001). While our study shows how insecticide impacted overall total soil arthropod numbers, it tells us nothing about the species assemblage. This would have been helpful in order to interpret results and impacts of soil arthropods on other factors (Fountain et al. 2007).

The lack of insecticide effect on soil and soybean parameters does not mean that soil arthropods are not important for crop production. Impacts of soil arthropods on soil and plants may be context dependent. In this case, although some pests may have been present, a producer would not have gained yield by applying an insecticide. In the long run, insecticide application may become detrimental to crop production as a result of decreased predators to control arthropod pests and decreased detritivores important for decomposition and nutrient cycling.

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# CHAPTER 4. CONCLUSION

Weed management in a glyphosate-tolerant soybean system had little effect on overall collembolan diversity or density. The trends that we saw indicated that the presence of weeds was beneficial to Collembola, specifically those that live on the soil surface. Our results show that the application of glyphosate has little to no detrimental effects on the density of Collembola, but did influence the diversity in some cases. Overall, the effects of weed management were marginal compared to the difference in diversity and density between the two experimental sites. The sandy site had higher densities and more diversity than the clayey site.

Application of a granular soil insecticide negatively affected aboveground total soil arthropod densities, but belowground densities were generally not impacted. Reduction of soil arthropod densities had no effect on soil nitrate or soybean yield, but nodule number was higher in plots with insecticide. It is unclear whether the reduction of nodules is a result of pests in the no insecticide plots consuming nodules, or overcompensation of the soybean plant in the +insecticide plots as a result of nodule herbivory. In the end, application of insecticide was not beneficial to overall soybean yield in our experiment. Although increased weed pressure was occasionally beneficial to Collembola and likely other soil arthropods, it had a negative impact on soybean yield.

Although this study offers insight into the soil arthropod community in agroecosystems, it only gives us a limited picture. Agricultural fields are usually in production for many years and similar chemicals are used each year. A longer term study would offer more complete answers to our questions about how weed management affects soil arthropods and how soil arthropods impact soil and plant parameters.