Influence of GM crops, aromatic crops, allelopathy and litter decomposition on species assemblages of meso-arthropods in cultivated soils of the Free State Province, South Africa

by

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Declaration

“I declare that the thesis hereby submitted by me for the Master of Science degree in Entomology at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore concede copyright of the dissertation in favour of the University of the Free State.”

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Jehane Smith

“Sand is for fun; Soil is for life!”

- Mehmet Murat ildan

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Abstract

Integrated methods in land use and land management are needed, in addition to traditional agricultural practices, to provide an increasing human population with the necessary food security. By conserving soil organisms in crop agro-ecosystems, farmers can in essence be practicing sustainable conservation agriculture, where soil biodiversity is responsible for soil health. Potential toxic plants, whether natural (allelopathic) or anthropogenic (GMOs), cause a concern regarding this biodiversity in agro-ecosystems. Maize that has been genetically engineered using the soil bacterium *Bacillus thuringiensis* (*Bt),* known as *Bt* maize*,* expresses the synthetically modified Cry1Ab, Cry1F, Cry1A.105 or Cry2Ab2 proteins that are toxic to some insects. The impact of *Bt*-maize on non-target soil organisms is an important aspect in soil health and agricultural sustainability. The same goes for allelopathic crops, which can influence other crops in their immediate vicinity or in succeeding seasons.

The aims of this study were to determine the possible effects of GMOs (*Bt*-maize), allelopathic crops (alfalfa and sunflower) and aromatic crops (onion) on soil meso-arthropod assemblages. A trial on humus decomposition rates and the potential occurrence of a Home field advantage (HFA) of decomposing litter was also conducted, the relevance being that decomposition is the driver of soil organic matter (SOM) production which enriches soil and, in turn, benefits soil organisms.

Soil samples were taken at the roots of the plants in the porosphere where the plant interacts directly with its environment. To extract soil mesofauna, the Tullgren extraction method was used. Samples were collected from the following localities in the Free State: Bainsvlei area (maize, onion, and decomposition samples on the farm Geluk), Bainsvlei area (alfalfa and decomposition samples on the farm Maranatha), Bloemdal area (maize – on the farms Karee Laagte and Feather Stone) and Petrusburg area (sunflower and onion – on the farm Thornberry). To analyse data statistically, the Shannon diversity index, Sørensen similarity index and Home field advantage index (HFAI) was used.

No immediate negative effects of *Bt* maize on soil faunal diversity were observed. However, in a 2012 study, a higher diversity of soil mesofauna was observed in the *Bt* fields, indicating that plants with the insect resistant gene may very well benefit soil faunal groups due to increased plant health and production of a

larger root mass (podosphere). The influence of allelopathic crops on soil meso-arthropods showed that stressed allelopathic plants had an overall lower diversity than non-stressed plants. However, there is some uncertainty here, since lower diversity can also be attributed to low soil humidity and exposure to external post-harvest factors during the trial. Overall diversity in onion fields was lower than in the control fields, whilst some species of soil organisms only occurred in the natural fields and not in the onion field. There was no indication that the toxins produced from these plants actually kill the soil fauna, but the assumption could be made that onion plants were at least repellent. Certain mesofaunal species specifically occurred only in the onion fields, indicating opportunism and resistance towards onion repellent odours.

The different sampling methods used in the decomposition trial showed some filtering effect in terms of the organisms allowed into the traps. The HFAI patterns for the four successive sampling dates (16, 24, 30 April and 07 May 2014) temporally correlate with the abundance of soil arthropods within the litter traps and litterbags at the given sampling date. Noteworthy during this trial is that certain trophic groups, such as microbes and predators, fulfil a vital role in decomposition and that this process is not only dependant on the litter producing plants as such. Furthermore, allelopathic alfalfa litter was seemingly also preferred by certain introduced, opportunistic collembolan species, indicating the important role alien species can play in the soil environment. In spite of all this and albeit that the sampling methods used in this trial created an unnatural scenario (to a certain degree) for litter decomposition agents by excluding certain size groups of soil arthropods, the overall conclusion is that a HFA (to a certain extent) was confirmed and demonstrated across all the sampling methods used for this short-term decomposition study.

All of these aspects in crop agriculture can play a significant role in determining soil fertility and productivity. A better understanding of these processes can provide farmers with the necessary expertise and knowledge to manage sustainable crop farming systems.

Uittreksel

Geïntegreerde metodes in die gebruik van land en grondbestuur word, tesame met tradisionele landboupraktyke, benodig om 'n toenemende menslike bevolking met die nodige voedselsekuritiet te voorsien. Deur die bewaring van grond biodiversiteit in gewas agro-ekostelsels kan boere volhoubare landbou-bewaring toepas, en sodoende die grond organismes wat verantwoordelik is vir grondgesondheid bewaar. Potensiële giftige plante, of dit nou natuurlik (allelopatiese) of antropogenies (GMOs) is, veroorsaak kommer oor biodiversiteit in agro-ekostelsels. Mielies wat geneties gemanipuleer is, met behulp van *Bacillus thuringiensis* (*Bt),* staan bekend as *Bt*-mielies en stel die sinteties veranderde Cry1Ab, Cry1F, Cry1A.105 of Cry2Ab2 proteien vry wat toksies is vir sekere insekte. Die impak van *Bt*-mielies op nie-teiken grondorganismes is 'n belangrike aspek in grond gesondheid en volhoubare landbou. Dieselfde geld vir die allelopatiese gewasse wat ander plante rondom hulle, of in daaropvolgende seisoene kan beïnvloed.

Die doelwitte van hierdie studie was om die moontlike gevolge van GMO (*Bt*-mielies), allelopatiese (lusern en sonneblomme) en aromatiese gewasse (uie) op grond meso-geleedpotiges te bepaal. ‘n Proef op die ontbindingstempos en die moontlike voorkoms van 'n tuisveldvoordeel vir ontbindende humus is ook uitgevoer om die ontbindingsproses as drywer van organiese materiaal produksie in grond te beklemtoon. Die proses verryk grond en bevoordeel vervolgens grondorganismes.

Grondmonsters is by die wortels van die plante in die porosfeer, waar die plant in direkte kontak met sy omgewing is, geneem. Mesofauna is met behulp van die Tullgren ekstraksie tegniek ge-ekstraeer. Monsters is op die volgende lokaliteite in die Vrystaat versamel: Bainsvlei area (mielies, uie, en ontbinding materiaal op die plaas Geluk), Bainsvlei area (lusern en ontbinding materiaal op die plaas Maranatha), Bloemdal omgewing (mielies op die plase Karee Laagte en Feather Stone) en Petrusburg area (sonneblom en uie op die plaas Thornberry). Om data statisties te ontleed is die Shannon’s diversity index, Sørensen similarity index en Home field advantage index (HFAI) gebruik. Geen onmiddellike negatiewe uitwerking van *Bt*-mielies op die grondfauna diversiteit was opgemerk nie. Daarenteen was 'n hoër diversiteit van grondmesofauna in die 2012 studie in die *Bt*

velde opgemerk, wat aandui dat plante wat die insekbestande gene bevat grondfauna groepe kan bevoordeel as gevolg van verhoogde plant gesondheid en dus die vorming van 'n groter wortelmassa (porosfeer). Die invloed van allelopatiese gewasse op die grond meso-geleedpotiges het getoon dat onderdrukte allelopatiese plante 'n algehele laer diversiteit toon as nie-onderdrukte plante. Hierdie verskynsel kan egter ook toegeskryf word aan lae grond humiditeit en blootstelling aan eksterne na-oes faktore wat gedurende die proef ondervind is. Algehele diversiteit in uie-lande was laer as in die kontrole lande en sommige grondorganisme spesies was slegs in die natuurlike land en nie in die uie-land versamel nie. Daar was geen aanduiding dat die gifstowwe wat hierdie plante produseer tot grondfauna mortaliteit lei nie, maar dit kan aanvaar word dat uie plante ten minste afwerend was. Sekere spesies het slegs in die uie-lande voorgekom, wat dui op opportunisme en weerstandbiedendheid teenoor uie se afwerende reuke.

Die verskillende versamelmetodes in die ontbindingstudie het 'n aantal grondfauna spesies in terme van die toegangklikheid tot die lokvalle gefiltreer. Die HFAI patrone vir die vier agtereenvolgende versameldatums (16, 24, 30 April en 7 Mei 2014) toon temporale korrelasie met die volopheid van grond-geleedpotiges binne die humus-lokvalle en humus-sakke tyens die gegewe versameldatum. Noemenswaardig is dat sekere trofiese groepe, soos mikrobes en predatore, ‘n belangrike rol vervul in ontbinding en dat hierdie proses nie alleenlik van die plant materiaal van die betrokke plante afhang nie. Nietemin, ten spyte hiervan en alhoewel die versamelmetodes wat in die proef gebruik is in ‘n sekere mate 'n onnatuurlike voorstelling van die ontbindingsagente van humusmateriaal geskep het deur sekere grond-geleedpotige grootteklasse uit te sluit, was die algemene gevolgtrekking tog dat 'n tuisveldvoordeel in ‘n sekere mate plaasgevind het oor al die versamelmetodes wat vir hierdie korttermyn ontbindingstudie gebruik is.

Al hierdie aspekte kan in landbou 'n belangrike rol in die bepaling van grondvrugbaarheid en produktiwiteit vervul. 'n Beter begrip van hierdie prosesse kan aan boere die nodige kundigheid en kennis verskaf om volhoubare gewasboerdery stelsels te bestuur.

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CHAPTER 1

The importance of mesofaunal diversity in soil

1.1. Introduction

Integrated methods in land use and land management are needed in addition to traditional agricultural practices to provide an increasing human population with the necessary products (Boserup 1975, Dias *et al.* 2014). Not only do farmers have to keep up with the current demand of quality and quantity of crops, they urgently need to adapt their land use methods for more sustainable farming. These crops feed a fast growing human population, their livestock and also provide energy in the form of bio-fuels (Dias *et al.* 2014). In this context soil management has become increasingly important. Over the past few years, since agricultural fields largely lack soil fertility for crop, fodder and forage production, extensive fertilizer application has to compensate for this. According to Kreuzer *et al.* (2004) and Eisenhauer *et al.* (2013), the functions that leads to soil fertility and nutrient availability are directly linked to vegetation diversity which, in turn, is linked to soil faunal diversity and function. Kreuzer *et al.* (2004) found that earthworms increased plant growth in some species. This effect was more commonly observed in grasses than legumes.

Changes in soil characteristics such as pH, nutrient availability, organic matter and structure are caused by agriculture (Powlson *et al.* 2011, Söderström *et al.* 2014). Because a vast range of functional and taxonomic organismal groups is responsible for soil formation and properties, it is important to manage agricultural soils in such a manner that will favour these organisms immensely (Powlson *et al.* 2011). Roger-Estrade *et al.* (2010) reviewed the influences of tillage as one of the factors negatively influencing soil biota. However, aside from reduced tillage there are many agricultural practises such as intercropping, crop rotation and the supplementation of organic matter that favours soil organisms and that can help famers worldwide to preserve soil biodiversity and obtain sustainable land use.

The aim of this chapter is to determine the importance of soil faunal diversity, as well as their ecological function and how it influences soil fertility. The chapter will include a discussion on trophic groups and functional classification of soil fauna and their

importance in agriculture. A brief summary will be given on soil processes such as decomposition, nutrient cycling and soil formation. In addition, the focus will be on allelopathic crops and GMO's and their influence on soil fauna. Finally, there is an overview on the ecological function of soil organisms.

1.2. Why preserve soil biodiversity in agricultural environments?

Diversity is defined as the relationship between abundance and quantity (richness) of species within an ecosystem (Bennett 2010). Land use, in general, causes a decline in either diversity or abundance of soil organisms (Wallwork 1976, Curry 1994, Edwards and Bohlen 1995, Didham *et al.* 1996, Widyastuti 2004). This decline is not only due to the alteration of the physical environment of soil organisms, but also due to reduced soil organic matter and an increased chemical input. When conserving biodiversity, in an agro-ecosystem, it is important to conserve the system as a whole. This includes the diversity of habitats, populations, species and genetic diversity within the ecosystem (Emmerling *et al.* 2002). The conservation of biodiversity relies highly on all of these entities combined.

All ecosystems include various trophic groups that influence one another, either directly or indirectly. This in turn leads to top-down and bottom-up effects, with the decline in organisms from one trophic level influencing the organisms from other trophic levels (Haddad *et al*. 2009). According to Wardle *et al.* (2005), predators feeding on prey can have cascading effects on lower trophic levels. This is known as top-down effects where higher trophic levels influence the levels below. These cascading effects are known as trophic cascades and occur naturally in all ecosystems (Wardle *et al.* 2005). Wardle *et al.* (2005) found that above-ground trophic cascades could influence food webs below-ground. In the case where predators managed primary consumers (phytophages), more plant material was available for decomposition and soil microfauna increased as a result. In the case where predators did not suppress primary consumer biomass, less foliage fell to the ground resulting in a decline in soil microfauna. Bottom-up effects are thus dependent on resource availability. In below-ground decomposer

food webs, resource availability is dependent on the quality and quantity of resources entering the system, in this case plant litter. Primary production or vegetation availability is the driver of all food webs, but at the same time is driven by all the other trophic levels. Soil decomposer fauna are responsible for maintaining a constant supply of nutrients to plants. These decomposer fauna are managed by below-ground predators (Wise *et al.* 1991, Wardle *et al.* 1998, Salmon & Ponge 1999).

In agro-ecosystems the vegetation type often changes, the quality of plant material varies and the supply of litter is not constant throughout the year. Monocrops also decreases litter diversity that influence the variety of nutrients that can be recycled back into the soil. In an agro-ecosystem, it is important to leave crop residues in the field, so that it can be reprocessed to increase availability of nutrients. An increase in crop diversity within a field will also promote a wider variety of nutrients available for plants. Another important aspect of biodiversity conservation, is spatial heterogeneity (Bennett 2010). Spatial heterogeneity influences ecological processes, including ecosystem function, the ability of a specific population to survive, animal mobility, as well as inter- and intra-species interactions. Figure 1 predicts the influences of heterogeneity within an agro-ecosystem.

In agro-ecosystems, landscapes tend to be more homogenous (Figure 1), due to monocropping (A or B in figure 1). By creating a heterogeneous landscape with a higher diversity of crop species (both crop species A and B in figure 1), and by planting these crops in a pattern that is spatially complex, overall diversity in agricultural landscapes can be improved (Fahrig *et al.* 2011). The isolation of populations due to agricultural practices can lead to insubstantial genetics within populations and ultimately the disappearance of species (Fahrig *et al.* 2011). According to Fisher *et al.* (2006), there are three main advantages to a more complex ecosystem. The first advantage, of a complex ecosystem, is the establishment of habitation for native species and the second is the improvement of landscape connectivity leading to a more complex genetic variability and the last advantage is the reduced edge effect (Fisher *et al*. 2009).

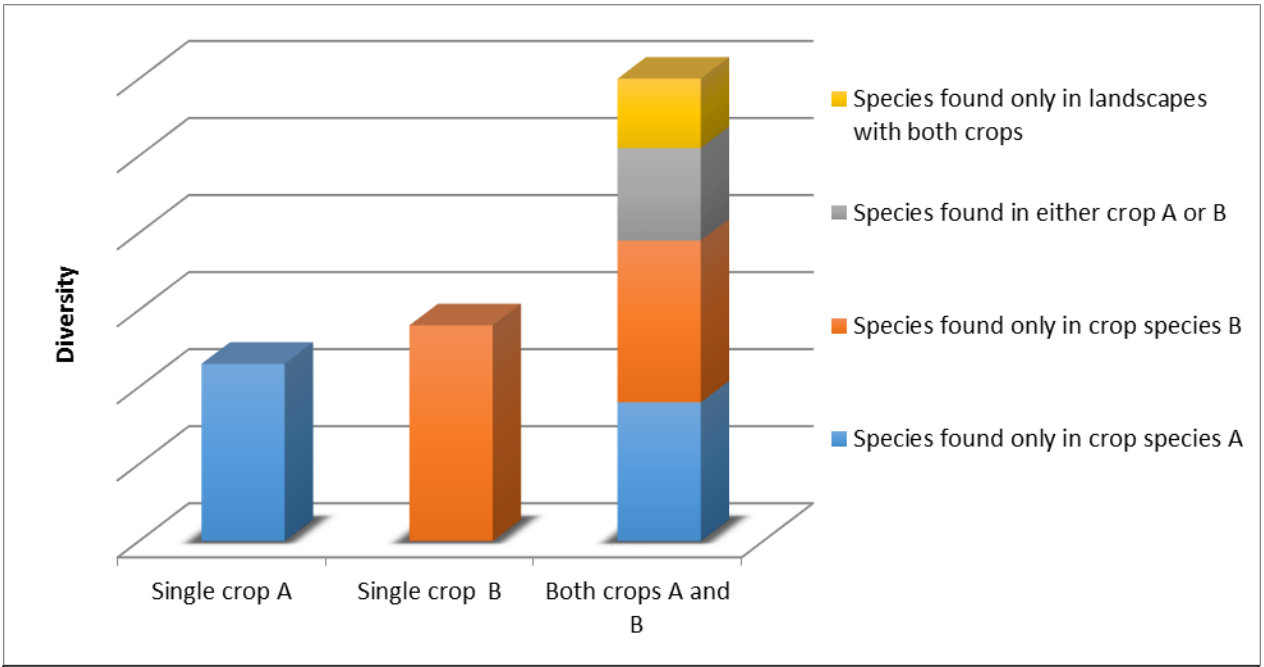

Figure 1: Predicted influences of heterogeneity in agro-ecosystems on biodiversity (adapted from Fahrig *et al.* 2011).

The key in preserving biodiversity lies in the knowledge and ability to preserve keystone species and functional groups (Altieri 1999). Some species play a bigger functional role than others and are therefore more important in both natural and agricultural systems (Davidson & Grieve 2006). The problem with soil mesofauna, in South-Africa, is that not enough research has been done up to date to know which the more important species are. Even though the function of all mesofaunal groups in soil is not certain, it is accepted that they play an important role in soil health and fertility. According to Emmerling *et al.* (2002), soil fauna is responsible for soil nutrient availability by breaking down organic matter into humus, mixing it into the soil, distributing nutrients though their movements and actions and regulating microbial activity. They are also mainly responsible for the formation of soil aggregates, drainage and ventilation (respiration) of soil, the formation of bio-pores that increases the water holding capacity and water infiltration rates. They also aid in the formation of the physical soil structure (Davidson & Grieve 2006). Altieri (1999) stated that future soil problems cannot be predicted and that any species might become useful at a later stage. It is thus important

to conserve biodiversity as a whole and to consider all organisms, regardless of the role they play.

Many soil ecologists have reached an understanding that a higher diversity promotes ecosystem functions and leads to higher decomposition and nutrient cycling rates (Bengtsson 1998, Schläpfer *et al.* 1999, Diaz & Cabido 2001, Hättenschwiler *et al.* 2005, Hooper *et al.* 2005). Functional groups, in soil ecosystems, can have both trophic and non-trophic effects on their surrounding environment. Both these effects are equally important since the non-trophic effects often make the trophic effects possible (Bengtsson 1998). Non-trophic effects include ecosystem engineers that are responsible for the modification of the soil environment and the distribution of carbon and nutrients. Ants, termites and earthworms are examples of such soil ecosystem engineers. Soil mesofauna are organisms that range between 100µm and 2mm in size (Briones 2014) and contribute a great deal to decomposition and nutrient cycling (= trophic effects). Most of these organisms are unable to restructure soil and use existing cavities in the soil to move from one space to another. They are thus reliant on ecosystem engineers to provide these changing spaces.

Biodiversity of soil fauna is related to the diversity of plant species and soil type (St John *et al.* 2006, Bennett 2010). According to Fowler & Mooney (1990), the entire 1440 million ha of land used for agriculture worldwide are cultivated with no more than 70 plant species, including 12 species of grain crops, 35 nut and fruit crop species and 23 vegetable crop species. In contrast, a single ha of tropical rain forest consists of over 100 plant species. As biodiversity directly and indirectly provides many ecosystem services, it is essential to create a level of diversity in agro-ecosystems that will not only contribute to the sustainability of these systems, but also provide ecological services concerning soil conservation, natural pest control and nutrient cycling. As such ecosystems include many interactions between organisms and the smallest disturbances can cause a modification in the system. This can either be positive or negative, depending on the modification.

Soils perform several functions that support essential ecosystem services. The quality of these functions and services is dependent on the composition of below-ground communities (Nielson *et al.* 2010). Soil biodiversity services play an important role in all agricultural systems (Beare *et al.* 1995, Agwunobi & Ugwamba 2013) and not only do they aid bio-geochemical cycling, but also physically reform the soil and play a significant role in plant health (Wood & Philip 1998). Soil health and soil quality are two closely related terms used to describe the condition of soil. According to Doran (2002), soil health can be defined as the ability of soil to function. This applies for both natural and man-made ecosystems and is essentially the ability of soil to sustain both plant and animal life. Soil health is thus the capacity of soil to function as a self-sustainable system. Soil quality, on the other hand, is the ability of soil to function in natural and man-made ecosystems to support human health and habitation (Doran & Zeiss 2000). Thus, soil quality can be divided into physical, chemical and biological properties of soil and soil health is only the biological properties of soil affecting the abiotic properties.

Natural occurring plant species in agro-ecosystems take part in many food web interactions and harbours valuable genetic material for future crop improvement (Harlan 1975). Natural biodiversity in agriculture has an influence broader than just simply the production of goods or income. Soil organisms serve as ecosystem engineers and take part in renewable processes such as the recycling of nutrients, the detoxification of harmful chemicals and controlling the abundance of unwanted organisms. According to Klironomos *et al.* (2000), soil communities also have an influence on plant productivity. These communities provide nutrients to the plants and thus play a role in important plant processes, such as stress tolerance and competitive ability (Bennett 2010). The loss of these functions can lead to considerable environmental and economic costs. These expenses include the supplementation of certain compounds necessary to the agro-ecosystem that is deprived of crucial functions and lacking the ability to produce soil fertility and regulate pests. Thus, the removal of biodiversity leads to an all-out artificial system where constant supplementation of basic functions must be done. These functions does not only include soil processes, but also above-ground processes such as pollination and natural predation (Price *et al.* 1980, Siemann 1998, Knops *et al*. 1999,

Perner *et al.* 2003). By creating an ecosystem that is only dependent on external inputs and does not function by itself, food security and sustainable food production will collapse due to synthetic chemical build-up in the soil (Altieri 1999).

Soil mesofauna (including Collembola, Acari, Isopoda, Diplopoda, Myriapoda and Insecta) play an important role in soil structure and nutrient cycling (Hendrix *et al.* 1990, Emmerling *et al.* 2002). They take part in the regulation of bacterial and fungal populations and many serve as natural control agents for these organisms that may become harmful. Many groups of soil mesofauna are involved in fragmentation of plant residues and produce faecal pellets that contain nutrients which can be directly utilized by plants (Hendrix *et al.* 1990).

Various agricultural practices can be applied to promote soil diversity, as well as add to crop health (Doran & Zeiss *et al.* 2000). The conservation of soil microbial activity and maintenance of soil organic matter can help preserve soil biodiversity that leads to more fertile and better quality soils in agricultural fields (Emmerling *et al.* 2002). The use of animal manures has proven to increase both richness and activity of soil fauna, whilst they also serve as an additional nutrient source for crops. According to Axelsen & Kristensen (2000) and Olla *et al.* (2013), Collembola populations respond positively to animal manure applications and an increase in population numbers has been observed after application. Similarly, Doran & Werner (1990) found an increase in earthworm biomass as a response to animal manure additions. A more stable soil environment will also promote soil fauna diversity and development. Tillage is one of the most common soil disturbances in agriculture and usually disturbs at least 15-25 cm of the soil surface (Altieri 1999). The disruption of the stratified soil microhabitat causes a decline in soil faunal abundance. Reduced tillage can be applied to create a more stable environment and to promote decomposer diversity. Mulch and crop residues, left in fields, support larger decomposer faunal numbers. Not only does mulch serve as a source of nutrients when there is an absence of soil sustenance, it also protects the soil surface from frost and other environmental extremes (Waddell 1975, Chalker-Scott 2007). In another study Kukkonen *et al.* (2004) found that soil supplementation with

peat, lead to dramatically increased numbers of *Aporrectodea caliginosa* over three growing seasons, but this was not true for all organisms within the soil system.

As mentioned earlier, soil fauna diversity is dependent on the diversity of plant species. By implementing agricultural techniques such as intercropping, shifting cultivation and agro-forestry, that mimics natural ecological processes, soil fauna diversity can be preserved. According to Altieri (1999), the status of biodiversity in an agricultural system is dependent on the diversity of vegetation within and surrounding the agro-ecosystem. The establishment of natural vegetation between fields is also important in providing pollinators and natural enemies for pest organisms (Zhang *et al.* 2007). The durability of the specific crops cultivated and the extent of their isolation from natural vegetation can also play a role in biodiversity within an agro-ecosystem. Thus by promoting the natural vegetation surrounding the crop field, one can increase diversity within the field. Living mulches and cover crops can also promote diversity of soil fauna, since they provide a more diverse environment for the survival of soil fauna and protect the upper soil layers from desiccation and other external factors (Abawi & Widmer 2000).

Presently the only motivation for human society to protect biodiversity is that preserving diversity has some kind of economic advantage (Bengtsson 1998). Farmers and researchers worldwide are looking for an agricultural system that's able to support itself with the lowest possible external inputs (= costs) (Altieri 1999). This can only be achieved by a diversified, energy-effective system. Because biodiversity provides many ecological services, the promotion of biodiversity can lead to a sustainable agricultural system that is able to self-control pests and diseases and produce optimal nutrient cycling and soil fertility. This system will thus lead to more sustainable yields with less dependence on external inputs (Altieri 1999). According to Louw *et al.* (2014) fundamental and applied research are needed to to generate climate smart management strategies to improve soil health.

1.3. Trophic interactions and functional groups in soil ecosystems

Soil is not only a resource, but it also serves as a habitat that should be able to support the activities of soil fauna and flora and sustain both plant and animal diversity (Emmerling *et al.* 2002). According to Agwunobi & Ugwamba (2013), arthropods play an important role in the functioning of soil ecosystems. Soil arthropods include micro-, meso- and macrofauna and their size (body length) range from 200 µm up to 20 mm. The five main groups found in the upper soil layers include Isopoda, Myriapoda, Insecta, Acari and Collembola. According to Behan-Pelletier (2003), Acari and Collembola are the most abundant and diverse of these five groups. Micro- and meso-arthropods play an important role in the energy flow of soil food webs channelling energy from soil microfauna and -flora to macrofauna on higher trophic levels. They serve as both predators and prey in soil food webs and form a middle link in these systems (Darby *et al.* 2011).

Collembola can be found in the upper soil profile of every biome across the world. According to Castaño-Meneses *et al.* (2004), the majority of collembolans feed on fungi associated with decomposition of litter. They mostly occur in shallow soil levels and leaf litter layers and certain species may act as biological control agents for certain fungal pathogens. The fungus pathogen *Rhizoctonia solani* that is associated with cotton roots, is one known plant pathogen on which they feed (Lartey 2006). Collembola tend to aggregate in clusters, although they have been sampled at random in soil samples. They are capable of fast reproduction rates, especially when conditions are favourable and food is abundant (Tully & Ferriere 2008). Unlike insects, they moult during their complete life-span and not just between instars. In a study done by Sechi *et al.* (2014) the gut content of collembolans can include fungi, plant debris and even animal matter. This indicates that some species are opportunistic feeders that will feed on a wide variety of food resources. A study done by Butcher *et al.* (1971), indicate that when given a choice they will always choose fungi as a food source. In the cases of predatory Collembola, feeding mostly on Nematodes, they tend not to be specialized and will feed on a range of nematode species.

Collembola can be more abundant than Acari in some soils, but the two groups are equally dependent on soil moisture for survival. Their diet includes microflora, such as fungi and bacteria, the protonema life stages of moss, pollen, faecal matter of other arthropods, other Collembola, decomposing plant litter and humus (Berg *et al.* 2004, Castaño-Meneses *et al.* 2004, Chahartaghi *et al.* 2005, Fiera 2014). Because of their small size, individual Collembola contribute only a small fraction to the energy flow in soil ecosystems, but since they aggregate in such large numbers their impression can be of much importance. They also play a pertinent role in soil respiration, plant health in general, mineralization of nitrogen and leaching of dissolved organic carbon (Bengtsson & Rundgren 1983, Bardgett & Chan 1999, Zanuzzi *et al.* 2009). One of their most important functions is that they feed on fungal hyphae associated with decomposition. Their fungal feeding is not necessarily negative and in some cases grazing stimulates fungal growth when a moderate number of Collembola is present (Bengtsson & Rundgren 1983). According to Sechi *et al.* (2014), most members of the group Poduromorpha, including species from Brachystomellidae and Hypogastruridae are mostly mycophagous, while *Isotoma* spp. (Isotomidae) are predacious on microfauna, such as nematodes. They also determined that *Lepidocyrtus cyaneus* feed on bacteria, fungi and micro-organisms based on their gut content. It therefore seems that Collembola shows high variation in feeding preferences, with some species tending to be specialists, while others are more generalistic or opportunistic.

Mites are minute to small sized arthropods closely related to spiders. According to Coleman *et al.* (2004), mites can be divided into four suborders, *viz.* the Oribatida, Prostigmata, Mesostigmata and Astigmata. Of all these groups the Astigmata is the least common in soil environments. Their population sizes are usually small, but they can reach high numbers in agricultural fields post-harvest or those in which rich manures or fertilizers have been used. They prefer to live in moist soils and most members are microbial feeders (Coleman *et al.* 2004). Some members of this group are able to chew vegetable matter, fungi or algae, whilst Anoetidae species are filter feeders with reduced chelae and adapted palpi. Mesostigmata mites are almost always predacious, with the

larger species feeding on small arthropods (Walter *et al.* 1988) and their eggs, *e.g. Hypoaspis* spp. are important predators on small insect larvae that spend a part of their life cycle in the soil (Coleman *et al.* 2004). Smaller species feed on nematodes and there does not seem to be a preference for any particular nematode species. These small predators can become very abundant in agricultural soils associated with high plant parasitic nematodes and they can also serve as a natural control to keep nematode populations at bay. Some species found in soil are parasites on above-ground vertebrates and invertebrates that sometimes seek refuge in soil (Walter & Proctor 2013).

The mesostigmatids are less abundant in soil than the Prostigmata and the Oribatida, but more abundant than Astigmata. Much like the mesostigmatids, the Prostigmata consist mainly of predators, but some members are known to feed on microbes (Seastedt 1984). These micro-phytophages (feeding on microflora) are opportunistic and reproduce rapidly after a disturbance or during an abundance of resources (Coleman *et al.* 2004). In conditions like this they may become more abundant than Oribatida.

As with the mesostigmatids, the prostigmatids have small species feeding on nematodes and can therefore play a role in regulating pests. The larger species feeds on other arthropods and their eggs (Buryn & Brandl 1992). One species, *Allothrombium trigonum*, feeds exclusively on grasshopper eggs and another (*Dolicothrombium* sp.) feeds only on termites (Coleman *et al.* 2004). Members of the Trombiculidae feed on Collembola and their eggs. According to Walter & Ikonen (1989), nematophagous mites can be more numerous in grassland habitats because of the abundance of nematodes in these ecosystems. Some species of this group also feeds on plant material or are parasites of larger organisms.

According to Seastedt (1984) and (Wallwork 1983) the oribatids play the most important role in decomposition processes and are the most abundant of the soil Acari. They play a vital role in the turnover of organic matter in grassland and forest

ecosystems. Unfortunately they are dependent on high soil humidity levels and are not as successful in drier soil habitats. Oribatids can be divided into 4 main feeding groups (Wallwork 1983): 1) macro-phytophages which feed on decomposing higher plant material, 2) micro-phytophages feeding on microflora including fungi and bacteria, 3) pan-phytophages which have a broader spectrum of food including plant matter, as well as microflora and 4) coprophages feeding on faecal matter of other organisms. The Phtiracaridae, or box mites, are largely macro-phytophagous feeding on decomposing plant matter. Some oribatids feed on woody substrates, but possess gut flora assisting with the digestion of these substrates. According to Hansen (2000), oribatids are primarily opportunistic mycophagous mites and have a broad spectrum of fungal species they feed on. As a group Oribatida contributes to decomposition, both indirectly and directly. Indirect influences include feeding on fungi and stimulating their growth the same manner that Collembola does. Another contribution, made to the soil by oribatids, is that they feed on fungal hyphae which contain calcium oxalate crystals. After feeding this is possibly stored in the exoskeleton, which is shown to be rich in calcium. When these organisms die and decompose this calcium is released in the soil which can be utilized by plants (Seastedt & Tate 1981).Oribatida's immature stages are morphologically quite different from the adults, but they feed on the same food source.

The arthropod Myriapoda that is important in soil environments includes; the Diplopoda (millipedes), the Symphyla (pseudocentipedes) and Chilopoda (centipedes). The Myriapoda in general tend to be most successful in soils that are moist with a high pH and they can be commonly found in the upper layers of these soils (Xylander 2009). According to Kime & Golovatch (2000), they also prefer calcium rich soils. Millipedes mostly feed on decaying plant material, but some species feed on fungi. They play an important role in calcium cycling due to their calcareous exoskeletons and in high abundance, they can contribute a considerate amount of calcium in forest soils (Seastedt & Tate 1981). Species feeding on leaf litter can be very selective and avoid eating litter high in polyphenols, but favour calcium rich litter (Osman 2013). Overall they feed on decomposing litter and are not commonly found feeding on fresh leaves.

The Symphyla consists of only two families and are a small group of arthropods. They have an elongated body, small in size, colourless and have no eyes (Podsiadlowski *et al.* 2007). Populations can reach high densities in some environments and they are known to be the most abundant in mixed managed agricultural environments (Osman 2013). As with Acari and Collembola they can only survive in soil with very high relative humidity levels. Symphyla feed on plant matter in the early decomposition stages, a resource that not many soil invertebrates exploit (McColl 1974). According to Coleman *et al.* (2004), some symphylans do not only feed on decomposing plant litter but are omnivores feeding on both plant and animal tissue.

Centipedes are active predators found in both soil and leaf litter and they also prefer habitats with a high humidity (Blackburn *et al.* 2002, Salmon *et al.* 2005). Depending on their size, they primarily feed on Collembola and other small soil fauna. Even though they are predacious, they occasionally feed on leaf litter (Coleman *et al.* 2004).Important Isopoda in soil environments include woodlice and sowbugs from the suborder Oniscidea. They are also dependent on high soil moisture and their survival in drier regions is mostly achieved through behavioural procedures. They feed mostly on wet leaf and wood matter, as well as their own faeces (Szlavecz & Maiorana 1998). This coprophagous behaviour is to recover inorganic copper and other vital nutrients (Szlavecz & Maiorana 1998). Oniscidea are able to fragmentize plant litter into smaller pieces, with their heavy, sclerotized mandibles giving them this shredding ability (Kautz & Topp 2000).

Insecta in soils are dominated by two orders, *i.e.* Isoptera (termites) and Hymenoptera, of which the Formicidae (ants) are the most abundant. Both termites and ants are social insects and serve as ecosystem engineers with the ability to modify their environment. They move soil from bottom layers to the top and take part in both above- and below-ground ecosystem activities. According to Jouquet *et al.* (2002), termites use finer soil from deeper soil layers to build their nests. Termites feed on humus, wood or plant litter depending on the species in question (Black & Okwakol 1997). Some species

of termites and ants also specialize in developing their own fungus colonies inside their nests (Aanen *et al.* 2002). Termites are one of very few arthropods referred to as tertiary feeders that are able to break down cellulose, a compound making up most of all plants (Aanen *et al.* 2002). This ability makes them a keystone species in many grassland habitats. Ants are one of the most successful soil arthropods due to their ability to exploit a wide variety of food resources. Being generalists they serve as both predators and scavengers in soil ecosystems and some feed on plant matter, such as leaves and seeds. Certain winged insects also take part in soil food web structure, with some even being permanent residents in the soil. In most insect orders the immature stages are dominantly present in soil and these include Diptera, Lepidoptera, Hymenoptera (excluding ants), Hemiptera, Thysanoptera, Neuroptera, Coleoptera and Orthoptera.

Coleoptera contains a wide variety of trophic groups that includes predators, phytophages, mycophages, saprophages and some are parasitic (Triplehorn & Johnson 2005). One of the most commonly found families of Coleoptera in soil is Staphylinidae (rove beetles). They are mostly predacious, but a few species feed on decaying matter. Scarabaeidae is another important Coleoptera family that feeds on carrion, dung or plant matter, such as leaves, flowers, pollen, roots and small saplings (Triplehorn & Johnson 2005). Coleoptera larvae found in soil usually feed on plant roots and decaying plant matter. Predatory Coleoptera are of high importance in agricultural and natural soils because they play a role in regulating pests.

Members of the Elateridae (click beetles), are phytophagous and are important in agricultural systems as pests, especially when they occur in large numbers. Schallhart *et al.* (2012), studied the dietary choice of soil insect phytophages and stated that their food choice is dependent on certain characteristics of the host plants, with some plants containing a certain set of nutrients that are preferred by certain insects. Soil fauna in agro-ecosystems are subdued by constant and rapid changes in vegetation type and microhabitat which leads to limited mobility. In order for them to survive they have to adapt to their ever-changing environment. In the light of this they questioned if dietary choice of these larvae is plant specific or availability related. Schallhart *et al.* (2012),

found that three species of Elateridae larvae preferred grass and legume litter. However, their dietary choice changed in accordance with the diversity of litter available. They preferred mixed plant litter that contained a wider variety of nutrients. They will also feed on low nutrient litter when only that is available, but preferred more nutritious plant species. Schallhart *et al.* (2012) concluded that dietary choice is availability related and that these larvae are adapted to feed on a wide variety of plant species and are thus actually opportunistic (Schallhart *et al.* 2012). Generalist behaviour in soil fauna, especially phytophages, is the norm because of the constant change in their environment. Hansen (2000) found that mixed plant litter has more successional stages at any one time because different litter qualities relate to decomposition at different rates. This ensures a more stable food source for soil fauna and also ensures a steady supply of nutrients to the surrounding environment.

1.4. Decomposition and nutrient cycling in soil environments and its importance to agriculture

Decomposition serves as a driver for below-ground food webs which in turn is responsible for nutrient turnover. The purpose of decomposition is to break down dead material into carbon dioxide (CO_2) and other nutrients (Swift *et al.* 1979). Plant productivity in many ecosystems is dependent on decomposition of litter which converts nutrients trapped in organic matter to mineral form in soil (Gartner & Cardon 2004). More than 90% of primary production is decomposed and reprocessed through the detritus food web (Guevara *et al.* 2002, Culliney 2013). Decomposition increases soil organic matter and fertility, as well as aiding in soil formation. These nutrients are then directly or indirectly absorbed by plants. Soil biota changes the composition of these chemicals into more accessible forms for plants to absorb. In other words, these decomposer organisms provide the surrounding vegetation with nutrients that would otherwise be trapped in dead plant litter. In nutrient poor soils the only source of nutrients for plants comes from the decomposition of plant litter (Freschet *et al.* 2013).

The most important group in decomposition of plant litter is the microfauna and -flora (Guevara *et al.* 2002). They can break down litter in the initial stages of decomposition. Because very few of the soil fauna possesses the ability to digest plant

litter, microbes are mainly responsible for this process. These microbes include gut-fauna which aids in the breakdown of plant litter in the digestive systems of some fauna (Watanabe & Tokuda 2010). Overall litter decomposition rates are the result of combined activities of a variety of soil fauna. Litter breakdown is a key component in soil ecosystems and soil fauna are as dependent on this activity as on the surrounding plants (De Deyn *et al.* 2008). Decomposition of plant litter in a soil system can be divided into four stages (Figure 2) with energy flowing in descending order through the system.

When fresh plant litter initially falls to the ground, physical weathering or fragmentation is necessary for utilization by microfauna and -flora (Harley 1971). This is the first stage of energy flow in decomposition food-webs (Figure 2). Physical weathering includes photo-degradation or exposure to solar radiation and exposure to water or wind. Physical fragmentation is mainly achieved by saprophages of plant material.

Soil decomposer fauna are a very important component in primary productivity. They are, however, depended on mycophagous fauna to stimulate their growth and manage their population dynamics by feeding (Gonzalez & Seastedt 2001). The most important role of arthropods in decomposition is the physical fragmentation or comminution of litter. They shred litter into smaller pieces and eliminate the protective leaf cuticle (Zimmer 2002), which exposes cell contents and makes it easier for microbes to utilize. According to Adl (2003), physical fragmentation results in a larger surface area of the litter exposed, thus aiding in decomposition.

The salivary excretions from macro-arthropods aid the decomposition process through active digestion (Adl 2003). They feed on this plant material, which then passes through their digestive system, and the waste is excreted as they move through the soil. Not only do they thereby redistribute litter, but these faecal pellets are smaller in size and differ in chemical composition than the initial product (Teuben & Verhoef 1992, Wolters 2000).

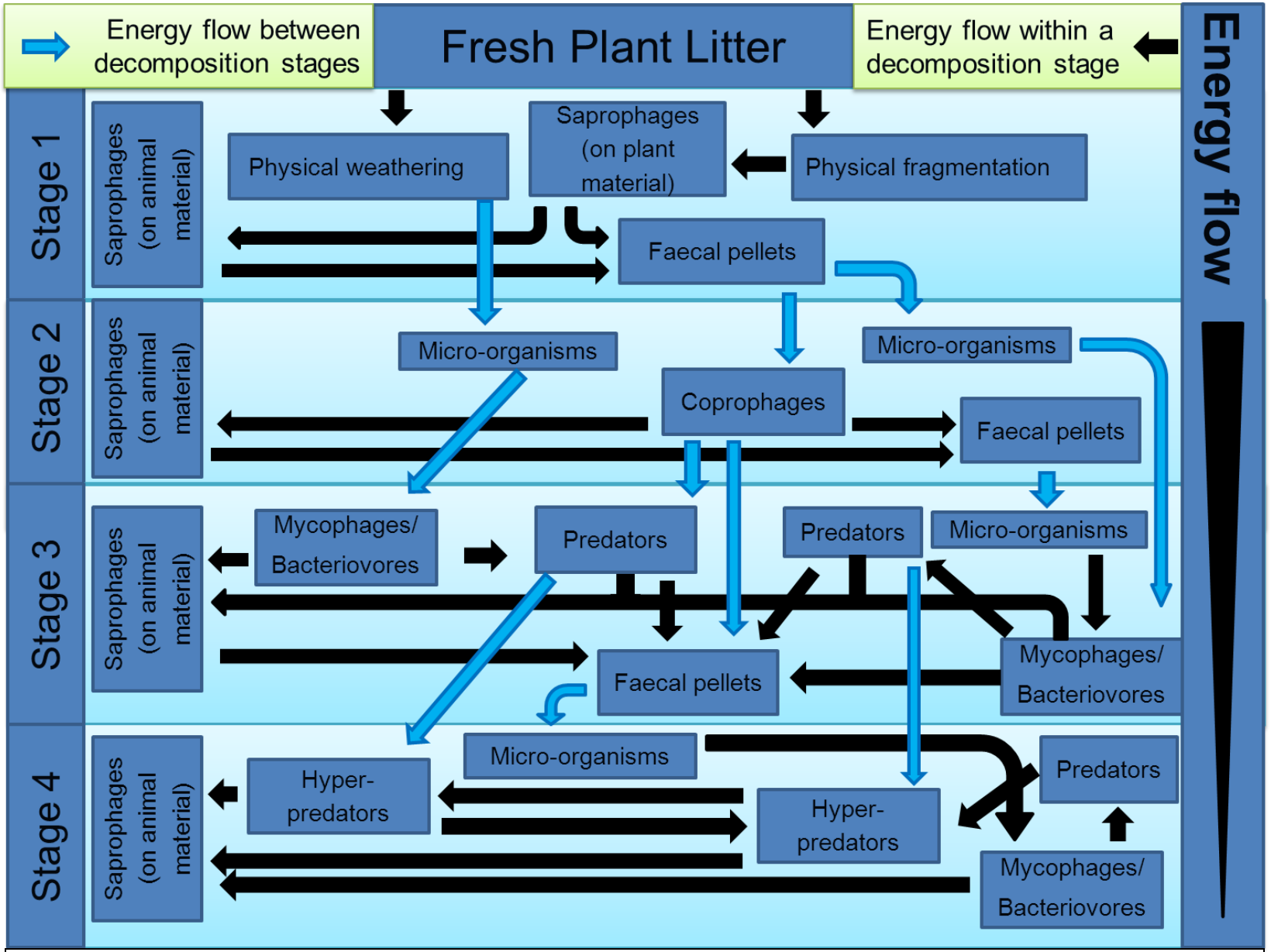

Figure 2: Hypothetical flow diagram within a decomposition food-web (based on Culliney 2013).

Faecal pellets also present a larger surface area for micro-organisms to exploit. Some of the nutrients in these faecal pellets can leach into the soil and become an immediate nutrient resource for plants. As with any food web, some of the energy is lost through respiration but another portion of the energy is used to break down litter into smaller pieces, mix litter with the surrounding soil, disperse litter and microflora inoculum and regulate microflora through feeding (Lavelle 1997). In this context the presence of millipede faeces in soil can increase the pH by up to 2.2 (McBrayer 1973). Faeces also contribute to soil moisture and create a favourable environment for microfauna and -flora. Some nutrients in faeces of arthropods are more concentrated than in the consumer's original food source. For example, Collembola faeces contain 40 times more Nitrate ($NO_{3)}$ than their fungal food source (Teuben & Verhoef 1992). Soon after the initial fungi colonization, bacteria follows and increases in importance (Culliney 2013). Both microbes feeding on weathered litter and faecal material are placed in the second stage of decomposition (Figure 2). In this stage of decomposition only micro-organisms, saprophages and coprophages are actively breaking down litter.

Coprophages play an important role in stage two of decomposition by digesting faecal material of saprophages and redistributing nutrients through their own faecal material. In the third stage (Figure 2), decomposition slows down and other arthropods start to appear (Culliney 2013). Arthropod mycophages and bacteriovores feed on fungi and bacteria and redistribute nutrients though their excrementa and soil activities. Once the saprophages, bacteriovores and mycophages are present, their predators soon follow. Predators also redistribute nutrients though their faeces. When considering the role of arthropods in decomposition and nutrient turnover, their effect is mainly indirect. According to Culliney (2013), less than 10% of the net primary production is consumed by oribatid mites, one of the most numerous groups in decomposition food webs. When considering the role of arthropod excrements in decomposition, nutrient turnover is said to be one of the most important contributions (Teuben & Verhoef 1992).

Stage four of decomposition (Figure 2) is where predators and hyper-predators play the most important role in nutrient turnover. Hyper-predators feeding on each other

redistribute nutrients through their faecal pellets that can have higher concentrations in elements such as Ca, found in the cuticula of some mesofauna, such as oribatids.

When analysing the overall decomposition food web, it is also important to take saprophages on animal litter (*e.g.* cadavers of decomposers) into consideration. Their faecal pellets also contribute to nutrient availability in soil. Saprophages feeding on animal material plays a role in energy flow in all of the decomposition stages. Soil arthropods thus have direct and indirect actions in digestion of plant litter and aid in the conversion of nutrient poor and/or difficult to digest substances into more nutrient rich and easier to break down substances respectively (Parkinson *et al.* 1979). Microbes convert low quality resources into easily digestible nutrients that can be utilized by consumers at low metabolic costs (Swift *et al.* 1979). Arthropod grazing on microbes stimulates their actions resulting in mineralization of nutrients, *e.g*. Collembola grazing on microflora increased the availability of N and Ca in soil (Filser 2002). It was also observed that Isopods feeding on oak and alder tree litter, increased microbial respiration up to 20-fold (Kautz & Topp 2000). The presence of Isopods may also increase the availability of nutrients such as C, N, P_2O_5-P, K^+, Mg^{2+} and Ca^{2+} through their faeces in topsoil (Kautz & Topp 2000). Microbial population regulation is also an important contribution of mycophages and bacteriovores in soil food webs. By grazing on microbes they ensure a slow but constant supply of nutrients to the surrounding vegetation and prevent microbial breakouts (Culliney 2013). Mycophages disperse fungal spores that stick to their cuticles and through their faeces that also contains viable fungal spores (Poole 1959).

Another important source of nutrients is held in what is referred to as ‘arthropod biomass’. According to Teuben & Verhoef (1992), a significant amount of K^+, PO_4^{3-}, N, Na^+ and Ca^{2+} is stored in arthropod biomass. Termites, together with their gut symbionts can digest polysaccharides and compounds, such as lignin, which are more difficult to digest. The termite diet is extremely high in N and fungal feeding termites feed on fungi that may fluctuate between 39.16 – 43.37 % protein (Sidde Gowda & Rajagopal 1990). Many of these nutrients are stored in their tissue, making termite

colonies a very rich nutrient store in grassland habitats (Sidde Gowda & Rajagopal 1990).

Because of their digestive adaptations termites can degrade almost any plant material leaving very little residue (Lee & Wood 1971). In addition termite mounds contain up to 76 times higher concentrations of NH_4^+, NO_3^-, N, Ca^{2+}, Mg^{2+}, K^+ and inorganic phosphorous than unaltered soil surrounding the mounds (Arshad 1982, Bagine 1984, Nutting *et al.* 1987, Abbadie & Lepage 1989, Martius 1994, López-Hernández 2004, Ndiaye *et al.* 2004, Ji & Brune 2006, Jiménez & Decaëns 2006, Ngugi & Brune 2012). Soil eroded from these mounds can contribute a great deal of nutrients to surrounding plants and play a significant role in agricultural soils. The same can be said for ant nests. Because they feed on both plant and animal material and also store these food sources in their nests, large amounts of organic matter can accumulate in nest chambers (Salick *et al.* 1983, Watson 1977). Microbes present in these nests break down their faeces, secretions and food material, leading to nutrients accumulating in the nests that in turn leaches out into the surrounding soil (Salick *et al.* 1983, Watson 1977).

Decomposition rates are the result of soil biota, litter and matrix quality, microclimate and the state or condition of the ecosystem (Sariyildiz *et al.* 2005, Freschet *et al.* 2012). The more diverse the organisms in a soil ecosystem, the wider the variety of litter that can be utilized by soil fauna. Some researchers suggest that litter will decompose faster in their area of origin (i.e. where the ‘mother plant‘ grows), than elsewhere (Ayres *et al.* 2009). Through physiological adaptation, soil communities can specialize in the decomposition of their native vegetation (Freschet *et al.* 2012). This phenomenon is known as ‘the home-field advantage’ (HFA) of decomposing litter (Ayres *et al.* 2009). According to Ayres *et al.* (2009), the outcome of experiments done on this phenomenon varies considerably and as such it is still unsubstantiated. Their study found some evidence that certain tree species have an effect on the soil community underneath their canopies. According to Gießelmann *et al.* (2011), specialization of decomposer fauna in such cases will only be helpful if litter is of low

quality, because high quality litter is decomposed by almost all decomposer fauna. In the case of high litter quality almost no adaptation or specialization is needed to break down litter.

According to Freschet *et al.* (2012), the HFA hypothesis only takes soil biota into account when predicting decomposition rate and this is only one of the litter quality-decomposer fauna interactions. They mention further that the HFA hypothesis suggests that in an ecosystem with high plant diversity, all soil fauna will be adapted to break down mixed litter of different qualities at the same rate. They therefore suggest an alternative hypothesis: the 'substrate quality-matrix interaction' (SMI) hypothesis. Matrix can be defined as the layer of litter in an ecosystem that drives decomposer fauna activity. The SMI hypothesis suggests that litter of low quality will decompose at a faster rate than expected in a low quality matrix. It makes sense that when only low quality litter is available, the decomposer fauna will have no choice but to feed on the available litter (Freschet *et al.* 2012). However, when high quality litter is placed in an area of low matrix quality, it will decompose at a faster rate than the low quality litter that originated in that area. This will be the same for low quality litter in a high quality matrix. It will decompose at a slower rate because decomposer fauna will favour the high quality litter. Of course in nature extremes of high or low quality only are not found. Intermediate litter qualities occur in most ecosystems. The SMI hypothesis thus suggests that decomposer fauna will always favour the litter that is of the highest quality and thus have no correlation to whether the litter originated in that area or not (Freschet *et al.* 2012).

According to Aber *et al.* (1990) and Aerts (1997), litter chemistry may have an influence on decomposition rates. As chemistry between different species of plants differs, it would make sense that different species of plants decompose at different rates. According to Strickland *et al.* (2009), and Taylor *et al.* (1991), litter with higher C:N and higher lignin content have slower decomposition rates than litter with lower C:N and lower lignin content. This is where litter quality comes in. When the litter contains many sugars and starches, it can easily be digested by microbes and soil fauna.

Sugars and starches are not only easily digestible, but provide a nutrient rich resource for soil biota (Coleman *et al.* 2004). On the other hand, if leaf litter is rich in polyphenols, such as tannins and lignins, only organisms specialized in the digestion of this litter type can utilize it directly (Coleman *et al.* 2004). Litter that is rich in cellulose and hemicellulose are intermediate when it comes to digestibility, since it is not as difficult to digest as polyphenols, but some specialization in decomposer fauna is needed. Decomposition rates are thus dependent on the percentage of these compounds in litter. Litter quality differs greatly between plant species, for example the leaves of dogwood (*Cornus florida*) is rich in calcium (Jenkins & White 2002), and the leaves of oak (*Quercus* spp.) and conifer needles (*Pinus* spp.) are high in lignin (Gholz *et al.* 1985, Morris *et al.* 2008). Litter quality even differs in the same plant, *e.g*. the leaves of maize (*Zea mays*) are more easily degradable than the stalks because of the difference in litter quality (Coleman *et al.* 2004). Bray *et al.* (2012) found that litter quality could also influence the microbial community associated with decomposition rates. This indicates that different decomposer fauna may be associated with specific litter characteristics.

Microclimate, which includes both temperature and humidity, surrounding litter material also influences decomposition rates. According to Gonzalez & Seastedt (2001), decomposition in colder regions will be slower due to limited respiration of decomposer fauna. Soil fauna, being invertebrates, are exothermic and are reliant on their surrounding temperature to generate body heat and determine their level of activity. Gonzalez & Seastedt (2001) found that decomposition rates were consistently higher in wet tropical forests compared to a dry subalpine forest. According to Culliney (2013), these increased decomposition rates are due to increased actions of microfauna and flora in the soil. Soil fauna such as earthworms was only found in the wet tropical forests. These faster decomposition rates are thus connected to increased soil moisture, which in turn influences densities and diversity of soil fauna (Gonzalez & Seastedt 2001). Their results also indicated that the micro-arthropods per gram of litter were higher in the wet tropical forest.

Another factor influencing decomposition rates is the state or condition of the ecosystem. Disturbed ecosystems' such as agro-ecosystems may have slower decomposition rates than predictions derived from the micro-climate of the area (Lavelle *et al.* 1993). Constant disturbances, such as tillage, can change the micro-climate, which in turn reduces soil faunal activities. Agro-ecosystems are an ever changing environment with the crop species and cultivation practices changing regularly. This gives soil fauna in these conditions less time to adapt to their environment and organisms with long life cycles are seldom found in these situations. Agricultural fields also lack the diversity of vegetation that natural ecosystems have, leaving soil fauna with no or little variety in their food source (Freschet *et al.* 2013).

1.5. Plant-induced chemicals in soil agro-ecosystems

Certain plants have the ability to influence surrounding plants by releasing chemicals into their environment (He *et al.* 2012). This phenomenon was first described as 'allelopathy' in 1937 by Hans Molish, an Austrian plant physiologist (Aliotta *et al.* 2006). Since then the definition of the term allelopathy was refined by Rice (1984) as the stimulatory and inhibitory effect of one plant on another and this definition also includes microbes (Aliotta *et al.* 2006). Sodaeizadeh & Hosseini (2012) describes allelopathy as any process concerning secondary compounds produced by organisms including plants, fungi, micro-organisms and viruses that influence the growth and development of another organism positively or negatively. This phenomenon can take place in both agricultural and biological systems. These interactions are primarily beneficial to the donor (allelopathic plant) and harmful to the receiver. The chemicals responsible for allelopathy are universally known as allelochemicals or allelochemics (Singh *et al.* 2001). According to Singh *et al.* (2001), the chemicals that are produced by plants, as secondary metabolites, seem to have no direct role in plant growth and development but rather provide the plant with defensive capabilities.

According to Ehlers (2011), aromatic plants, such as the Lamiacae, produces essential oils that can be high in compounds, such as monoterpenes, that can have

allelopathic effects. Even though it has been shown that monoterpenes can be digested by some microbes, it is said to have anti-microbial effects on bacteria and fungi (Ehlers 2011). Studies have shown that these monoterpenes act as growth inhibitors for a wide range of fungi and bacteria. Another aromatic plant that shows allelopathic potential is thyme (Ehlers 2011). These plants also produce monoterpenes that affect the growth of their surrounding plants negatively. However, it has been observed that plants that are in constant association with plants producing monoterpenes, can adapt to this allelochemical effect (Ehlers 2011).

One of the most debated issues in allelopathic research is distinguishing between competition and allelopathy. Even though these two terms are difficult to distinguish, Weidenhamer (2006) did so by using density as an example. When plant density is taken into account, it makes sense that there will be a maximum density at which plants can grow and develop optimally without competing for resources (Weidenhamer 2006). Allelopathy of plants also influences vegetation densities. Plants growing in these phytotoxic soils at low densities will have to cope with high concentrations of these toxins. But as plant densities increase the dosage of toxin absorbed by each plant will decrease. Thus the effects of competition increase with increasing densities and allelopathic effects decrease with an increase in plant density (Weidenhamer 2006).

Allelopathy causes a problem known as soil sickness in croplands, due to post-harvest plant residues left behind in fields (Singh *et al.* 2001). Allelopathic interaction is a chemical based reaction and is concentration specific (Singh *et al.* 2001) and the toxicity of these chemicals is dependent on the plants age, metabolic stage (Singh *et al.* 2001) and cultivar (Chung and Miller 1995). Environmental conditions, such as climate, season and humidity levels also influence the toxicity of allelochemicals (Blum *et al.* 1999). Another abiotic factor that influences the toxicity of allelochemicals is soil type and soil characteristics, such as pH, organic carbon, available nitrogen and organic matter (Singh *et al.* 2001). Allelopathic interactions may act as a repellent for insect pests, pathogens and it also reduces competition, thereby increasing the reproductive

fitness of allelopathic plants (Singh *et al.* 2001). Plants release allelochemicals into the environment by leaching, volatilizing, root exudation as well as the decomposition of fallen and dead plant material (Singh *et al.* 2001). According to Weidenhamer (2006), allelopathy also includes negative effects such as a decrease in plant growth and development and reduction in water and mineral absorption abilities.

According to Zhou & Yu (2006), researchers found a reduction in chlorophyll content in some plants that were treated with allelochemicals. Plant roots are often the first to come in contact with allelochemicals that can have a negative effect on water and nutrient absorption. The loss of these absorption abilities are mainly achieved by an interference of membrane function of roots by the allelochemicals (Zhou & Yu 2006). A reduction in water and iron uptake can lead to stomata closure and a decrease in turgor pressure within the receiver plant. The loss of these functions in plants can influence the plants' ability to perform photosynthesis (Zhou & Yu 2006). The duration of these effects by the donor plants can differ from one allelopathic plant to another. Cucumber plants affected receiver plants for a few hours, but sunflowers and tobacco allelochemicals may affect their receiver plants for several days (Zhou & Yu 2006).

According to Pedrol *et al.* (2006), plant stress can determine how donor and receiver plants respond. Stress can be biotic and abiotic, with abiotic influences including temperature, water, radiation, chemicals and other factors, such as wind or air-pressure and, biotic influences including pathogens, herbivores and other plants (Atkinson & Urwin 2012). The effects of other plants then include the already mentioned allelopathy and competition. Allelopathic plants respond to stress by producing more allelochemicals and also by increasing the concentration levels of these secondary metabolites (Sodaeizadeh & Hosseini 2012). On the other hand, plants targeted by allelopathic plants that are experiencing stress can be more susceptible to allelochemical effects (Pedrol *et al.* 2006).

Active allelopathy by plants is not a constant fuelling of allelochemicals into the soil because allelopathic plants respond to environmental changes throughout their life-time (Pedrol *et al.* 2006). According to Sodaeizadeh & Hosseini (2012), plants in nutrient rich soils with little environmental stress will not perform allelopathic activity as much as plants under stress. In some environments where plants are under constant stress throughout their life-time, tolerance towards stress will develop (Pedrol *et al.* 2006). Because plants in nature are exposed to stress at one time or another they tend to be less sensitive to allelopathy than plants grown under laboratory conditions (Pedrol *et al.* 2006).

Allelopathy can be affected by seasonal changes. Seasons affect the blooming, flowering and germination periods of plants (Pedrol *et al.* 2006). Some allelopathic plants produce more allelochemicals in the germination period to defend themselves against surrounding plants. Allelochemicals can be diluted by rain or irrigation that can change the concentration of toxins that reach the receiver plant (Sodaeizadeh & Hosseini 2012). In some studies it has been shown that continued release of allelochemicals into soil can take place over a short period of time to ensure that the receiver organism is exposed to the concentration of allelochemicals substances that are most effective (Pedrol *et al.* 2006). The transfer of allelochemicals to the receiving organisms also depends on the distance between the donor and the receiver. Furthermore, soil barriers can be physical, chemical and biological and limit the phytotoxicity of allelochemicals, where the barriers will influence the quantity and quality of the allelochemical that reach the receiver (Pedrol *et al.* 2006).

Plant residues that are phytotoxic or allelopathic can influence germination of seedlings and activity of soil microbes (Gawronska & Golisz 2006). This problem can be enhanced if the residues are from plants that were under stress. Such residues are said to have higher concentrations of allelochemicals (Gawronska & Golisz 2006). Allelochemicals from live plants or dead plant material can also influence microfauna and -flora that is responsible for the breakdown of these materials. Not all microfauna and -flora are involved in decomposition and there are some species of fungi and

bacteria that are beneficial to plant health and soil structure in a different manner (Gawronska & Golisz 2006). Vesicular arbuscular micorrhizae are a group of micro-organisms that form a mutualistic bond with plants, in particular plant roots (Gawronska & Golisz 2006). Certain allelochemicals are known to influence these fungi negatively and in so doing have an indirect negative influence on the health of surrounding plants (Gawronska & Golisz 2006). By illuminating these helpful fungi the donor indirectly places stress on the receiver. Allelopathic compounds of baldy grass (*Imperata cylindrica*) were tested on seven species of vesicular arbuscular micorrhizae and had a negative effect on all seven species (Gawronska & Golisz 2006).

According to Vokou *et al.* (2006), some allelochemicals can promote microbial infection in the receiver. It was found that extracts from cassava (*Manihot esculenta*) can stimulate the growth of diazotrophic bacteria under laboratory conditions. According to these authors there are some mutualistic relationships between microbes and allelopathic plants. In some cases the donor excreted the chemicals into the soil that weakened the roots of the receiver plants and was absorbed by microbes. Certain *Fusarium* species also increased the presence of phytotoxins in the surrounding environment (Vokou *et al.* 2006).

According to Halbrendt (1996), allelochemicals can also influence larger organisms such as nematodes. According to Toudert-Taleb *et al.* (2014) allelochemicals can act as a repellent or attractant for some nematodes and thus alter their behaviour towards the involved plant. Certain allelochemicals that occur in crops are said to have nematicidal compounds that can actively reduce nematode population numbers. In this regard it has been found that rapeseed can be used to manage nematode population levels. In this study cited by Halbrendt (1996) it was concluded that the decline in nematodes was post-harvest. This means that decomposition of rapeseed litter released the chemical compounds needed to suppress nematode population numbers. They speculate that the living plant could also have excreted some of these compounds at sub-lethal concentrations and that the decomposition of

the rapeseed material led to higher concentrations that resulted in the control of the nematodes.

Allelopathic plants can have various modes of action towards receiver organisms that includes direct disruption of essential functions and an indirect influence on other organisms in the soil. Direct disruption of essential functions includes the disruption in absorption of nutrients and water, the malformation of roots (such as clubbing), the disruption of communication, energy and cation flow within the plant and the interruption of seed germination (Gawronska & Golisz 2006). Indirect influences of allelochemicals include the mortality of beneficial organisms, promotion of harmful microbes and the indirect pathways of these chemicals. According to Blum (2006), soil fauna breaking down allelopathic plant litter can either amplify or reduce effects of the allelopathic compounds, *e.g.* in cases where the compounds released during decomposition are more harmful to the receiver plant than the compound initially released from the donor. According to Lankau (2010), microbes can also detoxify these chemicals completely, converting them to less harmful or harmless substances in the soil. These allelochemicals can furthermore influence the ability of organisms to compete and influence nutrient flow, microbial activity and some abiotic factors within a system (Iderjit & Weiner 2001). Nutrient flow within a system is one of the most important aspects of soil ecology. A restriction in nutrient flow can be caused by soil pH which can be the result of the organisms in the system as such. According to Inderjit & Weiner (2001), allelopathic plants can influence pH, as well as nutrient flow, within an ecosystem.

Allelopathic plants that are invasive can have an advantage over native plant species. According to Lankau (2010), surrounding plants will be more seriously affected by these plants because they did not evolve together. Without an evolutionary history together the receiver will not have any counter measures to protect them from the allelopathic plants (Lankau 2010). This may be one of the reasons why invasive plant species thrive in their new environment. Not only do their allelochemicals hold detrimental repercussions for native species, but in most cases they don't have natural enemy phytophages that feed on them.

When considering allelopathy one would think that allelopathic plants of the same species will not have an influence on one another, this, however, is not the case. Auto-allelopathy is allelopathy between plants of the same species. This phenomenon can have serious effects on agro-ecosystem management where replacement of crops is often needed. According to Gawronska & Golisz (2006), this occurs within fruit trees orchards such as cherries and citrus, perennial crops such as alfalfa and grapes, as well as annual crops such as sunflowers. Allelopathy can also be implemented as a beneficial tactic in agriculture (Weidenhamer 2006). Allelochemicals hold the key in the development of new herbicides and other organisms such as nematodes (Halbrendt 1996) and microbes can also be managed by using these toxic compounds in pesticides. By implementing management practices such as allelopathic cover crops, mulches, cropping rotations and crop varieties one can more naturally control weeds in agricultural systems (Gliessman 1983, Liebman & Dyck 1993, Weston 1994, Bond & Grundy 2001, Weidenhamer 2006). By understanding allelopathy and choosing the correct cultivar, farmers can furthermore reduce the effects of allelochemicals that causes soil sickness (Williams & Wise 1997, Weidenhamer 2006).

1.6. Genetically modified crops and their influence on soil and soil fauna

Pests occur on every known crop and pesticides must be used to control these pests. These pesticides end up in the soil and have to be degraded into non-toxic forms by micro-organisms (Digrak & Özcele 1998). Chemical insecticides have been used globally to decrease yield losses, but this control method has proven to be expensive and cause extensive environmental pollution and degradation (Obonyo *et al.* 2008). The most recent control method for crop pests is to introduce transgenic cultivars. The cultivation of crops that are genetically modified to resist insect pests is not only an effective method to control pests, but can potentially reduce production costs (Obonyo *et al.* 2008). According to Shankar *et al.* (2008), genetically modified (GM) or genetically modified organisms (GMO) crops can provide many solutions in agriculture regarding pest control and general crop quality of crop. GM crops were first introduced

in South-Africa in 1998, where especially *Bacillus thuringiensis* (*Bt*) cotton played an important role (Shankar *et al.* 2008). This initial GM crop was developed for the control of bollworm that is a major pest in cotton. The first company to produce GM cotton cultivars in South-Africa was Monsanto, Inc (Shankar *et al.* 2008).

Not only is GM crops used to manage pest, such as insects and weeds, but it can contribute to genetic improvements in yields, environmental adaptation and specific quality features demanded by farmers and the public (Conner *et al.* 2003). Despite these benefits GM crops raise questions by researchers and farmers worldwide concerning environmental and consumer safety. Naturally, consumer safety received a lot of attention over the past decade, but environmental impacts and especially impacts on soil were not thoroughly studied. According to Conner *et al.* (2003), when considering the effects of GM crops in an environmental perspective, it is firstly important to determine whether GM crops are quantitatively and qualitatively different from non-GM crops. Environmental effects can be direct and indirect, delayed or immediate and extensive studies on environmental impacts need to be conducted to determine if there are any changes when planting these crops (Conner *et al.* 2003).

One of the greatest concerns involving environmental effects of GM crops is their effect on non-target organisms (Craig *et al.* 2008). Other environmental effects include gene flow, cross pollination and insemination, as well as resistance and the development of super-pests. Because these GM crops are planted in close proximity with natural and non-GM crops, it is unavoidable that they would come in contact with these plants at one stage or another (Craig *et al.* 2008). They are also planted in environments where they come in contact with non-target organisms and the respective soil environment (Craig *et al.* 2008). These non-target organisms include above-ground pollinators and parasitoids, above- and below-ground non-target herbivores, and predators, and below-ground beneficial microbes. One of these non-target effects are tri-trophic influences where the organism that is in immediate contact with the GM crop is not affected, but organisms higher in the food chain are influenced negatively (Craig *et al.* 2008). This is known as bioaccumulation and several studies have been done on

this phenomenon regarding a pesticide known as DDT. According to Craig *et al.* (2008), many studies have focused on non-target effects in food webs, but none of these studies provided promising results as to actual negative effects of GM crops. Overall the conclusions made from most of these studies pointed out that those non-target effects are mainly because of decreased quality of prey available for predators (Craig *et al.* 2008). In studies where herbicide resistant cultivars were used the conclusions made pointed to less diverse resource availability for primary consumers because of herbicide usage. These results are more herbicide than GM crop related and provide no hard evidence regarding non-target effects of GMO's (Craig *et al.* 2008).

Craig *et al.* (2008), also stated that soil organisms have the greatest and longest exposure to GM crops. Certain species live in the rhizosphere in close proximity to plant roots and are constantly exposed to root exudates. They are also exposed to plant litter and are responsible for the decomposition of this GM litter. Problems in this regard include potential exposure to toxin, *e.g. Bt* toxins, and changes in plant metabolites associated with GM crops. According to Craig *et al.* (2008), studies have shown that GM crops affects microbes, but these effects are not primarily negative and in some cases no change between GM and non-GM crops was observed concerning microbes. According to Kumar *et al.* (2008), fungi associated with *Bt* wheat produced less mycotoxins making it more acceptable for international trade. They also proposed that a decreased level of mycotoxins will make these crops safer for human and animal consumption.

Bt is a gram positive bacteria mainly found in soil that produces crystalline inclusion of proteins when forming spores (Das *et al*. 2009). These authors conducted a decomposition study on *Bt* and non-*Bt* plant material and concluded that *Bt* material took the longest to decompose. However, further studies are needed to determine the difference in litter quality, *e.g.* lignin content, between *Bt* and non-*Bt* plant material. They also determined that the *Bt* toxin is persistent in soil, but when they added these toxins to soil it did not influence decomposition rates of non-*Bt* litter. They also proposed that *Bt* plant litter can have a direct influence in soil fertility due to the slower

break-down of *Bt* material. According to Marutescu (2012), *Bt* toxins are specific and should therefore not affect non-target organisms. The *Bt* toxins of Cry1Ab, Cry1F, Cry1A.105 and Cry2Ab2 genes are specific to Lepidoptera and even though these toxins targets more than one species of Lepidoptera they are still order specific (Marutescu 2012). *Bt* toxins from the genes mCry3A, Cry3Bb1, Cry34Ab1 and Cry35Ab are specific to Coleoptera and more specific to only one species, namely *Diabrotica virgifera virgifera* (Maize rootworm)(Marutescu 2012).

As with all chemicals and toxins *Bt* toxin can accumulate in soils. The extent of this accumulation is dependent on a wide variety of factors. These factors include clay and mineral content, soil pH, broader soil type, nutrient levels and environmental factors such as temperature (Marutescu 2012). Other factors also include the type of GM crop, the type of GM toxin and agricultural practices (Marutescu 2012). According to Saxena & Stotzky (2003), the persistence of *Bt* toxins in the soil are dependent on clay content of soil and other substances such as humus. These particles adsorb toxins and make it more difficult to break down in soil environments. Clays can become saturated and only a certain fraction of the surrounding toxins can therefore be adsorbed. Sand and silt particles don't have the same adsorption abilities and are therefore not important in the persistence of *Bt* toxins. *Bt* proteins produces both toxins and protoxins, with protoxins regarded as those chemicals that were previously non-toxic, but that have changed in some way to become toxic (Saxena & Stotzky 2003). According to Saxena & Stotzky (2003), these toxins and protoxins can be adsorbed within a period of 30 minutes. This can be a problem since microbes have a very short time-frame to disintegrate these compounds when they are released into the soil as root exudates. Specialization in rapid decomposition of these substances is thus needed to ensure sufficient break down.

Saxena *et al.* (2010), conducted studies on earthworms and exposed them to *Bt* toxins for 40 days. They determined the mortality percentage and body weight of all the individuals. No significant differences in earthworm mortality or weight were found between *Bt* toxins and the control. They also conducted studies on nematodes,

protozoa, bacteria and fungi and found no significant differences in the quantity of these organisms exposed to either *Bt* and non-*Bt* wheat. This indicated that although there is a persistence of *Bt* toxins in the soil, they are not harmful to these few specific groups of non-target organisms. According to Marutescu (2012), Cry1Ab proteins have a lower persistence in soil than Cry2Bb1 because they decompose at a faster rate. Other studies have shown that the concentrations of *Bt* toxin of Cry1Ab are initially high for the first 6-9 weeks into decomposition, thereafter decreasing rapidly over the next few months. According to Marutescu (2012), studies done by various researchers indicated that the persistence of numerous *Bt* toxins in soil ranges from nine weeks up to 21 weeks. During this break down of the toxin it reaches a concentration that is not lethal to the target organism. Margarit *et al.* (2008) also recorded the Cry1Ab protein in fields cultivated with both *Bt* and non-*Bt* maize indicating that these proteins occur naturally in soil and that their presence in soil environments cannot be directly pinpointed to *Bt* crops. There was also no significant difference during 12 months in the concentrations between *Bt* and non-*Bt* fields.

At the moment sustainable agriculture forms the key element in both food production and conservation (Digrak & Özcele 1998) and thus studies concentrating on this issue are of extreme importance. Huesing & English (2004) stated that transgenic crops are used in over 18 countries across the world to control various agricultural pests. GM crops are said to be more environmentally friendly than synthetic pesticides (Phipps & Park 2002) and with this technology farmers can achieve maximum yields without the additional application of pesticides against major crop pests. According to Huang *et al.* (2003), the use of *Bt* cotton in China reduced the need of pesticides dramatically and by implementing such modified crops worldwide we can reduce the need for expensive and detrimental pesticides. Not only are GM crops more cost effective, but the genes used in the process are from naturally occurring organisms and should theoretically be more easily degradable than synthetic pesticides. Kumar *et al.* (2008) focused on GM crops as an environmentally friendly solution to pest management and crop improvement. They agreed that GM crops reduced the need of insecticides thus saving framers money on the pesticide itself, labour, fuel and water.

1.7 Ecological function of soil organisms

Soil organisms can be divided into 4 functional categories depending on the intensity of their presence in soil. These categories are: permanent, periodical, temporary and transient. Some organisms complete their entire life cycle in the soil and these organisms fall in the permanent soil fauna category and include mites, collembolans, and earthworms (Coleman *et al.* 2004). Transient species are organisms that only hibernate in soil, *e.g.*: Coccinellidae species that can spend the cold winter months in the soil (Coleman *et al.* 2004). These organisms do not directly take part in soil processes, but can indirectly contribute to energy flow when they are consumed by other organisms. Some soil fauna are only temporary members of the soil system and spend only a part of their life cycle in the soil environment (Coleman *et al.* 2004). For the duration of this period they feed and form part of the energy flow in soil ecosystems. An example of temporary soil fauna is Tipulidae larvae (Diptera) and Tenebrionidae larvae (Coleoptera). Periodic soil fauna spend most of their lives below-ground and are directly linked to soil food webs. These organisms complete their entire life cycle in the soil, but the adults move in and out of the soil. Two examples of periodic fauna are ants and termites that are dependent on the soil environment for survival, but adults move in and out of the soil to forage for food. Some organisms occur in soil accidentally or in enemy-free space and don't usually take part in soil processes. These can include certain insect larvae and adults that feed on above-ground plant parts.

An alternative method employed to provide functional classification of organisms is by analysing reproductive strategies (Briones 2014). The reproduction and development rates of soil fauna reflects how a species will respond towards environmental change. Root feeders that have to survive on a low quality food source tend to have extended life cycles. Reproductive strategies are dependent on environmental factors such as temperature, rainfall, resource availability and quality (Briones 2014). In anthropogenic environments such as agricultural systems, organisms with slow reproduction and long life cycles will be more seriously affected by change or disturbance. Organisms with fast reproduction rates and repeated

generation per season stand a better chance to adapt and survive due to these additional generations per year. According to Briones (2014), the use of reproduction strategies in functional classification is not very useful for soil fauna because very little is known about the biology of most soil arthropods. Another method used to characterise soil fauna into functional groups is according to body size (length) (Table 1). In this regard functional groups include micro-biota or microfauna, mesofauna and macrofauna.

Table 1: Characterization of soil fauna according to body width, in order to distinguish different functional groups (based on Briones 2014).

Functional group	Size	Examples
Micro-biota **Microfauna**	< 100µm	Bacteria, fungi, protozoa, nematodes
Mesofauna	> 100µm and < 2mm	Acari, Collembola, insects
Macrofauna	> 2mm	Spiders, Mollusca, insects, earthworms

When using body length of organisms to characterize soil fauna, the emphasis is on their microhabitats (Coleman *et al.* 2004). Microfauna are only able to survive in water films in the soil and are thus restricted to these environments (Coleman *et al.* 2004). Mesofauna are limited to air filled spaces and are dependent on macrofauna to provide these living spaces (Coleman *et al.* 2004). They are thus not able to alter their own living spaces in the soil. Macrofauna construct their own living spaces by means of burrowing and therefore fulfil a significant role in soil structure and displacement (Coleman *et al.* 2004). According to Briones (2014), the influence of soil fauna occurs

at a range of spatial scales because of this variability of body size. However, one disadvantage of this method is that not all organisms fall directly into these groups (Briones 2014). For example, Collembola have very small and very large individuals. The family Neelidae consists of only minute members that, according to size, should fall into the microfauna group and Entomobryidae have larger members that should be categorized into the macrofauna. However, the Collembola falls into mesofauna because most of its members fall into the size range of this functional group. Despite this problem, this method is still being used to date and has been used in the past to answer some ecological questions regarding soil organic matter and decomposition (Briones 2014). According to Thurnbull *et al.* (2014), body size analysis is a universally easy method to use when predicting soil function. Size can be measured in body length, body width and body mass. According to Thurnbull *et al.* (2014), body mass expresses the organism's metabolism and resource usage more confidently than other body size measuring methods.

Microflora, such as fungi and bacteria, contribute to soil processes and decomposition a great deal. Despite their small size they are able to digest all kinds of animal and plant material (Briones 2014). Micro-arthropods include spiders and other small predators that are periodic residents of the soil system (Coleman *et al.* 2004). Some spiders are active hunters, while others are 'sit-and-wait' predators. These 'sit-and-wait' predators usually have an underground retreat from where they catch prey. As with most predators they are opportunistic feeders and will eat almost anything they can overpower (Coleman *et al.* 2004). Collembola serves as an important food source, especially for juvenile spiders. It has been found that spiders feeding on Collembola can have a top-down effect on decomposition rates (Coleman *et al.* 2004). Studies showed that if spiders where removed from experimental areas, decomposition rates accelerated due to the increase of Collembola numbers (Coleman *et al.* 2004). Macro-arthropods also have a significant role to play in soil ecosystems. Being the largest of the soil arthropods many are predacious on meso-arthropods (Coleman *et al.* 2004) and this interaction forms the all-important link between meso- and macro-arthropods. Meso-/microfauna, such as Collembola and mites feeding on microfauna and –flora, in

turn create the link between micro- and mesofaunal groups. All in all a micro- meso-macrofaunal link in the soil food web is therefore formed (Coleman *et al.* 2004). The interactions between size orientated functional groups are, however, more complex than just described, but one can judge the interdependency of these groups on one another. Together they are responsible for energy flow below-ground and also link this whole system to above-ground food webs (Coleman *et al.* 2004).

Soil fertility is a term used to describe the ability of soil to meet the needs of plants, including demands for water, nutrients and physical matrix for optimal root development and other biological processes (Aweto 1981). According to this author, soil arthropods contribute to the soil food web with two main functions; firstly they serve as litter transformers where they fragment litter material, which passes through their digestive systems and are excreted in the form of smaller, more humid faecal pellets. These faecal pellets then serve as a high quality, moist food resource for microbial populations which can more easily be broken down. Secondly, soil arthropods serve as ecosystem engineers. As mentioned before ants and termites structurally modify the soil environment, making it more accessible for smaller organisms which are not able to create their own living spaces. They are also responsible for redistributing soil, organic matter and minerals (Jouquet *et al.* 2006).

Soil organisms also provide services that are essential for natural soil processes. They help with drainage, ventilation, stabilization of soil aggregates, bioturbation, as well as mixing and degradation of organic matter, thus improving soil quality and fertility (Lavelle *et al.* 1997, Barros *et al.* 2001, Hunter 2001, Lavelle *et al.* 2001, Emmerling *et al.* 2002). Soil fauna is also partly responsible for the stabilization of soil structure. They contribute towards the spatial distribution of soil particles and create pore spaces and voids in the soil (Wilkinson *et al.* 2009). It is also important when considering adequate root penetration, as well as the prevention of soil erosion and the largely investigated phenomenon of surface crusting (Culliney 2013). Soil organisms are major role players in the displacement of soil particles. This mixing of soil by organisms is known as bioturbation and is mainly conducted by macrofauna when tunnelling through the soil (Canfield & Farquhar 2009).

According to Culliney (2013), termites can work soil up to 50 m in depth and old nest material of termites also has higher infiltration rates than surrounding soil. Ants and termites form a network of tunnels and chambers that improves organic matter content (food storages and excrements). Millipedes also form burrows that aids in soil structure. Some mesofauna, such as oribatids, are strong enough to form channels in deeper soil layers and they leave behind faecal matter and mixes into lower soil layers (Maruan & Scheu 2000, Caruso *et al.* 2006). Symphylans also modify deeper soil layers by their rapid movements up and down the soil profile (Culliney 2013).

Another important component in soil structure is soil aggregates (Bronick & Lal 2005). This is where soil particles bind together and form clumps that will eventually result in a change in soil classification. A major component of soil classification is soil organic matter (Skjemstad *et al.* 1998 and Kögel-Knabner 2000), and the mixing and distribution of soil organic matter are largely accomplished by soil organisms (Kögel-Knabner *et al.* 2008). By shredding organic matter and redistributing nutrients through faeces and other activities these organisms contribute to soil aggregation (Brussaard 1997). Humus that is partly broken-down organic matter makes up the upper layer of the soil and serves as a reservoir for nutrients. This layer supports the largest quantity of soil biota and increases nutrient availability to vegetation. It also buffers the lower soil from desiccation and sudden pH fluctuations and weather changes, it chelates metals, bind to clay minerals to promote soil structure and increase the cation exchange ability of soil (Bollag & Loll 1983, Skjemstad *et al.* 1998, Sauer 1999).

As already mentioned, fertility is a result of the actions of soil biota. Fertility can be defined as the ability of soil to provide the physical and chemical foundation for optimal root penetration, as well as a favourable medium for plant growth and development. This includes adequate nutrient availability, sufficient respiration and limited erosion of soil (and by implication limited root exposure). Diversity of beneficial arthropods plays a major role in soil fertility, rendering both trophic and non-trophic influences.

1.8 Conclusion

Soil should not only been seen as a resource, but also as a habitat were various organisms live and contribute to ecosystem function. Soil faunal diversity in agriculture is dependent on various inputs such as organic matter and vegetation diversity. A more diversified agro-ecosystem, as opposed to a monoculture, can provide soil fauna with a larger diversity of resources, thereby resulting in a larger variety of nutrients placed back into the soil. Soil fertility is a natural by-product of the interactions of soil organisms with the surrounding vegetation. This vital interaction is therefore a principle of inefficient management that is causing soil functions and soil systems to collapse. Agricultural practises, including intercropping, crop rotation, shifting cultivation, cover crops and the addition of mulches and other organic matter, can improve the occurrence and survival of soil fauna.

Soil fauna play a significant role in the decomposition of plant litter and recycling nutrients back into the soil. They contribute to these processes not only by direct feeding, but also through their interactions with other soil fauna. They stimulate the growth of microfauna and -flora and regulate pathogenic populations of fungi and bacteria. Their faeces contain a wide variety of nutrients that can be higher in concentration than their actual food source. The nutrients in these faecal pellets leaches out into the soil and become an immediate nutrient source for plants. Soil fauna are also responsible for soil formation and soil characteristics through their activities and movement in soil. Ecosystem engineers, such as ants and termites, are responsible for displacement and spread of soil and nutrients. Soil fauna contribute to soil quality and fertility by breaking down and mixing (bioturbation) of organic matter in soil and aids in the formation of soil aggregates, as well as ventilation and drainage of soil systems.

Soil ecosystems include various trophic and functional groups that support essential soil processes. These trophic groups are in continuous interaction with one another and the soil around them. A decrease on one trophic level can have an effect on the levels above and below. These top-down and bottom-up effects ultimately

influences primary production. In the case of agriculture, crops are the primary producers that can be affected by these top-down and bottom-up effects. It is also important to classify soil organisms into functional groups to determine their ecological role in ecosystems. Various researchers in the past used methods, such as body size (length and width), reproductive strategies and the degree of presence in soil, to classify soil fauna into functional groups. Recent research on this matter suggests that body weight is the most efficient strategy to determine functional groups of soil fauna.

Potential toxic plants, whether natural (allelopathic) or anthropogenic (GMOs), cause a concern regarding biodiversity in agro-ecosystems. Thus far the influences of GM crops have not proven to have any negative impacts on soil fauna, however, previous research focused only on microbes and nematodes, omitting the influences on soil arthropods. Studies done on allelopathic and aromatic plants indicated that these plants influence soil organisms both positively and negatively, pointing out that more studies are required to determine the effects of these potentially toxic plants.

The key in preserving ecosystem functions provided by these organisms lies in our understanding of their biology and general behavioural activities. Even though soil arthropods are very small in size, their contribution to ecosystem services is large and meaningful. By conserving these organisms in agro-ecosystems, farmers can in essence be practicing sustainable conservation agriculture where soil organisms are responsible for soil fertility, health and quality. Thus by conserving biodiversity in agricultural fields, farmers can obtain a self-sustainable system with minimal outside inputs and expenses. It is also said that agro-ecosystems with a high biodiversity should be able to self-control pests and supplement nutrients necessary for optimal yields.

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CHAPTER 2

Genetically modified maize and its environmental impact on soil mesofaunal diversity

2.1. Introduction

Genetically modified organisms (GMO's) are the result of scientific advances in cell and molecular biology, where DNA from any source can be transferred to a particular plant or crop (Nap *et al.* 2003). This transfer of genes is said to improve the crops ability to resist pests, disease, herbicides and environmental stress (Nap *et al.* 2003). It also prolongs crop shelf life and presents the crop with improved colour, flavour and nutrient content. This technique allows plant breeders to present improved cultivars of plants that can keep up with the increasing consumer demand (Nap *et al.* 2003). Maize (*Zea mays*) is one of the most cultivated crops in South Africa, whilst Kruger *et al.* (2012) states that South Africa is ranked eighth in the world in the cultivation of GM crops. *Bt* maize expresses the synthetically modified Cry1Ab, Cry1F, Cry1A.105 or Cry2Ab2 protein that have been isolated from the soil bacterium *Bacillus thuringiensis* (*Bt)* (Obonyo *et al.* 2008). GM maize was initially used in South Africa to control lepidopteron pests, especially the stem borers *Busseola fusca* and *Chilo partellus* (Kruger *et al.* 2011).

According to Balog *et al.* (2010), the effects of the *Bt* toxin on non-target organisms have not been efficiently studied. However, many above-ground studies have showed no significant difference in survival and development of non-target organisms treated or exposed to the *Bt* toxin. Most of these studies focused on predators and parasitoids and the tri-trophic effects of the *Bt* toxin. Balog *et al.* (2010) found that there were no significant differences in activity and densities of Staphylinidae beetles exposed to *Bt*- and non-*Bt* crops. Similarly Peterson *et al.* (2011), found that spiders are not positively or negatively affected by *Bt* crops. They do however, report some differences in foliar spider populations in less studied *Bt* crops such as rice and potatoes. But they agreed that the number of observations made to obtain these data was not sufficient to make accurate conclusions.

Alfarez-Alfageme *et al.* (2009) found the *Bt* toxin present in predatory Carabidae adult and larvae tissue. These beetles don't feed on the crop plant and must have

obtained these toxins in their tissue from prey that fed on the *Bt* plants or material. They also found that the concentration of the *Bt* toxin decreased along the trophic chain indicating no accumulation. Other tests done on these beetles also show no negative effect of the *Bt* toxin on Carabidae survival, development time and growth, although the toxin is present in their tissue. Garcia *et al.* (2010) found that phytophagous mites (*Tetranychus urticae*) feeding on *Bt* maize can transfer the *Bt* toxin to their predators (rove beetle, *Atheta coriara*) without a decline in *Bt* toxin concentration. This indicated that the mites do not have the ability to process and break down the *Bt* toxin. Their study also showed that the toxin and their incapability to break down this toxin did not affect the mites negatively and further, 48 hours after digestion of a mite containing the *Bt* toxin, the toxin was no longer traceable in the rove beetle tissue. The rove beetle thus has the ability to digest these toxins and the toxin seems to have no negative effect on the beetle itself. According to Whitehouse *et al.* (2005), the only significant difference of diversity of arthropods between *Bt* and non-*Bt* cotton fields was the fewer Lepidoptera found in *Bt* fields. As Lepidoptera is the target organisms of the *Bt* toxin, it is to be expected that only this group will be influenced.

A study done by Marutescu (2012) proved that the *Bt* toxin binds to humus and clay in soils and can be present in soil long after the crop has been removed. Craig *et al.* (2008) mention that soil fauna are exposed to the *Bt* toxin for the longest period of time, since they live among the roots of the living plants and are responsible for plant litter decomposition. If any organisms should be affected by the *Bt* toxin it should be the soil fauna. Some studies on the effects of the *Bt* toxin on soil fauna have been done and mostly include microbes and earthworms. Tan *et al.* (2011) found no significant differences in mycorrhizal colonization between *Bt* and non-*Bt* treatments. According to Shu *et al.* (2011), *Bt* proteins have no effect on earthworm survival and growth. A study done by Emmerling *et al.* (2011) found that 75.80% of *Bt* maize litter disappeared from the soil surface during the first 2 weeks of a microcosm study using earthworms. They also observed that the Cry1Ab protein from *Bt* maize material decreased in the foregut and midgut of earthworms. They established that a decline in *Bt* protein concentration,

of up to 99%, occurred in the foregut of earthworms. This study therefore demonstrates that some soil fauna can digest and fragment *Bt* maize material.

Since the effect of *Bt* maize on soil arthropod diversity has not been studied efficiently it is important to address this issue. The aims of this study will be to determine differences in soil arthropod diversity between *Bt* and non-*Bt* maize fields to determine possible impacts of this toxin on soil communities and trophic groups. The focus will be on Collembola and Acari, since they are the most numerous arthropods found in soil, but notes about other soil organisms will also be made.

2.2. Material and methods

2.2.1. Soil sampling procedure

Soil samples were taken at the roots of the involved plant, in the porosphere, where the plant interacts directly with its environment (Figure 1). All samples were taken randomly at least 10m form the edge of the field to eliminate any edge effects. A small shovel was used to take ± 2 kg samples at a depth of ± 15 cm.

Figure 1: Soil samples were taken in the plant porosphere by using a small shovel (Sunflower field, Petrusburg, January 2013).

In situations such as the natural veld where the soil was too hard and compact a garden fork was initially used to loosen the soil. All soil samples were placed in brown paper bags and transported inside a cooler box to prevent overheating and desiccation.

2.2.2. Extraction and sorting methods

To extract soil mesofauna Tullgren extraction funnels (Figure 2) were used. This method consisted of a sieve or grid placed inside a funnel with a preservative liquid in a container at the bottom of the funnel snout. Soil was placed on top of the grid and a light source was mounted above the soil. The principal of this method was for the light source to heat and dry out the soil from the top downwards over time. Behavioural studies have shown that soil fauna tended to move downward in soil, to deeper more moist regions, to prevent drying out (André *et al*. 2002). When they move downwards they reach the grid and fall through and down the funnel, landing in the preservative.

Figure 2: Tullgren extraction funnels was used throughout the study to extract soil mesofauna.

The Tullgren funnels used in this research had a diameter of 28 cm and a depth of 11 cm. The light source used was 14 W 220-240 V and 110 ml of 70% ethanol was used as a preservative in a 250 ml plastic bottle. The mesh or sieve size was 3 mm x 3 mm and the light source was situated ± 7 cm above the soil surface.

Soil samples were taken from the cooler box and the brown paper bags were emptied on top of the grid. Large lumps of soil were broken into smaller pieces to ensure that the soil dried out evenly. The grids were sprayed with water beforehand to prevent large amounts of soil falling through. At the bottom where the preservative was to be placed, an empty bottle was initially placed to collect excessive soil falling through. The bottle was emptied on the grid before the 70% ethanol was poured in and the bottle was fastened at the bottom of the device. The soil samples were kept on the Tullgren funnels for 7 days, whereafter the preserved fauna was sorted and qualitatively and quantitatively analysed.

In the 2012 preliminary study on *Bt*- and non-*Bt* maize, the preserved material was poured through white filtration paper and then picked up with a needle and placed into micro-tubes. This method proved to be insufficient since the smaller colourless fauna was overlooked because of the fixed white background. The 2012 study thus only included fauna ranging from approximately 1 mm to 2 cm in body length. This method was improved in 2013 to include fauna ranging across the spectrum of mesofauna size, *i.e.* from 100 µm to 2 cm in body length. Preserved soil fauna was emptied directly into a small glass petri-dish and identified under the microscope. With this method the background could be changed to black or white to make all material visible, which was then sucked up by using a pipette and placed in micro-tubes. Trophic guilds were assigned to organisms using a wide variety of literature (Krantz & Walter 2009, Fjellberg 2007, Triplehorn & Johnson 2005, Fjellberg 1988)

2.2.3. Humidity and compaction analyses

Before soil samples were taken, a hygrometer (Lutron Electronic Enterprise Co., LTD, Model: PMS-714) was used to determine the humidity percentage of the soil

(Figure 3a). The hygrometer was inserted into the soil and left for a few seconds to obtain a stable humidity reading. A soil compaction meter (Dickey-John) was also used to determine compaction of soils before samples were taken (Figure 3b).

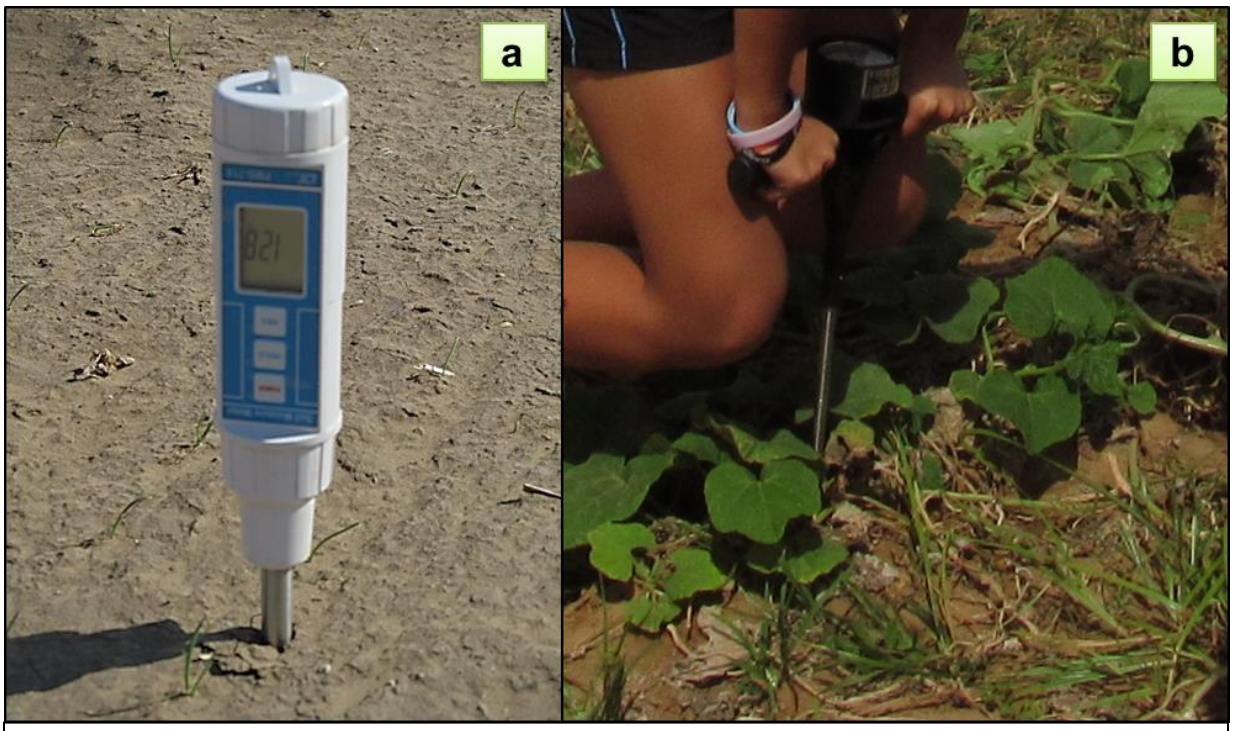

Figure 3: Apparatus used during fieldwork - (a) Soil hygrometer; (b) Dicky-John soil compaction meter.

Because samples were taken in agricultural soil, the ¾-inch tip was used for compaction readings throughout the studies to ensure that this data was uniformly attained and therefore comparable. The soil compaction meter measured the compaction in relation to the depth of the rod. Since samples were only taken at a depth of 15 cm, it was only necessary to measure compaction up to 15cm, but the soil compaction layer depth was also determined. The compaction meter measured compaction in pounds per square inch (psi). Values ranging between 0 and 199 psi represented an area where root development and growth was optimal, between 200-300 psi represented an area where root growth and development was fair and in areas with a reading above 300 psi root growth and development was poor. The compaction meter can also be used to determine the compaction layer of soil to give farmers an

indication of how deep down their soil preparations must be done. It gives two values *e.g.* 200 psi and 3", where 200 is the compaction measurement in psi and 3" determines the compaction layer's depth. The depth of the compaction layer is important because it can give an indication of the mobility of soil organisms within the soil profile.

2.3. Test Statistics

2.3.1. Shannon's Diversity and Evenness Index

According to Allen *et al.* (2009), the Shannon's index was developed and published by Claude Shannon in 1948. This index was based on the number of species, or species richness in order to determine diversity. The equation also took the local spread of species or evenness (E) into account that gives researchers a better understanding of how species are spaced within this diversity. According to Spellerberg & Fedor (2003), the Shannon's index of species diversity is the most commonly used statistical equation in determining diversity. The Shannon's Index of Diversity was used throughout this study to determine diversity and evenness of soil arthropods in agricultural and natural soils.

The equation is as follows:

$$H = \sum_{i=1}^{S} (\mathrm{Pi} \times \ln[\mathrm{Pi}])$$

Pi = proportion of individuals in the i-th category

S = number of categories

Throughout this study the statistical program PAST designed by Hammer *et al.* (2001), was used to calculate Shannon's diversity and evenness.

2.3.2. Sørensen Similarity Index

According to Diserud & Ødegaard (2007), the Sørensen similarity index uses species found in only the 'first' study site and species found only in the 'second' study site and compares that to species found in both study sites to give a value of similarity. Similarity indices have been used for many years to compare species at two study sites (*i.e.* beta or gamma diversity) in order to observe the difference in species composition. The equation is as follows:

$$C_s = \frac{2ab}{a+b}$$

a = species found at site A

b = species found at site B

ab = species found at sites A and B

The Sørensen similarity index gives a value between 0 and 1. The closer the value is to 1 the more similar the species composition of the two sites is. Throughout this study the statistical program PAST designed by Hammer *et al.* (2001), was used to calculate Sørensen's similarity.

2.4. Study layout

2.4.1. Study sites

This study was divided into two sections (see Table 1 and 2). The first section was the preliminary survey on *Bt*- and non-*Bt* maize that was conducted in 2012 (Table 1). The second section was a survey of three cultivars of *Bt* maize and one of non-*Bt* maize that was conducted in 2013 (Table 2). These studies had a control veld that consisted of two natural veld areas in 2012 and two natural veld areas and a 10 year old Smuts finger grass (*Digitaria eriantha*) field in 2013.

Table 1: A summary of the 2012 localities and sampling sites.

Sample	Town	Farm	Crop	Figure
BT	BFN, Bainsvlei	Geluk	Maize	Figure 4
NBT	BFN, Bainsvlei	Geluk	Maize	Figure 4
C	BFN, Bainsvlei	Geluk	Natural field	Figure 4
BT	BFN, Bloemdal	Karee Laagte	Maize	Figure 5
NBT	BFN, Bloemdal	Feather Stone	Maize	Figure 5
C	BFN, Bloemdal	Karee Laagte	Natural field	Figure 5

Table 2: A summary of the 2013 localities and sampling sites.

Sample	Town	Farm	Crop	Figure
RRBT	BFN, Bainsvlei	Geluk	Maize	Figure 6
BTa	BFN, Bainsvlei	Geluk	Maize	Figure 6
BTb	BFN, Bainsvlei	Geluk	Maize	Figure 6
NBT	BFN, Bainsvlei	Geluk	Maize	Figure 6
CS	BFN, Bainsvlei	Geluk	Smuts finger and natural field	Figure 6
CN	BFN, Bainsvlei	Geluk	Natural field	Figure 6
CL	BFN, Bainsvlei	Maranatha	Natural field	Figure 7

2.4.1.1 The 2012 study

The two study sites that were used for the 2012 study were 23 km apart from one another by the shortest possible route. The first study site was on the farm Geluk, in the Bainsvlei area (Figure 4) outside of Bloemfontein (29°14'04.38"S, 26°07'52.24"E). The two maize fields (*Bt*- and non-*Bt*) were situated adjacent to each other and were roughly

similar in size. Next to the non-*Bt* field was a natural veld which was used as a control. The natural veld consisted mostly of the following grass species: Red Grass (*T. triandra*), Narrow-leaved Turpentine Grass (*C. plurinodis*), Spear Grass (*H. contortus*), Dropseed grass (*S. fimbriatus*), and Common Finger Grass (*D. eriantha*) (Van Oudtshoorn 2012).

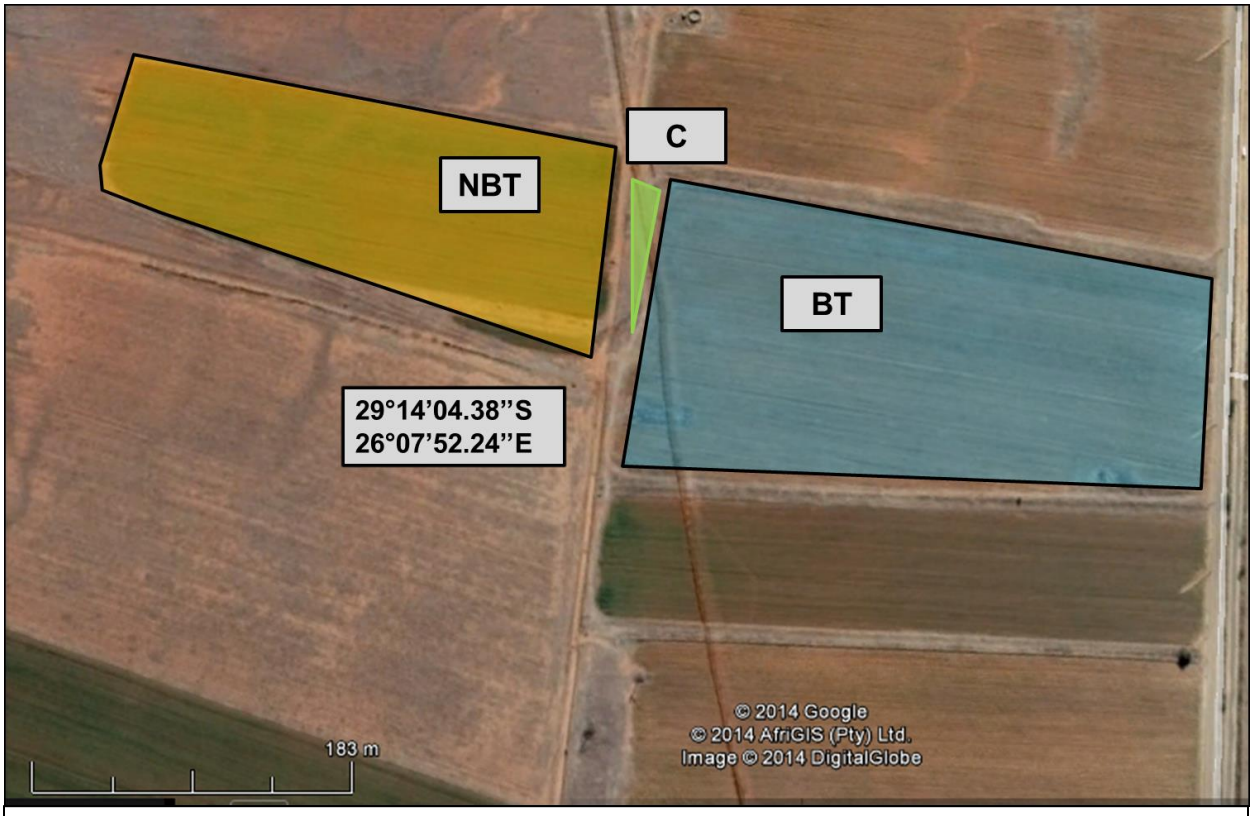

Figure 4: Bainsvlei study site (2012) on the farm Geluk showing adjacent *Bt* (BT) and non-*Bt* (NBT) maize fields, separated by a natural control veld (C). (Image adapted from Google Earth).

The second study site was on two adjacent farms (Feather Stone and Karee Laagte) in the Bloemdal area (Figure 5) outside of Bloemfontein (29°14'04.38"S, 26°07'52.24"E). Here the two fields differed in size, where the *Bt* maize field was bigger than the non-*Bt* maize field. Both maize fields border on the natural veld, which was used as the control veld. The natural veld consisted mostly of the following grasses: Red Grass (*Themeda triandra*), Narrow-leaved Turpentine Grass (*Cymbopogon*

plurinodis), Spear Grass (*Heteropogon contortus*) and Dropseed grass (*Sporobolus fimbriatus*) (Van Oudtshoorn 2012).

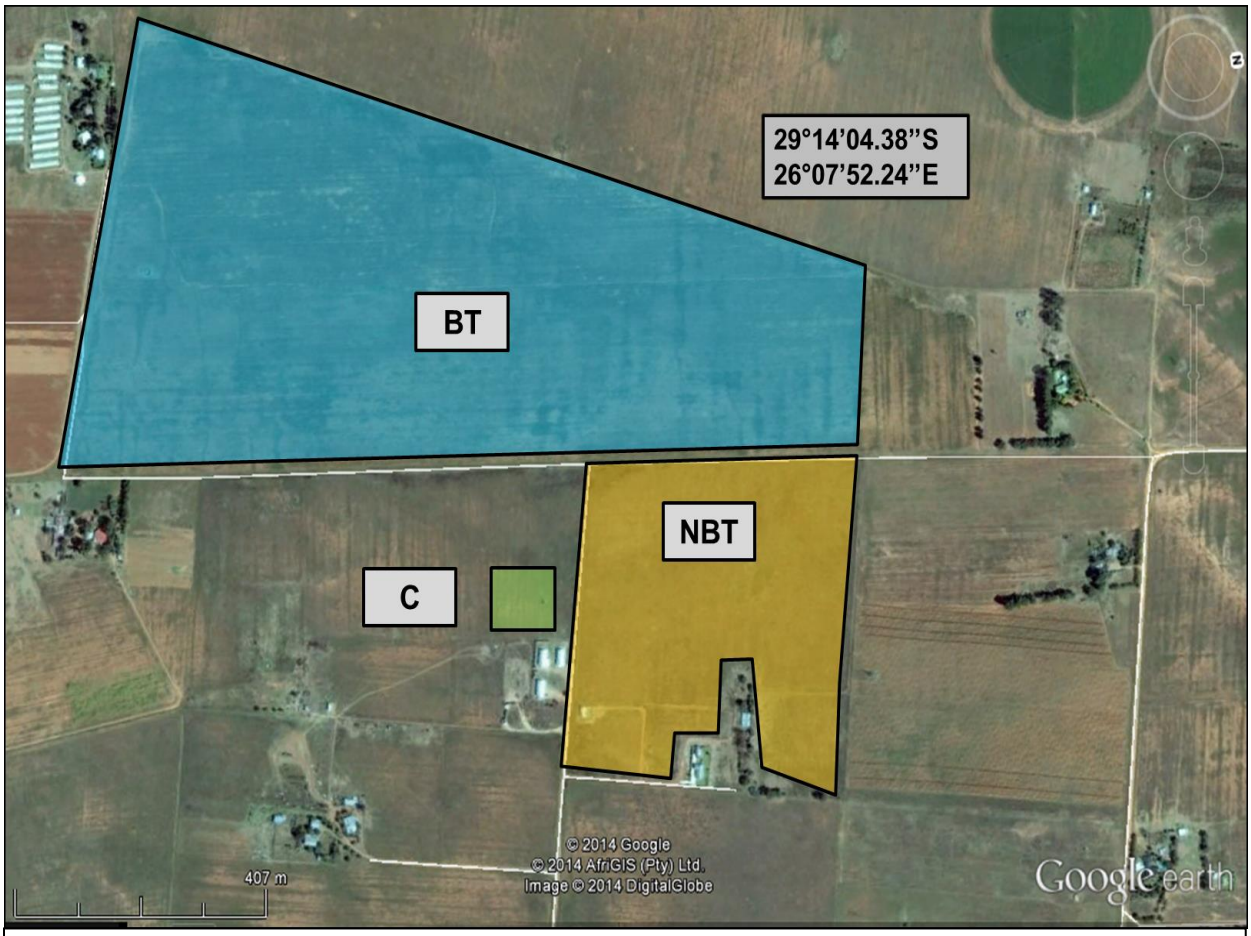

Figure 5: Bloemdal study site (2012), on the farms Feather Stone and Karee Laagte showing adjacent *Bt*- (BT) and non-*Bt* (NBT) maize fields and a natural control veld (C). (Image from adapted Google Earth).

The cultivars for the 2012 study were not available for all the fields. Pioneer cultivars, of which the specifics are unknown, were planted in all the maize fields. Omnia 3:1:0 fertilizer was used early in January 2012, for all of the cultivated fields and all of the fields were planted between 10 and 20 December 2011. The only field that had additional chemical application, was the Bloemdal *Bt* maize field, that was treated with herbicides late in March 2012.

2.4.1.2 The 2013 study

The two study sites where the 2013 study was conducted were 3.8 km apart from one another. The largest part of the study was conducted on the farm Geluk in the Bainsvlei area (Figure 6) outside of Bloemfontein (28°59'30.32"S, 26°05'47.58"E) and the last control sample was taken on the farm Maranatha (Figure 7).

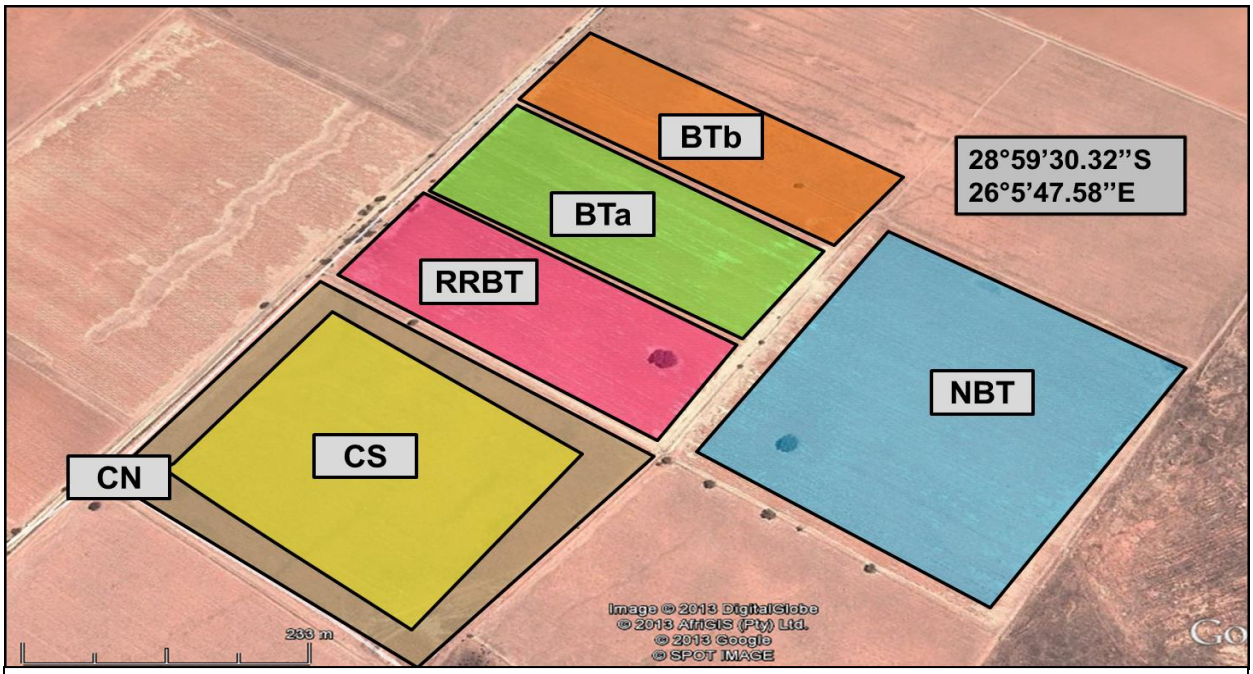

Figure 6: Bainsvlei study site (2013) on the farm Geluk showing *Bt* (BTa), *Bt* (BTb), *Bt* and herbicide resistant maize (RRBT) and non-*Bt* (NBT) fields. Natural veld served as first control (CN) and surrounded a 10 year old Smuts finger grass field (CS) that served as second control (Image adapted from Google Earth).

All of the maize fields were planted from 12 to 14 December 2012 and Omnia 3.1.0. fertilizer was added to the soil on 20 January 2013 (more or less a week later). The following maize cultivars was planted: 1) Non-*Bt* maize (Pioneer-Phb3442) - NBT (Figure 6); 2 and 3) *Bt* maize (Pioneer – Phb33H52B and Pioneer - Phb32W72B) - BTa and BTb respectively (Figure 6); 4) *Bt* and herbicide resistant maize (Pioneer – Pbh31D46BR) - RRBT (Figure 6). The control samples were taken in a natural veld (NC in Figure 6 and CL in Figure 7) and a 10 year old Smuts finger grass (*D. eriantha*) field (SC in Figure 6). The natural veld (NC) mainly consisted of the following grass

species: Red Grass (*T. triandra*), Narrow-leaved Turpentine Grass (*C. plurinodis*), Spear Grass (*H. contortus*) and Dropseed grass (*S. fimbriatus*) (Van Oudtshoorn 2012). The Smuts finger grass field (SC) only contained Smuts finger grass (*D. eriantha*) with Red Grass (*T. triandra*) and Spear grass (*H. contortus*) occurring meagrely spread throughout the veld.

Figure 7: Second Bainsvlei study site (2013) outside of Bloemfontein showing a small patch of natural field (CL) that served as third control field (Image adapted from Google Earth).

The second study site was on the farm Maranatha in the Bainsvlei area (Figure 7) outside of Bloemfontein (29°01'36.31"S, 26°05'04.32"E). Only the last control site was at this study site and this was a small patch of natural grass situated between animal forage crops. The control site mostly contained the following grasses: Red Grass (*T. triandra*), Narrow-leaved Turpentine Grass (*C. plurinodis*), Spear Grass (*H. contortus*) and Dropseed grass (*S. fimbriatus*) (Van Oudtshoorn 2012).

2.4.2. Methodology

2.4.2.1. The 2012 survey

Soil sampling was conducted monthly (from February 2012 to June 2012) at the two study sites (Figure 4 & 5) outside of Bloemfontein. Soil samples were taken randomly throughout the fields. Temperature and rain data was also recorded for all the sampling dates (Table 3) and included minimum and maximum temperatures, as well as the occurrence of rain the week before sampling and the day of sampling. Even though the soil forms a buffer for external climatic changes, the top layer of the soil was still exposed to the above-ground conditions.

Table 3: Sampling dates and climatic conditions during soil sampling from February 2012 to June 2012 in the Bloemfontein area.

Sampling Date	**15 Feb 2012**	**14 Mar 2012**	**18 Apr 2012**	**16 May 2012**	**13 Jun 2012**
Temperature (max / min)	26°C / 18°C	33°C / 15°C	25°C / 5°C	21°C / 3°C	10°C / 0°C
Rain on sampling date	YES	NO	NO	NO	YES
Rain prior to sampling date	YES	YES	NO	NO	NO

Since soil samples were taken at a depth of ± 15 cm the above-ground conditions could still have an effect on soil fauna activities and this data were therefore included for accuracy. During all five sampling dates, 30 soil samples from each field site were taken, thus totalling 90 soil samples per study site (30 in the *Bt* maize field, 30 in the non-*Bt* maize field and 30 in the natural veld control). Once the particular 30 soil samples were taken they were combined and mixed in containers and 4 sub-samples were taken to represent each field condition. The end result was 24 soil samples in total (4 for each field) per sampling date. Because the control veld in the Bainsvlei area was very small, a combined sample between the Bainsvlei and Bloemdal area was used to represent the control. Once the soil samples were collected they were placed separately on Tullgren funnels for seven days and organisms were filtered, sorted and

identified. The data were analysed by using the Shannon's diversity index and the Sørensen similarity index. The four samples of each field site, on each date were combined, prior to the statistical analysis to give a single representative value for each date at each field. No soil humidity or compaction data is available for 2012.

2.4.2.2. The 2013 survey

Soil collections were conducted monthly from February 2013 to June 2013 (Table 4) at the two study sites outside of Bloemfontein. Environmental parameters similar to 2012 as shown in section 2.2.3 were recorded (Table 4). Soil samples were taken randomly throughout the field. During all five samplings, three soil samples from each field were taken. Once the soil samples were collected they were placed separately on Tullgren funnels for seven days. The preserved organisms was sorted and identified directly from the ethanol. Data analysis was similar to that of 2012 as shown in section 2.3.1 and 2.3.2. Additionally soil humidity and compaction analyses were done during each of the sampling dates.

Table 4: Sampling dates and climatic conditions during soil sampling from February 2013 to June 2013 in the Bloemfontein area.

Sampling Date	**12 Feb 2013**	**20 Mar 2013**	**17 Apr 2013**	**15 May 2013**	**12 Jun 2013**
Temperature (max / min)	27°C / 16°C	26°C / °18C	24°C / 19°C	23°C / 17°C	21°C / 10°C
Rain on sampling date	NO	NO	NO	NO	NO
Rain prior to sampling date	YES	YES	NO	NO	NO

2.5. Results and discussion

2.5.1. The 2012 study

Diversity can be measured on a variety of different scales and levels of biological organization (Spellerberg & Fedor 2003). The Shannon's index of diversity (H') and evenness (E) for the *Bt* maize fields (BVBT and BDBT), non-*Bt* maize fields (BVNBT and BDNBT) and the control veld (C) can be seen in Figure 8. According to Wenninger & Inouye (2008), plant productivity can also play a role in the diversity and abundance of organisms. Plant productivity is dependent on many factors and one of these is plant condition in general. Because maize plants are seasonal crops they deteriorate towards the end of the season and this decreases plant productivity. In Figure 9 the deterioration of maize plants from February 2013 to June 2013 can be seen, which is also similar in 2012.

A Sørensen similarity test was done to determine the difference or similarity in species composition between the five sites (Table 5). The closer the value was to one, the more similar the two sites were. This played a major role when comparing different treatments, as in this case with *Bt*- and non-*Bt* maize plants. It could be an important factor in giving an indication whether species are affected negatively or positively by different treatments. Fields that showed high similarity might have had some features in common, such as land use history or plant characteristics.

Table 5: The Sørensen similarity index for soil mesofauna at the Bainsvlei and Bloemdal study sites for all five different fields (2012). BVBT- Bainsvlei *Bt* maize, BVNBT- Bainsvlei non-*Bt* maize, BDBT- Bloemdal *Bt* maize, BDNBT- Bloemdal non-*Bt* maize, C- Control.

	BVBT	**BVNBT**	**BDBT**	**BDNBT**	**C**
BVBT		0.5	0.5	0.54	0.39
BVNBT	0.5		0.52	0.47	0.45
BDBT	0.5	0.52		0.51	0.45
BDNBT	0.54	0.47	0.51		0.39
C	0.39	0.45	0.45	0.39	

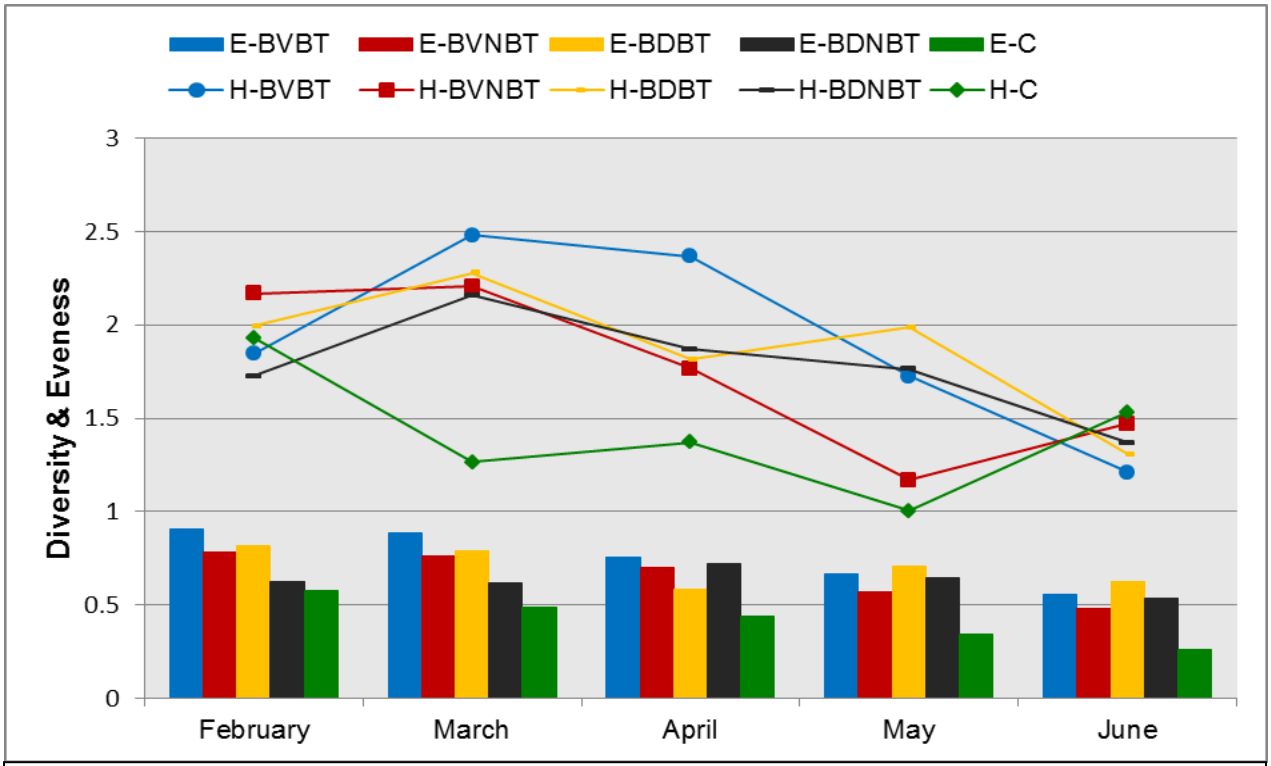

Figure 8: The Shannon's index for diversity and evenness of soil mesofuana in maize fields and the natural veld in the Bainsvlei and Bloemdal areas outside Bloemfontein over a period of five months in 2012. The evenness (E) is represented by the bars and the diversity (H') is represented by the graph lines. BVBT- Bainsvlei *Bt* maize, BVNBT- Bainsvlei non-*Bt* maize, BDBT- Bloemdal *Bt* maize, BDNBT- Bloemdal non-*Bt* maize, C- Control.

Figure 9: Deterioration (left to right) of maize plants from February 2013 to June 2013 at the Bainsvlei study sites

For the largest part of the study, the H' values were higher in the *Bt* maize fields than in the non-*Bt* maize fields (Figure 8). This could possibly be explained on the basis of better crop health, higher nutrition levels and competitive exclusion which were associated with *Bt* maize. The ability of *Bt* maize to show resistance to certain insect pests enable the plants to withstand damage, thus promoting overall crop health. Healthier crops have a larger root mass (porosphere), encouraging higher soil mesofauna diversity as far as both richness and abundance are concerned. The *Bt* toxin is present in plant tissues and specifically targets Lepidoptera, which is the key pest of maize crops. Overall soil mesofauna in both the cultivated fields was more diverse compared to that of the natural veld (Figure 8). Although the plant diversity was higher in the natural veld, the soil was less favourable than in the cultivated fields due to higher compaction and lower soil humidity. A sudden plunge in diversity in the *Bt* maize field in the Bloemdal area (BDBT, Figure 8) was observed in April 2012 and this might have been caused by herbicide application in late March 2012. According to Perucci *et al.* (2000), the use of herbicides could be harmful to soil fauna and this in turn could have a negative effect on general soil health. Studies done by Perucci *et al.* (2000), indicated that herbicides had a direct toxic effect on microbial activities and it caused the C: N ratio in the soil to change. This could probably explain the sudden decline in soil mesofauna diversity after herbicide application in the *Bt* maize field.

The diversity of soil mesofauna in the natural control veld (C, Figure 8) was the highest in February 2012 (H' = 1.932) and June 2012 (H' = 1.532). This is the only days that it had rained on the day of sampling (Table 2) and soil fauna responded positively to higher soil humidity. Briones *et al.* (1997) studied general trends in soil fauna responses to different climatic conditions in the UK. In their research they found that the number of individuals recorded increased with rainfall. Although their research focused mainly on Diptera, Enchytraeidae and Tardigrada, the concept could also be applied to other soil fauna.

The soil of the natural control veld was usually very dry, compact and difficult to penetrate for sampling purposes. Dowdy (1994) completed a study that indicated the

migration of soil fauna to deeper parts in unfavourable climatic conditions. Dowdy (1994) reported that soil fauna seemed to have had a daily migration pattern, where they occurred closer to the surface during the night and tended to migrate deeper into the soil during sunny (hot) days to avoid desiccation. This tendency for soil organisms to migrate up and down in soil relative to circumstances, might explain the low diversity of fauna recorded in the natural control veld during the dryer periods between February 2012 and June 2012. The higher diversity of fauna in February 2012 and June 2012 might have been attributed to rain on both of these days. According to Gonzalez & Seastedt (2001), soil faunal activities were directly related to soil humidity and more moist soil would have had an increased faunal activity as a result. Soil organisms were also directly and indirectly affected by cultivation practices (Beghum *et al.* 2013). The diversity in the cultivated fields (Figure 8) for February 2012 was lower than in March 2012. This might have been due to the extent of the agricultural practices prior to the February 2012 sampling. From March 2012, the diversity declined gradually, due to the change in temperature and the condition of the maize plants towards June 2012 (Figure 9). Kuryakov & Cheng (2001) found that a decrease in photosynthesis results in a decrease in CO_2 efflux from soil. Kuryakov & Cheng (2001) found that respiration is strongly connected to photosynthesis. As the maize plants deteriorated and died off photosynthesis declined and thus the CO_2 efflux. Trumbore (2000) found that annual leaf litter imputs band metabolic respiration of live roots contributes towards CO_2 in soils. Thus both live and decaying plants tissue forms an important source for soil CO_2. When maize plants died off towards the end of the season, respiration and CO_2 from live plants were lost form the maize field, but with the increase in SOM, other reactions such as decomposition were triggered. Soil fauna could have been influenced by the decrease in soil respiration, even though more SOM was available. The spike in diversity for all the fields in June 2012 could have been attributed to the rain (Table 3).

Data sets initially indicated high E values for BVBT (0.9051) and BVNBT (0.7786) fields in February 2012 (Figure 8). This might be an indication that the agricultural practices used on the Geluk farm site in the Bainsvlei area favoured soil faunal activities more than the practices used at the other two sampling sites, or rain could have been

the determining factor. A gradual decline in E values was observed in the latter two fields towards June 2012 as the season progressed (Figure 8). The E values for the BDBT field was also initially high (0.8178), but dropped suddenly in April 2012 to a value of 0.5817. The decline in E values may be attributed to the increased dominace in certain mite species. This phenomenon might have been due to herbicide applications late in March 2012.

A relatively fast recovery of evenness was observed towards May 2012, with an E value of 0.7102, but the evenness declined again towards June 2012 (Figure 8) due to deterioration of plant condition (Figure 9) and possible changes in soil respiration and CO_2 levels in soil. The evenness of the BDNBT field (Figure 8) started off in February 2012 with a low value (0.5373) and increased towards April 2012 (0.7119). This might have been due to the effect of agricultural practices in the beginning of the season, with soil fauna populations possibly recovering from some kind of disturbance. The E values of all the cultivated fields (BVBT, BVNBT, BDBT and BDNBT) were even (>0.5) throughout the study, except for the June 2012 value of BVNBT of 0.4846. The control veld's evenness showed a low value in February 2012 (0.5751) that declined throughout the season reaching 0.2611 in June 2012 and this could once again be ascribed to the effects of soil humidity and compaction on soil faunal populations, favouring some species more than others.

As the season progressed, temperatures dropped and the maize plants deteriorated (Figure 9). When comparing the deterioration of maize plants with the H' and E values of the Bainsvlei area (Figure 8), one can see that the soil fauna was most likely affected by changes in soil respiration and CO_2 fluxes, as well as descending temperatures. The BVBT field differed the most from the control (C) veld with a value of 0.39. Thus only 39% of the organisms found between these fields were similar (Table 3). There were no substantial similarities between the four cultivated fields (BVBT, BVNBT, BDBT and BDNBT). Furthermore the BDNBT field differed considerably from the C field with a value of 0.39. Because of the different field histories, soil types and

humidity levels in the respective soils, it was understandable that there would have been a difference in soil faunal composition between the five fields.

The food web or trophic structure in the soil was important in terms of soil system functions. Detritivores mostly made up the biggest part of these trophic structures, but phytophagous, omnivores and predatory insects were also included (Hunter 2001). By dividing soil organisms into trophic groups their function and importance in soil processes could be documented. One of the most important functions of soil fauna is the breaking down or decomposition of organic matter (Culliney 2013). Soil arthropods are mainly responsible for shredding of plant litter and dispensation of nutrients through their faeces (Culliney 2013). According to a study done by Adeduntan & Adeniyi (2009), mites and Collembola made up the largest proportion of soil fauna. Collembola were the most commonly associated with microbes, but some species were also predatory on nematodes. Mites have a wide range of trophic levels and could be phytophages, mycophages, bacteriovores, predators and parasites. Together with the Collembola they made up the largest part of the below-ground mesofaunal trophic structure (Addendum 1). The data on the soil mesofauna found at the five sampling sites were lumped for the five sampling dates to represent the trophic groups of the organisms recorded in each field setting (Figure 10). Mycophages and bacteriovores were the most abundant for all five sampling sites sampled (Figure 10). Their presence or absence in *Bt* maize fields indicated their level of activity in the decomposition of *Bt* plant litter. Mycophages and bacteriovores were less abundant in both the *Bt* maize fields (BVBT and BDBT). Organisms mainly consists of the Collembola *Brachystomella* sp. 1 and *Folsomides* sp.1, as well as Oribatida mites (*Oppiella* sp. 1). These dominant species were one of the reasons why the diversity of both non-*Bt* maize fields were slightly lower than the *Bt* maize fields (Figure 8).

Collembola in general play an important role in the soil trophic structure and areone of the most abundant soil detritivores (Coleman *et al.* 2004). In a larger context of matters, they are, together with other soil invertebrates, responsible for nutrient cycling in soils, thereby increasing soil fertility levels. According to Rusek (1998), they

also serve as disseminators of soil micro-biota and transport fungal spores in decaying organic matter through the soil. They also serve as an important food source for many predators including nematodes, mites, other collembolans, ants, as well as macrofauna such as carabid beetles and their larvae

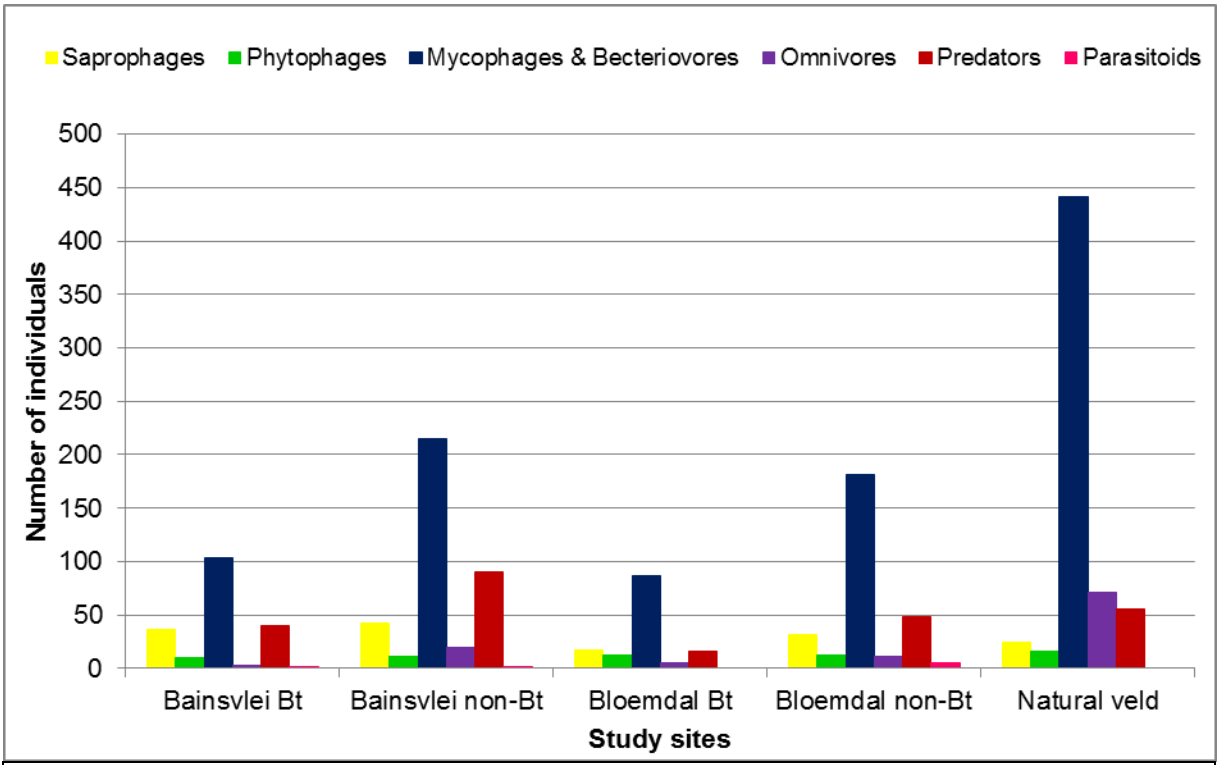

Figure 10: Trophic structure of five sampling sites from February 2012 to June 2012 (BVNBT - Non-*Bt* field in Bainsvlei, BVBT - *Bt* field in Bainsvlei, BDNBT - Non-*Bt*-field in Bloemdal, BDBT - *Bt* field in Bloemdal and C - Control for both study sites).

For an effective manner in which to link Collembola biodiversity to ecosystem functions it was necessary to relate and interpret them in terms of different soil types, stages of ecosystem succession, human activities and other soil system stresses. According to Tan *et al.* (2011), no difference in mycorrhizal colonization between *Bt* and non-*Bt* treatments was observed. Even though their study proved that the *Bt* toxin did not influence mycorrhizal colonization, they have not investigated other fungi and bacteria involved in decomposition of plant litter that might be influenced by *Bt* toxin. The *Bt* maize field at Bloemdal had the lowest number of mycophages and

bacteriovores (Addendum 1, Figure 10) and according to Rusek (1998), the usage of pesticides, mineral fertilisers and intensive farming could result in low Collembola densities. The low number of Collembola might therefore be as a result of herbicide application late in March 2012. The other trophic levels in this field were also low and this might have been due to a trophic cascade as a result of herbicide usage. The lower levels of mycophages and bacteriovores were thus not able to support a substantial number of predators.

Soil phytophages were mainly made up of Cicadellidae, Thysanoptera and Coleoptera larvae (Addendum 1, Figure 10). Plant hoppers and thrips were only temporary members of the soil food web and pupate in the soil. Even though they did not actively feed on plant tissue, they served as a food source for larger predators in soil thus contributing to energy flow in the larger food web. Coleoptera larvae could feed on plant roots and when occurring in large numbers they could become primary pests. Mycophagous arthropods were represented by Liposcelidae bark lice (Psocoptera), Oribatida mites (*Oppiella* sp. 1) and the two Collembola species (*Brachystomella* sp. 1 and *Folsomides* sp.1, Figure 10, Addendum 1). These organisms played an important role in the larger trophic structure, feeding on fungi and returning these nutrients to the soil. Omnivores were primarily represented by Formicidae and occurred in the highest numbers in the control location (C, Figure 10, Addendum 1). According to Lavelle *et al.* (2006), ants played an important role in physical engineering of the soil structure. Ants are primarily responsible for altering soil structure by creating voids and pores and forming soil aggregates, thereby improving the hydraulic properties of soil. They play an important role in ecosystem functioning not only by regulating soil structure and feeding on various organisms, but by serving as a food source for predators as well. According to Botes *et al.* (2006) ants are sensitive to plant structure and the presence of bare soil. Tis could also explain the high numbers of Formicidae in the natural field. Agricultural practices such as fertilisation and tillage were found to reduce colony densities of ants (Folgariat 1998). This might be the reason for the low numbers of ants found in all four the cultivated fields (BVBT, BVNBT, BDBT and BDNBT, Figure 10). Despite this reduction in population size it seemed as if ants re-invaded the same areas

after disturbance. Ants were usually one of the first (pioneer) species that were observed after disturbance and populations seemed to recover rapidly in comparison to that of other organisms (Folgariat 1998).

Predators form part of the upper levels of the trophic structure and are usually less numerous than lower trophic groups. Predators found during this study were mostly represented by Mesostigmatida mites, and Carabidae and Staphylinidae beetles (Addendum 1, Figure 10). The lowest number of predators was found in the *Bt* maize field in the Bloemdal location (BDBT) and this could possibly be attributed to the herbicides sprayed in this area at the end of March 2012. When there was a cascading trophic affect in an ecosystem the lower trophic levels would have recovered first and only then higher trophic levels such as predators could recover. Predators in general and therefore also those in soils were generalist feeders (polyphages that often opportunistically prey on a wide range of organisms (Kajak 1995)). Predator populations were mainly supported by lower trophic levels such as saprophages, phytophages, mycophages and omnivores, and these organisms determine the size of predator populations (Kajak 1995). This is known as the bottom-up effect where lower trophic levels have an effect on the higher trophic levels (Power 1992).

At the control location (C, Figure 10), it was observed that some lower trophic groups (phytophages and omnivores) were the most abundant compared with that of the cultivated fields, but the predators were not so numerous. A reason for this may be the high numbers of omnivorous organisms that were recorded. According to Schoener (1983), omnivores might act as predators in an ecosystem in the presence of enough resources. Interspecific competition between these two groups may have led to the lower numbers of predators. Small numbers of parasitoids were also present in the soil samples taken and this trophic group was only represented by Braconidae and Platygastridae wasps. These are flying insects and don't usually occur in the soil, but some species pupate in soil environments. Pupa could have been collected during soil samples and adults possibly emerged during the Tullgren extraction process. Although they did not usually parasitise soil organisms, they could still have served as a food

source for soil organisms. These insects thus only spend a part of their life cycle in the soil and were a temporary member of the soil trophic structure.

2.5.2. The 2013 study

The Shannon's index of diversity (H') and evenness (E) for the *Bt* maize fields (BTa and BTb), non-*Bt* maize field (NBT), the insect and herbicide resistant maize (RRBT) and the control (C) is presented in Figure 11. These datasets includes smaller soil arthropods that were not included in the 2012 study (Addendum 1). According to Battigelli (2011), soil mesofauna responds rapidly to any changes in their soil environment. They can serve as bio-indicators of soil quality and health and because of their rapid reaction to soil disturbances can be a faster indicator of 'unhealthy' soil than physical or chemical tests. As mentioned earlier, soil humidity and compaction influence the activities and mobility of soil fauna. According to Coleman *et al.* (2004), many soil organisms are dependent on soil moisture for survival. Furthermore, some species are limited to areas with high humidity and cannot survive in dry environments at all. It's thus important to include data on compaction and humidity as influencing factors when examining soil faunal diversity. The average soil humidity for all the fields over the five month sampling period can be seen in Table 6. Soil compaction controls soil fauna mobility and is depended on soil moisture as well as soil type. In Table 7 the average soil compaction and the depth of the soil compaction layer for all the cultivated fields and the control is provided. The depth of the compaction layer needs to be included in agricultural studies because extensive land use results in a compaction layer that may influence root development. Root growth and development in turn influence soil mesofauna, since many soil organisms are depended on CO_2 leaching from plant roots, as well as root respiration.

When comparing different treatments (in this case different cultivars of maize), it is important to include a similarity test to compare the species found in each treatment to each other. Some cultivars may be favoured by certain species of soil fauna and this

may prove to be helpful in determining the effects of different plant cultivars on soil faunal species assemblages. A Sørensen similarity test was done to determine the degree of similarity in species composition between the five sites (Table 8).

The H' values of February 2013 for the RRBT (1.631), BTa (1.695) and C fields (1.784) were very close to each other (Figure 11). In the case of the RRBT and BTa fields a low number of species (10 and 11 respectively) and a relatively high abundance of mite individuals could explain these results. In the C veld, 27 species were recorded, but the number of Formicidae and *Protogamasellus* sp. mites was very high, thus causing a *pro rata* lower diversity (Addendum 1). This was also the reason for the low evenness (E) in the control veld for February 2013 (Figure 11). The BTb and NBT maize field had the highest diversity (H') of 2.335 and 2.330 respectively, when considering only the cultivated fields (Figure 11). The BTb field contained 19 species of soil arthropods and the NBT field 20 species. However, both these fields showed a low abundance.

Table 6: The average soil humidity of each field (NBT - non-*Bt* maize, RRBT - *Bt* maize that's insect resistant, BTa - *Bt* maize, BTb - *Bt* maize, C - Control) over a period of five months in 2013.

	Feb	Mar	Apr	May	Jun
NBT	6.40	8.07	0.83	0.83	0.83
RRBT	4.57	7.23	7.60	4.40	2.40
BTa	7.90	4.13	7.87	5.50	5.40
BTb	5.53	2.43	4.87	5.20	2.70
C	2.97	9.57	0.60	0.57	0.20

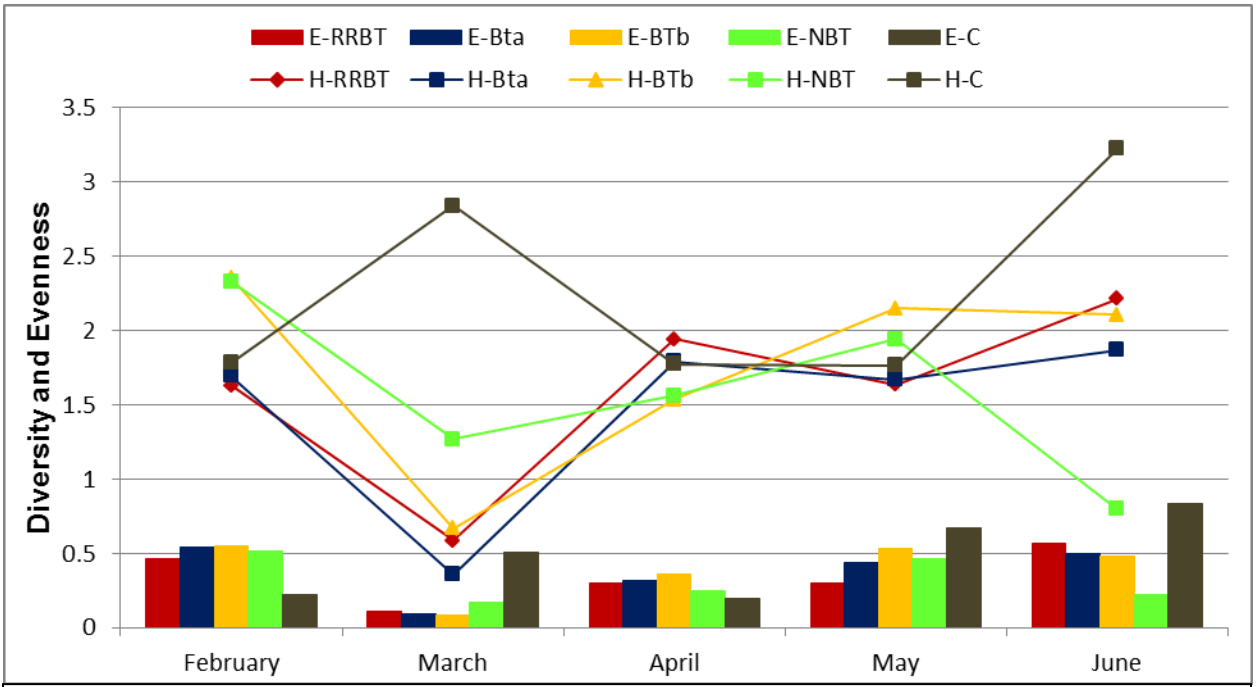

Figure 11: The Shannon's index for diversity and evenness of soil mesofauna in maize fields and the natural veld in Bainsvlei outside of Bloemfontein over five months in 2013. The evenness (E) is represented by the bars and the diversity (H) is represented by graph lines. RRBT - *Bt* insect resistant maize, BTa - *Bt* maize, BTb - *Bt* maize, NBT - non-*Bt* maize, C - Control.

Table 7: The average soil compaction in pounds per square meter and the depth of the compaction layer in inches of each field (NBT - non-Bt maize, RRBT- Bt insect resistant maize BTa - Bt maize, BTb - Bt maize, C - Control) over a period of five months in 2013.

	Feb	Mar	Apr	May	Jun
NBT	<200, 3"	<200, 3"	>200, 0"	>200, 3"	>200, 0"
RRBT	<200, 6"	<200, 6"	<200, 6"	<200, 12"	>200, 6"
BTa	<200, 6"	<200, 6"	<200, 6"	<200, 12"	>200, 12"
BTb	<200, 3"	<200, 3"	<200, 12"	<200, 12"	>200, 6"
C	<200, 3"	>200, 0"	>200, 0"	>200, 0"	>200, 0"

Table 8: The Sørensen similarity index for the Bainsvlei sites for all 5 different fields. (NBT - non-*Bt* maize, RRBT - *Bt* insect resistant maize, BTa - *Bt* maize, BTb - *Bt* maize, C - Control) over a period of five months in 2013.

	RRBT	**BTa**	**BTb**	**NBT**	**C**
RRBT		0.5	0.58	0.4	0.5
BTa	0.49		0.45	0.5	0.4
BTb	0.58	0.5		0.4	0.4
NBT	0.39	0.5	0.39		0.5
C	0.46	0.4	0.43	0.5	

A sudden plunge in diversity was observed for all of the cultivated fields in March 2013 (Figure 11). This may be as a result of soil ridging in the plant rows that was done the week before sampling. Even though it had rained the days prior to sampling, no increase was seen in the diversity of the cultivated fields. The control veld, however, showed a noteworthy increase in diversity (2.841) towards March 2013 (Figure 11) and this may be due to higher soil humidity (Table 6). An increase in soil humidity was observed from February to March 2013 for the C field after it had rained (Table 6). In the cultivated fields the humidity of only the NBT and RRBT fields increased towards March 2013 (Table 6).

The non-*Bt* maize field's (NBT) diversity was the less affected by the soil disturbance of ridging, but the relatively high diversity (Figure 11) can also be attributed to higher soil humidity. The RRBT field was treated with herbicides after the February 2013 sampling date that may be a possible explanation why the diversity of this field stayed low even though there was an increase in soil humidity (Table 4). The diversity of the five fields was relatively similar to one another for April 2013 and May 2013 (Figure 11). It also seemed as if the diversity of all the cultivated fields, including the RRBT field that was treated with herbicides, recovered towards April 2013. The diversity and soil humidity of the C veld decreased towards April (Figure 11).

When considering the compaction and compaction layer data recorded in each field for each of the sampling dates, it can be seen that the three *Bt* fields (BTa, BTb and RRBT) had the lowest compaction and the deepest compaction layer of the five fields (Table 7). The relative humidity data (Table 4) for these three fields was also the highest when compared to the non-*Bt* (NBT) and control (C) veld. When correlating this to the diversity data (Figure 11) it can be seen that these three *Bt* fields had more or less the same H' values throughout the five sampling dates. The H' and E values in Figure 11 cannot be directly attributed to the humidity and compaction data because of the soil disturbance in March 2013. The control veld (C) and non-*Bt* fields also did not correlate directly with compaction and humidity because in June 2013 both these fields were very dry and compact. The control (C) veld, which was natural and undisturbed, had the highest H' and E values during this time (Figure 11). The NBT field, however, had roughly the same compaction and humidity in June 2013 than it had in May 2013 (Table 7). The NBT field showed a sudden drop in both the E and H' values on the last sampling date (Figure 11) in June 2013. No soil preparation or soil application was done prior to this sampling date. The general climate and rainfall for this field was the same as for the other trial plants, so this could not have been an explanation for the decline in diversity. This particular maize cultivar was, however, the smallest in size and already started lodging in May 2013. The decline in soil fauna might be attributed to the fact that these maize plants dried out faster in the root area causing the plants to fall over. As discussed previously the dying off, of plants causes a change in soil respiration and CO_2 levels in soil. As these plants fell over they also exposed the inner soil layers where organisms that found refuge to above ground predators.

According to Ekschmitt *et al.* (2003), the measurement of soil biodiversity poses a challenge when considering sampling, processing and extraction efficiency. When comparing the biodiversity in Figure 8 and 11, its clear how big a difference sorting and filtration techniques can make in the results obtained from soil studies, in this case 2012 *vs* 2013. The results indicated that *Bt* maize despite the cultivar did not have any immediate negative effect on soil mesofauna, and in some cases reflected the opposite, where soil fauna diversity was higher in *Bt* maize fields than in non-*Bt* maize fields.

Thus, not only did this study indicate that *Bt* maize has no direct negative influence on soil faunal diversity and occurrence, it in fact reflected the opposite to a certain extent.

The trophic groups that dominate soil ecosystems in this part of the study were saprophages, phytophages, mycophages, bacteriovores, omnivores, predators, parasitoids and parasites. Mycophages were mostly represented by Collembola and Acari that are always abundant and fairly diverse in soil ecosystems (Addendum 1). In 2013 phytophages included both active and non-active species. Some fauna found in soil, such as the Thysanoptera, feed on above-ground plant parts, but can occur in the soil because they spend time here to pupate and overwinter. They are thus not active members of the soil food web, but contribute to energy flow when they fall prey to predators.

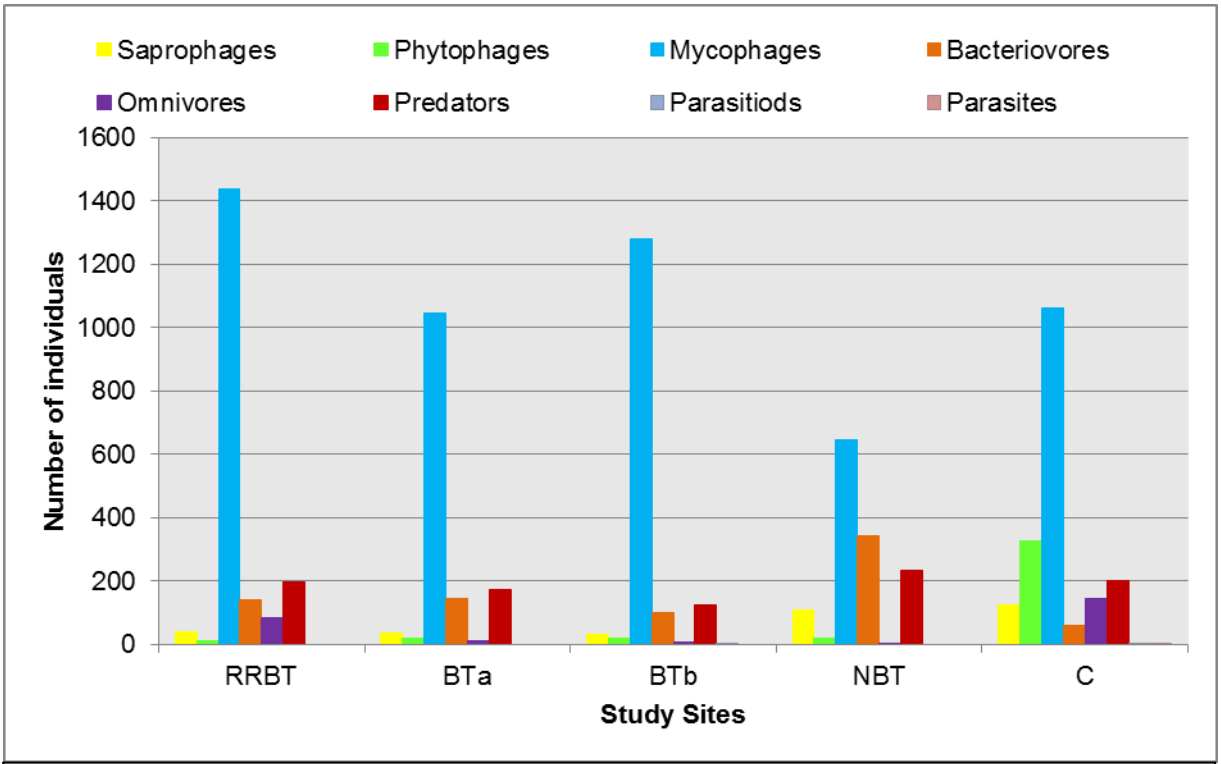

Figure 12: Trophic structure of the five sampling sites sampled in 2013. RRBT - *Bt* insect resistant maize, BTa - *Bt* maize, BTb - *Bt* maize, NBT - non-*Bt* maize, C - Control.

Active phytophages in soil environments include Cydnidae, burrowing bugs and other root feeding fauna (Addendum 1). Saprophages play a very important role in litter

decomposition by breaking down plant material. Members of this trophic group include a wide variety of insect larvae across different orders and Coleoptera beetles. Predators feeding on prey in soil environments can be made up of numerous species from many faunal groups. They mostly include spiders, mites, collembola, insects and millipedes. Many parasitoids and parasites are merely accidentally in the soil or spend an inactive life stage there. As with Thysanoptera they also contribute to energy flow in the food web when consumed by predators. The data on the soil mesofauna found at the five sampling sites in 2013 were lumped for the five sampling dates to represent the trophic groups of the organisms found in each of the trial fields (Figure 12).

Omnivores were represented by various ant species (Formicidae) and were most numerous in the control veld (Figure 12). According to Richards (2009), ants together with termites, contribute a great deal towards bioturbation of soil. They construct nests or mounds and form a close relationship with the soil in which they nest. Agricultural activities and soil preparation have a huge impact on ant occurrence since they need to rebuild their nests every time a destructive activity has occurred. Conventional tillage was applied in all the cultivated fields and possibly influenced ant populations. This can be a possible explanation for the high ant numbers in the control veld compared to the cultivated fields. According to, tillage of soil and other soil preparation procedures have a direct effect on soil biodiversity. In their study they examined how soil fauna reacts to different soil tillage methods. They experimented with conventional tillage, conservation tillage and no tillage. They found that tillage broadly influenced soil fauna trophic groups, but that there was no significant effect on predators and omnivores. In this study the results differed from Van Capelle *et al.* (2012). In 2013 in this study ants were almost completely absent in the cultivated fields, except for the RRBT field (insect and herbicide resistant maize). This might be explained by the close proximity of this field to the natural veld. Ants can reinvade agricultural fields close proximity easily and over time will also inhabit fields further away (Haddad *et al.* 2011).

Mycophages was the most abundant in all five different sampling sites (Figure 12). This group was not only abundant, but diverse as well. Mycophages were made up

of 16 species of mites and 13 species of Collembola. Some members of the Coleoptera and Diptera (mostly larvae) also contributed to this trophic group but were not as abundant (Addendum 1). Mycophages are a good indication of the dynamics of microbes such as fungi in the agro-ecosystem. The mycophages were the most abundant in the insect and herbicide resistant maize crop (RRBT, Figure 12). This might have been on account of the higher humidity in this field (Table 6). The other two *Bt* maize fields (BTa and BTb) also had a higher humidity level compared to that of the non-*Bt* field (NBT) and control (C), which could explain the higher numbers in mycophagous fauna. Mycophages in all the cultivated fields had a high abundance, except for the non-*Bt* maize field (that had lower soil humidity) and the control veld which showed no significantly higher abundance of this fauna (Figure 12). According to Van Capelle *et al.* (2012), conservation tillage will favour mycophages and bacteriovores which can explain the lower abundance in the control veld where no tillage was applied.

The bacteriovores, however, did not follow the pattern discussed here. Bacteriovores were only represented by a single mite species, *Speleorchestes meyeri*, which is known to feed on bacteria and algae (Russell *et al.* 2010) and they were most abundant in the non-*Bt* maize field (Addendum 1, Figure 12). The high presence of bacteriovores indicates a high presence of bacteria that probably influenced the growth and development of plant roots, which could explain the weak anchoring ability of the non-*Bt* maize plants during the last two sampling dates. Saprophagous mesofauna did not occur in large numbers and was mostly made up of Coleoptera and Diptera maggots. It seemed that they were the most abundant in the drier and more compact soil of the non-*Bt* maize field and the control (Figure 12). Van Capelle *et al.* (2012) also found that phytophages feeding on plant roots were strongly influenced by soil disturbances because they occur close to the soil surface. Phytophages were less abundant in all the cultivated fields compared to the control natural veld (Addendum 1). Phytophages had the highest number of individuals present in the control and were primarily represented by Thysanoptera. Phytophages were to a lesser extent represented by Hemiptera, such as Cydnidae that feed on plant roots, and various

other nymphal stages of this order, as well as a wide range of Coleoptera of which the most were either accidental or temporary residents in soil (Addendum 1).

Predators found throughout this study were very diverse and were represented by Araneae (spiders), Coleoptera (Carabidae and Staphylinidae) and 11 species of predatory mites. There seemed to be no substantial difference in the abundance (Figure 12) or diversity of predators found at the five sampling sites. The three *Bt* maize fields (BTa, BTb and NBT) had the fewest predators, whilst the non-*Bt* maize field (NBT) and control veld (C) had slightly more (Figure 12).

2.6. Conclusion

Soil meso-arthropods play an important role in soil functioning based on their role in decomposition processes, ecosystem engineering and nutrient cycling. Because agriculture all over the world is intensifying, GMO crops are needed to help producers to reach ever-increasing demand. The impact of *Bt* maize on non-target soil organisms is an important aspect in soil health and agricultural sustainability. The results of this study show that there were no immediate negative effects of *Bt* maize on soil faunal diversity during two growing seasons. In the 2012 season, the results showed slightly higher soil-arthropod diversity in *Bt* maize fields. In this study it is clear that soil preparation and not *Bt* or non-*Bt* maize influenced soil faunal diversity the most, followed by humidity and compaction. In both the 2012 and 2013 surveys the similarity between the different fields was non-significant.

The trophic structure of both surveys showed mycophages to be the most abundant, followed by higher trophic levels in ascending order. Disturbance could possibly allow mycophages to better access the fungal biomass to feed upon, leading to greater abundance. Omnivores represented by ants were most abundant in the controls because of the soil disturbance factor in the cultivated fields. Ants reinvade cultivated soils but at a relatively slow rate.

The *Bt* maize cultivars used in this study showed no significant influence on soil meso-arthropod diversity or trophic structure. In the 2012 study, a higher diversity of soil mesofauna can be seen in the *Bt* fields indicating that plants with the insect resistant gene may very well favour soil faunal groups due to increased plant health and a larger root mass (porosphere). Thus, not only did this study indicate that *Bt* maize has no direct negative influence on soil faunal diversity and occurrence, it in fact reflected the opposite to a certain extent.

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CHAPTER 3

Soil meso-arthropod diversity in allelopathic alfalfa and sunflower cultivations

3.1. Introduction

Several agricultural crops have been reported to have allelopathic potential (Ahmed & Wardle 1994, Djurdjevic *et al.* 2004, Hao *et al.* 2007, Ali & Mezori 2008, Alsaadawi *et al.* 2011). Sunflower (*Helianthus annuus*), being one of these crops, contains phenols and turpentine's in their tissue responsible for their allelopathic abilities (Anjum & Bajwa 2005). Alfalfa (*Medicago sativa*) is also known for its allelopathic abilities, but researchers have yet to determine the active ingredient responsible for this ability. According to Ayub *et al.* (2012), the best known effect of alfalfa allelochemicals is the negative influence on seed germination. Because alfalfa is a perennial legume that can be cultivated for up to seven years on the same rootstock, allelochemicals in soil may reach high concentrations and this may influence succeeding crops (Ayub *et al.* 2012). The inability of seeds to germinate because of the presence of allelochemicals in soil is referred to as soil sickness.

Sunflower is an important annual crop and is native to North America (Irons & Burnside 1982). Sunflower belongs to the family Asteraceae and is cultivated world-wide for its seeds that are used in various foods. In certain countries it is also utilized as biofuel. Researchers have discovered some variations in weed susceptibility to allelopathic crops depending on crop varieties/genotypes (Alsaadawi *et al.* 2011). A study done by Anjum & Bajwa (2005) concluded that allelopathic compounds in sunflower leaves affect the biomass of a range of weed plants negatively. According to Oracz *et al.* (2007), sunflower phytotoxins targets the antioxidant system in the receiver plants. This in turn damages plant cells that restrict permeability of the cell-membrane. Due to this action the receiver plant cannot absorb nutrients and water which reduces seed germination rates, as well as plant growth. Oracz *et al.* (2007) also found that toxins present in sunflower leaves damage the fat store of germinating seeds reducing their overall viability.

Alfalfa, also known as lucerne, belongs to the Fabaceae and is cultivated around the world as a forage crop for cattle (De Albuquerque *et al.* 2011). Despite being a

forage crop, alfalfa is also recognised for its allelopathic ability. The allelochemicals associated with alfalfa are water-soluble and the precise chemical composition is still unidentified (Chon & Kim 2002, De Albuquerque *et al.* 2011). Chon & Kim (2002) conducted a study on the effects of different alfalfa plant part extracts on alfalfa, lettuce and Chinese cabbage seedlings. They determined that chemicals extracted from alfalfa leaves, influenced root length of alfalfa, lettuce and Chinese cabbage plants the most. The plant part that influenced root length of receiver plants the second most, was the stems and thereafter roots and seeds respectively. Contrary to previous findings (Weissinger *et al.* 2001, Jasicka-Misiak *et al.* 2005), seeds did not have the overriding influence on root lengths. Chung *et al.* (2000) found alfalfa leaves to have the highest concentrations of chlorogenic acid that is an inhibitor of seed germination and influences seedling growth and weight. Chon *et al.* (2002) also determined that coumarin found in alfalfa plant tissue had the highest concentrations in the leaves and that this compound is responsible for inhibiting longitudinal growth of the receiver plants roots due to the swelling of seminal roots. Coumarin is only one of the autotoxins found in alfalfa that influence other plants.

Dornbos *et al.* (1990) found that medicarpin in alfalfa plants are also one of the many allelopathic compounds. When studying allelopathic potentials and influences on receiver plants, it is also important to know the extent of the area around the plant that is influenced. Jennings & Nelson (2002) determined that the allelopathic zone surrounding alfalfa plants ranges between 20 and 25 cm. In this zone, germination and growth of other plants are influenced. According to Romeo *et al.* (1996), the occurrence where a plant diverts all its energy into defence whilst stressed is coined the optimal defence hypothesis and states that energy resources are diverted from growth into defence. According to Karlovsky (2008), the extent of energy diversion depends on the severity of stress. It is also stated that plants will use the cheapest means of defence to be more energy efficient. Allelopathy is one of the defence methods used by plants that include the use of secondary metabolites to compensate for stress. The severity off allelopathic ability in plants can be turned off and on, depending on the circumstances and severity of stress.

Many studies have been done on the effects of allelopahic alfalfa on seed germination and plant survival (*e.g.* Miller 1983, Amal & Showcat 1989, Dornbos *et al.* 1990, Ells & McSay 1991, Wynman-Simpson *et al.* 1991, Seguin *et al.* 2002, Xuan & Tsuzuki 2002). In a study by Golawska *et al.* (2010) it was found that apigenin glycosides found in alfalfa plants, modify the behaviour of the pea aphid and that the aphid was less abundant on alfalfa plants with a high concentration of this compound. The apigenin glycosides have a deterring effect on aphid feeding and also stunted their growth. To date research on the effects of allelopathic plants on soil fauna diversity and abundance have not been done. It is thus the aim of this study, to determine the effects of alfalfa and sunflower allelopathy on soil meso-arthropod diversity. Because the concentration of secondary metabolites are higher in stressed plants (Pedrol *et al.* 2006), this study will also include the effects of stressed alfalfa plants on soil meso-arthropod diversity. The first hypothesis will be that undisturbed natural ecosystems (possibly also containing allelopathic plants) will support a greater diversity of soil fauna than disturbed agro-ecosystems (in this case alfalfa and sunflower fields). It is also hypothesized that stressed allelopathic alfalfa plants will influence soil faunal diversity negatively due to their increased allelopathic potential. Lastly it is hypothesized that newly planted young alfalfa plants, will not influence soil meso-arthropod diversity negatively because their alleopathic potential could be lower than that of mature plants.

Farooq *et al.* (2010) focused on the phytotoxic effect of allelopathic plants on weeds with the goal to produce more environmentally safe herbicides to be used in agriculture. The question then arises that if these natural products will be used, will they be environmentally friendly? As soil and the organisms that is sustaining soil health and quality is of the utmost importance in agricultural production and sustainable land use, the effects of allelopathy on these organisms must be studied thoroughly before these products can actually be labelled environmentally friendly. This study will furthermore also focus on the allelopathic potential of male and female sunflower plants on soil meso-arthropods occurrence with respect to different soil types. Soil mesofauna trophic structure analysis will also be considered in this regard.

3.2. Study layout

3.2.1. Study sites

The first part of this study was conducted in 2013 at two localities in the Bainsvlei area outside of Bloemfontein that were situated 3.8km apart from one another (Figure 1 & 2). The largest part of this study was conducted on the farm Maranatha (Figure 1), (29°01'36.31"S, 26°05'04.32"E) and two of the control samples were taken on the farm Geluk (Figure 2), (28°59'30.32"S, 26°05'47.58"E). The Maranatha study site consisted of three alfalfa fields (named field 1, 2 and 3: Figure 1). All three of these fields were planted with S.A. Standard alfalfa cultivars. Fields 1 and 2 were planted in 2007 and were approximately 6 years old when the study was conducted. Field 1 had mostly sparse vegetation and was ripped for re-cultivation in June 2013. This field was sampled from mid-February to mid-August 2013. Field 2 was sampled from mid-February to the first week of October 2013.

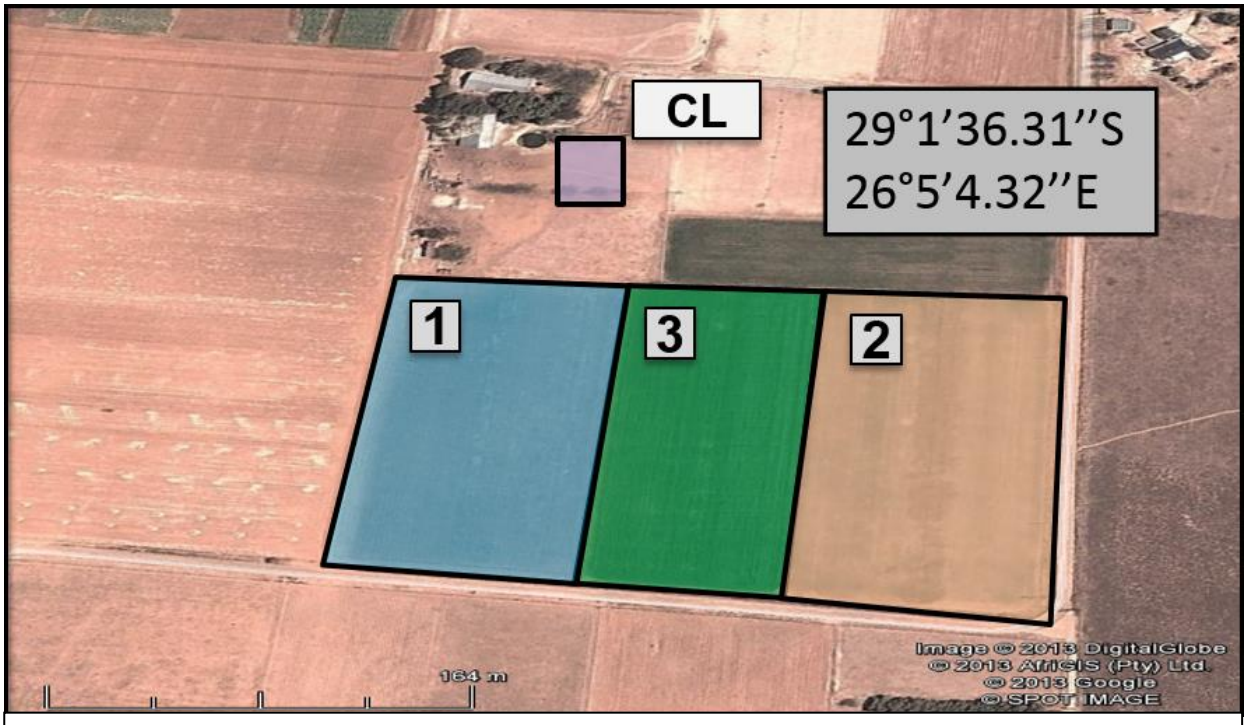

Figure 1: Maranatha farm outside Bloemfontein in 2013 showing the three alfalfa fields (Field 1, 2 and 3) and a small patch of natural field (CL) that served as first control (Image from Google Earth).

Field 3 was planted at the end of April 2013, and the study was conducted from mid-April to the first week of October 2013. Three control sampling sites were used. The first was on Maranatha (CL; Figure 1) and was a small patch of natural grass situated between animal forage crops. This control site mostly contained the following grass species: red grass (*Themeda triandra*), narrow-leaved turpentine grass (*Cymbopogon plurinodis*), spear grass (*Heteropogon contortus*) and dropseed grass (*Sporobolus fimbriatus*), (Van Oudtshoorn 2012). The other two control sites were on Geluk (CN and CS; Figure 2) and consisted of natural veld (CN) surrounding a 10 year old smuts finger grass (*Digitaria eriantha*) field (CS). The natural veld (NC) mainly consisted of the following grass species: red grass (*T. triandra*), narrow-leaved turpentine grass (*C. plurinodis*), spear grass (*H. contortus*) and dropseed grass (*S. fimbriatus*), (identified using Van Oudtshoorn 2012).

Figure 2: Geluk farm outside of Bloemfontein in 2013 showing the natural field (CN) and the Smuts finger grass field (CS) that served as the second and third control fields (Image from Google Earth).

The smuts finger grass field (SC) only contained smuts finger grass with red grass (*T. triandra*) and spear grass (*H. contortus*) occurring sparsely spread throughout the field. These grass species are commonly found in the Free State area and make up the majority of the Free State grassland vegetation.

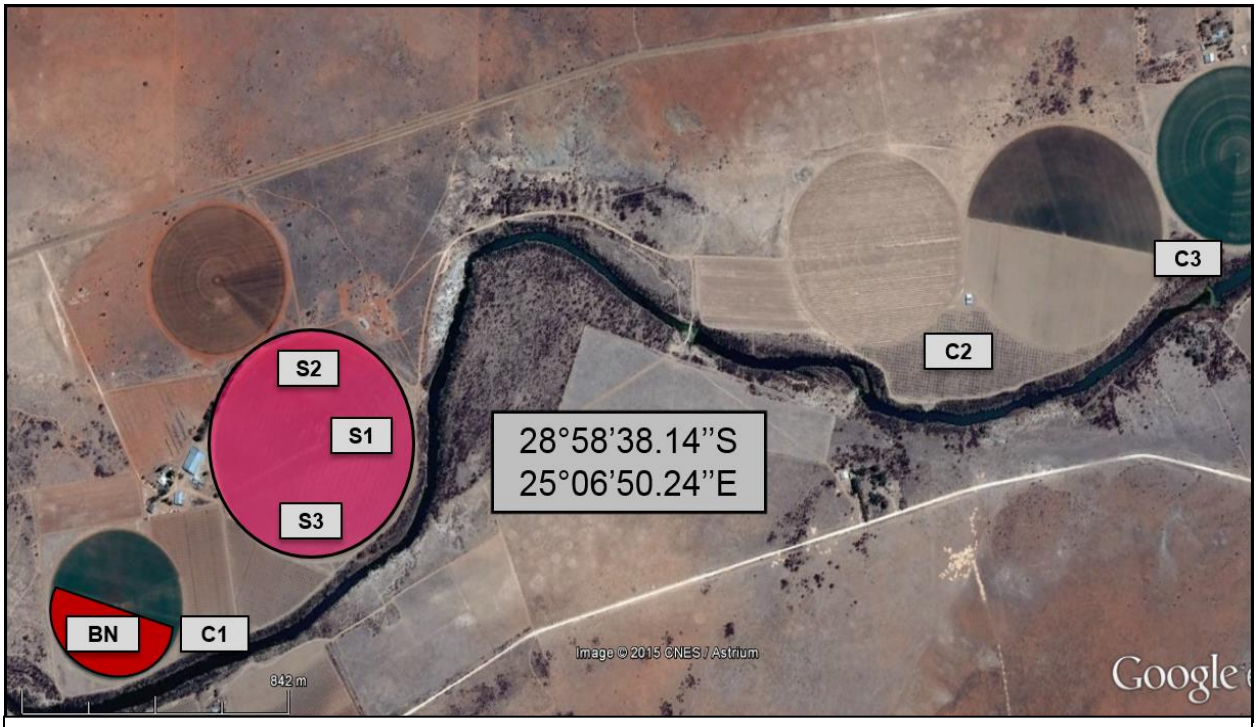

Figure 3: Thornberry farm near Petrusburg in 2013, showing the sunflower field divided into three sections according to soil type (S1: mixure of red sandy and dark soil with higher clay content, S2: red sandy soil & S3: darker soil with a high clay content). Three control sampling sites (C1, 2 & 3) and a butternut field (BN) in close proximity to the sunflower field are also indicated in the figure (Image adapted from Google Earth).

3.2.2. Methodology

Soil samples were taken monthly at the three localities (Figure 1, 2 & 3) outside of Bloemfontein and Petrusburg (see summary of study sites at Table 1). In the Bainsvlei area sampling of Field 1 occurred from February to August 2013, in field 2 from February to October 2013 and in Field 3 from April to October 2013. Since the fields were in close proximity to one another, the same control fields were used to

compare all three alfalfa fields. In the Petrusburg area samples were taken from February to June 2013.

Table 1: A summary of the localities and sampling sites.

Sample	Town	Farm	Field	Crop	Figure
L1	BFN, Bainsvlei	Maranatha	Field 1	Alfalfa	Figure 1
L2	BFN, Bainsvlei	Maranatha	Field 1	Alfalfa	Figure 1
L3	BFN, Bainsvlei	Maranatha	Field 1	Alfalfa	Figure 1
L4	BFN, Bainsvlei	Maranatha	Field 2	Alfalfa	Figure 1
L5	BFN, Bainsvlei	Maranatha	Field 2	Alfalfa	Figure 1
L6	BFN, Bainsvlei	Maranatha	Field 2	Alfalfa	Figure 1
L8	BFN, Bainsvlei	Maranatha	Field 3	Alfalfa	Figure 1
L9	BFN, Bainsvlei	Maranatha	Field 3	Alfalfa	Figure 1
L10	BFN, Bainsvlei	Maranatha	Field 3	Alfalfa	Figure 1
CL	BFN, Bainsvlei	Maranatha	NA	Natural field	Figure 1
CN	BFN, Bainsvlei	Geluk	NA	Natural field	Figure 2

Table 1 (Continues): A summary of the localities and sampling sites.					
CS	BFN, Bainsvlei	Geluk	NA	Smuts finger and natural field	Figure 2
S1♀	Petrusburg	Thornberry	NA	Sunflower	Figure 3
S1♂	Petrusburg	Thornberry	NA	Sunflower	Figure 3
S2♀	Petrusburg	Thornberry	NA	Sunflower	Figure 3
S2♂	Petrusburg	Thornberry	NA	Sunflower	Figure 3
S3♀	Petrusburg	Thornberry	NA	Sunflower	Figure 3
S3♂	Petrusburg	Thornberry	NA	Sunflower	Figure 3
BN	Petrusburg	Thornberry	NA	Butternut	Figure 3
C	Petrusburg	Thornberry	NA	Natural field	Figure 3

Temperature and rain data was also recorded for all the sampling dates (Table 2 and 3) and included minimum and maximum temperatures, as well as the occurrence or absence of rain the week before sampling and the day of sampling. Three soil samples were taken in each field on each sampling date in the porosphere of the plant (see Chapter 2.2.1). Because the allelopathic ability of plants are influenced by stress, two samples in Field 1 (Bainsvlei) were taken at plants that appeared healthy and unstressed (L1 and L2). One sample was taken at a plant that appeared sick, weak and stressed (L3). In Field 2 (Bainsvlei) samples were taken at stressed plants (L4 and L5) and one was taken at an unstressed plant (L6). Because Field 3 (Bainsvlei) was a

newly planted field, all plants within the field appeared healthy and no variation in plant condition was observed during the course of this study.

Three samples were taken at random in this field (L8, L9 and L10). These were newly planted saplings and stressed and non-stressed sections could not be distinguished. No L7 sample was collected and the samples of Field 3 started off at the L8 sample. In the Petrusburg area two samples were taken in each section of the sunflower field (S1, S2 and S3), one at male plants and the other at female plants. The samples will be referred to S1♀, S1♂, S2♀, S2♂, S3♀ and S3♂ for the remainder of this chapter. Three samples were taken at random in the butternut field and were lumped to represent the butternut field. Both the alfalfa field and sunflower field were under irrigation during the study. The sunflower and butternut field used a pivot irrigation system while the alfalfa field was irrigated with a sprinkler system.

Table 2: Sampling dates and climatic conditions during soil sampling from February to October 2013 in the Bloemfontein area.

Sampling Date	**12 Feb 2013**	**20 Mar 2013**	**17 Apr 2013**	**15 Mar 2013**	**12 Jun 2013**	**16 Jul 2013**	**14 Aug 2013**	**17 Sept 2013**	**22 Oct 2013**
Temperature (max / min)	27°C / 16°C	26°C / 18°C	24°C / 19°C	23°C / 17°C	21°C / 10°C	19°C / 11°C	21°C / 16°C	24°C / 19°C	28°C / 19°C
Rain on sampling date	NO	NO	NO	NO	NO	NO	NO	NO	NO
Rain prior to sampling date	YES	YES	NO	NO	NO	NO	NO	NO	YES

Table 3: Sampling dates and climatic conditions during soil sampling from February to June 2013 in the Petrusburg area.

Sampling Date	**16 Jan 2013**	**20 Feb 2013**	**03 Apr 2013**	**22 May 2013**	**19 Jun 2013**
Temperature (max / min)	30°C / 21°C	28°C / 19°C	24°C / 17°C	21°C / 17°C	17°C / 10°C
Rain on sampling date	NO	NO	NO	NO	NO
Rain prior to sampling date	YES	YES	YES	NO	NO

Soil samples taken were then placed on the Tullgren funnels for seven days, sorted and identified directly from the ethanol (see Chapter 2.2.2). The data were analysed by using the Shannon's diversity index (see Chapter 2.3.1). The data for the three control fields in Bainsvlei (CN, CS & CL) and Thornberry farm samples (C1, C2 & C3) were lumped respectively to give a single representative value for each sampling month. Thornberry farm was also managed with more of an environmentally friendly approach, *i.e.* applying animal manures and microbial additives, compared to Maranatha farm that was managed with conventional farming practices.

3.3. Results and Discussion

By measuring the diversity of soil meso-arthropods in stressed and unstressed allelopathic alfalfa soils, one can determine if stressed plants affect soil fauna more dramatically than non-stressed plants. On the other hand, soil fauna can be influenced by the same stresses as plants. These stress factors include humidity, compaction and temperature of soil. Other factors include phytophagy or mechanical injury and nutrient availability. In cases where plants are stressed by these factors, one should keep in mind that diversity is not exceptionally influenced by the stressed plants only, but that these factors also play a direct role. Stressed plants are a relative concept, because all

plants are stressed from time to time. Stressed plants in this trail refer to a weakened condition in plants where growth is slower and plants appear smaller and are sparsely spread.

Plants can be stressed due to many direct and indirect influences: 1) allelopathy (production of secondary metabolites) in plants can increase to defend plants against stress and this may cause stunted growth, 2) leaching of soil nutrients and minerals can appear in patches in a field where there is more concentrated rainfall or water (irrigation), 3) phytophagous nematodes can cause patchiness in vegetation, 4) even in the same cultivar plants, stress can be genetically or physiologically different, which can result in strong and weak plants within the field and 5) supplements that are artificially added to fields can sometimes end up in too low or too high concentrations resulting in a variation in plant condition within a field.

In this study all these factors was considered, but the focus was primarily on allelopathy and its influence on soil meso-arthropods. Because no research has been done to determine whether alfalfa autotoxins influence soil faunal diversity, it is important to mention that both stressed and unstressed plants could have an influence on diversity indices. Sunflower, also being an important allelopathic crop, may also influence soil biodiversity. In this section of the study the role of stress on plants will not be a focus point. The focus will rather be on the influence of allelopathy in three different soil types and the difference in allelopathy between male and female sunflower plants.

3.3.1. Bainsvlei (alfalfa)

The Shannon's index for diversity (H') and evenness (E) was used to determine the difference in diversity between the three alfalfa fields (Field 1, 2 and 3). The H' value represents the diversity that expresses a value that takes both abundance and species richness into account. The E value represents the evenness, indicating the distribution of species within diversity. A value of 1 represents total evenness and a

value of 0, total unevenness. The Shannon's index of diversity H' and evenness E for Field 1 (L1, L2 and L3) and the control (C) can be seen in Figure 4. The Shannon's index of diversity (H') and evenness (E) for Field 2 (L4, L5 and L6) and the control (C) can be seen in Figure 5. Surveys in Field 2 continued over a period of nine months. The last part of this study was in Field 3 which was a new field planted in May 2013. The Shannon's index of diversity H' and evenness E for Field 3 (L8, L9 and L10) and the control (C) can be seen in Figure 6. The correlating humidity, compaction and compaction layer data of all three fields can be seen in Tables 4 and 5.

Alfalfa is a perennial crop and needs to be harvested from time to time. The alfalfa in Field 1 was harvested in January 2013 and May 2013 and Field 2 was harvested in January 2013, May 2013 and September 2013. Field 1 was ripped in June and most plants were de-rooted in the process. The purpose for this soil preparation was to demolish the current field and prepare the field to be cultivated with new alfalfa plants the following year. Field 1 also did not receive any irrigation after this date. The first two samples in Field 1 (L1 and L2) were taken at unstressed plants and the third sample (L3) was taken at a stressed plant (Figure 4). Samples L4 and L 5 were sampled at stressed plants, while sample 6 was taken at unstressed plants in Field 2 (Figure 5). Stressed and non-stressed plants in this study relates to the plant condition at the beginning of the study. After the study began, all plants (both stressed and non-stressed) were harvested in January 2013 and May 2013 (Field 1 and 2) and September 2013 (only Field 2). This additional stress is constant for all the plants and even though a plant was labelled non-stressed it was still exposed to the same additional disturbances and stresses of agriculture. According to Kruidhof *et al.* (2014), mechanical wounding to allelopathic plants may enhance their allelopathic ability. Sampling in Field 1 took place from February 2013 to August 2013 and in Field 2 from February 2013 to October 2013. Both fields (Field 1 and 2) showed an overall low E value (< 0.5) for February 2013 (Figures 4 and 5). This may be due to the harvesting in January 2013, with increased stress on the plants coupled with soil being more exposed to the sun that could result in more dramatic micro-climate changes below-ground. The

control field (C) also had a low E value for February 2013, but this value was higher than all of the alfalfa samples, except for L5.

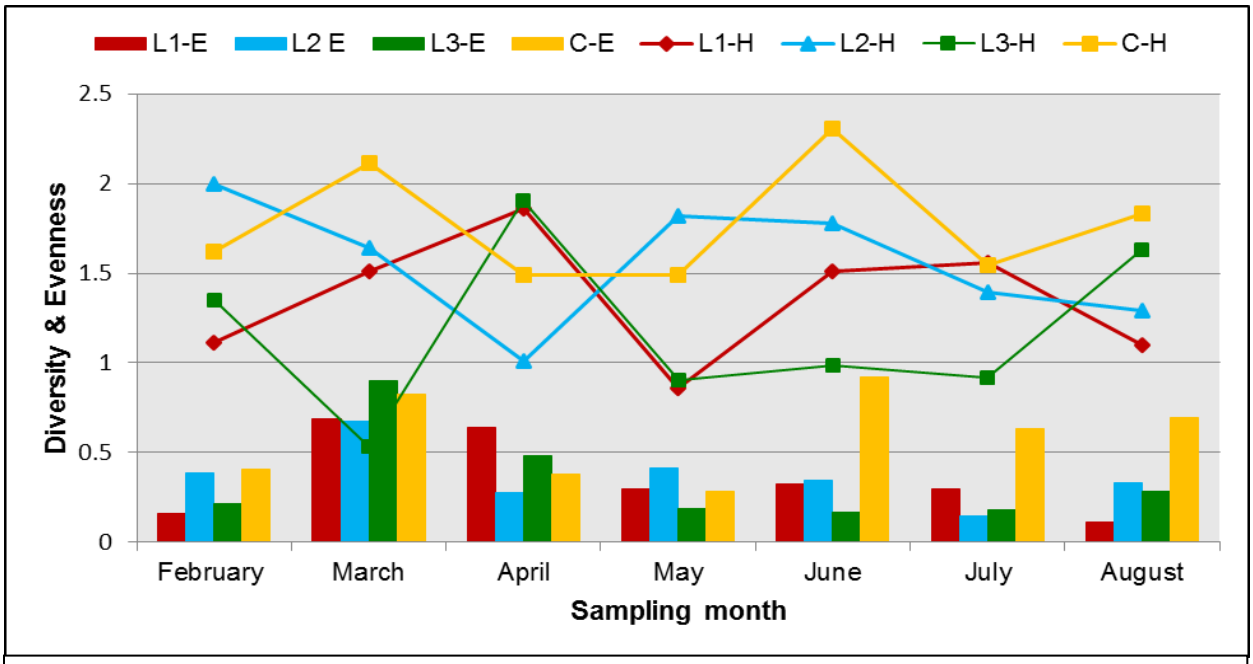

Figure 4: Shannon's index for diversity and evenness of soil meso-arthropod in alfalfa Field 1 and control sites in the Bainsvlei area outside of Bloemfontein over a period of seven months in 2013. Evenness (E) is represented by bars and diversity (H') is represented by graph lines. L1: non-stressed plants, L2: non-stressed plants, L3: stressed plants and C: control.

The low E values in the cultivated fields and control fields in February 2013, can be attributed to the large numbers of certain genera of Collembola and Acari (Addendum 1) found in these samples. *Brachytydeus, Nanochestes* and *Cryptopygys* are mycophages, suggesting an increase in soil fungal activity during this period. *Brachytydeus* sp. 1 mites reached high numbers of individuals in the L1 and L3 samples, while *Nanorchestes* sp. 1 mites were also recorded in high numbers (200-396 individuals) in all the other alfalfa samples. connected to the wetter soil promoting fungal growth. Field 3 was still in the early recovering stage and fungi were possibly not as established as in the old fields. The compaction meter used (see Chapter 2.2.3) measured compaction in pounds per square inch (psi).

Table 4: Average % soil humidity of each sample in three fields (Field 1: L1, L2 & L3, Field 2: L4, L5 & L6, Field 3: L8, L9 & L10 and control field: C) over a period of nine months in 2013.

	Feb 2013	Mar 2013	Apr 2013	May 2013	Jun 2013	Jul 2013	Aug 2013	Sep 2013	Oct 2013
L1	13.50	7.70	9.50	0.80	4.10	2.30	2.80	-	-
L2	10.80	1.10	3.00	0.90	7.30	1.90	1.80	-	-
L3	13.50	3.80	8.90	1.20	1.10	1.90	0.90	-	-
L4	7.20	4.30	7.90	2.30	13.30	12.50	11.60	10.20	2.50
L5	12.90	7.40	3.90	2.10	10.50	3.50	13.30	12.40	1.40
L6	12.50	10.00	7.20	0.30	16.50	7.10	11.90	9.20	4.20
L8	-	-	14.50	14.50	14.20	12.90	7.20	10.10	10.60
L9	-	-	13.90	13.90	14.90	10.00	3.80	8.30	11.90
L10	-	-	12.10	12.10	13.80	12.30	4.30	10.30	1.20
C	9.57	2.97	0.60	0.57	0.20	0.23	0.30	0.20	0.53

Table 5: Average soil compaction value (in psi) were 200 for all samples and depth of compaction layer (inches) of each sample in three fields (Field 1: L1, L2 & L3, Field 2: L4, L5 & L6, Field 3: L8, L9 & L10 and control field: C) over a period of nine months in 2013.

	Feb 2013	Mar 2013	Apr 2013	May 2013	Jun 2013	Jul 2013	Aug 2013	Sep 2013	Oct 2013
L1	200,0	200,1	200,1	200,0	200,2	200,0	200,0	-	-
L2	200,0	200,0	200,1	200,0	200,1	200,0	200,0	-	-
L3	200,1	200,0	200,0	200,0	200,0	200,0	200,0	-	-
L4	200,1	200,1	200,1	200,0	200,1	200,1	200,1	200,1	200,1
L5	200,1	200,0	200,0	200,0	200,1	200,0	200,1	200,1	200,1
L6	200,2	200,1	200,1	200,0	200,1	200,1	200,1	200,0	200,0
L8	-	-	200,3	200,3	200,3	200,3	200,3	200,3	200,3
L9	-	-	200,3	200,3	200,3	200,3	200,3	200,2	200,2
L10	-	-	200,3	200,3	200,3	200,4	200,4	200,1	200,0
C	200,1	200,0	200,1	200,0	200,1	200,0	200,0	200,0	200,0

The increase in Collembola and Acari can be attributed to two variables. The first is the disturbance due to harvesting that may cause a surplus of organic matter and the second, is the high humidity (Table 4). Decrease of these two groups can be due to a decline in the above mentioned variables. Entry *et al.* (1986) studied the effect on microbial populations after a disturbance that alters the quantity of organic matter. They found that in cases where organic matter was left in the area, microbial biomass increased dramatically compared to cases where organic matter was removed. Plant residue was not left on the field intestinally, but during harvest, considerate quantities of plant matter remained in the field. This largely includes plant material that is not collected during baling. Remnants left behind post-harvest, could account for the increase in some organisms resulting in lower E values. This sudden increase in numbers of Acari and Collembola was followed by a reduction in their abundance in March 2013 for Field 1 (L1, L2 and L3, Figure 4) and Field 2 (L6, Figure 5) resulting in an increase in E value. The two stressed samples in Field 2 (L4 and L5) did not show the same inclination as the other field and had a lower E value in March 2013. This may be due to the severity of stress in the alfalfa plants post-harvest. Stress in plants can trigger defence responses that can influence soil fauna directly.

Defence can include many aspects, but chemical defence is the most common. This is where plants produce secondary metabolites to prevent stress by becoming less palatable or even toxic. These defences are costly and can only be obtained by plants for short periods of time. Plants thus use the same energy resources for growth and defence and while a plant is under stress, optimal growth is compromised (Lind *et al.* 2013). This phenomenon is known as an energy trade-off, where plants compromise one aspect of essential functions for another (Messina *et al.* 2002). Once plants are not stressed anymore the energy will once again be directed into growth. Allelopathic potential of plants may be enhanced during stressful periods. Once the stress on a plant is no longer present the allelopathic potential may become less. In April 2013 L1, L2 and L3 (Field 1, Figure 4) showed a decline in E value due to the increased numbers of certain genera of Acari and Collembola. No additional organic matter was added to this field but an increase in soil humidity (Table 4) from March 2013 to April 2013 could

be responsible for higher microbe activity that resulted in these high numbers in mycophages.

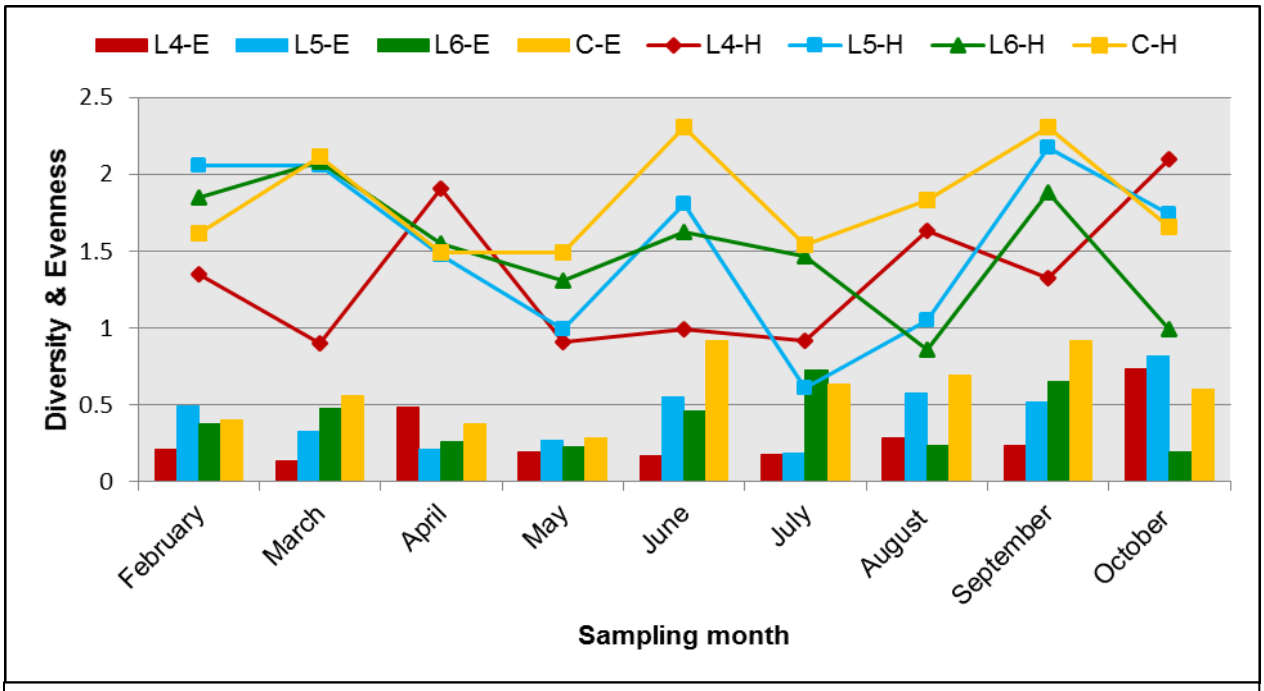

Figure 5: Shannon's index for diversity and evenness of soil meso-arthropod in alfalfa Field 2 and control sites in the Bainsvlei area outside of Bloemfontein over a period of nine months in 2013. Evenness (E) is represented by bars and the diversity (H) is represented by graph lines. L4: stressed plants, L5: stressed plants, L6: non-stressed plants and C: control.

Field 1 and 2 was harvested in May 2013 again and all six samples (L1, L2, L3, L4, L5 and L6) showed a decline in E value (Figure 4 and 5) due to this disturbance. In June 2013 the field was ripped and the E values of all three samples in Field 1 stayed uneven up to August 2013 when the last sample was taken. The E values for the control field (C) from June 2013 to August 2013 was much higher than in cultivated Field 1. The E values for the L4 sample (Field 2, Figure 5) was the lowest throughout the whole study except for April 2013 and October 2013 after recovery from harvest when diversity spiked as well. Being one of the stressed samples, this could have played a role in the evenness of soil meso-arthropods. As discussed earlier mechanical

damage to the alfalfa plants could enhance allelopathic ability and this could possibly also explain the results found in Field 2 (Figure 5).

The E value of sample L5 declined towards March 2013 and April 2013, but showed a slight increase in May 2013 when the field was harvested for the second time. This could once again be attributed to the increased organic matter in the field post-harvest. In June 2013 the E value of sample L5 spiked again just like in February 2013. This response is once more post-harvest and indicates a recovery or positive response a month after harvest. The E value dropped drastically from 0.5549 in June 2013 to 0.1845 in July 2013 (Figure 4). The stressed L5 sample had less vegetation coverage, which could have led to exposure of soil to external climatic conditions. An increase of E value for the following three months (August 2013, September 2013 & October 2013) was observed in the L5 sample. The reason for the increase in evenness post-harvest could have been due to a decline in the number of individuals of certain generain Acari and Collembola (Addendum 2). These two groups (Acari and Collembola) can reach high numbers in soil ecosystems and causes lower evenness when occurring in large quantities. Approximately a month after harvest their numbers decreased, resulting in a more even spread. Sample L6 started off at 0.3748 in February 2013 and increased up to 0.4730 towards March 2013 (Figure 4). This is an opposite response to that of both the stressed samples (L4 and L5) for this period. This can be attributed to the fact that the soil system of unstressed plants is less susceptible to change. Meaningful in this regard is that, according to Freedman (1995), an ecosystem under constant stress can reach a point where it starts absorbing stress without presenting measurable changes.

In June 2013 the E values of sample L6 spiked post-harvest which occurred in May 2013. This spike could be once again attributed to the lower numbers in Collembola and Acari. This increased E value continued towards July 2013 where values of both stressed samples (L4 and L5) was very low. Higher densities of alfalfa vegetation could buffer the soil from direct external climatic conditions and this could be an explanation for this decline. The E value increased once more post-harvest in September 2013, but declined towards October 2013 due to the presence of large

numbers of Acari and Collembola (Figure 4). According to Fulbright (1999), stress tolerant microbial species will out-compete other species during unfavourable conditions. This could possibly explain the high numbers of certain genera of Acari and Collembola post-harvest (Addendum 1). If certain species flourish when plants are under stress and plant defence is increased, while others become dormant, the diversity of food resources for soil fauna belowground could be limited to stress tolerant microbes. This in turn can favour certain organisms feeding on these microbes. *Brachytydeus, Nanorchestes* and *Cryptopygus* are mycophages that could possibly take advantage of specialized microbes as a food source during stressed periods (Addendum 1). A sudden decline in E value for sample L6 in August 2013 does not correlate with either soil humidity (Table 4) or compaction (Table 5). It is rather connected to lower H' values and thus a decrease in diversity. Many of the larvae and immature life stages that were found in July 2013 were absent in the August 2013 samples. As discussed in Chapter 1 many of the soil inhabitants only spend a part of their life cycle in soil and emerge as adults in the warmer months, a factor which could explain the lower E value and the decrease in diversity (Figure 4).

The L1 and L3 samples (Figure 4) started off at a low H' value of 1.114 and 1.348 in February 2013 which may be due to harvesting in January 2013 resulting in unusually high numbers of *Brachytydeus, Nanorchestes* and *Cryptopygus* species. Mechanical wounding of plants may have led to an increase of allelopathic ability of plants causing a decline in soil faunal diversity due to some species being able to exploit these stressful situations. Another explanation is that, because soils are more exposed to external factors such as sun (heat intensity) and wind post-harvest, soils could have dried out and became more compact. According to Ballard (2000), removal of the canopy in northern temperate forests alters soil microclimate and can, amongst others, cause imbalances in nutrient inputs. Harvesting of the alfalfa field could have led to these changes but on a much smaller scale. Ballard (2000) also stated that harvesting machinery increases soil compaction. Since the soil was exposed to harvest machinery for the past six years before samples were taken, the compaction was already very intense and there was no accurate way to measure whether soil was more

compacted post-harvest. Alfalfa Field 3, however, was a newly planted field and showed a gradual decrease in soil compaction depth (Table 5) towards October 2013.

The L2 sample (Figure 4) did not follow the same pattern as the other two samples and had the highest H' value of all the samples for February 2013. The high H' value for this sample cannot be attributed to either higher humidity (Table 4) or less compaction (Table 5). The reason for the high diversity in sample L2 for February 2013 could be due to a more diverse composition of Acari and Collembola (Addendum 1) compared to samples L1 and L3. All of the Acari and Collembola recorded in L1 and L3 were also recorded in the L2 sample for February 2013, but the L2 samples included an additional four species of Acari and four species of Collembola. These Acari include: *Marcocheles, Cunaxa, Chelyletus* and *Nanochestes* species (Addendum 1). The Collembola recorded only in the L2 sample for February 2013 included: *Seira* sp. 3 and two different species of *Entomobrya* (*Entomobrya* cf. *multifasciata* and *Entomobrya* sp. 3).

According to Coleman *et al.* (2004), springtails and mites tend to aggregate in soils. Sampling for soil fauna in a field can thus be a "hit or miss" scenario. If high levels of resources are available in a microhabitat, it is possible to sample high numbers of soil fauna due to their aggregation behavioural patterns in these areas. This occurrence may be a possible explanation for the unusual high diversity for the L2 sample in February 2013 (Figure 4). In March 2013 the L2 sample showed a decline in diversity and this continued up to April 2013. This decline can be attributed to a decrease in soil humidity (Table 4) and an increase in compaction (Table 5) due to long term exposure to external factors post-harvest. The same can be seen in the L3 sample for March 2013 (Figure 4). Plant densities in samples L2 and L3 were lower than in sample L1 and the soil was exposed to external factors for a longer period of time. The compaction (Table 5) and humidity (Table 4) data correlated with this. In areas with a more dense vegetation cover, soils dry out slower which promotes soil faunal activity. The H' value of the L1 samples in March 2013 and April 2013 showed an increase due to higher soil humidity (Table 4). The H' value of the L3 sample in April

2013 increased dramatically and this can also be due to an increase in humidity (Table 4). Another explanation might be that plants are less stressed and allelopathy or chemical defences, which would affect the fauna, is declining. Subsequently plants are less stressed and convert the energy that would have been used in chemical defences into growth.

In May 2013 Field 1 was harvested again and a sudden decline in H' values was observed in samples L1 and L3. Soil humidity also declined towards this sampling date. The H' values of the L2 sample did not seem to be affected by the disturbance of harvesting, similar to what was recorded in February 2013. The further decline in soil humidity (Table 4) did not seem to have an effect on sample L2 either (Figure 4). Since this was the second harvest disturbance that sample L2 did not respond to, it might be possible that more plant litter might have accumulated in this area during harvesting, promoting soil organic matter (SOM) and subsequent soil faunal activities. The decline in temperatures from June onwards (Table 2) did not seem to have a direct effect on soil faunal diversity in sample L1. Sample L3 (Figure 4) seemed to recover much slower towards June 2013 and July 2013 than with the previous harvest. This might have been due to the slower growth of the alfalfa plants in the winter and because this sampling area was temperature stressed. The allelopathic ability of these plants might be more than with the unstressed plants. Field 1 recovered slowly towards August and seemed to have a positive response to the increase in daily temperatures (Table 2) in August 2013. Because vegetation coverage in the L2 and L3 sampling area was sparse, the soil temperature could have been lower and more unfavourable. The L2 field did not recover as fast as the L3 field in August 2013 (Figure 4). Field 1 was ripped to be replanted in June 2013 and the slow recovery of all three samples (L1, L2 and L3) could have been attributed to the removal of most vegetation in this field coupled with the soil disturbance of ripping.

The alfalfa plants in the L4 sampling area (Field 2) recovered towards April 2013 because of a reduction in stress and thus a reduction in the need for allelopathic propensity. In other words, the plant was stressed in January 2013 when it was

harvested and mechanically damaged and allelopahic ability (plant defences) increased possibly resulting in a lower diversity in February 2013 and March 2013. Thereafter the plant did not receive any stimuli for defence and invested its energy into plant growth rather than defence. This may have relieved the allelopathic pressure on soil organism diversity thus resulting in an increase in diversity in April 2013. The H' values of samples L5 and L6 started off in February 2013 with initial high readings after the January 2013 harvest. These sampling areas had higher soil humidity in February 2013 compared to that of L4 (Figure 5). The H' values of the L5 and L6 samples (Figure 5) remained high in March 2013 and showed no direct negative effect post-harvest. The stressed sample (L4, Field 2) showed a further decline in diversity towards March 2013 and this correlated with the decline in soil humidity (Table 4) and increase in compaction (Table 5) that may have occurred due to exposure to external factors because of harvesting in January 2013 (Figure 5).

In April 2013 both L5 and L6 samples (Figure 5) showed a decline in H' value and this might have been due to a decrease in humidity (Table 4). L4, however, showed a strong increase in H' value in April 2013 which could have been attributed to possible reduced allelopathic potential due to reduced stress (Figure 5). This, however, was difficult to prove, but numerous (Mittler 2002 & Ma 2004) have connected lower stress levels with lower defence levels of plants. According to Coleman & Hendrix (2000), plants growing under optimal conditions will have energy and nutrient stores to support various metabolic processes. Plants respond to stress by diverting this energy usually used for growth and development into defence or reproduction. In severe stress situations where plants are exposed to more permanent stress or long term stress such as drought, the plant could place all its energy into reproduction before it dies off. However, in the case of phytophagy or mechanical damage the plant usually tends to switch energy towards defence. All these strategies have a direct or indirect bearing on the soil organisms inhabiting the soil in which such plants grow.

In May 2013 the diversity of all three samples (L4, L5 and L6, Figure 5) responded negatively to harvesting. During this time temperatures were beginning to

drop (Table 2) and the soil was now exposed to colder winter conditions. Samples L4 and L6, especially the former, recovered slightly towards June 2013 in spite of the colder climate. According to Van Breemen & Buurman (2003), above-ground temperatures are strongly buffered due to the low heat conductivity properties of soil. This buffer could be a reason for the increase in H' values of these two fields. The H' values of the L5 sample showed a sharp increase towards June 2013 which was again correlated with humidity levels (Table 4). Humidity could not be the only factor responsible for this increase, since the L4 and L6 samples also had an increase in humidity and still showed a decline in diversity. A possible explanation for this phenomenon could be the higher density of alfalfa plants in the L5 sampling area that could have acted as a buffer against climate fluctuations. In July 2013 all three these alfalfa samples showed a decline in diversity. This could once again be attributed to lower soil humidity (Table 4) concurrent with soil cool down later into the winter season (Table 2). Both these factors trigger stress in plants, which in turn could encourage allelopathy levels to rise, which may affect soil faunal diversity. As mentioned earlier stress on plants may enhance defensive and allelopathic abilities thus resulting in lower soil arthropod diversity. The soil fauna, however, were also exposed to the same stress as the plant, in July 2013 these stresses was lower soil humidity and a colder climate.

The L5 sample had the most drastic decline in soil diversity of all three alfalfa samples and this strongly reflected in the general soil meso-arthropod diversity indices. In August 2013 both the stressed fields showed an increase in diversity that yet again could be accredited to the lower stress in plants based on an improvement of conditions (Figure 5). The unstressed sampling area (L6) showed a decrease in soil meso-arthropod diversity and this occurrence did not correlate with either humidity or compaction data (Tables 3 and 4). This decrease in diversity was detected as an overall decline in all groups making up the meso-arthropod community. Only 24 individuals of seven morpho species were sampled. A high level of SOM scarcity may explain this atypical situation. As previously discussed, soil fauna tend to aggregate and form "hot spots" of increased activity and abundance. The abundance of food resources could play a role in this aggregation behaviour explaining the decrease in

diversity at this sampling site (L6). In September 2013 the field was harvested again and only the one stressed sample (L4) responded negatively post-harvest. A response delay to harvest disturbance could be seen in the second stressed sample (L5) and the unstressed sample (L6) in October 2013 (Figure 5). By October 2013 the L4 sample already showed signs of recovery to the disturbance, since an increased H' value occurred. The H' values of the control field (Figures 4, 5 and 6) were 1.5 and higher for the entire study. For the largest part of the study in all three fields (Field 1, 2 & 3), the H' values of the control field was higher than the alfalfa field. This supports the first hypothesis that natural undisturbed soils could sustain a higher diversity of soil meso-arthropods. This phenomenon could be seen in especially Field 3 (Figure 5) were agricultural soil disturbance was most recent. The control field (Figure 3, 4 and 5) also showed high evenness throughout the study and, supported by diversity indices, therefore reflects a more stable community make-up.

Field 3 in the alfalfa field (Bainsvlei) laid fallow for six months prior to planting in May 2013 and was tilled and prepared for planting during this period. The H' values of all three samples (L8, L9 and L10) in Field 3 (Figure 6) for April 2013 were above 1. This is a high diversity considering the frequent disturbances and absence of vegetation in this field for several months. The soil humidity (Table 3) was high in this field for all three samples from April 2013 to July 2013. The high diversity in April 2013 could be explained by the fact that there was still plant debris (the source of SOM) in this field and the soil humidity was high. In May 2013, Field 3 was planted with alfalfa seedlings, but during the May 2013 sampling date there were no seedlings visible. The diversity of all three samples (L8, L9 & L10, Figure 6) declined quite dramatically towards May 2013 which could be attributed to the recent disturbance during this time when the seedlings were planted. Albeit that the humidity for this date was high for all three samples, soil disturbance impacts apparently had a bigger affect on the soil meso-arthropod diversity. Much research has been done over the past 70 years on the effect of allelopathic compounds on germination of receiver plants. But the effect of germination of allelopathic plants on surrounding plants, and for that matter associated soil organisms, have not been thoroughly studied, if at all (Bazzaz 1979, Bogatek *et al*. 2006).

Germination of the seeds is one of the most important phases of plant growth and it makes sense that seeds could be allelopathic while germinating to promote their survival success. It could thus be possible that germination of seeds might have influenced soil diversity in May 2013 in all three samples (L8, L9 and L10, Figure 6).

A slight increase in diversity could be seen in June 2013 in two of the three samples (L9 and L10, Figure 6) and one possible explanation for this could be population recovery or succession. Lopez-Lozano *et al.* (2013) conducted a field trail with microcosms to determine successional patterns of microbes in disturbed soils compared to undisturbed soil. Lopez-Lozano *et al.* (2013) suggest that succession in soils starts off with initial rapidly growing communities that are generalist feeders who are thereafter out-competed by specialist's communities. Their study was done over a longer period of time compared to this study and they found that after a disturbance the diversity (Shannons Index / H' value) increased. This was followed by a decrease in diversity at nine months and once again an increase at 12 months. They also found that the communities in the disturbed field did not recover to consist of the same composition as the undisturbed area within 12 months. They thus concluded that 12 months after a single soil disturbance was not enough time for soil communities to recover to the condition prior to disturbance. In this study, the same pattern of succession was recorded, albeit over a much shorter period of time.

Excluding other variables, such as climate, humidity and compaction, an initial decline in diversity after soil disturbance in Field 3 (L8, L9 and L10) between April 2013 and May 2013 was recorded. This correlated with the results reported by Lopez-Lozano *et al.* (2013). Samples L9 and L10 showed an increase in diversity towards June 2013 (the same increase prior to month nine in Lopez-Lozano *et al.* (2013)) and thereafter a sudden decrease of L8, L9 and L10 in July 2013 (diversity at nine months by Lopez-Lozano *et al.* (2013)), followed once again by an increase in diversity for the rest of the study (diversity at 12 months by Lopez-Lozano *et al.* (2013)). This study was undertaken over seven months, but instead of the diversity constantly increasing as with

the Lopez-Lozano *et al.* (2013) study, the same fluctuations in diversity over seven months was recorded that they experienced over 12 months.

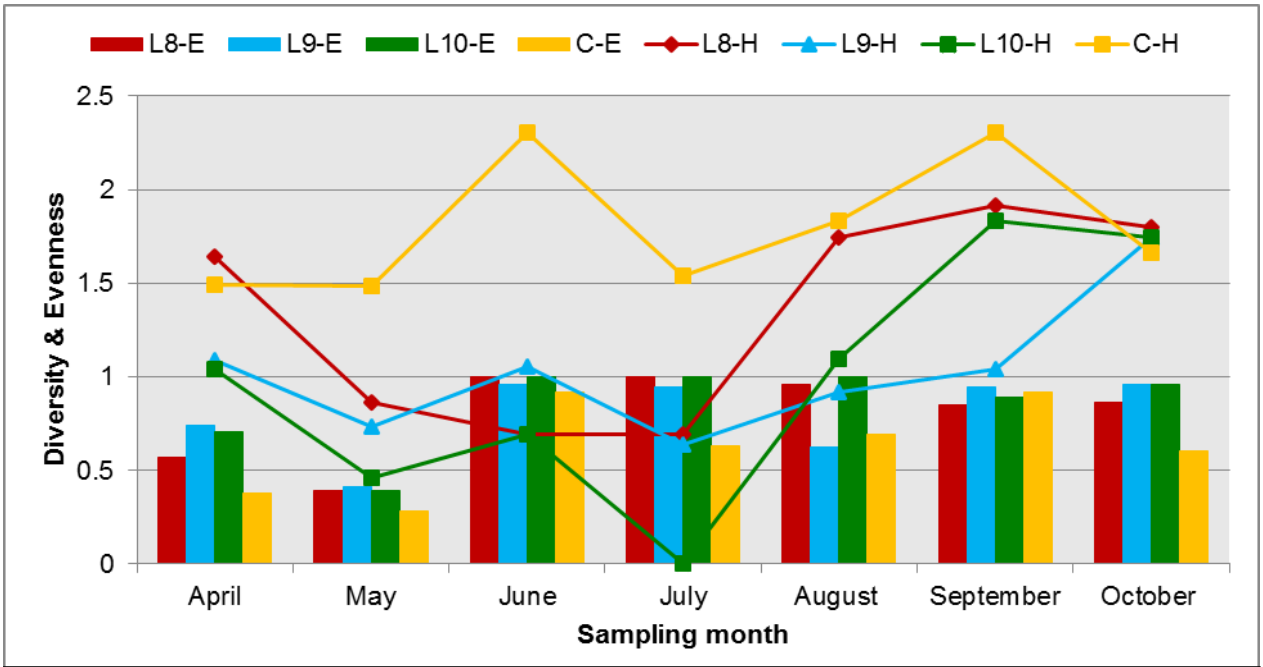

Figure 6: Shannon's index for diversity and evenness of soil meso-arthropod in alfalfa Field 3 and control fields in the Bainsvlei area outside of Bloemfontein over a period of seven months in 2013. Evenness (E) is represented by bars and the diversity (H) is represented by graph lines. L8, L9 and L10 are samples taken in the alfalfa field and C is the control.

The negative response of soil meso-arthropods diversity to harvest was not as drastic in Field 3 (Figure 6) as in Field 1 (Figure 4) and Field 2 (Figure 5). That said, it is possible that populations could not establish completely causing it to look like no apparent effects was caused by disturbance. This could be explained on the basis of repeated disturbance and unfavourable conditions in Field 3 over the previous 13 months. These unfavourable conditions included the fallow field conditions for 6 months where no vegetation was present, followed by soil preparations, fertilizers and soil additives, followed by planting of allelopathic alfalfa. After all these agricultural activities ecological succession could follow its normal course. According to Lopez-Lozano *et al.* (2013), succession of disturbed soil could take longer than 12 months and the field was harvested at 6 months after planting. This was once again an agricultural disturbance and although soil was not directly disturbed, soil micro-climate and soil properties

(chemical compositions which could have changed due to allelopathic properties in alfalfa plants) was altered. Disturbance was just so constant that normal ecological packing in the succession process could not have occurred. The E values in Field 3 were very high throughout the study except for May 2013, immediately after the alfalfa seeds were planted. The high E values could be contributed to the fact that there was low richness and abundance during this particular survey. The findings in May 2013 could once again be attributed to the possible negative or allelopathic effect of germinating alfalfa plants.

The data of all the soil meso-arthropods sampled during the seven months was lumped to compile the soil trophic structure for Field 1 (Figure 7). In all four samples of Field 1 (L1, L2, L3 and C) mycophages made up the largest portion of all the soil fauna. Mycophages were more abundant in the alfalfa field and this might be due to the higher humidity and continued availability of dead plant material post-harvest. They mainly consist of Collembola and Acari. Mycophagous Acari included: *Brachytydeus*, *Eupodus*, *Hemitarsonemus*, *Tyrophagus putrescentiae*, *Pronematus*, *Nanorchestes*, *Rhizoglyphus*, *Hypozetes*, *Oppiella*, *Oribatula*, *Epilohmannia*, *Galumna* species and Oribatidae immatures. *Bachytydeus*, *Eupodes* and *Pronematus* species made up the largest percentage of all the mycophages recorded in Field 1, as well as in the control (Table 6). *Pronematus* sp. 1 was the most abundant of all the mite species and made up 60% of all the mycophages in samples of Field 1 and 45 % in the control sample (Table 6). No association could be made with certain mycophagous Acari species being associated with stressed or unstressed alfalfa plants.

The second most dominant trophic group in all four samples (L1, L2, L3 and C) was the predators which included numerous Acari species, of which *Protogamasellus* was the most abundant. Other predators included Staphylinidae beetles and spiders. *Protogamasellus* was the most abundant in the stressed sample (L3) and made up 86% of the predators found in these samples (Addendum 1).

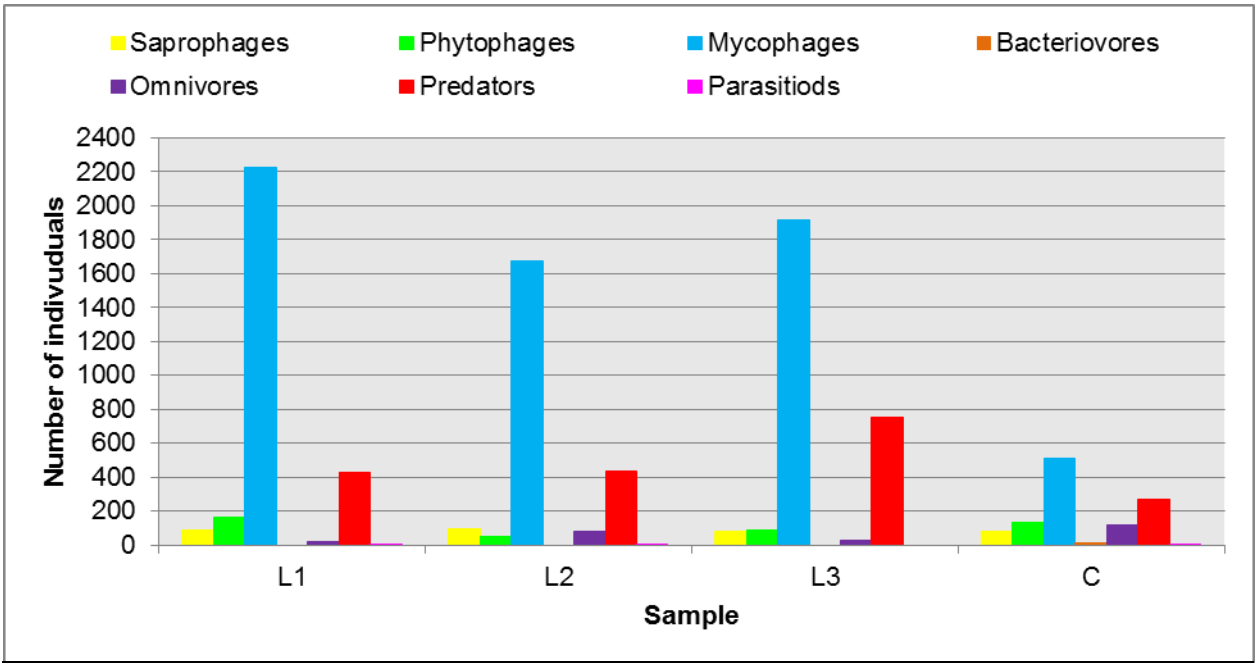

Figure 7: Mesofaunal trophic structure of Field 1 (L1 – L3) and Control (C) in the Bainsvlei area outside of Bloemfontein from February 2013 to August 2013 (L1: non-stressed plants, L2: non-stressed plants, L3: stressed plants and C: control).

Table 6: The dominant mycophagous mite species expressed as % of total found in Field 1 and the control in the four samples taken in the Bainsvlei area outside of Bloemfontein from February 2013 to August 2013 (L1: non-stressed plants, L2: non-stressed plants, L3: stressed plants and C: control).

	L1	**L2**	**L3**	**C**
***Brachytydeus* sp.1**	28%	<1%	11%	<1%
***Eupodes* sp.1**	14%	10%	24%	13%
***Pronematus* sp.1**	29%	60%	28%	45%

This Acari species also made up the largest proportion of the predators found in the other three samples, but was not as abundant as in sample L3. The high numbers of this *Protogamasellus* species may also explain the plant condition that was classified as stressed. By comparing predators between sample L3 and the other alfalfa samples (L1 and L2) it is clear that *Protogamasellus* was not directly linked to mycophagous prey. According to Emmerson and Raffaelli (2004), in some cases the presence of predators could be associated with the body size of the predator in relation to the body

size of available prey. *Protogamasellus* is much smaller in body size than most of the above-mentioned mycophagous Acari and it is most unlikely that the high quantity of *Protogamasellus* could be attributed to the high numbers of mycophages. The Tullgren extraction method does not extract soil nematodes, but *Protogamasellus* is a small predator that feeds on micro-arthropods and nematodes. The plants in the L3 sample could possibly also be stressed due to high numbers of phytophagous nematodes which explains the abundance of *Protogamasellus* species. However, this explanation for the high abundance of *Protogamasellus* could not be confirmed because of the extraction method used.

Omnivores only comprised of Formicidae (eight morpho species) and were the most abundant in the control field. This could be explained in the context of soil disturbance and the fact that ants showed preference to building nests and becoming established in areas with a low degree of disturbance. Phytophages were the most abundant in sample L1, but the fauna that represents phytophages were not mostly permanent soil residents and only found refuge in the soil environment during metamorphosis. Other trophic groups included saprophages that were made up out of numerous larvae and a few beetles. No clear difference between the four samples was observed for this feeding group. Bacteriovores and parasitoids were not found in large numbers in any of the samples. Overall the control sample showed the most even spread of trophic groups within the Field 1 trial.

Similar to Field 1, the data of all the soil meso-arthropods sampled for Field 2 over the nine months was lumped to compile the soil trophic structure (Figure 8). As with Field 1, mycophages were also the most abundant trophic group in all four samples (L4, L5, L6 and C). The control field (C) had the least mycophages and this might be due to the lower soil humidity and higher compaction factors (Tables 3 and 4). Mycophagous arthropods mostly consisted of Collembola and Acari. In Field 2, the mycophages were made up by less species than in Field 1. In Field 1, 13 species of mycophagous Acari were present and in Field 2 only eight (Addendum 1). Species of the following genera were recorded in Field 2: *Oppiella*, *Bachytydeus*, *Eupodes*,

Hemitarsonemus, *Pronematus*, *Nanorchestes* and *Rhizoglyphus*. Here the *Rhizoglyphus* species was the most dominant mycophagous mite in all four samples throughout the study. The *Bachytydeus* species was the second most dominant in all four samples. The reason for the high number in mycophages could once again probably be atributed to beneficial circumstances, especially high quantities of soil organic matter (SOM) post-harvest. All three the alfalfa fields were under irrigation and the high humidity in these fields, together with the availibitity of SOM could have provided the ideal conditions for fungal growth, hence the high numbers of mycophages (Figures 7, 8 and 9). Compared to Field 1 bacteriovores were higher in Field 2 and reached the highest numbers in the L4 stressed sample (Figure 8).

Bacteriovores were represented by a single mite species, *i.e. Speleorchestes meyeri*. Bacteriovores were more abundant in Field 2, because this field was under irrigation throughout the nine months, compared to Field 1 that did not recieve any irrigation after May 2013. Field 2 was sampled up to the early summer months (October 2013) in contrast with Field 1, which was only sampled up until August 2013. The data also indicated that *S. meyeri* was more abundant after the winter. This could be because warmer months were more advantageous for bacterial growth and that growth would be slower in the colder winter months.

Predators were the most abundant in the L5 and L6 samples (Figure 8). As in Field 1, predators were dominated by *Protogamasellus* sp. 3 and was evenly made up by numerous Acari species (Addendum 1). These included the genera: *Protogamasellus* sp. 1 and 3, *Caeculus* sp. 1, *Cunaxa* sp.1 and 2, *Microthrombidium* sp. 1, *Typhlodromus* sp. 1*, Spinibdella* sp. 1, *Cheyletiella* sp. 1, *Anoplocheylus* sp. 1 and *Hypaspis* sp. 1 and some families (which could not be identified up to generic level) namely Bdellidae msp. 2 and 3 and Erythraeidae immature msp. 2. Discussing Figure 7, (Field 1, L3) it was argued that the high abundance of *Protogamasellus* sp. 3 could have been related to the high abundance of nematodes. A wide variety of nematodes in soil environments are free living and the high numbers of *Protogamasellus* could again possibly be explained on the basis of high nematode abundance.

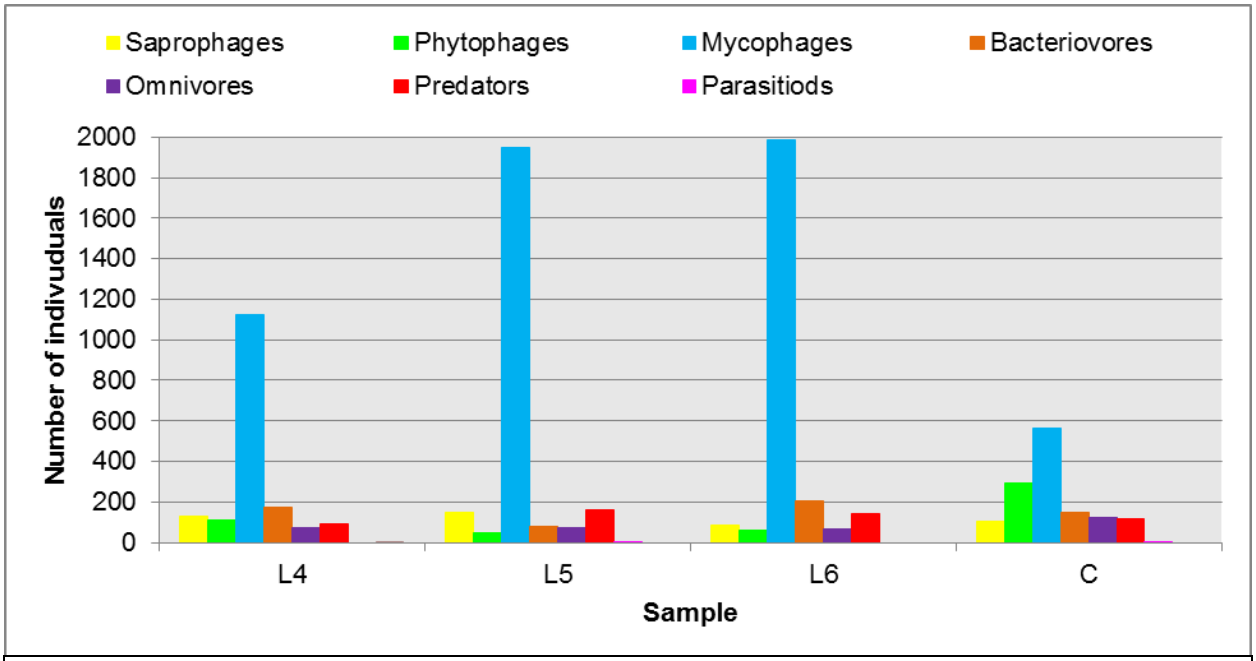

Figure 8: Mesofaunal trophic structure of Field 2 (L4 – L6) and Control (C) in the Bainsvlei area outside of Bloemfontein from February 2013 to October 2013 (L4: non-stressed plants, L5: non-stressed plants, L6: stressed plants and C: control).

Once again the omnivore trophic level was only represented by Formicidae (i.e. 15 morphospecies) and was the most abundant in the control sample. The difference in Formicidae abundance between the natural and cultivated field, was not as clear as that of Field 1, which is ascribed to the fact that Field 2 did not receive any soil preparation or disturbance interference. Once again saprophages were very low in numbers and no substantial difference was observed in quantities between the four samples. Phytophages were mostly made up of non-permanent soil residents and was the most abundant in the natural field.

The data of all the soil meso-arthropods sampled in the seven months was again lumped to compile the soil trophic structure for Field 3 (Figure 9). Field 3 had extreme disturbances prior to the first sampling date, as well as in April 2013 when the alfalfa was planted and also when the field was harvested in September 2013. Compared to the other two fields (Figures 7 and 8), Field 3 had a clearly lower abundance of all individuals. According to Kardol *et al.* (2009), succession can be described as

organisms striving to repopulate an area to the level of diversity prior to disturbance. When an area is constantly disturbed, as with Field 3, normal species packing or succession cannot take place because organisms cannot establish long enough for successful reproduction.

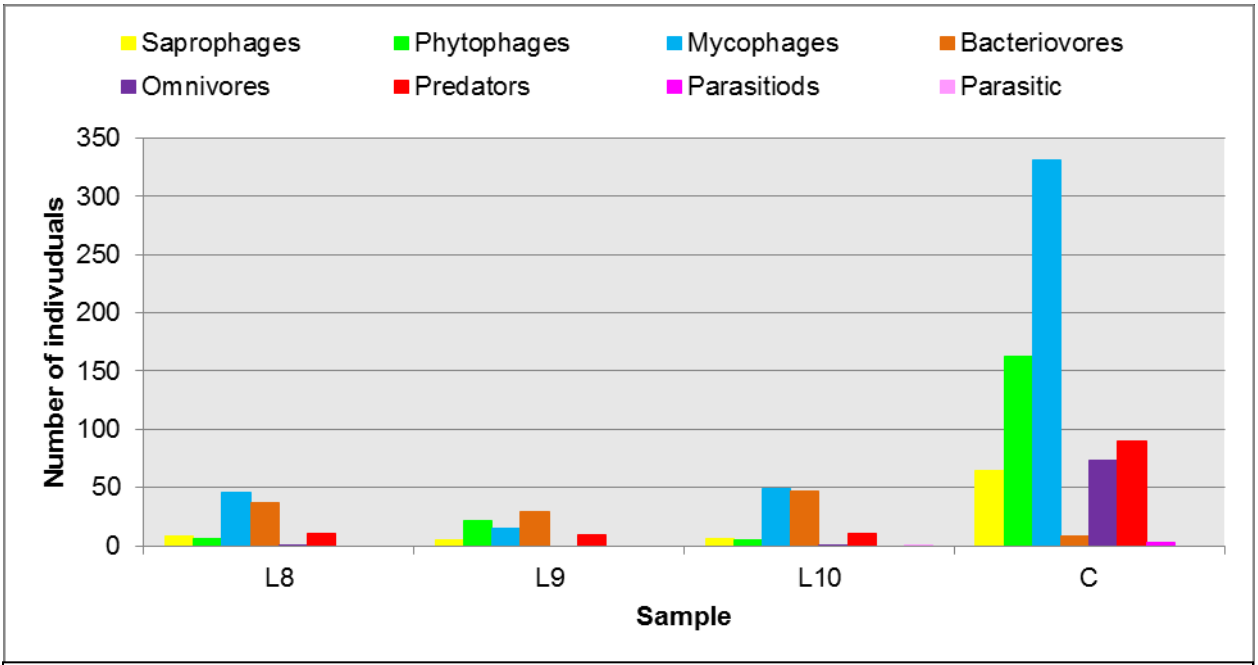

Figure 9: Mesofaunal trophic structure of Field 3 (L8 – L10) and Control (C) in the Bainsvlei area outside of Bloemfontein from April 2013 to October 2013 (L8, L9 & L10: alfalfa field and C: control).

Except for sample L9, mycophages was once more the most dominant feeding group in Field 3 (Figure 9). The reason for the decreased number of mycophages in the L9 sample could possibly be related to the establishment of fungi in this young field. Similar to Field 1 and 2 (Figures 7 and 8), mycophages were mostly made up of Collembola and Acari. Mycophagous mites included species in the genera: *Oppiella*, *Eupodes*, *Hemitarsonemus*, *Tyrophagus putrescentiae*, *Pronematus*, *Imparipes*, *Oribatula, Nanorchestes* and *Galumna*. Similar to Field 1 (Figure 7), *Pronematus* was the most abundant mycophage. The most abundant mycophagous Collembola were two morphological species of *Cryptopygus* sp. 1 and 2. Both these species were

present in very high numbers in Fields 1 and 2 (Figures 7 and 8), but although also dominant in Field 3 (Figure 9), they occurred in lower numbers.

The second most abundant trophic group in alfalfa Field 3 (Figure 9) was bacteriovores that was only made up by *S. meyeri*. Their presence in the newly cultivated field could be due to the high humidity throughout the sampling period (Table 4). Damp environments create the perfect conditions for most bacteria and fungi species to survive. Explaining why the mycophages and bacteriovores in combination were the most dominant in alfalfa Field 3, albeit in relatively low numbers (Figure 9).

Mycophages were the most abundant in the control which was taken in a more established environment, but bacteriovores were not as dramatically abundant as in the alfalfa field (Figure 9). The absence of bacteriovores in the control sample can be attributed to a lack of soil moisture (Figure 9). Predators and omnivores were not as numerous in the alfalfa field as in the control field. This could be explained on the basis of extensive soil disturbances prior to planting. Saprophages, phytophages and parasitoids were more abundant in the control field (Figure 9). Even though the control fields were drier and more compact than the cultivated field it supported a greater diversity of trophic levels. This once again confirms our hypothesis that undisturbed natural ecosystems, whether containing allelopathic plants or not, will support a greater diversity of soil fauna than cultivated (disturbed) fields.

3.3.2. Tornberry farm (Sunflower)

The Shannon's index for diversity (H') and evenness (E) was also used to determine the difference in diversity between the three sections within the sunflower field (S1, S2 and S3). The Shannon's index of diversity H' and evenness E for Section 1, 2 and 3 (S1♀ and S1♂, S2♀ and S2♂, S3♀ and S3♂), the control (C) and the butternut sample (BN) can be seen in Figures 10, 12 and 13. Corresponding soil humidity (Table 7) and compaction levels (Table 8) for the Thornberry farm over the five month sampling period are also provided.

The H' values of both the sunflower samples for Section 1 (S1♀ and S1♂) and Section 2 (S2♀ and S2♂) started off high in January 2013 (Figures 10, 12 and 13). The sunflower seedlings were still very small in January 2013, with the male plants much smaller than the female plants (Figure 11). The reason for this was poor germination of male plants during the first planting and they were replanted two weeks before sampling.

As mentioned S1♂ and S2♂ (Figures 10 and 12) had a surprisingly high diversity in January 2013, taking into consideration that the sunflower seeds were replanted and that soil disturbances were intense as recent as 4 weeks prior to sampling. Soil humidity was high in February 2013 and soil compaction was low. These favourable conditions, together with the additives of microbes and animal manures applied to the soil prior to planting, could possibly be responsible for the high H value in these samples. The H value for both Section 1 and 2 (S1♀ and S1♂, S2♀ and S2♂) declined dramatically in February 2013 (Figures 10, 12 and 13). This was not the case for Section 3 (S3♀ and S3♂), the control (C) or the butternut (BN) samples which relates to the physical nature of the first two sections of the sunflower field (Figure 13). This decline in H values does not correlate with soil humidity or compaction data. The soil humidity for February 2013 was slightly higher and the compaction lower.

In February 2013 the sunflower field was already in bloom. This suggests that the plants were directing all of their energy into growth and the decline in diversity in Section 1 and 2 (Figures 10 and 12) could not have any correlation to allelopathy of sunflower plants. Sunflowers are well known as a soil nutrient depleting crop that puts heavy demands on soil nutrient stores, especially N and P (Reddy *et al.* 2005, Krishna 2010).

Table 7: Average % soil humidity of each sample in three sampling sections (S1♀, S1♂, S2♀, S2♂, S3♀, S3♂: sunflower samples in three sections taken at male and female plants; BN:

butternut field; C: control field on Thornberry farm over a period of five months in 2013.					
	Jan 2013	**Feb 2013**	**Apr 2013**	**May 2013**	**Jun 2013**
S1♀	14.8	18.2	13.4	11.4	3.4
S1♂	16.2	29.8	22.7	19.2	3.6
S2♀	13.4	15.2	16.7	5.1	1.3
S2♂	15.6	16.6	20.5	13.1	1.1
S3♀	20.7	19.6	24.9	12.7	5.3
S3♂	21.3	34.1	20.1	7.7	4.6
BN	19.8	21.1	24.6	44.6	9.9
C	11.3	10.5	16.3	14.3	0.3

Because male plants developed slowly, growth hormones was used to boost their growth since both male and female plants needed to reach maturity at the same time to ensure cross pollination. A possible reason for the decline in H' value for Section 1 and 2 (Figures 10 and 12) could be that the soil was depleted of nutrients during this rapid growth period. Section 1 and 2 both had lower soil clay content than Section 3. According to Waugh (2002), soils with higher clay content can retain water and nutrients for a longer period of time. Furthermore, Section 3 was situated slightly downhill from Section 1 and 2 and nutrients that leached out from these sections could have accumulated in Section 3 explaining the higher stability in H value (Figure 13). This leaching out of nutrients together with the high nutrient demand during this period, could have had a great impact on soil pH levels. According to Bryant (1986), soil fauna are extremely sensitive to changes in soil pH. Because of the high nutrient demand and the leaching out of nutrients downhill in the sunflower field, the soil pH could have been altered, also explaining lower H' values for S1 and S2 during this period (Figures 10 and 12)

Table 8: Average psi value of soil compaction and depth of compaction layer (in inches) of each sample in three sampling sections (S1♀, S1♂, S2♀, S2♂, S3♀, S3♂: sunflower samples in three

sections taken at male and female plants; BN: butternut field; C: control field on Thornberry farm over a period of five months in 2013.					
	Jan 2013	**Feb 2013**	**Apr 2013**	**May 2013**	**Jun 2103**
SF♀	200,6	100,6	150,1	200,1	200,1
SF♂	200,5	100,1	200,3	200,1	200,1
SF♀	200,2	100,2	150,1	200,2	200,1
SF♂	200,6	100,1	200,2	200,2	200,1
SF♀	200,5	100,1	150,2	200,2	200,1
SF♂	200,6	100,6	200,1	200,2	200,1
BN	200,6	100,5	150, 5	150, 5	200,5
C	200,0	200,1	200,0	200,0	200,0

In April 2013 all three sections of the sunflower field (Figures 10, 12 and 13) showed an increase in H'. This could possibly be due to the pollen and other plant material that has fallen to the ground increasing SOM which lead to an increase in soil faunal activity. The rows where male plants were present was ripped in April 2013, since their seeds were not harvested and by this time all the sunflower plants had started to dry out. At the beginning of May 2013 the female sunflower seeds were harvested and all plant material was worked into the soil. The only sample that reacted to this disturbance was S3♂, presumably due to severely hardened soil in Section 3, coupled to harvesting disturbance and soil management activities.

All the H' values of the sunflower samples, except for S3♂, decreased towards June 2013 (Figures 10, 12 and 13) when the soil was disturbed repeatedly by tillage. Soil humidity (Table 7) decreased and soil compaction increased (Table 8) towards June 2013. This reversed reaction of the S3♂ sample may be due to allelopathic compounds being released during decomposition.

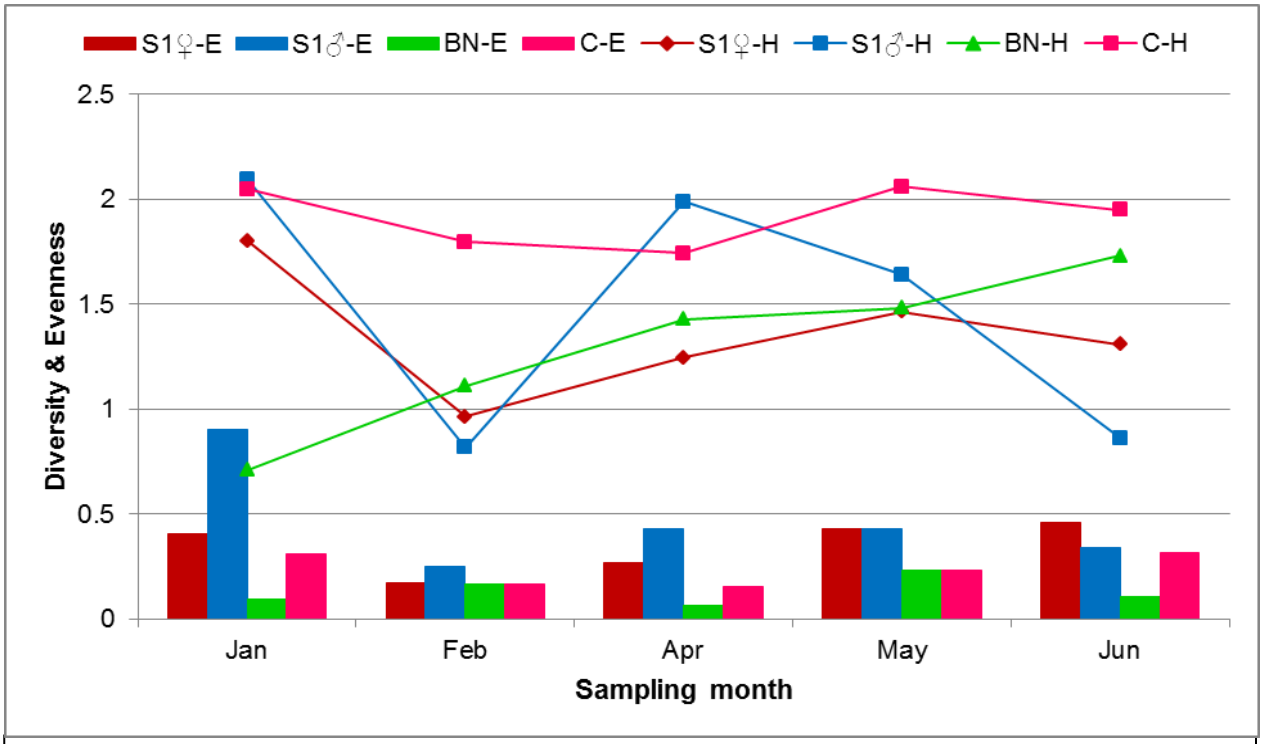

Figure 10: Shannon's index for diversity and evenness of soil meso-arthropod in the sunflower field Section 1, and control sites on Thornberry farm over a period of five months in 2013. Evenness (E) is represented by bars and the diversity (H) is represented by graph lines. ♀: samples at female plants, ♂: samples taken at male plants, BN: butternut field soil samples and C: control.

Figure 11: The size of male (left) and female (right) sunflower plants in January 2013 on Thornberry farm (Free State).

Section 3 were situated on the downside of a slope, leading to a possible higher concentration of allelopathic compounds. Allelopathic compounds could have accumulated here and became fixed in the clay soil. It would make sense that the mesofauna of the S3♂ sample of Section 3 (Figure 13) declined in this matter and that the S3♀ only responded later since the male plants were tilled into the soil almost a month before the soil of the female plants was tilled. The butternut field (Figures 10, 12 and 13) started off with a low H' value and kept increasing even after harvest in May 2013. The variation in soil type in this field was less than in the sunflower field and it would seem that soil humidity played a larger role in sustaining soil arthropod diversity as the field was moist throughout the sampling period. The control field did also not experience dramatic changes in H' value and remained pretty much constant throughout the sampling period (Figures 10, 12 and 13).

The E values of both male and female plants in Section 1, 2 and 3 (Figures 10, 12 and 13) was overall the highest for the entire sampling period (from Jan 2103 to Jun 2013). This increase in E values can also be connected to high soil humidity (Table 6) and low soil compaction (Table 8) favouring soil arthropod activities. The butternut field which also showed high soil humidity and low compaction (Tables 7 and 8), had a lower E value than the sunflower field, but it was still higher than the natural field (Figures 10, 12 and 13). The natural field had the lowest soil humidity and the highest compaction off all the sampling fields which explains the lower E value throughout the survey (Figures 10, 12 and 13).

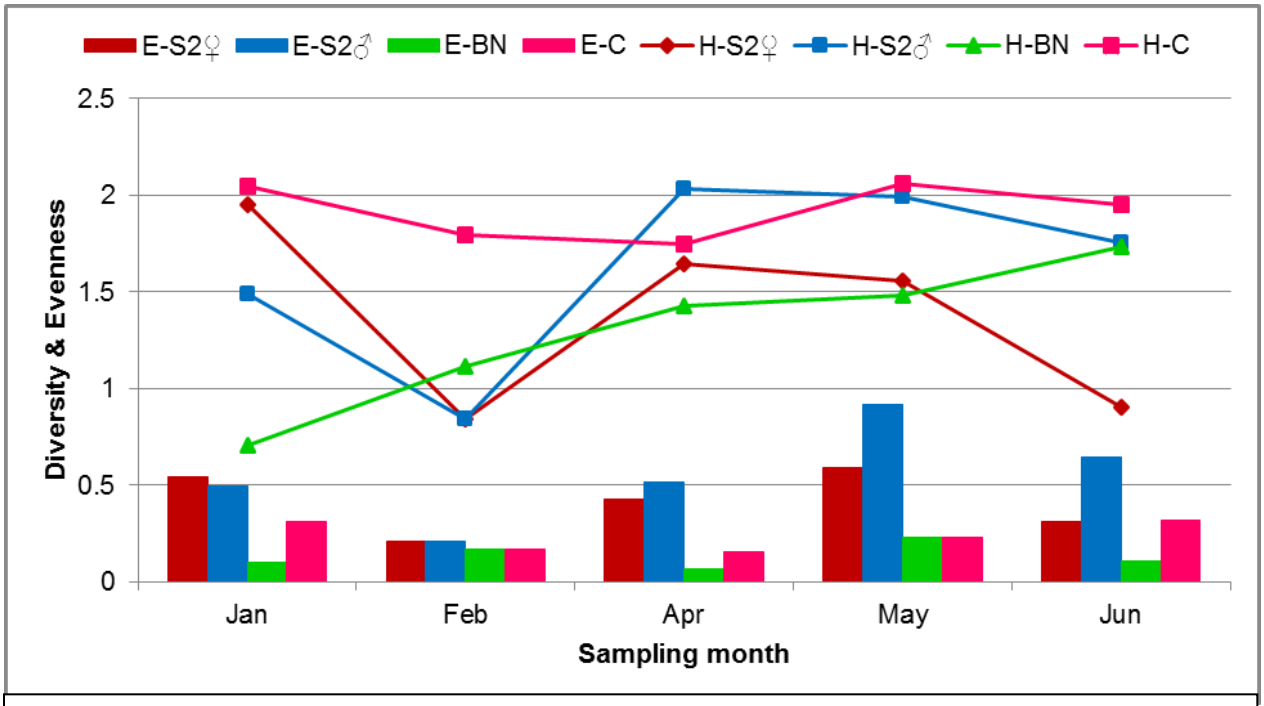

Figure 12: Shannon's index for diversity and evenness of soil meso-arthropod in the sunflower field Section 2, and control sites on Thornberry farm over a period of five months in 2013. Evenness (E) is represented by bars and the diversity (H) is represented by graph lines. ♀: samples at female plants, ♂: samples taken at male plants, BN: butternut field soil samples and C: control.

The data of all the soil meso-arthropods sampled in the eight months was lumped to compile the soil trophic structure for Thornberry farm (Figure 14). Similar to the Bainsvlei area (Figures 7, 8 and 9), the mycophages were the most abundant in all the samples. Yet again, Acari and Collembola made up the largest proportion of mycophages. Acari were represented by 25 species and Collembola by nine species (Addendum 1). Compared to Bainsvlei Field 1 and 2 (Figures 7 and 8), the mycophages were not as numerous in the allelopathic sunflower field (Figure 14).

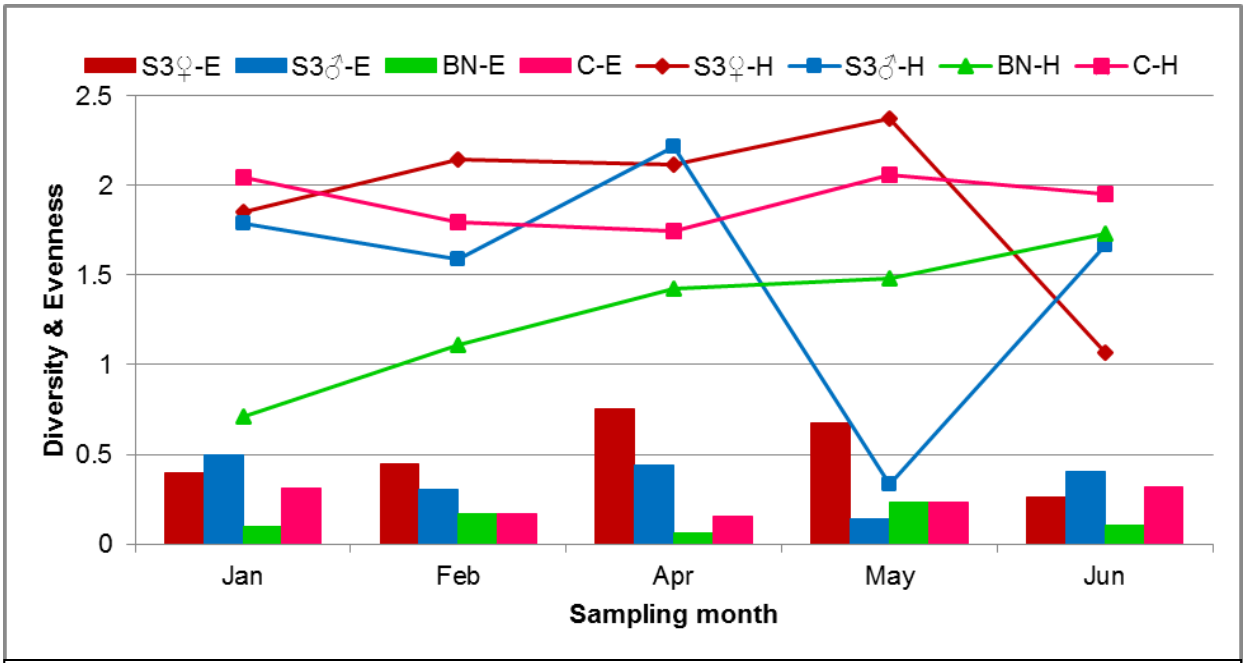

Figure 13: Shannon's index for diversity and evenness of soil meso-arthropod in the sunflower field Section 3, and control sites on Thornberry farm over a period of five months in 2013. Evenness (E) is represented by bars and the diversity (H) is represented by graph lines. ♀: samples at female plants, ♂: samples taken at male plants, BN: butternut field soil samples and C: control.

Mycophages were the most abundant in the butternut field (BN), followed by the control (C) and thereafter the samples taken in the sunflower field (Figure 14). In the butternut field (BN) the Oribatida had the highest adundance, with *Oribatula* (adults and nymphs) the most abundant followed by *Acrotritia* and *Oppiella*. The collembolan *Bourletiella* also occurred in high abundance, but was not as abundant as the abovementioned oribatids. The control samples had a higher abundance of mycophagous, Collembola and the mites were not as abundant as in the butternut field. Mycophagous Collembola found in C, included Folsomia, *Cryptopygus,* and *Bourletiella* species in that order of abundance. Acari found in C were also dominated by Oribatida mites, but only one species of *Oppiella* was found in great abundance.

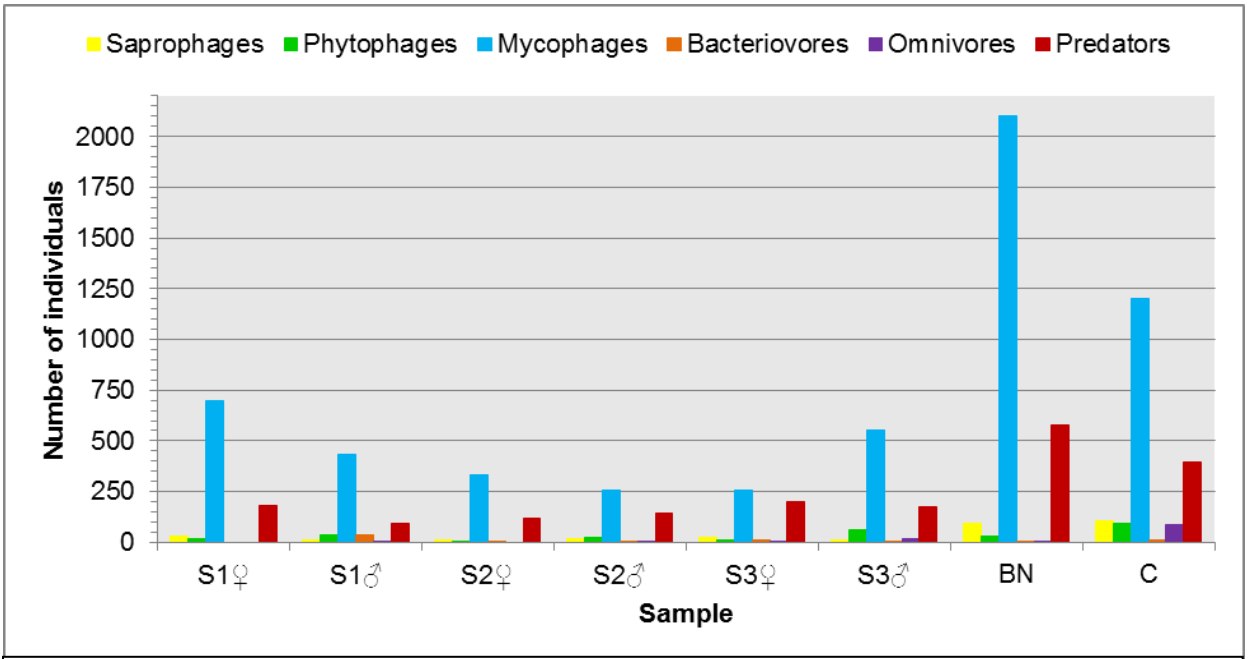

Figure 14: Trophic structure of samples in the sunflower field on Thornberry farm from January to June 2013. S1♀ & S1♂: samples taken at female and male plants in Section 1; S2♀ & S2♂: samples taken at female and male plants in Section 2 ;S3♀ & S3♂: samples taken at female and male plants in Section 3; BN: samples taken in the butternut field and C: samples taken in the control field.

The sunflower field showed high abundance in Acari and here the Collembola were not as abundant as in BN and C. It seemed that Sections 1 (S1♀ and S1♂) and 3 (S3♀ and S3♂) showed a strong similarity in terms of dominant Acari. *Oppiella* (Oribatida) and a species of *Arotritia* (Oribatida) was both found in high abundance in both Section 1 and 3, while an *Oribatula* species (adults and nymphs) was abundant in all three sections of the sunflower field. The most abundant mite species in Section 2 (S2♀ and S2♂) was *Oppiella* (Oribatida). This difference of species within the sunflower field could be attributed to soil type since Section 2 (S2♀ and S2♂) had red sandy soil. Sandy soils tend to dry out much faster than both clay and loam soil. The *Oppiella* species appears to be a generalist and occurred in high abundance in the control field that was overall drier (Table 7) than the cultivated fields.

The second most abundant trophic group was predators which are mostly generalist feeders on a wide variety of other soil animals. All predators were found in

more or less even abundance throughout all the different samples. Predatory insects included: Coleoptera larvae, Carabidae, Staphylinidae and Silvanidae species. A greater diversity of predatory mite species was found in all the samples and included: *Macrocheles, Gamasellevans, Saxidromes, Lealaps, Anystis, Microtrombidium,* 3 morpho-species of *Protogamasellus,* two morpho-species of *Cunaxa* and two morpho-species of *Spinbdella.* Predatory mites also included several species in the Rhodacaridae, Ascidae, Caeculidae, Anystidae, Bdellidae and Erythaeidae that could not be identified to generic level (Addendum 1).

Saprophages was the most abundant in the BN and C samples and mostly included Diptera and Coleoptera larvae (Addendum 1). Since these organisms only complete a part of their life cycle in soil, it would make sense that they would be most abundant in the control fields. These soils were subjected to lower disturbances such as tillage and soil applications as well as natural population of more diverse plant populations. The high abundance in the BN field could possibly be explained by the less intense soil preparations together with higher plant diversity in this field. Omnivores were only represented by Formicidae and a total of four morpho-species was present, most of them occurring in C. This could once again be explained by the degree of soil preparation in cultivated field that prevents ants from establishing properly. Phytophages was found in low abundance in all the fields, but were slightly more numerous in the S3♂, BN and C fields. The S3♂ field had many Thripidae that made up 95% of the phytophages in this field (Addendum 1). This high abundance was recorded during February 2013 and April 2013 when the crop was in bloom and the thrips were feeding on the pollen. The reason for the low number of thrips in the other sunflower samples could be attributed to aggregation and preference of these organisms in the most favourable microhabitat. Bacteriovores occurred in low abundance in all the samples and were represented by only one Acari species, *S. meyeri.*

3.4. Conclusion

Overall the results confirmed the first hypothesis in that natural veld will sustain a higher diversity of soil meso-arthropods than the alfalfa field. This was the case in both the Bainsvlei and Petrusburg localities. Soil meso-arthropods were more abundant in the cultivated alfalfa fields (Bainsvlei), but they harboured lower species richness than the natural veld. This was not the case in the sunflowers of Thornberry farm samples suggesting that environmentally friendly farming practices could improve overall diversity and evenness of soil fauna. Omnivores were less abundant in the cultivated fields of both localities due to soil disturbances that made it difficult for Formicidae to establish. Natural veld had a higher diversity of plants, less disturbance and constant vegetation coverage compared to the crop fields that underwent soil preparation, were under irrigation, contained a single plant species and, in some cases, were left fallow.

The second hypothesis stating that stressed allelopathic alfalfa plants would influence soil meso-arthropod diversity negatively was not confirmed. In some cases diversity was lower after mechanical disturbance (harvest), but this could also be due to soil desiccation and higher compaction. In most cases, however, it seemed that stressed plants had an overall lower diversity than non-stressed plants, but this could also be attributed to low soil humidity and exposure to external post-harvest factors. When considering trophic structure, it seemed that stressed plants had a lower number of individuals in all trophic groups and did not support all trophic levels as well as non-stressed plants. No dramatic differences were found in the mesofaunal diversity or evenness between male and female sunflower plants and it was clear that soil type rather than allelopathy played a role in species distribution and possibly aggregation.

Throughout this study mycophages were the most dominant feeding group and consisted mainly of Acari and Collembola. The dominant mite species differed between fields in both localities and considering the close proximity of the three Bainsvlei fields and the three Thornberry farm sections, this was an unusual occurrence. In the case of the Bainsvlei locality it could probably be explained on the basis of a combination of

subtle species preferenda, allelopathic potential of plants and soil disturbance factors and on Thornberry farm, on the basis of these factors, as well as differences in soil type. In addition soil compaction and humidity levels would have also played a role in soil arthropod diversity. However, in some Bainsvlei cases when humidity was high and compaction low, diversity was still low, which could be explained by the allelopathic ability of alfalfa plants and their allelopathic response to stress factors and mechanical injury, such as the harvesting procedure.

The third hypothesis could be discarded on the basis of increased allelopathy in seedlings of alflafa while germinating. In spite of actual soil disturbance prior to planting, diversity was the lowest during the seed germination and seedling period. This could be explained on the basis of a fight for survival in the presence of competition in the early stages of plant development. As the plants grew larger soil faunal diversity increased independent of soil humidity and compaction. Trophic group analysis revealed that predictions could be made on other groups such as nematodes not sampled in this survey. The weaker condition of the plants in Field 1 at Bainsvlei, could also be due to nematode root damage, if the high number of a *Protogamasellus* species, a predatory mite of nematodes, was taken into consideration. Both localities showed mycophages to be the most abundant. Fungi and other microbes could reach high numbers especially in soil environments that were buffered from extreme climatic changes and could stay humid for long periods of time. Judging from plant condition the soil microbes that were deemed to be present in large numbers were not harmful to plant growth, thus promoting overall crop health.

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CHAPTER 4

The influence of aromatic onion on soil mesofauna

4.1. Introduction

Allium cepa (onion) belongs to the plant family Amaryllidaceae that include many perennial and bulbous plant species (Block *et al.* 2011). This plant family is most commonly known for its pungent and irritating qualities due to electrophilic sulphur that is excreted when the plant is injured (Block *et al.* 2011). Recent studies have indicated that aromatic plants such as *A. cepa*, *A. sativum* (garlic) and various others possess insecticidal properties (Denloye 2010, Dauda *et al.* 2012, Meles *et al.* 2012, Mousa *et al.* 2013, Souguir *et al.* 2013). Onion, which is cultivated world-wide, is also known to possess medicinal properties (Kim 1997). Han *et al.* (2013), found that *A. sativum* has allelopathic potential in high concentrations. However, according to Han *et al.* (2013), it was also found that low concentrations of *A. sativum* extract can promote growth in lettuce shoots.

In cultivated fields of *A. cepa*, the concentrations of soil volatiles may become very high. According to Meles *et al.* (2012), aromatic plants, such as *A. sativum*, may influence the ability of insects to detect other food plants because of their pungent smell. The life style of soil fauna is generally strictly soil bound and despite constant changes in agricultural soils (due to various anthropogenic activities), this fauna is dependent on the particular cultivated crop as food source. Most species lack the mobile capabilities to search for alternative food sources. Meles *et al.* (2012), also states that aromatic plants act as insect repellents and are toxic in high concentrations. This explains why so few non-specialized organisms are found on these plants. These bio-insecticides and bio-repellents (natural plant products) are non-specific and can be harmful to beneficial organisms, as well as pest species.

According to D'Alessandro & Turlings (2006), plants can emit odours in their environment that are known as volatile organic compounds (VOC's). These VOC's can indicate a level of physiological or physical stress in plants and can alter the chemical composition of the soil and air surrounding the plant. Most of these VOC's prevent herbivory and oviposition of phytophagous insects. VOC's released above-ground are

diluted in the air and can drift away via wind currents. In soil, however, these compounds are largely locked in and thus alter the chemical composition of the soil. The levels and activity, of these compounds in the soil, is influenced by soil type as well as other biotic and abiotic factors (Wenke *et al.* 2010). Since soil fauna tend to occur in close proximity to plant roots (in order to utilize the plant itself), as well as the photosynthetically fixed carbon that plants excrete from their roots (Wenke *et al.* 2010), it seems obvious that these VOC's may have an influence on these organisms.

Bengtsson *et al.* (1991), established that Collembola can distinguish between a range of volatiles to detect fungal food sources in soil. Even though this is not the only stimuli used when searching for nutrition, it appears that olfaction is the dominant sensory method used in soil environments. Nilsson & Bengtsson (2004) found that Collembola also make use of olfaction to avoid predators. They react to the smell of dead or dying conspecifics and change their direction of movement based on the odour. Collembola, being one of the most dominant and important groups in soil ecosystems, are thus sensitive to odour cues and it is only logical that a sharp odour, such as that of the onion, will influence their presence in soils. Bucheli (2014) stated that even though these phytotoxins, produced by plants, may be more successful than synthetic pesticides in suppressing organism activity, the consequences on human and animal health are not yet known. It is thus important to study the influence of these chemicals in soil environments in order to determine their influence on soil fauna and in turn soil health.

The aim of this study was to determine whether soil fauna and mainly Collembola and Acari are influenced by *A. cepa* odours and phytotoxins in an agricultural onion field. Shannon's diversity and evenness index, as well as Sørensen's similarity index, will be applied to determine and compare the mesofauna of onion fields in two regions in terms of accompanying control veld.

4.2. Study layout

4.2.1. Study sites

The study was conducted in 2013 at two different localities (see Table 1 for a summary on study sites); the first was a small onion field in the Bainsvlei area outside Bloemfontein (Figure 1), accompanied by two nearby locations used as a control and the second was a large onion field (Thornberry farm) near Petrusburg (Figure 2), accompanied by three nearby control patches. The control veld used in this study, for the Bainsvlei area, are identical to the control veld used in Chapter 2 and 3 (see Figure 1 & 2, Chapter 3 and Figure 6 & 7 Chapters 2).

Table 1: A summary of the localities and sampling sites.

Sample	Town	Farm	Crop	Figure
U	BFN, Bainsvlei	Geluk	Onion	Figure 1
CL	BFN, Bainsvlei	Maranatha	Natural field	Figure 1, Chapter 3
CN	BFN, Bainsvlei	Geluk	Natural field	Figure 2, Chapter 3
CS	BFN, Bainsvlei	Geluk	Smuts finger and natural field	Figure 2, Chapter 3
U	Petrusburg	Thornberry	Onion	Figure 2
C1	Petrusburg	Thornberry	Natural field	Figure 2
C2	Petrusburg	Thornberry	Natural field	Figure 2
C3	Petrusburg	Thornberry	Natural field	Figure 2

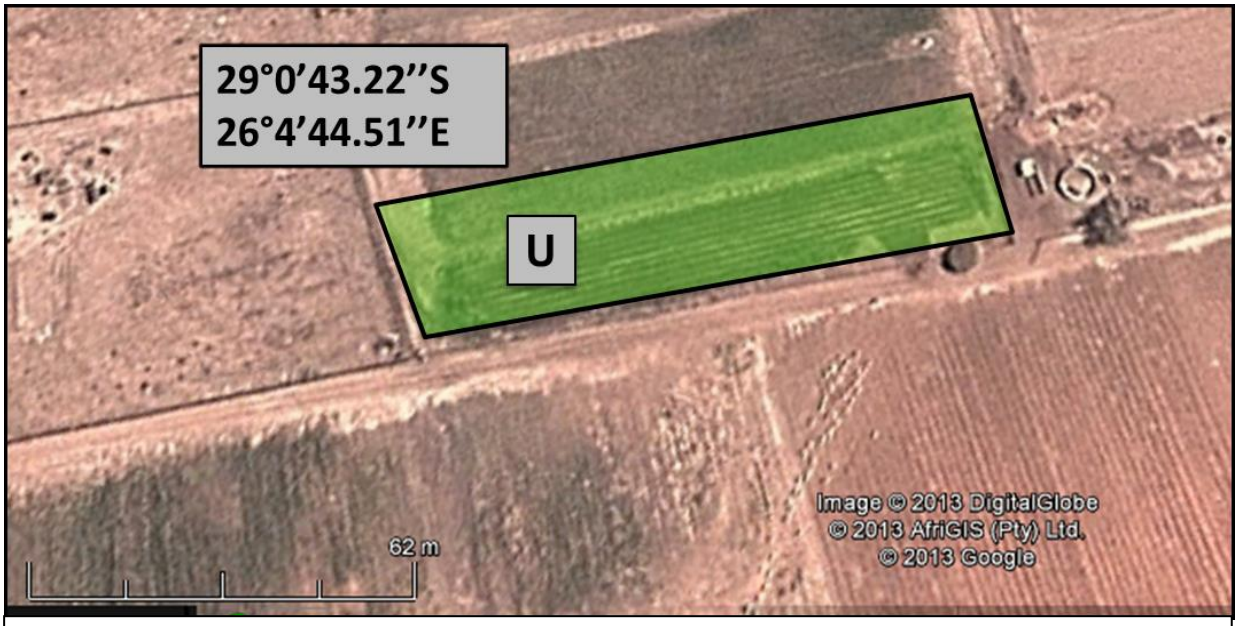

Figure 1: Geluk farm outside Bloemfontein, showing the onion field (U), (Image adapted from Google Earth).

The first site, used in this study, was on the farm Geluk (Figure 1) (29°0’43.22’’S, 26,4’44.51’’E) and consisted of a single small onion field, accompanied by two control areas. The first of these was also on the farm Geluk (see Chapter 3 & 2), (28°59’30.32”S, 26°05’47.58”E) and the second on the farm Maranatha (see Chapter 3 & 2), (29°01’36.31”S, 26°05’04.32”E). The Geluk onion field was planted with Texas Grano onion and was much smaller that the Thornberry field.

Thornberry farm contained a large onion field (Figure 2) planted with Shinju 200 This field was also accompanied by three control sites (C1, C2 & C3, Figure 2). Control number one (C1) was the furthest away from the onion field in a small patch of natural veld. This area was overgrown with short grass types with no trees. This control site mostly contained narrow-leaved turpentine grass (*Cymbopogon plurinodis*) and dropseed grass (*Sporobolus fimbriatus*), (Van Oudtshoorn 2012). The weed *Tribulus terrestris* was the most dominant other plant type in this area and covered most of the soil surface. The second control site (C2, Figure 2) was a pecan orchid that was rarely exposed to intensive agricultural disturbances. This area was predominantly populated by pecan trees and the soil below the trees was covered in leaf litter and plant debris.

However, narrow-leaved turpentine grass (*C. plurinodis*) and dropseed grass (*S. fimbriatus*) occurred in patches between trees.

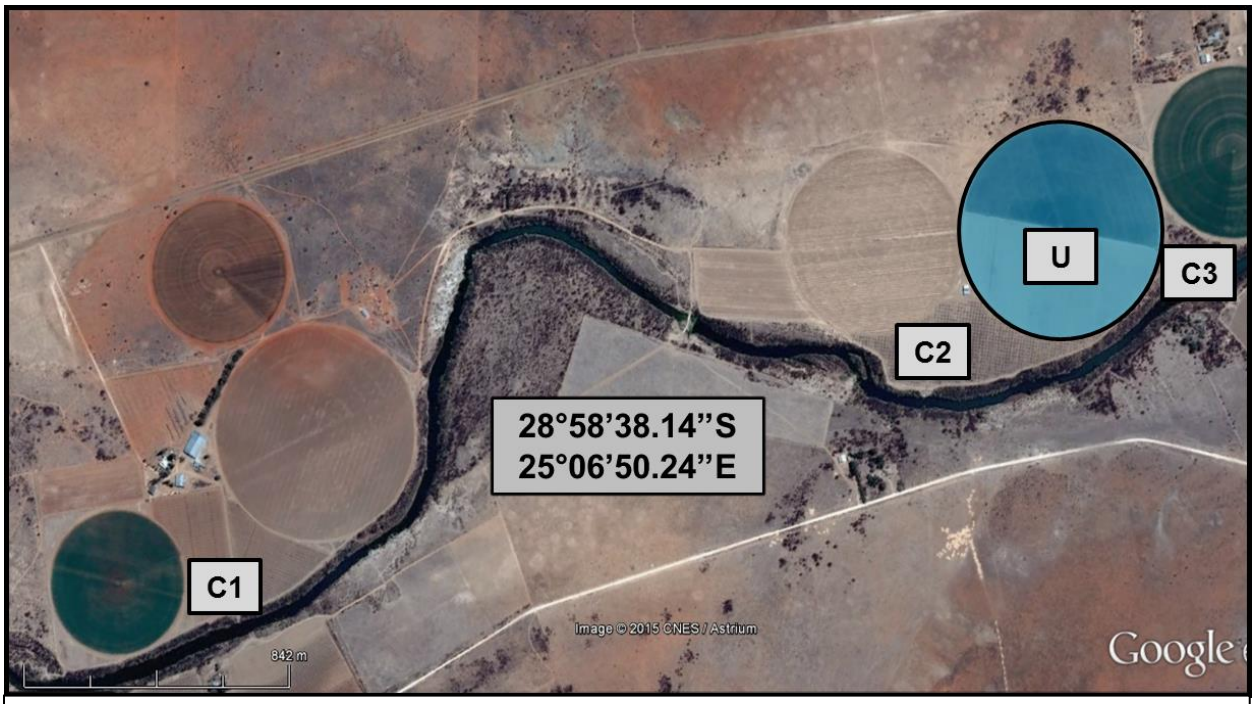

Figure 2: Thornberry farm near Petrusburg showing the onion field (U) used to conduct the study and the three control sampling sites (C1, 2 & 3) in varying degrees of proximity to the onion field (Image adapted from Google Earth).

The last control site (C3, Figure 2) was occupied by narrow-leaved turpentine grass (*C. plurinodis*), spear grass (*Heteropogon contortus*) and dropseed grass (*S. fimbriatus*), *Acacia karroo* (sweet thorn tree) was also found close to the sampling site. At the control sites with trees, the samples were taken as far away as possible from the edges of the natural patch, but not directly under trees. Some of the control veld included trees while others did not and this sampling method was employed for consistency. As illustrated in Figure 2, all the sampling sites at Thornberry farm was situated close to a river (Modder River). At the Bainsvlei sampling site three, control sampling sites were used, with the first on Maranatha (CL, Figure 1, Chapter 3) which consisted of a small patch of natural grass situated between forage crops. This control site mostly contained red grass (*Themeda triandra*), narrow-leaved turpentine grass (*C. plurinodis*), spear grass (*H. contortus*) and dropseed grass (*S. fimbriatus*). The other

two control sites were on Geluk (CN and CS, Figure 2, Chapter 3) and consisted of a natural veld (CN) and a 10 year old Smuts finger grass (*Digitaria eriantha*) field (CS). The natural veld (NC) mainly consists of red grass (*T. triandra*), narrow-leaved turpentine grass (*C. plurinodis*), spear grass (*H. contortus*) and dropseed grass (*S. fimbriatus*). The Smuts finger grass field (SC) only contained Smuts finger grass, with red grass (*T. triandra*) and spear grass (*H. contortus*) occurring sparsely spread throughout the field.

Both the onion fields were under irrigation up until the beginning of November 2015, with the Bainsvlei site under sprinkler irrigation and the Petrusburg field under pivot irrigation. As mentioned they differed in size with the Petrusburg onion field undergoing more organic management, while the Bainsvlei field undergoing conventional farming practices. Organic in this case refers to the application of manure rather than synthetic fertilizer, whilst a natural supplement (Microbial™) was used to provide nutrients for the plants. Conventional tillage was conducted in both fields, whilst the Bainsvlei field had manual weed removal during the trail and the Thornberry farm had no weed removal.

4.2.2. Methodology

Soil samples were taken monthly at both study sites from June to November 2013 (see Table 1 for study site summary). Climatological data was also recorded for all the sampling dates (Table 2 & 3) and included minimum and maximum temperatures, as well as rainfall figures the week before sampling and the day of sampling. In each field three soil samples were randomly taken inside or as close as possible to the porosphere (see Chapter 2.2.1.) and soil organisms were extracted by means of the Tullgren extraction method. The organisms collected was then sorted and identified directly from the ethanol (see Chapter 2.2.2.). The majority of the samples were collected in the cold winter months (Jun-Aug 2013), with Sept 2013 announcing spring and then summer (Oct-Nov 2013). All samples were collected in the Free State Province (classified as a summer rainfall region).

Table 2: Sampling dates and climatic conditions during soil sampling from June to November 2013 in the Bloemfontein area.

Sampling Date	**12 Jun 2013**	**16 Jul 2013**	**14 Aug 2013**	**17 Sep 2013**	**22 Oct 2013**	**20 Nov 2013**
Temperature (max / min)	21°C / 10°C	11°C / 03°C	21°C / 16°C	24°C / 19°C	28°C / 19°C	30°C / 23°C
Rain on sampling date	NO	NO	NO	NO	NO	NO
Rain prior to sampling date	NO	NO	NO	NO	YES	YES

Table 3: Sampling dates and climatic conditions during soil sampling from June to November 2013 at the Thornberry farm.

Sampling Date	**19 Jun 2013**	**17Jul 2013**	**7 Aug 2013**	**18 Sep 2013**	**16 Oct 2013**	**20 Nov 2013**
Temperature (max / min)	21°C / 12°C	14°C / 07°C	16°C / 11°C	26°C / 19°C	29°C / 21°C	32°C / 24°C
Rain on sampling date	NO	NO	NO	NO	NO	NO
Rain prior to sampling date	NO	NO	NO	NO	NO	YES

4.3. Results and Discussion

Since onion plants have deterring and toxic abilities, it is important to determine whether these properties influence soil mesofauna and (indirectly) soil health. Many organisms are adapted to override the effect of certain plant toxins and then feed selectively on these plants. The phenomenon where organisms selectively feed on

specific plants or favour certain food resources more than others has long been recognised (Bonkowski *et al.* 2000).

In below-ground soil environments the variety of food resources may not always be as extensive as that of the above-ground systems. This is even more so from an agricultural viewpoint. Soil fauna do not have the mobility and/or the 'closed' soil medium prevents them from searching over extensive distances for food and they have to make do with what is available in their immediate environment. In agriculture, soils are firstly and mainly disturbed by means of tillage and other soil preparation methods, secondly by the process of planting seeds or seedlings and lastly by removing these plants from the system. Regardless of all the other disturbances, such as pesticide and fertiliser application, these three disturbances severely influence the direct environment which the soil organisms inhabit. Adaptation towards plant toxins has followed an extended evolutionary path where the plants, and the organisms feeding on them, have co-evolved. By cultivating a potentially toxic plant in an agro-ecosystem, as is the case with planting onions, it is important to determine whether soil organisms are able to survive in such environments, since this has a bearing on the ecological function and health of the soil and ultimately affects the crop yield. The mentioned Shannon's index for diversity and evenness, was subsequently used to determine this biodiversity survival potential at the different study sites. (Figure 3 & 4). Supportive data included soil compaction and soil humidity parameters (Table 4 & 5).

At the Bainsvlei site, the diversity was higher in the control veld than in the onion field throughout the sampling period (Figure 3). Despite the high humidity and low compaction readings of the onion field (Table 2 & 3), soil faunal diversity was very low (Figure 3). Because many soil fauna species are dependent on high soil humidity for survival (Coleman *et al.* 2004), this particular soil environment should be ideal for them to flourish in. Instead their richness and abundance is low. This low diversity could be due to low winter temperatures (Table 2) or the phytotoxins produced by the onion plants, or both. The claim that it might be due the colder winter temperature is discarded on the basis that the control veld are exposed to the same environmental

conditions and should therefore also have a lower diversity. Cold temperature could be a factor in July 2013 when the diversity in both field types suddenly dropped and recovered shortly after in August 2013. The insulation effect of weeds was removed from Bainsvlei onion field in July 2013, that could possibly have influenced diversity, but no such activities took place in the control veld and therefore this activity cannot explain the simultaneous decline in diversity for both field types. After the decline in diversity in July 2013, the diversity of the control veld showed a slight increase and remained stable for the rest of the sampling dates.

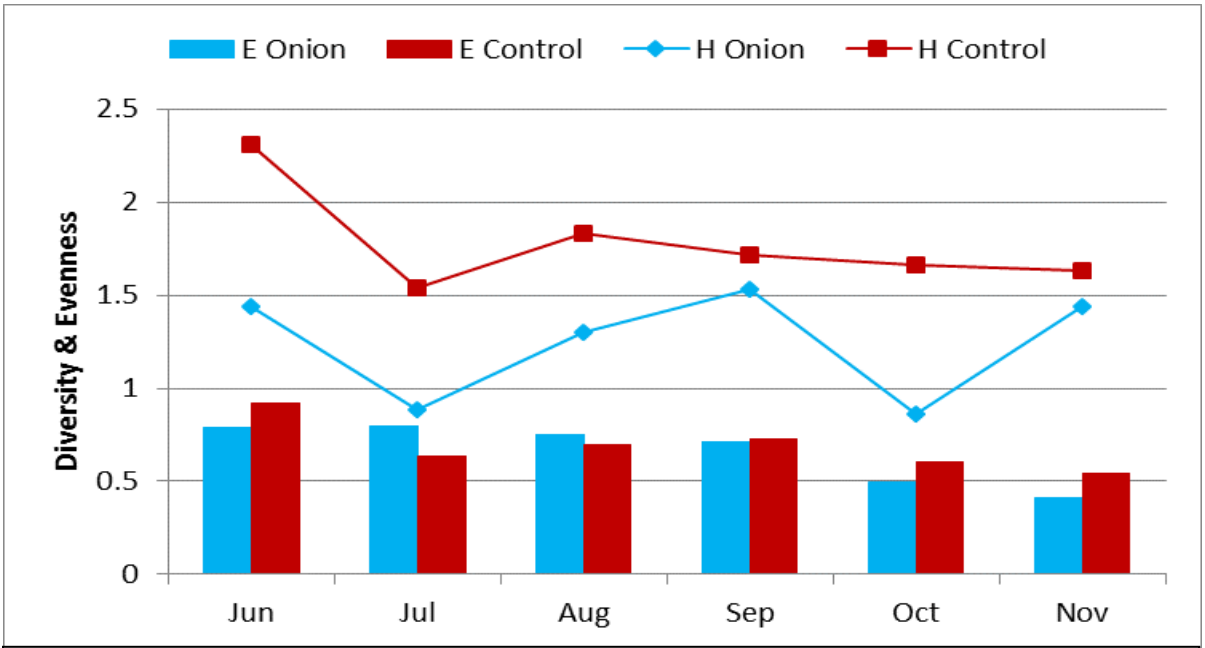

Figure 3: The Shannon's index for diversity and evenness of soil mesofuana in onion and control veld in the Bainsvlei area, Bloemfontein, over a period of six months in 2013. Evenness (E) is represented by bars and diversity (H') by graph lines.

The diversity of the onion field increased in August 2013 and September 2013 but declined suddenly in October 2013 (Figure 3). The initial increase could have been due to a combination of warmer temperatures and a change in population dynamics as new generations of organisms increased towards spring. This increase did not reflect in the control veld, in spite of the fact that new species were starting to occur as the season progressed.

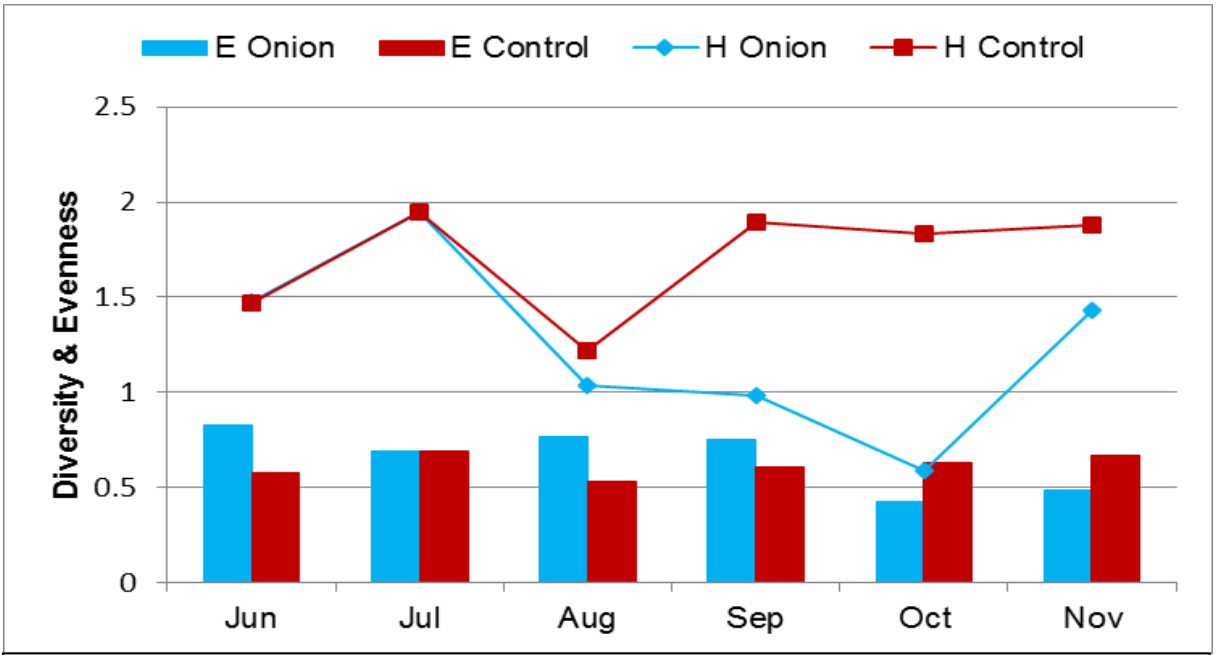

Figure 4: The Shannon's index for diversity and evenness of soil mesofuana in onion fields and control veld in the Thornberry area, Petrusburg, over a period of six months in 2013. Evenness (E) is represented by the bars and the diversity (H') by the graph lines.

Table 4: The average soil compaction (in pounds per square meter) and the depth of the compaction layer (in inches) of each field over a period of six months in 2013 (P = Petrusburg location and B = Bainsvlei location).

	Jun 2013	Jul 2013	Aug 2013	Sep 2013	Oct 2013	Nov 2013
B-Onion	200;3	200;3	200;5	200;4	200;3	200;3
B-Control	200;0	200;0	200;0	200;0	200.1	200;1
P- Onion	200;5	200;6	200;6	200;4	200;3	200;2
P-Control	200;0	200;1	200;1	200;1	200;1	200;1

Table 5: The average soil humidity (in %) of each field over a period of six months in 2013 (P = Petrusburg location and B = Bainsvlei location).

	Jun 2013	Jul 2013	Aug 2013	Sep 2013	Oct 2013	Nov 2013
B-Onion	16.8	17.1	18.9	18.2	18.9	19.3
B-Control	0.2	0.3	0.2	0.2	0.7	0.4
P-Onion	22.2	26.2	21.0	39.3	28.4	18.4
P-Control	0.8	7.1	7.9	7.3	6.5	6.3

The sudden drop in diversity in October 2013 is not because fewer organisms occurred but because more individuals of a single Collembola species were recorded (Figure 3, Addendum 1). Abundance-wise *Cryptopygus* sp. 2 never exceeded 50 individuals from June 2013 to September 2013 regarding the three samples taken monthly, but reached high densities in October 2013 when 567 individuals were present in the three samples taken from the onion field. New records that occurred in the onion field, in the warmer months only, included the insects Dermestidae (*Anthrenus* sp. 1), Scarabaeidae (*Aphodius* sp. 3), two morphologically different Coleoptera larvae (Scarabaeiform larvae msp. 3 & Elateriform larvae msp. 3) and Gryllidae nymphs, as well as Symphyla (Scolopendrellidae), (Addendum 1). New mites during the summer months included the genera *Gamasellevans* sp. 1, *Protogamasellus* sp. 1 & 2, *Oppiella* sp. 1, *Pergamasus* sp. 1, *Anoplocheylus* sp. 1 and *Oribatula* sp. 1. These were species additional to that of the winter months. All the species that were found in the winter samples (Jun-Jul 2013) were also found in the summer months (Oct-Nov 2013). These new species indicate that some soil arthropods are inactive during the winter months, but also that winter specialist species is adapted to the changes in temperature, thus they can be found in summer and winter months. Thus, they are tougher than the species only occurring in warmer conditions. *Oppiella* sp. 1, *Anoplocheylus* sp. 1 and *Oribatula* sp. 1 had a much higher abundance in the control veld samples but were not completely absent from the onion fields (Addendum 1). Of the 88 species of organisms found in total in the Bainsvlei area, 36 was only found in the control veld, 22 only in the onion field and 30 occurred in both the sampling sites (Addendum 1). The evenness (E)

for the Bainsvlei site was overall more even in the control veld, except for June 2013 and August 2013, which is ascribed to the high numbers of Formicidae found on both of these dates.

At the Thornberry farm the diversity of both the fields had the same H'-value (1.47) in June 2013 (Figure 4). In July 2013 the diversity of both fields increased to 1.94 despite the decrease in temperatures, which may be ascribed to an increase in soil humidity and lower soil compaction (Table 4 & 5). A sudden decrease in diversity can be seen towards August 2013 (Figure 4), which may be due to the low temperatures during this time (Table 3). Since both sampling fields showed this decrease in diversity, the causative factor has to be a locality parameter. Temperatures increased towards September 2013 (Table 3) and the diversity in the control veld increased in parallel (Figure 4). The onion field, however, did not show the same tendency. This can possibly be explained on the basis of onion phytotoxins influencing the occurrence of soil fauna negatively. This onion *vs* control trend stayed the same from August 2013 to November 2013. From September 2013 to November 2013 the diversity in the control veld stabilized and stayed more or less the same. However, the diversity of the onion field spiked towards November 2013 when the onions were harvested. Onion harvesting involves loosening and turning of the soil allowing aeration and lessening of compaction, both factors which could have benefited faunal movement and occurrence in the soil.

The Sørensen similarity index for Bainsvlei indicates very little similarities between the two fields and within the same field, for different sampling dates (Table 6). Significant differences can be seen between the onion field and the control veld for all sampling dates and between sampling dates. Not a single soil fauna species that was found in the onion field in June 2013 was found in the November 2013 control sample. This was also the case for July 2013 and November 2013. In August 2013 the greatest similarity between the two fields for the same sampling date was found to be 0.55. The control of September 2013 correlated strongly with the June control (0.60). The July 2013 onion fields correlated strongly with the August 2013 onion fields (0.67). These

were the largest similarities found for these sampling sites. Overall, there are remarkable differences that indicate how much the soil faunal species composition can vary over short time spans at the same sampling site. Temperature, humidity and vegetation type can have a substantial influence on these organisms and their occurrence in soil environments. The trophic structure in which these organisms operate is also meaningful and will be discussed below and shown in Figure 5. Of the 78 species found in both fields, 9 were found in the onion field only, 49 only in the control and 20 species was found in both sampling sites (Addendum 1). This explains the low similarity (Table 2) between these two sampling sites. There is also a low similarity between different dates in the same field, which may be due to the differing seasonal and climatic conditions at the time of sampling.

In the majority of similarity indices for Thornberry the onion fields differed the most from the control veld, but in some cases (such as November 2013 control and June 2013 control, 0.67) the main difference is between two samples taken in the same field (Table 7). Thus, the control samples were dissimilar between two samplings four months apart. Because June is in the winter months and November is in the summer this could explain the dramatic dissimilarity. This can be explained based on temperature and general climatic conditions as the season progressed. The Thornberry farm shows a much closer resemblance between the sampling dates (November 2013 and June 2013, Table 7) than the Bainsvlei site for the same dates (November 2013 and June 2013). This might be due to the more conservation orientated cultivation practices at Thornberry farm. By using more natural fertilizers the difference between natural field and agricultural field is less heterogeneous. Thus despite the temperature and general climatic conditions that varied between the two sampling dates the onion field on Thornberry farm had similarities in species between the onion field and the control field.

Trophic structure analysis at the different sites at the two localities was also analysed (Figure 5). For the largest part, mycophages were the most dominant trophic group and occurred in all samples taken from both localities (Figure 6). Mycophages

were only made up by Acari and Collembola (Addendum 1). It is important to notice that both these groups increase in abundance in the warmer months. One explanation could be that they migrate into the deeper soil layers during cold winter periods and return to the top soil in the summer. The individuals recorded were mostly adult stages of both Collembola and Acari indicating that they are not overwintering as egg- or immature stages. Predators, mainly small spiders and predatory mites, occurred in low numbers in all samples. They too prefer warmer temperatures and increased slightly as mycophages increased. Omnivores were only represented by Formicidae and they only occurred in high numbers in the control veld. This can partially be explained by the ants being able to establish colonies in the control field because of less anthropogenic disturbances. Regular soil disturbances in the onion field may have hampered nest construction by ants, explaining their absence in these fields. Ants are also influenced by the availability of bare soil and plant structure (Botes *et al.* 2006). This could possibly explain the higher number of omnivores, primarily represented by ants, in the natural fields or control sites.

Table 6: The Sørensen similarity index for the Bainsvlei onion field (U) compared to the control veld (C) over a period of six months in 2013.

		JUN		JUL		AUG		SEP		OCT		NOV	
		U	C	U	C	U	C	O	C	U	C	U	C
JUN	U	1.00	0.27	1.00	0.09	0.55	0.27	0.27	0.18	0.45	0.18	0.45	0
	C	0.27	1.00	0.33	0.55	0.45	0.53	0.41	0.60	0.33	0.52	0.38	0.38
JUL	U	1.00	0.33	1.00	0.11	0.67	0.33	0.33	0.22	0.56	0.22	0.33	0
	C	0.09	0.55	0.11	1.00	0.27	0.42	0.24	0.45	0.25	0.40	0.30	0.40
AUG	U	0.55	0.45	0.67	0.27	1.00	0.55	0.18	0.36	0.45	0.36	0.45	0.36
	C	0.27	0.53	0.33	0.42	0.55	1.00	0.18	0.47	0.42	0.42	0.37	0.37
SEP	U	0.27	0.41	0.33	0.24	0.18	0.18	1.00	0.29	0.25	0.29	0.12	0.12
	C	0.18	0.60	0.22	0.45	0.36	0.47	0.29	1.00	0.33	0.45	0.30	0.50
OCT	U	0.45	0.33	0.56	0.25	0.45	0.42	0.25	0.33	1.00	0.25	0.58	0.33
	C	0.18	0.52	0.22	0.40	0.36	0.42	0.29	0.45	0.25	1.00	0.29	0.38
NOV	U	0.45	0.38	0.33	0.30	0.45	0.37	0.12	0.30	0.58	0.29	1.00	0.29
	C	0	0.38	0	0.40	0.36	0.37	0.12	0.50	0.33	0.38	0.29	1.00

Saprophages mainly represented by Coleoptera and Diptera larvae and a few Coleoptera (Scarabaeididae and Dermestidae) occurred throughout the year in both fields (Addendum 1). Their numbers were very low for the duration of the study, which can be attributed to firstly, the soil acting as a temporary shelter and secondly relatively little organic matter to feed on.

Table 7: The Sørensen similarity index for the Thornberry onion field (O) compared to the control veld (C) over a period of six months in 2013.

		JUN		JUL		AUG		SEP		OCT		NOV	
		O	C	O	C	O	C	O	C	O	C	O	C
JUN	O	1.00	0.56	0.67	0.78	0.57	0.56	0.44	0.67	0.43	0.67	0.56	0.67
	C	0.56	1.00	0.67	0.45	0.43	0.45	0.23	0.50	0.29	0.50	0.38	0.27
JUL	O	0.67	0.67	1.00	0.67	0.33	0.67	0.67	0.50	0.50	0.50	0.50	0.50
	C	0.78	0.45	0.67	1.00	0.71	0.55	0.38	0.35	0.29	0.56	0.46	0.30
AUG	O	0.57	0.43	0.33	0.71	1.00	0.43	0.43	0.43	0.29	0.43	0.43	0.57
	C	0.56	0.45	0.67	0.55	0.43	1.00	0.31	0.45	0.29	0.44	0.38	0.30
SEP	O	0.44	0.23	0.67	0.38	0.43	0.31	1.00	0.54	0.43	0.38	0.38	0.54
	C	0.67	0.50	0.50	0.35	0.43	0.45	0.54	1.00	0.43	0.67	0.46	0.52
OCT	O	0.43	0.29	0.50	0.29	0.29	0.29	0.43	0.43	1.00	0.29	0.43	0.29
	C	0.67	0.50	0.50	0.56	0.43	0.44	0.38	0.67	0.29	1.00	0.54	0.67
NOV	O	0.56	0.38	0.50	0.46	0.43	0.38	0.38	0.46	0.43	0.54	1.00	0.54
	C	0.67	0.27	0.50	0.30	0.57	0.30	0.54	0.52	0.29	0.67	0.54	1.00

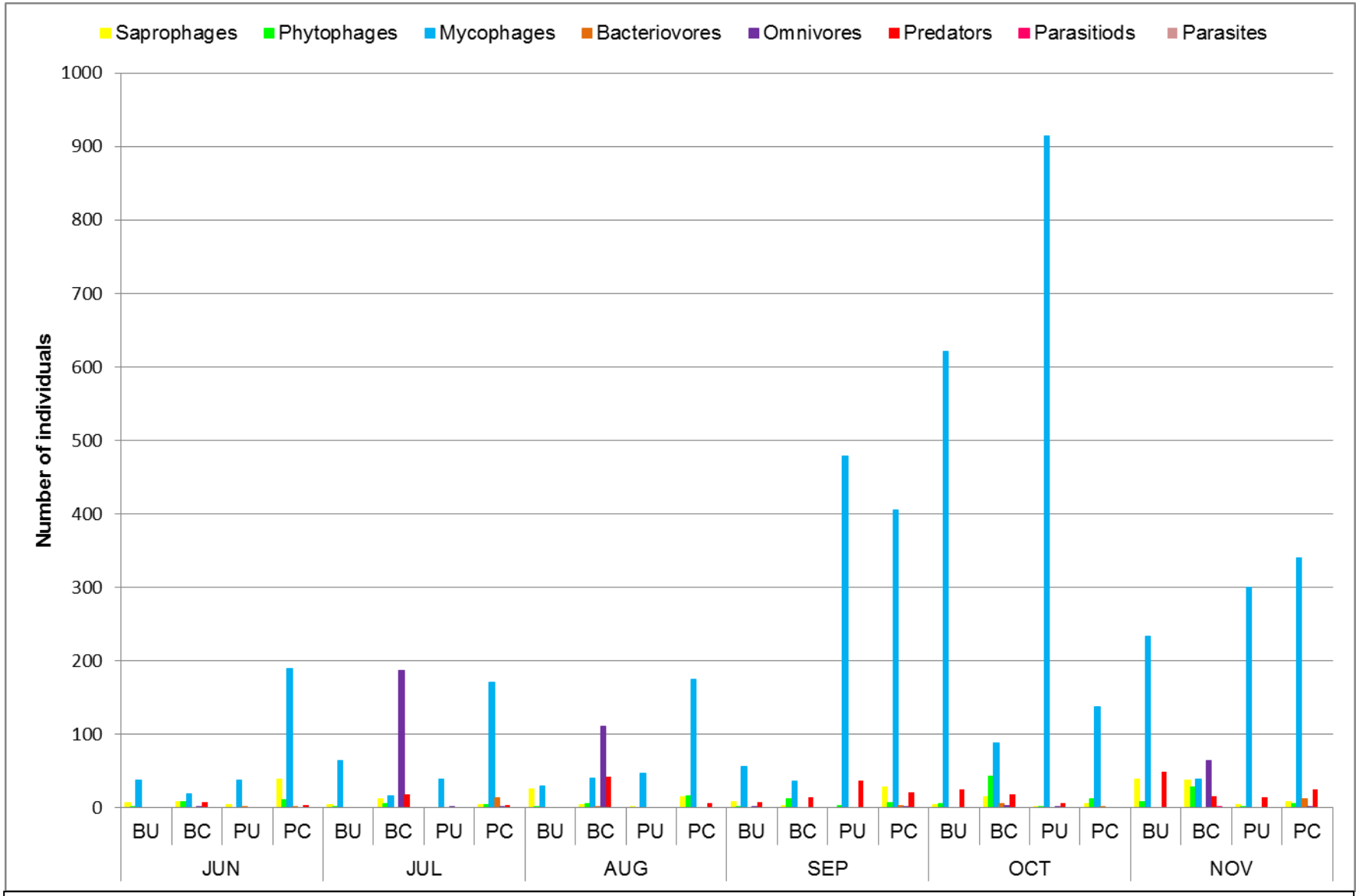

Figure 5: Trophic structure of sampling sites at both localities from June to November 2013 (B- Bainsvlei, U-Onion, C-Control and P-Petrusburg) on farms Geluk (Bainsvlei) and Thornberry (Petrusburg).

4.4. Conclusion

The data have shown that the overall diversity in onion fields is lower than in the control veld. Some species of soil organisms only occurred in the natural fields and not in the onion field. There are no traces that the toxins produced from these plants actually kills soil fauna, but it can be assumed that onion plants are at least repellent. Some species specifically occur only in the onion fields, indicating resistance towards onion repellent odours. The Collembola *Cryptopygus* sp.1 occurred in very high numbers in both onion fields and it would seem that this species are generalist feeders that have adapted to an array of chemical cues in the soil. They also occurred in the control veld of both localities and overall their increase in abundance also reflects wide temperature tolerances. Many soil faunal species were more abundant in the summer months which refutes the argument that soil is a 'closed system' in terms of temperature preferences, since, after a lag period, below ground soil organisms are influenced by above-ground temperatures.

Soil fauna similarity was very low between and within fields in the Bainsvlei area. At Thornberry farm, there was a remarkably higher similarity between and within fields. This may be due to the conservation management practices applied at the Thornberry farm where manures and microbial supplements are added to the soil. This increases the beneficial microbes in soil and boosts soil health. Compared to the Bainsvlei study site it is clear that the mesofaunal biodiversity gap between the cultivated field and natural fields is smaller at Thornberry farm. This practice and its outcome support responsible soil health management and is strongly recommended. Mycophages were the most abundant of all trophic groups and did not seem to be affected as much by the onion phytotoxins. Instead they are rather season or climate specific and reached high numbers as the temperatures increased. The other trophic groups were abnormally low in numbersin onion fields while controls were higher, which is probably attributed to the fact that they could not cope with the onion plant toxins as well as the mycophages. Omnivore ants were only found in the control veld because they were not able to establish colonies in the cultivated fields due to tillage practices.

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CHAPTER 5

Alfalfa litter decomposition in alfalfa and grassland fields: Testing the home field advantage hypothesis

5.1. Introduction

Plant litter has many functions in soil that is important for natural processes (Bhalawe *et al.* 2013). Litter functions as a reserve source for plant nutrients and increases both cation-exchange capacity and the ability of soil to hold water. According to Bhalawe *et al.* (2013), decomposer organisms and physiochemical properties of plants influence litter decomposition, which is a natural process and one of the most important ecosystem functions supporting life. Three factors can primarily influence decomposition rates; the physicochemical environment (*e.g.* humidity, pH), litter quality (*e.g.* lignin; N) (Gholz *et al.* 2000, Parton *et al.* 2007) and decomposer fauna, which includes micro- and meso-organisms (Ayres, *et al.* 2009a). Obvious but important is that the relative significance of these factors varies in different environments. Recent studies show that a great deal of attention is focusing on soil biota and their importance in decomposition and soil function (Butenschoen *et al.* 2014, Cleveland *et al.* 2014, Graham *et al.* 2014, Jiang *et al.* 2014, Castro-Huerta *et al.* 2015).

One hypothesis suggests that soil fauna is adapted to the specific plants that grow in their habitat and will decompose litter from their “native” vegetation at a faster rate than litter from elsewhere (Hunt *et al.* 1998, Gholz *et al.* 2000). This phenomenon is known as the Home-Field Advantage (HFA) of decomposing litter. Numerous studies have focused on how above-ground biodiversity is influenced by plants growing in their environment, but studies on below-ground communities have been neglected. Because soil fauna does not have the mobility of above-ground fauna, it makes sense that they would adapt to utilize immediately surrounding plant material more efficiently. Many studies have confirmed that HFA does occur (Hunt *et al.* 1998, Gholz *et al.* 2000, Ayres *et al.* 2009a, Ayres *et al.* 2009b, Veen *et al.* 2015), but some authors have also argued the opposite (Ayres *et al.* 2006, Chapman and Koch 2007, Gießelmann *et al.* 2011, St John *et al.* 2011, Kagata and Ohgushi 2013, Perez *et al.* 2013*)*.

Some authors suggest that the allelochemicals of different plant parts differ and therefore decomposition rates of this plant material may be influenced. For example,

Chon & Kim (2002) found that alfalfa seed compounds had the greatest effect on root lengths of other alfalfa plants. Stems and roots also had a strong effect on root lengths, whilst leaves had the smallest effect.

The aims of this study were: i) To test the HFA principle by determining whether a HFA pattern emerges in a four week trail using alfalfa litter and mixed grass litter in natural environment. ii) To determine whether levels of access to litter by different kinds of soil fauna influences decomposition.

iii) To determine the decomposition rates of different allelopathic alfalfa plant parts (*viz.* leaves, stems, roots and flowers).

5.2. Study layout

5.2.1. Study Sites

The study was conducted on two farms in the Bainsvlei area near Bloemfontein, *i.e*. Maranatha (29°01'36.31"S, 26°05'04.32"E) and Geluk (28°59'30.32"S, 26°05'47.58"E) (Figure 1). An alfalfa field, approximately six years of age, was used at Maranatha and a natural field was used at Geluk (Figure 1). The alfalfa field was planted with SA standard cultivar alfalfa seeds and the natural veld was mostly covered in Smuts finger grass (*Digitaria eriantha*), that was planted in this area in 2003.

The sampling sites were roughly 14 km from Bloemfontein and 4.2 km apart from one another as the crow flies. As can be seen in Figure 1, the Bainsvlei area is a highly disturbed area in terms of agriculture but in-between the agricultural fields, natural veld patches can also be found. The natural veld used in this study was surrounded by bare fallow fields and maize fields, planted between December 2013 and January 2014. The alfalfa field was under sporadic irrigation and the natural veld only received moisture in the form of rainfall.

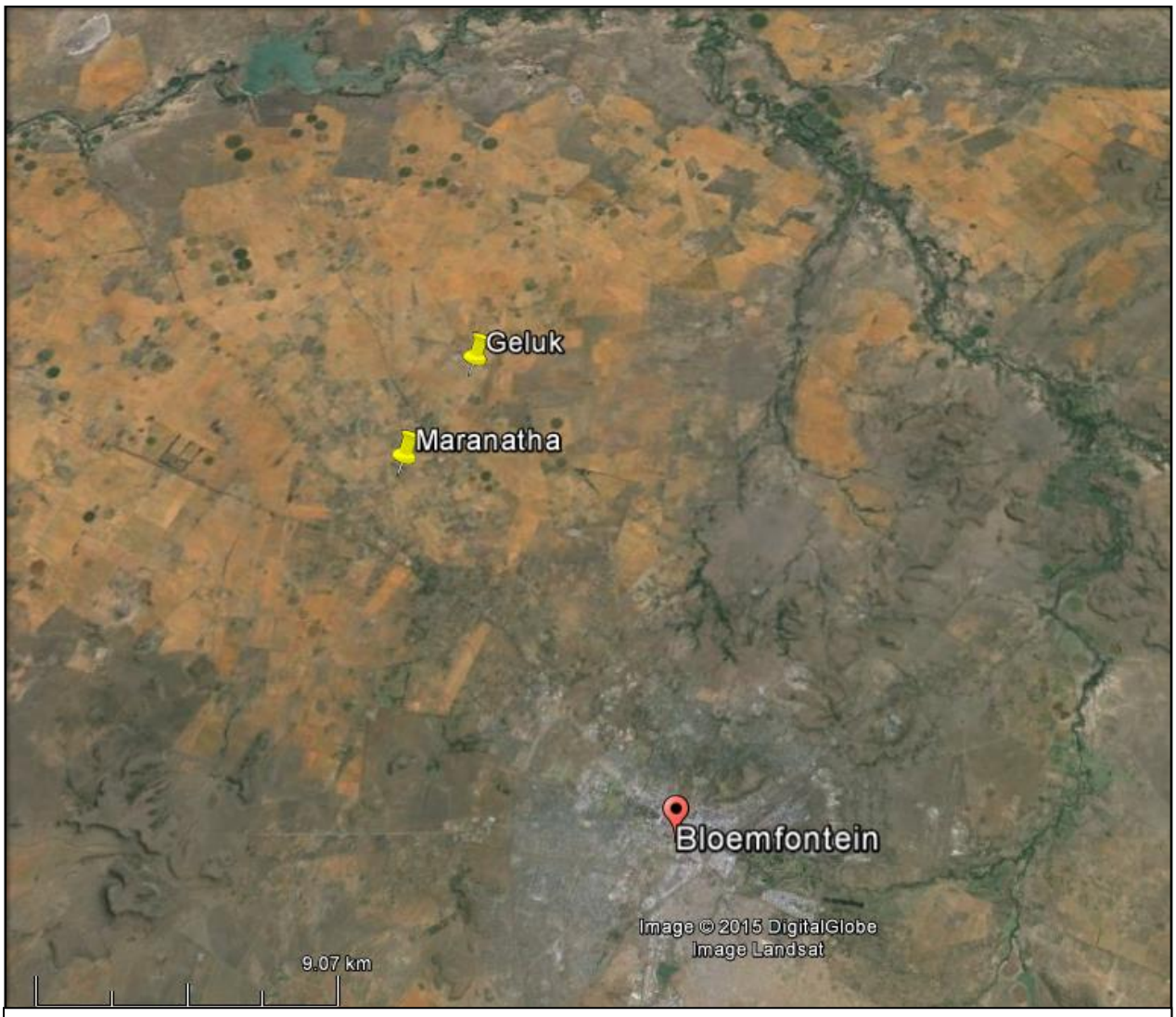

Figure 1: The two study sites, on the farms Maranatha and Geluk in the Bainsvlei area, 14km north-west of Bloemfontein (Image adapted from Google Earth).

5.2.2. Litter sampling and preparation

Alfalfa plant material (leaves, stems, flowers and roots), as well as mixed grasses from a natural grassland field, was collected during March 2014. Leaf material included petioles and flower material included pedicels, flowers and seeds. Roots at different stages of development and size were collected to include all categories of alfalfa roots. Stems and roots were cut into smaller pieces (±3 cm long) and thick roots were cut to a diameter of 0.5 cm. Grass material was also cut into 3 cm pieces. All material was dried at room temperature for a week and then placed in direct sunlight for a day (to allow for

natural photo- degradation) and to ensure the material was completely dried out, it was also placed in an oven at 70° F for an hour. According to Adl (2003), the loss of moisture from cells and tissue of decomposing litter leads to clumping of denatured cytoplasmic molecules. This causes material to become more frail and contributes to the physical fragmentation of plant material. In nature, the plants would extract nutrients and water from such plant matter before it falls off the plant. The manipulated drying of plant litter in this study is a key component since it alters the initial qualities of the plant litter thus simulating natural degradation. The extreme drying out of the litter is also important in order to eliminate all organisms already present in the litter which could affect the trial. Three grams of each material type was placed in the different litter bags/traps used in the study (see below). The litter was weighed to an accuracy of three decimals.

5.2.3. Litter traps and litter bags

Three types of litter traps and bags were used during this trial. The first type was a litter trap (Figure 2) that was sunk into the soil with the top rim level with the ground surface. The bottom of the trap is sealed-off with micro-mesh and the top with meso-mesh. This allows above ground mesofauna into the trap, but once organisms fall in they cannot escape. This design of the trap also allows for water and air to move through the trap and specializes in capturing free-living and periodically free-living above-ground fauna.

The second trap type was litter bags constructed from meso-mesh (Figure 3a) to allow below-ground mesofauna to freely move in and out of the bag, whilst the third type was litter bags constructed from micro-mesh (Figure 3b) which exclusively allowed microfauna to move in and out. The mesh size of the meso-bags was 4 mm x 4 mm and the micro-bags 0.2 mm x 1 mm. Both litterbag types therefore allowed water and air flow through the bag. Both bag types were 12 cm in length and 5 cm in width.

Figure 2: A litter trap planted in an alfalfa field.

Figure 3: The two litterbag types used in the decomposition experiment - (a) meso-mesh bag; (b) micro-mesh bag.

5.2.4. Decomposition study setup

In total 40 litter traps and 80 litter bags (40 meso-mesh and 40 micro-mesh) were placed in the soil of the sites ± 30 cm from one another (Figure 4). There were five types of plant material namely alfalfa leaves, alfalfa stems, alfalfa flowers, alfalfa roots and mixed natural grasses. Three grams of each material type was placed in the bags/traps. Half of the traps was buried in the alfalfa field (four of each material type for each of the

traps and bags), and the other half in the natural grassland, in the second week of April 2014. Al the traps were placed level with the soil surface and bags were placed 20 cm under the soil at least 20 m from the edges of the field to eliminate edge effect. The trap/bags of each material type and sampling type was collected weekly (in April and May 2014) in each of the two fields. The traps and bags were placed upside down on Tullgren funnels, for seven days, to extract soil fauna and to dry out the litter. The meso-arthropods, in all the different material types, were extracted and identified. This diversity (richness and abundance) was compiled into a species list (Addendum 2).

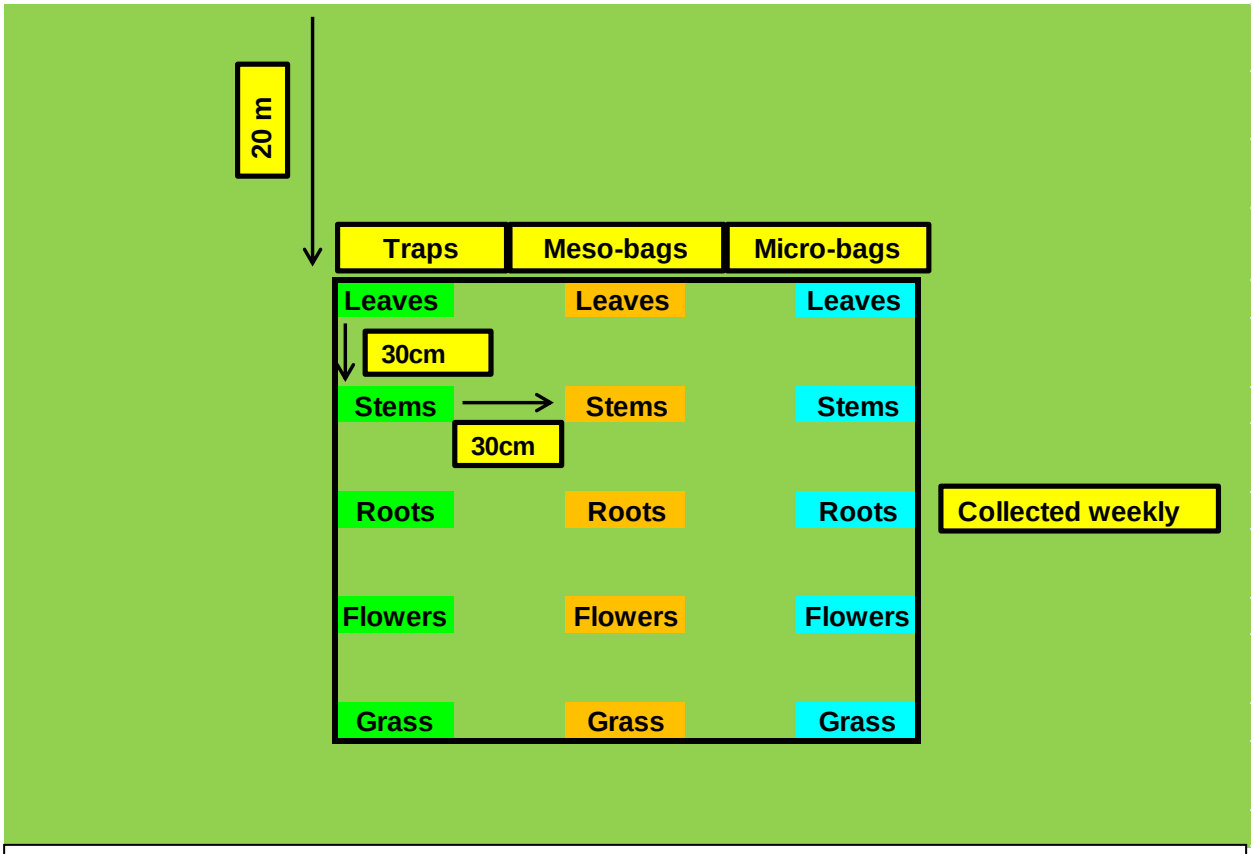

Figure 4: Decomposition trial setup in a field. Twenty litter traps and 40 litter bags, placed 30 cm apart from one another and 20 m from the field edge. Five samples of each sampling method, *re* the plant type/part, were collected weekly, to determine decomposition rates.

5.2.5. Determination of litter mass loss

After the litter bags and traps were placed on the Tullgren funnels for the extraction of soil fauna, the bags were cut open. The soil was brushed off the litter with a

soft brush. Subsequently the material was placed in warm water to get rid of any remaining soil and dried in an oven at 70° F for 6 hours and left overnight. It was then weighed to compile decomposition indices. To prevent litter from being lost in the process, litter was washed through a fine sieve and afterwards the water was checked under the microscope to confirm that no litter was washed away. The wet litter was then placed onto a tray with different compartments and dried in an oven at 70°F for a day. After this the biomass of the different litter types was determined. Percentage weight loss for each sample was determined by using the following formula ([b]Ayres *et al.* 2009):

$$\frac{\text{Current weight of litter}}{\text{Initial weight of litter}} \text{ X } 100 - 100 = \text{Percentage weight loss}$$

All the data on alfalfa plant litter (leaves, stems, roots and flowers) were combined (single sample) for the purpose of compiling the Home-field Advantage Index. This data was, however, recorded separately to determine the decomposition rates of different plant litter with respect to allelopathy.

5.2.6. Home-field Advantage (HFA) of decomposing litter

For most studies that focused on the HFA of decomposing litter, only the presence or absence of HFA was determined. [b]Ayres *et al.* (2009), however, proposed that it is important to determine the degree of HFA in order to determine whether the particular site advantage is significant or not. They used a formula to determine if litter of plant species A or B decomposed at a faster rate at site a (area dominated by species A) than at site B (area dominated by species B) and *vice versa*. This formula can therefore be used to determine the degree of HFA at two study sites in comparison to one another. The equation is as follows:

$$A_{RMLa} = \frac{A_a}{A_a + B_a} \text{x } 100$$

A_{RMLa} = relative mass loss of species A at site a.

A_a and B_a = represents relative mass loss of species A and B at site a.

This formula is then used to determine B_{RMLb}, B_{RMLa}, A_{RMLb} and A_{RMLa} which are used to determine the HFAI (Home-field Advantage Index).

The equation is as follows:

$$HFAI = \left[\frac{\frac{A_{RMLa} + B_{RMLb}}{2}}{\frac{A_{RMLb} + B_{RMLa}}{2}} \right] x\ 100 - 100$$

The HFAI represents the percentage faster mass loss at home *versus* away and is a net value for both species A and B. The advantage of this equation is that it quantifies plant litter decomposition, whereas ANOVA would not.

5.3. Results and discussion

5.3.1. HFAI of decomposing litter

The HFAI of decomposing litter is not the same concept as the litter decomposition rate. The HFAI is a net value for alfalfa litter (A) and grass litter (B) representing the percentage faster mass loss of litter at "home" vs. "away" and can be related to certain arthropod or other decomposer organisms. When studying the decomposition food web, it is important to distinguish between primary and secondary decomposers. The primary decomposer fauna feed directly on the litter material and form part of the initial decomposition processes, such as physical fragmentation. The secondary decomposer fauna feeds on microflora such as fungi and bacteria and in so doing break nutrients up into particles that can easily be absorbed by surrounding plants. Secondary decomposers can also be coprophages that feed on faecal pellets of macro- and meso-invertebrates. Predators within these food webs may also influence decomposition rates, since they are mostly opportunists that feed on any other organism they are able to subjugate. The HFAI for the three different sampling methods over the four weeks can be seen in Figure 5.

Figure 5 depicts that, on all sampling dates and for all sampling methods, litter decomposition is faster at the study site of origin than elsewhere, with the exception of the first week (litter traps) and the third week (micro-bags). Each of the sampling methods had a unique HFAI pattern over the four weeks. According to [a]Ayres *et al.* (2009) and Wang *et al.* (2013), the HFAI should increase with time during the initial stages of litter decomposition. This can only be seen in the HFA pattern of the litter traps. [a]Ayres *et al.* (2009) conducted a laboratory experiment in association with a field trial and the study was conducted over a period of 734 days that consisted of five sampling dates. Wang *et al.* (2013) conducted a review on 30 litter decomposition papers and the studies in these papers ranged for 3 to <12 months of litter decomposition. This study only focused on the first four weeks of decomposition and HFA rates, thus only the initial stages of decomposition were considered.

The HFAI of litter traps started at a negative value (Week 1) and increased over the four-week decomposition period. The meso-litter bags started with a significantly high HFAI and decreased gradually towards the third week, but increased again in Week 4. This was the only sampling method that had an incessantly positive HFAI over the four-week period. The micro-litter bags had a zigzag pattern with a low HFAI followed by a high HFAI for every two-week cycle. The three sampling methods were designed to target specific organisms, whether it was the size of the soil fauna or their behaviour and applied to above- and below-ground species. The meso-bags served to sample larger below ground and the micro-bags was based on the same below ground principle, but served to sample the smaller fauna. Overall, the meso-bags had the highest abundance of organisms, thereafter the litter traps followed by the micro-bags. The main reason for this phenomenon was the accessibility for organisms into these traps

In Week 1 of decomposition the HFAI for the litter traps was -7.22%, implying that both litter types decomposed faster away from their fields of origin. This phenomenon cannot be observed for either the meso- or micro litter bags in Week 1 and can be explained by mobility issues of organisms out of these traps. Because litter traps function in the same

manner as standard pit fall traps, the organisms recorded in these traps are above-ground and may be incidental.

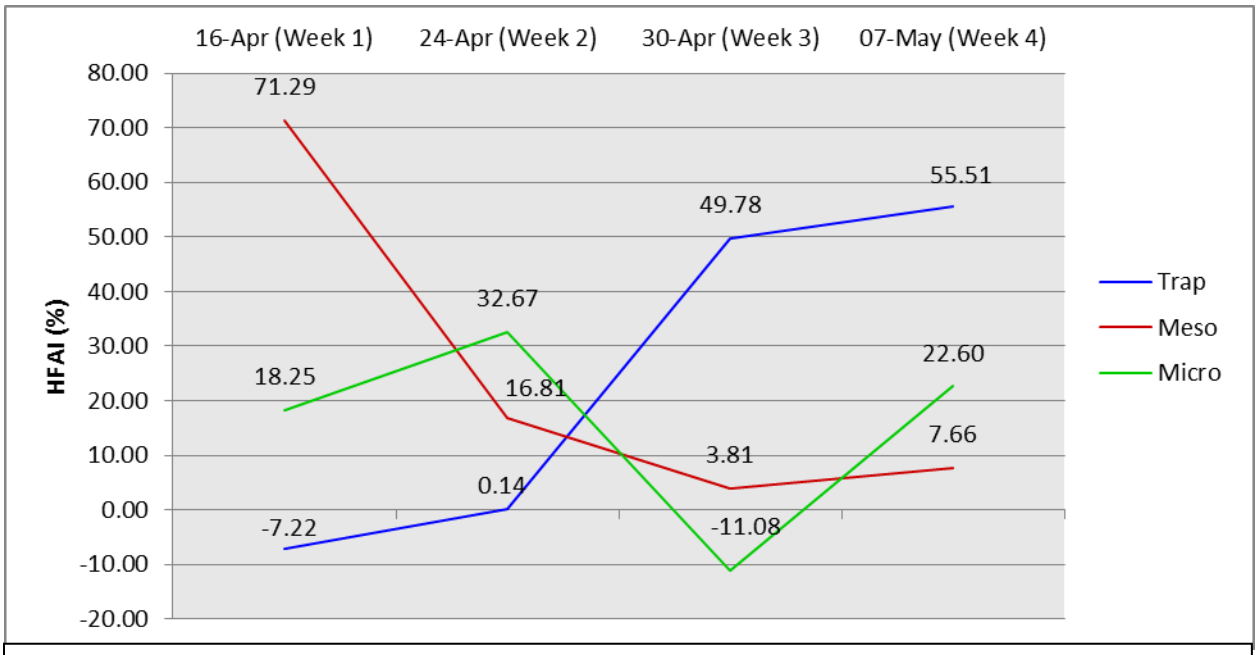

Figure 5: The home-field advantage index (HFAI) over a four week period (2014) compared decomposition rates of alfalfa and grass litter in an alfalfa and natural veld, showing variation between the three different methods used to facilitate litter decomposition (meso = coarse mesh litter trap; micro = fine mesh litter trap).

The litter traps were specifically designed to sample below-ground decomposer fauna. Intermediate decomposer fauna refers to organisms that can either occur above- and below-ground (See Chapter 1). According to Bernays and Chapman (1994), phytophagous insects primarily make use of olfaction to locate their food. This is the same for most arthropods, especially soil dwelling organisms that are small in size and generally have limited vision. Litter traps attract decomposer fauna by means of decomposing leaf odour. The organisms move towards and fall into the traps, the majority of which cannot escape. They are forced to feed on the available resources within the trap in order to survive. These were the initial phases of litter decomposition and the competition for survival in these traps was presumed to be very high.

For all three sampling methods, the general abundance of organisms was low during the first sampling date. In Week 1 litter traps had the highest abundance of organisms and this was mainly because of the high numbers of Collembola and Acari. The collembolans *Isotoma* sp.1 and *Hypogastrura* sp. 1 & sp. 2 (Figure 6) were common throughout the study for all sampling methods, especially the traps and meso-litter bags. Abundance of all three Collembola species was significantly higher in the alfalfa field compared to the natural field. These species were most probably introduced and the reason for their high abundance in the alfalfa field is most probably due to the opportunistic nature of such species. Janion *et al.* (2011) showed that at least 25% (34 out of 136 species) of the Collembola species recorded in the Western Cape (South Africa) are suspected of being introduced or considered widespread species. Among the Collembola families found to be widespread/introduced, Hypogastruridae and Isotomidae had a number of species that fell in this category. According to Greenslade & Convey (2012) the term naturalised species is the correct term to use when referring to an introduced or exotic species that successfully established in a new area. Introduced species are known to be opportunistic and can displace native species. In agricultural soil with continuous disturbances and fluctuations in resource availability and type of plant species, indigenous species are often outcompeted by these naturalised species.

According to Janion-Scheepers *et al.* (2015) approximately 20% of the Collembola species found thus far in South-Africa are introduced. According to Greenslade & Convey (2012), Collembola can be introduced to new study sites through the import of fresh vegetables and other kinds of plants. These introduced species become established rapidly, especially in agricultural fields, thus becoming naturalised species. The reason for this fast establishment is their ability to respond rapidly to agricultural and anthropogenic changes and their potential to persist in intensively managed ecosystems (Rebek *et al.* 2002). Rebek *et al.* (2002) show that these species were not unique to a certain cropping system or crop plant and suspected that the species composition in the agricultural fields studied were already a result of the introduced species outcompeting the native species. However, they did not have a reference of the species that occurred in that area before agricultural disturbances took place.

The collembolans *Willowsia* sp.1, *Tullbergia* sp. 1 and *Brachystomella* sp. 1 (Figure 7) mostly occurred in the grassland samples (Addendum 2). *Willowsia* and *Tullbergia* never reached the high population numbers that the three species of Collembola in the alfalfa field (*Isotoma* sp.1 and *Hypogastrura* sp. 1 & sp. 2) did. *Tullbergia* was found from Week 1 and thereafter in low abundance for all the sampling dates in both litter types in the natural field. *Willowsia* only appeared from Week 2 onwards, but were mostly recorded in greater abundance in the grassland veld, except during Week 4 when abundance started to increase in the alfalfa field of both litter traps and meso-bags. *Brachystomella* also appeared in Week 2 of sampling, but reached high numbers in Week 3, especially in the litter traps. These three species were also recorded in the alfalfa field, but only in much lower densities, as if they were straddlers.

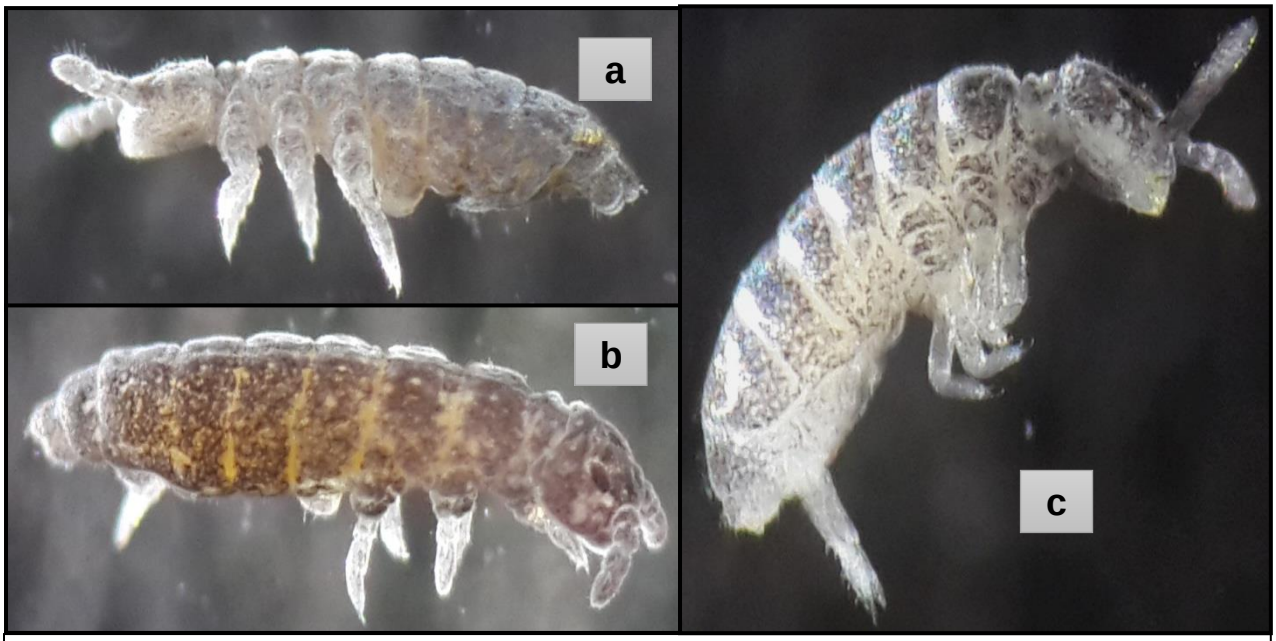

Figure 6: The three possibly introduced Collembolla species that were recorded most often in the alfalfa field. (a - *Hypogastrura* sp. 1, b - *Hypogastrura* sp. 2 and c - *Isotoma* sp. 1).

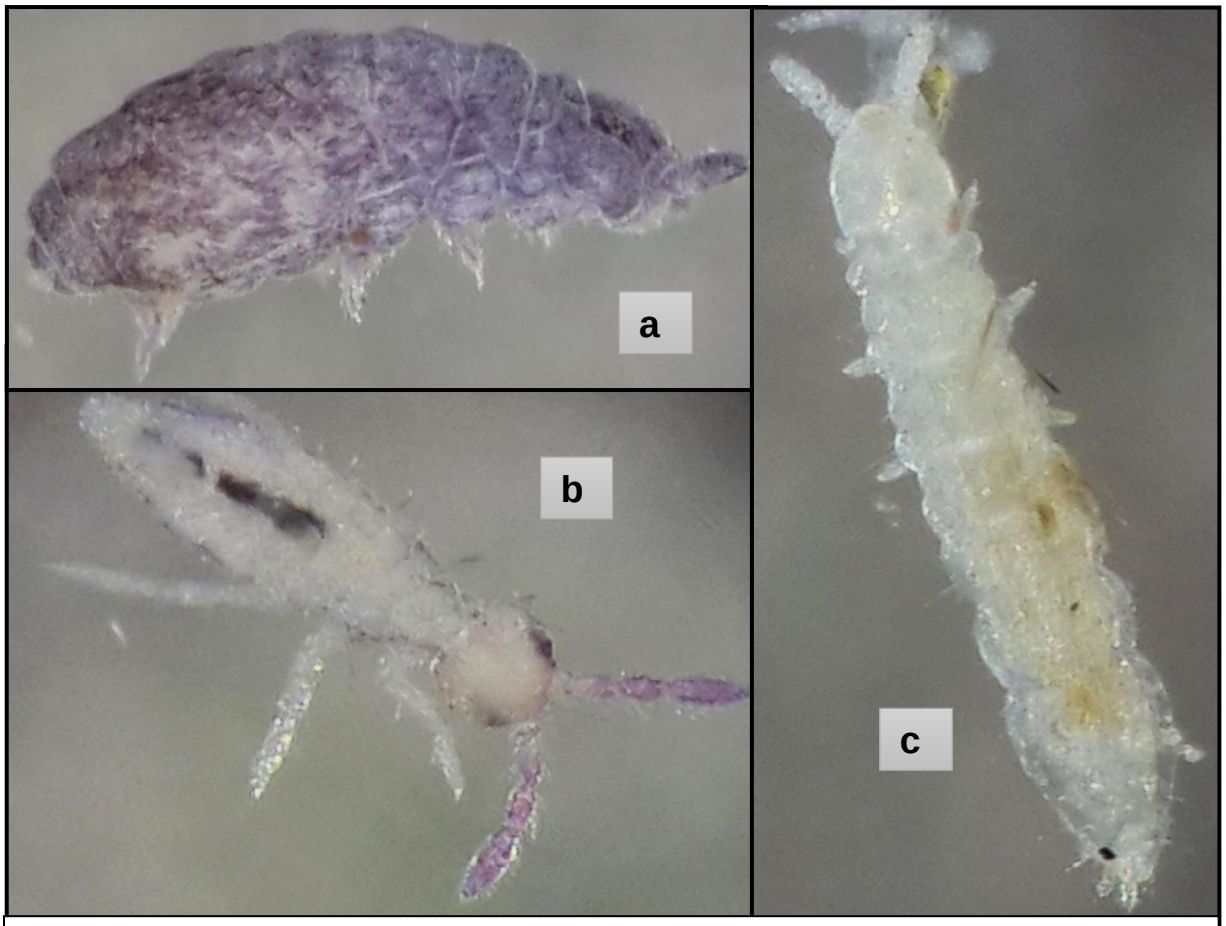

Figure 6: The three Collembolla species recorded most often in the grassland. (a - *Brachystomella* sp. 1, b - *Willowsia* sp. 1 and c - *Tullbergia* sp. 1).

Other Collembola species recorded include *Xenylla* sp. 1, *Brachystomella* sp. 1, *Parisotoma* sp. 1, *Capbrya* sp. 1, *Entomobrya* cf. *multifasciata*, *Entomobrya* sp. 1, *Entomobryoides* sp. 1, *Seira* sp.1 and *Sphaeridia* sp. 1. *Brachystomella* reached high abundance in the litter traps of the natural field for the last three sampling dates. *Parisotoma* reached high abundance in Week 3 in the meso-bags. According to Janion-Scheepers *et al.* (2015) *Capbrya* is one of the genera that is most likely endemic to South Africa. They occurred in low numbers during Weeks 1 – 3, but showed a sudden increase in abundance during Week 4.

Population sizes of the three dominant Collembola species, mentioned above, gradually increased from Week 1 to Week 2, reached a peak in Week 3, but plummeted in the last week of sampling. Micro-organisms, such as fungi and bacteria, are primary

decomposers and soil fauna, such as Collembola, aid litter decomposition by stimulating the growth of these microflora through their grazing preferences. During the first week of decomposition, mycophages were found in high densities especially in the meso-bags and litter traps. *Brachytydeus* sp. 1, a mycophage, was found most commonly in the litter traps in either the alfalfa field (Addendum 2: AB and AA) or alfalfa litter in the grassland veld (Addendum 2: BA). This species could very well be associated with fungi involved with alfalfa litter decomposition. *Rhizoglyphus* sp. 1, another mycophage, only appeared in Week 1 in the litter traps in the natural veld (Addendum 2). This species could be a pioneer species during litter decomposition, as they were not found for the rest of the trial. Given the nature of litter traps, *Rhizoglyphus* sp. 1 was either outcompeted by the vast abundance of other mycophages from the second week or preyed on by predators since they were not able to exit the traps.

Another mycophage that reached high densities in the litter traps, during the first week, was *Gamasellopsis* sp. 1. They were also more abundant in the alfalfa samples (Addendum 2) than in the grassland veld. The high abundance of mycophages corresponded with predator densities in both the litter traps and the meso-bags and it could be possible that the high abundance in predators may have delayed decomposition. Especially in the litter traps where organisms could not escape, the high densities of predators could have caused slower decomposition rates. Because predators prey on decomposer fauna (such as saprophages, mycophages and other decomposers), they are considered as part of the decomposition food web. Predators preyed on mycophages that could otherwise have consumed fungi. According to Wise *et al.* (1999), spiders can alter decomposition rates through cascading top-down effects. This should then be true for all predators especially those that occur in high abundance in confined spaces such as litter traps and bags.

The predators recorded in the litter traps and meso-bags reached high densities. *Protogamasellus* sp. 1, a predatory mite, was found in high numbers in both litter types from the alfalfa field (Addendum 2: AA and AB). They were more abundant in the AA sample, but not significantly so. Week 3 was the only sampling date that these mites

were found in such high numbers and they were completely absent from the natural veld (Addendum 2: BA and BB). During Week 1, *Pergamasus* sp. 1 was found in the litter traps only in the alfalfa field (Addendum2: AA and AB). The succeeding sampling dates contained *Pergamasus* sp. 1 for almost all the litter and sampling types, but they occurred in higher abundance at the alfalfa field samples. There is a strong correlation between abundance in *Pergamasus* sp. 1 and the Collembola, *Hypogastrura* (sp. 1 & sp. 2). It is possible that *Pergamasus* sp. 1 favoured these springtails as prey items, or they could have just been more available. Rhodacaridae immatures, also predacious, were only found during Week 1 in the AA sample of the litter traps (Addendum 2). As with *Pergamasus* sp. 1, *Gammasellevans* sp. 1 was also quite abundant through all the sampling dates, but during Week 1, they were only found in the traps, indicating that they prefer more free-living conditions. Another predatory mite, that was abundant in the traps during Week 1, was *Eupodes* sp. 2. The above-mentioned mites vary considerably in size and could therefore prey on a wide variety of decomposer fauna.

Despite the differences in the sampling methods and the mesh size of the litter bags, species found in the three different traps did not differ significantly between the litter traps and meso-bags. Differences between the meso-bags and the litter traps mainly arise in the abundance of certain species within these traps on different sampling dates. Being confined to restricted space with no escape could have influenced the ability of the decomposer fauna to break down plant litter in the litter trap and micro-bags. The litter trap differs from the meso-bag method in one important aspect, namely that the fauna are able to move in and out of the bags but this is not the case with the traps. Micro-bags excluded mainly the larger soil insects, but smaller immature stages could enter the traps and reach maturity. These adults or later developmental stages were then unable to exit the traps again due to their larger size, which lead to population collapses of organisms lower in the food chain causing an irregular pattern of the HFAI. *Pergamasus* sp.1 is one of the bigger predatory mites and the micro-bags were only accessible to immature individuals of these predators. Together with the partial exclusion of other large predators, such as the larger Staphylinidae species, mycophages such as *Eupodes* sp. 2 and *Hypogastrura* could reach high population

densities (Addendum 2). Larger predators, such as the Staphylinidae, were mainly found in the meso-bags and litter traps, but some smaller species were able to enter the micro-bags. Staphylinidae beetles are generally large-bodied predators, compared to the predatory mites, and even in low numbers are able to influence population dynamics.

Meso-bags were the only sampling method that started off with a considerably high HFAI. Of the three sampling methods, this was the only method that combined the accessibility by larger arthropods with natural movement in and out of the bags. The accessibility by larger arthropods allowed for larger predators to enter and prey on decomposer fauna that could have, if not preyed upon, resulted in overpopulation of the restricted area and over exploitation of resources. Not only did this sampling method allow larger predators, but it also allowed larger decomposer fauna. The movement in and out of these bags, allowed arthropods to remove small particles of litter from the bags.

Most soil arthropods are not able to break down plant litter in the initial phases of litter decomposition (Coleman *et al.* 2004). Vermiform Diptera larvae (Addendum 2) were found in high numbers in the alfalfa field and occurred in both the grass and alfalfa litter at this study site, but they were most abundant in the alfalfa litter. These larvae most likely fed on decomposing plant litter and as adults laid their eggs in or near the litter traps. According to Bernays and Chapman (1994), female insects may lay their eggs near or on suitable host plants or, in this case, decomposing litter. They select the oviposition site that will be most suited for larval survival. Because Diptera are flying insects and have advantages in distribution and mobility that wingless soil dwelling fauna do not have, their presence in the litter traps cannot be seen as incidental but rather as opportunistic. Prevalence in food source, *i.e.* alfalfa litter rather than grass litter, stands out and overall they were more abundant in the alfalfa field than in the natural veld. According to Adl (2003), immature stages of various invertebrates can play significantly different roles than the adults of those species and many of these immatures or larvae can contribute a great deal to decomposition processes. During the first three weeks of decomposition during the trial, these larvae were abundant (Addendum 2). They were

not as abundant four weeks into decomposition, which could indicate their importance in the initial stages of litter decomposition.

Vermiform larvae msp. 4 reached high numbers in the litter traps and were more abundant in the alfalfa field, predominantly in the AA samples (Addendum 2). This once again indicates the difference in soil arthropods found at the two study sites and their favouring of alfalfa litter. Larvae that were more abundant in the micro-bags were Elateriform larvae msp. 2. These larvae are also categorized as saprophages and they only appeared from Week 2 onwards. No preference to litter type was observed here (Addendum 2). Liposcelidae msp. 1 was also found more frequently in the alfalfa field of both litter types in all the traps and bags. *Tetranychus urticae* mites were found predominantly in the meso- and micro-bags (Addendum 2). This mite species is known for their polyphagous feeding behaviour as plant parasites and their tendency to become a pest on a wide variety of crop species. According to Marinosci *et al.* (2015), these mites are polyphagous on more than 1100 plant species and have a fast life cycle, leading to fast adaptation to changes in host plant or environmental factors. They are thus able to adapt fast to agricultural disturbances. They usually occur above ground laying their eggs on and feeding on plants. Their dominance in the grassland veld is very interesting, since it would make more sense to have found them in the agricultural field. Being phytophagous and occurring on dead or decaying plant material classifies them as primary (live plant) and secondary (plant litter) phytophages. They were predominantly recorded in the meso- and micro-bags. These mites feed and breed above-ground, but their low numbers in the litter traps suggests that they were feeding on the decaying plant material and/or had found refuge in the litter bags.

An overall increase in soil arthropod abundance can be seen in Week 3 in all the sampling methods (Addendum 2) which may be ascribed to a combination of two factors. One being that it had rained the days before the samples were collected. Secondly the soil arthropod populations were starting to establish after the initial disturbance of placing the litter traps and bags in the soil. During Week 4 most arthropod numbers started to decline. Exceptions include *Tetranychus urticae* (micro-bags), *Capbrya* (litter traps),

Willsowsia (micro-bags and litter traps) and Formicidae msp. 2 (litter traps) (Addendum 2). A decline in arthropod abundance, in Week 4, can possibly be attributed to the small volume of litter left in the samples, in combination with the high abundance the week before. It could be possible that the high abundance of some organisms in Week 3 resulted in resource over-exploitation which resulted in a collapse in populations, in turn leading to a decline in overall abundance.

Another factor, influencing decomposition rates, is fungal growth. As mentioned earlier these micro-organisms are mainly responsible for primary decomposition of litter. Fungal growth was observed in micro- and meso-bags, in the first and second week of decomposition, and was more conspicuous in the micro-bags. This was only a visual observation and no quantification or identification of fungi was included in this study. It could be the presence of fungi that led to the high abundance of mycophages in Week 3 (Addendum 2). Fungal growth in general probably resulted in faster decomposition rates since, as primary decomposers, they break down tough plant fibre in the litter, paving the way for other decomposers to follow. Fungi also attract mycophages that feed on them which, in turn, stimulates their growth but can also stunt decomposition rates when overgrazing occurs. This trophic scenario may explain the zigzag HFAI pattern in the micro-bag sampling method.

5.3.2. Decomposition rates of different allelopathic material

The percentage of litter mass loss, of different allelopathic plant parts in litter traps, can be seen in Figure 7. During the first week of decomposition, in the litter traps from alfalfa leaves, decomposition occurred the fastest at both study sites. Overall, most samples decomposed faster in the alfalfa field for the first week the exception being the flower litter sample at the alfalfa study site. As discussed earlier, flower material includes petioles and seeds. Seeds are one of the most sturdy litter parts to decompose since, for eventual germination purposes, they have to survive in soil for a longer period than other plant parts. Furthermore, since litter traps may collect arthropod species that are soft fibre feeders, the seeds could have taken longer to experience the initial phases of

decomposition. This was not the case in the grassland veld. One explanation for this may be that the organisms at this study site are adapted to utilize drier and more nutrient poor litter. Although the alfalfa field had the highest abundance of species, the grassland veld had a more complex diversity of soil arthropods, which may have contributed towards their success in breaking down the seeds in the flower litter traps in these initial stages.

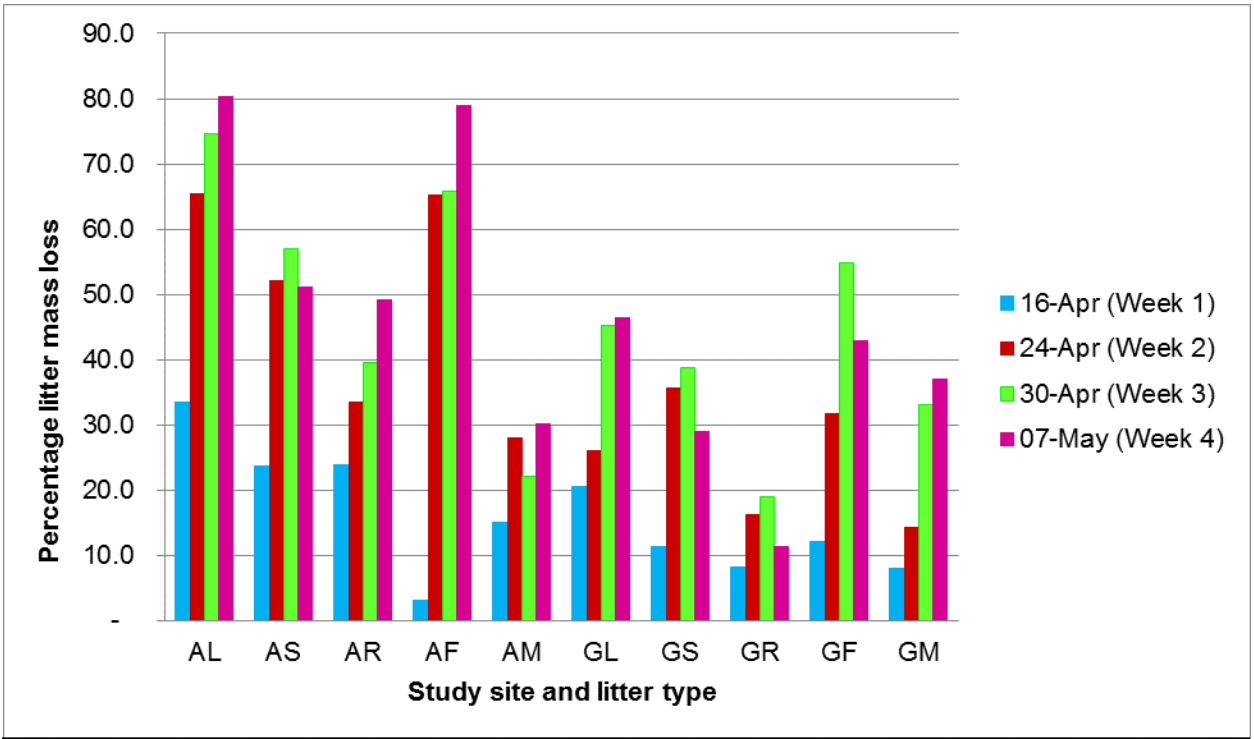

Figure 7: The percentage of litter mass loss of different allelopathic plant parts (alfalfa and mixed grass) at their study site of origin and the opposed field on four sampling dates (2014) in litter traps. (A - Alfalfa field, G – Grassland field, L – Leaves, S-Stems, R - Roots, F - Flowers and M - Mixed grass litter) The first letter on the X-bar represents the study site and the second the litter type.

Chon & Kim (2002) studied the impact of allelopathic alfalfa material on the root length of alfalfa plants. They found that seeds had the greatest influence in this regard, followed by stems, roots, and leaves in that order of significance. This is exactly the pattern observed in the decomposition of litter in traps for the alfalfa study site in Week 1. Flower material decomposed the slowest, followed by mixed grasses, alfalfa

roots and stems and the fastest decomposition was observed with the alfalfa leaves (Figure 7), probably because this plant part is the least fibrous. In Week 1 the grassland study site showed a pattern, with mixed grasses having the least litter mass loss, followed by roots, stems, flowers and leaves in that order. This decomposition pattern is not much different from the pattern observed at the alfalfa study site, except that the flower material decomposed second fastest.

In Week 2 the litter traps at the alfalfa study site showed mixed grasses to decompose the slowest, followed by roots, stems, flowers and leaves (Figure 7). The initially slow decomposing flower litter were then one of the fastest. The grassland field also showed the mixed grasses to decompose the fastest, followed by stems, leaves, flowers and stems. It was in the second week of decomposition that arthropod abundance at both study sites started to increase. In Week 3, mixed litter had the least mass loss at the alfalfa field and thereafter roots, stems, flowers and leaves, just as the week before. The grassland study site showed roots to be the slowest in decomposition, then mixed grasses, stems, leaves and flowers. The pattern stays more or less the same for Week 4, with leaves and flowers once again showing a faster decomposition rate than the other litter types (Figure 7).

Except for the seeds in the flower litter, the leaf and flower litter had the largest physical surface area exposed for decomposition. This could explain the rapid mass loss in these two litter types. Similar to what Chon & Kim (2002) suggested, the leaves had the lowest allelopathic potential and would decompose at a faster rate than plant parts with a higher allelopathic potential. The mixed grasses sample contained a variety of grass species. According to Gartner & Cardon (2004), mixed litter repeatedly decompose at faster rates than single species litter in a range of studies. However, it was not the case in this short-term study. Here litter quality and microclimatic conditions could have had a more determinant effect on litter decomposition rates, causing the mixed litter to decompose slower. The alfalfa study site was exposed to periodic irrigation which meant that soil was more regularly moist than in the case of the grassland study site. This could have promoted fungal growth, which led to faster

decomposition rates. Even when taking microclimate into consideration, it is clear that litter quality played a dominant role in decomposition, since the mixed grass litter on average decomposed the slowest at both study sites, regardless of the absence or presence of irrigation.

Overall, the largest quantity of litter mass was lost in the litter traps, for the leaf sample, in the alfalfa study site. The percentage of litter mass loss for this sample was 80.4%. The percentage mass loss on average was very high taking into account that this study was conducted over a four week period. The literature shows that most decomposition studies last between three to twelve months, with between 50% - 80% mass loss within this period. This study made use of an extremely small volume of litter (3g), which could have contributed to the faster decomposition rates. Some of the samples also did not decompose evenly, meaning that the mass loss in the consecutive week in some cases was less than that of the previous week. Even though these samples are placed a short distance from one another, soil fauna between samples differed significantly in diversity and abundance. One reason for this is the aggregation behaviour of some soil fauna.

Collembola, being one of these aggregating groups, produces a pheromone known as the aggregation pheromone to attract other individuals of the same species (Mertens & Bourgoignie 1977, Verhoef *et al.* 1977, Manica *et al.* 2001). This is believed to improve survival through 'safety in numbers' and the sharing of food resources. According to Manica *et al.* (2001) these pheromones do not seem to be water soluble since Collembola aggregated on the same spot of filter paper after it had been washed with water. Verhoef *et al.* (1977) found that that this pheromone is not strictly species specific and that the behaviour of the Collembola receiving the stimulus is orthokinetic, meaning that the response of other Collembola will be dependent on the intensity of the stimulus, in turn meaning that the more Collembola aggregate the more Collembola will be attracted. This explains why some samples had a high abundance of Collembola, whilst others only had a few. Considering this may explain why some samples of the same litter type and sampling method would not have the same decomposition pattern.

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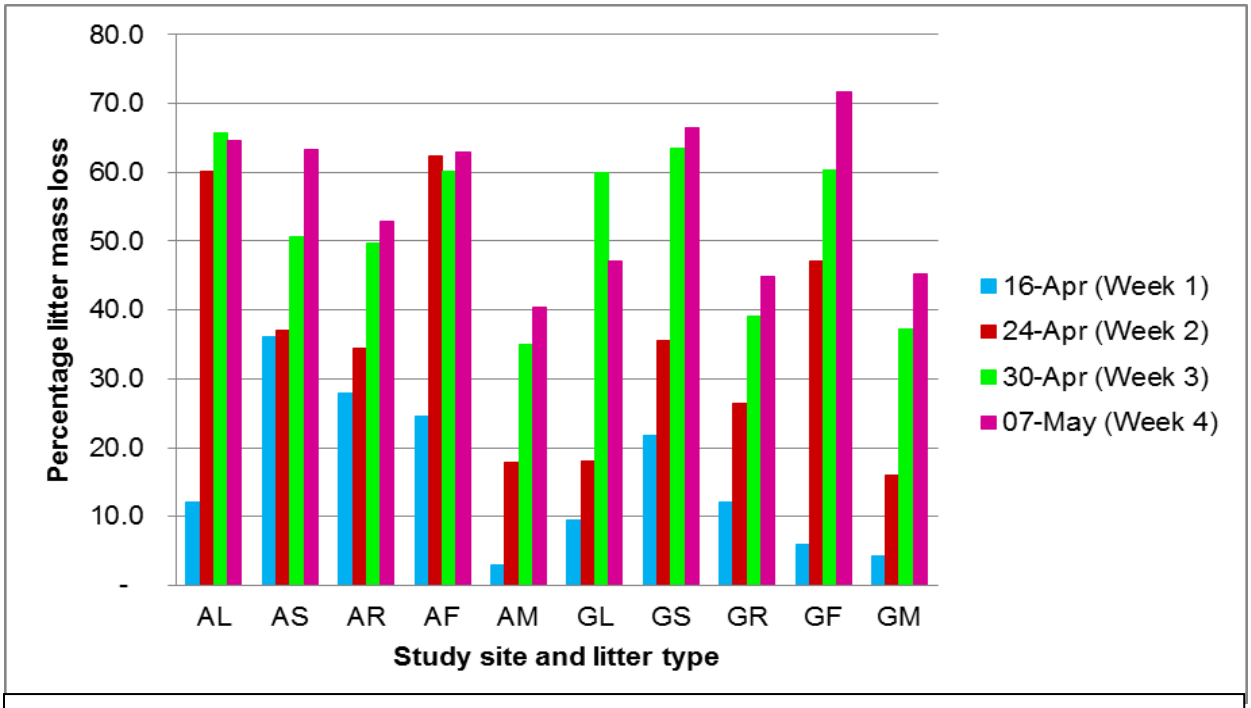

Figure 8: The percentage of litter mass loss (2014) of different allelopathic plant parts (alfalfa and mixed grass) at their study site of origin and the opposed field on four sampling dates in meso-bags. (A- Alfalfa field, G – Grassland field, L – Leaves, S-Stems, R- Roots, F- Flowers and M- Mix grass litter) The first letter on the X-axis represents the study site and the second the litter type.

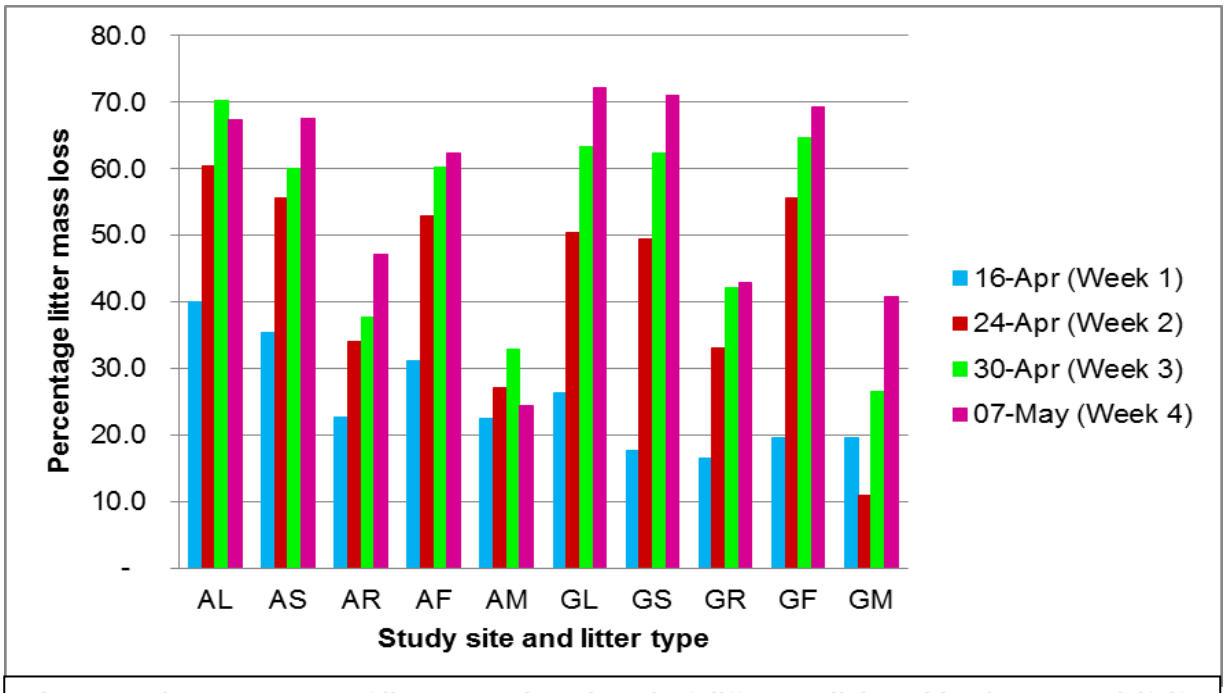

Figure 9: The percentage of litter mass loss (2014) of different allelopathic plant parts (alfalfa and mixed grass) at study site of origin and the opposed field on four sampling dates in micro-bags. (A- Alfalfa field, G – Grassland field, L – Leaves, S-Stems, R- Roots, F- Flowers and M- Mix grass litter) The first letter on the X-axis represents the study site and the second the litter type.

The percentage of litter mass loss of different allelopathic plant parts in meso-bags can be seen in Figure 8. The mixed grass litter and roots in the meso-bags showed very slow decomposition rates throughout the study at both study sites. Stems decomposed faster in the first two weeks than it did in the litter traps but leaves and flowers mostly decomposed the fastest of all litter types, especially from the third week onwards. As opposed to above, seed break-down now happens more rapidly because of the material being completely covered by soil during the trial. The meso-bags had the greatest abundance of arthropods, with Collembola and mites reaching very high population densities, especially in the third week (Addendum 2). Despite this high dominance of Collembola and mites, no significant mass loss can be observed for Week 3 (Figure 8). This goes to show that higher arthropod abundance within these litter samples does not necessarily mean that the decomposition rates will increase immediately. The percentage of litter mass loss of different allelopathic plant material in micro-bags can be seen in Figure 9.

On average mixed grass litter once again decomposed the slowest in the micro-bags as well (Figure 9). Thereafter roots, stems, flowers and leaves decomposed from slowest to fastest, in roughly that order. This decomposition pattern is not significantly different from that of the other two sampling methods. The average litter loss of the micro-bags was between litter traps and meso-bags taking second place. It would therefore seem that litter decomposition rates of allelopathic plant material is not as strongly dependant on the allelopathic potential of the plant, but rather on micro-climate, surface area of plant material exposed when decomposing and litter quality. Litter quality would differ significantly between hard woody roots and thin plant leaves. This can explain why leaves and flower material decomposed the fastest save for a few exceptions.

5.4. Conclusion

Decomposition of plant litter is a complex process that includes micro-, meso- and macrofauna. The different sampling methods used in this decomposition trial showed some filtering effect in terms of the organisms allowed into the traps. The HFAI patterns for the four successive sampling dates (16, 24, 30 April and 07 May 2014) temporally correlate with the abundance of soil arthropods within these traps or bags at the given sampling date. Even though all sampling methods created an unnatural representation of litter decomposition agents, by excluding certain size groups of soil arthropods, the overall conclusion is that a HFA to certain extent was confirmed across all the sampling methods for this short-term decomposition study. However, instabilities were found where soil arthropods were confined to the restricted space of the traps or bags. Meso-bags were the most natural, allowing free movement for the soil arthropods. *Isotoma* and *Hypogastrura* collembolan species reached a high abundance especially in the alfalfa field. These widespread or possibly introduced species of Collembola flourished in the disturbed area of the alfalfa field. Although this field was not subjected to regular soil disturbances, the alfalfa is still harvested regularly, causing stress in plants and certain changes in soil properties. *Willowsia*, *Tullbergia* and *Brachystomella* were the dominant Collembola species in the grassland field, suggesting that certain species may

survive better in the presence of specific vegetation types and that they can be more sensitive to agricultural disturbances than widespread or possibly introduced species.

Fungi, the key primary decomposers of litter, can influence litter decomposition significantly. The presence of fungi can speed up the decomposition process and depend on mycophages to stimulate their increase in abundance and richness. Both the bag types (micro and meso) had visible fungal growth from the second week of decomposition onwards. Since this sampling date, mycophages started to increase, but population densities declined towards the fourth sampling date suggesting that the volume of litter or competition between arthropods influenced abundance indices. Predators also form part of the decomposition food web and increased in parallel with the number of mycophages. The only association between a specific predator, and its prey preference, was *Pergamasus* that presumably preyed on both species of *Hypogastrura.* It is thus clear that HFA and decomposition rates are dependent on more than just the dominant plant species, with other factors such as agricultural disturbance and organism trophic interactions also playing an important role.

Litter decomposition rates varied between litter types and the study sites where the litter was placed. It is clear that litter decomposition had a small advantage in the more moist alfalfa field. The harder or tougher litter material seemed to be, the slower their decomposition rates except for roots that decomposed faster than grass litter. Litter decomposition rates between the three trap types were also considered insignificant since litter decomposition rate comparison between the traps and bags were more or less the same, with litter traps showing faster decomposition rates by a small margin.

When comparing decomposition rates of the different allelopathic material parts with previous research done in this regard, data across the sites and trapping methods correlated quite strongly in terms of the allelopathic potential of the different plant parts. However, this might also be due to litter properties and not solely because of the allelopathic potential.

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Chapter 6

Chapter summary, final conclusion and recommendations

Chapter 1:

- Soil is a living resource containing various organisms that contribute to ecosystem function and soil health.
- In agriculture, an integrated management system is needed to improve the survival and occurrence of soil organisms. Practices such as intercropping, crop rotation, shifting cultivation, cover crops and the addition of mulches and other organic matter, can improve the occurrence and survival of soil fauna. These are only a few of the management practices that can be implemented to directly improve soil biodiversity and indirectly contribute to soil fertility and soil health.
- Agro-ecosystems with a high biodiversity should be able to self-control pests and produce nutrients necessary for optimal yields. Amongst others, soil fauna play a significant role in the decomposition of plant litter and recycling nutrients back into the soil.
- Allelopathic plants (natural) and GMOs (anthtopogenic) can possibly be harmful and thus might have a negative impact on soil biodiversity in agroecosystems. Allelopathic and aromatic plants influence soil organisms both positively and negatively, creating an uncertainty that requires more study to determine the effects of these potentially harmful types of plants.
- Plant litter decomposition and the factors that influence the process are also important in conserving ecosystem function and services in agro-ecosystems.

Chapter 2:

- To supply in the ever-increasing demand for food and other crop-related resources, GM crops are needed to help producers meet the demand.
- *Bt* maize, being one of the most popular GMOs, has been intensively studied over the last decade, but studies on the effects below-ground has been largely neglected.

- The results of a 2012 study showed that soil surrounding *Bt* maize plants sustained a higher biodiversity than the soil of non-*Bt* maize plants. This could be because of increased crop health and associated larger root mass that benefit the soil organisms. The 2013 study showed no immediate negative effect of *Bt* maize, but the higher biodiversity found in 2013 was not observed as clearly.
- Even though the cultivated field underwent intensive soil disturbance, it appeared as if this encouraged soil fungal growth, since fungal-feeding mycophages were the most abundant of all trophic groups. Disturbance could alternatively allow mycophages to better access the fungal biomass to feed upon, leading to greater abundance.
- Omnivores, mostly represented by ants, had a higher abundance in the control field. Because the soil in cultivated fields is frequently disturbed, ants have difficulty in establishing, resulting in lower numbers in these fields.

Chapter 3:

- A number of crops cultivated world-wide are known to be allelopathic. If these plants are toxic to other plant species, they may also as well be harmful to soil mesofauna. Since allelopathy is enhanced in stressed plants as a survival strategy, the necessity arises to test the response of soil faunal diversity to such a situation.
- No negative impacts on the biodiversity of soil organisms due to allelopathy were recorded in the alfalfa or sunflower trails. It is obvious that soil organisms are exposed to the same stresses as the plant (*e.g.* soil humidity) and that this had a stronger influence.
- The only stress factor that influenced diversity negatively was mechanical disturbance (harvest) in alfalfa fields, but even here, soil humidity and compaction played a more dominant role.
- Stressed alfalfa plants overall harboured a lower diversity of species and did not support all trophic levels as pertinently as non-stressed plants did.

- There were no clear dissimilarity in diversity or evenness of soil fauna associated with male and female sunflower plants and it would seem that once again external factors such as soil type played a more important role.

Chapter 4:

- The onion is known for its strong scent and irritating qualities due to electrophilic sulphur contained in the tissue. Onion also shows insecticidal properties and could possibly be harmful to soil organisms.
- In this trial, a lower diversity was observed in the onion field than in the natural field. Certain species showed low tolerance towards either the onion plants and/or the cultivation and disturbance practises, and only occurred in the natural field. However, the Collembola, *Cryptopygus* sp.1 showed resistance towards these odour and chemical effects and was recorded almost exclusively in the onion field. This possibly suggests that this species is a generalist feeder and adapted to a variety of chemical cues below-ground. *Cryptopygus* sp. 1 is a mycophage and this was the most abundant trophic group.
- Here, once again, external abiotic factors played an important role, with biodiversity increasing as the weather got warmer.
- As in the GM maize study ants were once again more abundant in control fields confirming their sensitivity to soil disturbances, plant structure and the availability of bare soil.

Chapter 5:

- When considering decomposition and the organisms associated with this process, it is important to bear in mind size functional groups (*i.e.* micro-, meso- and macro-fauna). The ability of these different organisms to decompose litter originating from plants in the same field at a faster rate referred to as the home field advantage (HFA). A HFA could possibly increase nutrient turnover in certain areas, thus promoting soil health.

- In this short term decomposition study, a HFA was confirmed to a certain extent. Certain mesofaunal species could also be linked to certain vegetation types and localities, suggesting that some species are adapted to utilize specific plant litter.
- Collembola namely: *Isotoma* and *Hypogastrura* species, reached a high abundance, especially in the alfalfa field. *Willowsia*, *Tullbergia* and *Brachystomella* species were dominant in the grassland field. *Isotoma* and *Hypogastrura* species are possibly introduced or widespread and could possibly have advantages in adapting to agricultural disturbances. The *Willowsia*, *Tullbergia* and *Brachystomella* species are most likely specific to the grassland biome in the Free State and could be more sensitive to agricultural disturbances than widespread or possibly introduced species.
- Except for the Collembola species, associated with different vegetation types, the presence of fungi and pressure from predators also proved to have a noteworthy influence on decomposition rates and also determined the HFA outcome. The only association between a specific predator and its prey preference was a *Pergamasus* mite species that presumably preyed on both species of *Hypogastrura,* since the abundance of these two groups correlated for the largest part of the study.
- Litter quality also played a role, with tougher litter decomposing at a slower rate. Decomposition rate in terms of the different sampling methods showed no noteworthy differences.
- The litter quality factor overruled the expected results of decomposition rates associated with different kinds of allelopathic material and no definite conclusion could be made from this part of the study.

Recommendations

- Recommendations in relation to agriculture include the addition of microbes to the soil as in the case of the Thornberry (Petrusburg) location. This “soil supplement” does not only promote a more complex soil diversity, but favours plants and may reduce stress in plants that trigger a higher release of allelochemicals.

- Concerning GM crops (*Bt*-maize) no specific recommendations can be made. The study showed that soil compaction and humidity played a substantial role in soil faunal diversity. Both these factors are difficult to control in agriculture, especially in dry fields with no artificial irrigation. The addition of organic matter may increase soil humidity and lessen the compaction and will serve as a food source that can increase soil biodiversity.
- When planting aromatic plants (such as onions) soil fauna is influenced negatively. By intercropping with another non-aromatic plant and by altering cultivation practices, soil fauna could possibly be preserved.

Future research

- Include the study of other GM crops over a longer period to determine long-term effects on soil mesofaunal diversity.
- Conduct greenhouse experiments on a wide variety of allelopathic crops with artificial stress conditions to determine the release of chemicals, as well as the subsequent effect on soil mesofauna.
- Investigate the possible tolerance of soil mesofauna to long term aromatic plant exposure, *e.g*. fields that are planted with the same aromatic crop year after year.
- Further investigate leaf litter decomposition and Home Field Advantage using seasonal crops. Because alfalfa is an annual crop, mesofaunal species could have adapted over time. In the case of seasonal crops, this could not have happened.

Addendums

Species lists

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

A - Alfalfa Field 1 (2013, L1, L2 & L3), **B** - Alfalfa Field 2 (2013, L4, L5 & L6), **C** - Alfalfa Field 2 (2013, L8, L9 &L10), **D** - Alfalfa Control Fields (2013, CN, CS & CL), **E** – Maize fields (2013, NBT, RRBT, BTa & BTb), **F** – Maize Control Fields (2013, CN, CS & CL), **G** – Bainsvlei Onion Field (2013, U), **H** – Bainsvlei Onion Control (2013, CN, CS & CL), **I** – Maize fields (2012, BT & NBT), **J** – Maize Control Fields (2012, BT & NBT in Bainsvlei and Bloemdal), **K** – Petrusburg Onion Field (2013, U), **L** – Petrusburg Onion Control (2013, C1, C2 & C3), **M** – Butternut Field (2013, BN), **N** – Sunflower Field (2013, S1♀, S1♂, S2♀, S2♂, S3♀ & S3♂), **O** – Sunflower Control (2013, C1, C2 & C3).

Identification	Sapling sites														
	Bainsvlei								Bainsvlei & Bloemdal		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Kingdom: Animalia															
Phylum: Arthropoda															
Class: Arachnida															
Order: Araneae															
Family: Theridiidae															
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Euryopis immature sp. 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Latrodectus geometricus immature	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Family: Linyphiidae															
Metalepthyphantes sp. 1 immature	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Pelecopsis janus	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Family: Amaurobiidae															
Immature msp. 1	1	1	0	1	0	0	5	0	0	0	0	0	0	0	0
Family: Lycosidae															
Immature msp. 1	3	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Immature msp. 2	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Family: Gnaphosidae															
Camillina cordifera	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Camillina sp. 1 immature	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Drassodes sp. 1 immature	1	1	0	1	0	1	0	0	1		0	0	0	0	1
Pterotricha sp. 1 immature	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Setaphis sp. 1 immature	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Xerophaeus sp. 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zelotes sp. 1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Family: Salticidae															
Heliophanus sp. 1 immature	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pellenes bulawayoensis	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Tanzania sp. 1 immature	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Order: Acari															
Suborder: Ixodida															
Family: Ixodidae															
Amblyomma hebraeum	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Suborder: Mesostigmata															
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Family: Parasitidae															
Pergamasus sp. 1	0	0	0	0	0	0	19	1	0	0	0	0	0	0	0

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

A - Alfalfa Field 1 (2013, L1, L2 & L3), **B** - Alfalfa Field 2 (2013, L4, L5 & L6), **C** - Alfalfa Field 2 (2013, L8, L9 &L10), **D** - Alfalfa Control Fields (2013, CN, CS & CL), **E** – Maize fields (2013, NBT, RRBT, BTa & BTb), **F** – Maize Control Fields (2013, CN, CS & CL), **G** – Bainsvlei Onion Field (2013, U), **H** – Bainsvlei Onion Control (2013, CN, CS & CL), **I** – Maize fields (2012, BT & NBT), **J** – Maize Control Fields (2012, BT & NBT in Bainsvlei and Bloemdal), **K** – Petrusburg Onion Field (2013, U), **L** – Petrusburg Onion Control (2013, C1, C2 & C3), **M** – Butternut Field (2013, BN), **N** – Sunflower Field (2013, S1♀, S1♂, S2♀, S2♂, S3♀ & S3♂), **O** – Sunflower Control (2013, C1, C2 & C3).

Identification	Sapling sites														
	Bainsvlei								Bainsvlei &		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Family: Rhodacaridae															
Immature msp. 1	3	2	5	9	0	0	0	0	0	0	1	0	0	1	3
Protogamasellus sp. 1	49	167	1	49	314	201	1	4	0	0	3	4	8	4	2
Protogamasellus sp. 2	0	0	0	74	55	75	35	22	0	0	60	36	13	27	64
Protogamasellus sp. 3	1265	2528	42	580	0	2	0	2	139	59	10	0	16	2	0
Family: Macrochelidae															
Macrocheles sp. 1	1	0	0	1	3	1	0	0	1	1	3	1	4	4	0
Family: Phytoseiidae															
Typhlodromus sp. 1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Family: Ascidae															
msp. 1	2	0	0	15	0	0	0	0	0	0	0	0	1	0	0
Gamasellevans sp. 1	5	0	0	0	22	1	19	38	1	0	0	0	2	25	1
Family: Dermanyssidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Family: Laelapidae															
Hypoaspis sp. 1	2	0	0	12	0	0	0	1	0	0	0	0	0	0	0
Family: Uropodidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	37	2
Suborder: Prostigmata / Trombidoformes															
Immature msp. 1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Family: Microtrombidiidae															
Microtrombidium sp. 1	7	7	0	35	107	13	0	0	4	8	0	0	0	0	0
Suborder: Prostigmata / Trombidoformes															
Immature msp. 1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Family: Microtrombidiidae															
Microtrombidium sp. 1	7	7	0	35	107	13	0	0	4	8	0	0	0	0	0
Family: Erythraeidae															
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	5	0	2	11
Immature msp. 2	13	8	0	3	0	1	0	0	0	0	0	0	0	0	0
Immature msp. 3	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Leptus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Leptus sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Erythraeus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Family: Bdellidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	5	0	1
msp. 2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

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	Sapling sites

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

Identification	Bainsvlei								Bainsvlei & Bloemdal		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Spinibdella thori	1	0	0	3	0	4	0	8	0	0	0	1	1	0	6
Spinibdella sp. 1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Bdellodes sp. 1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bdelta sp. 1	5	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Cunaxidae															
Cunaxa sp. 1	19	2	0	4	2	0	0	0	0	0	0	5	0	49	0
Cunaxa sp. 2	1	0	0	0	9	32	0	2	0	0	0	0	1	0	0
Dactyloscheles sp. 1	0	4	6	4	0	0	0	0	0	0	0	0	0	0	0
Family: Ragidiidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Family: Eupodidae															
Eupodes sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eupodes sp. 2	969	447	5	86	6	2	3	9	0	0	2	6	18	27	5
Family: Tydeidae															
Brachytydeus sp. 1	871	26	13	28	133	4	46	94	0	0	2	207	251	102	294
Brachytydeus sp. 2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Pronematus sp. 1	2188	492	1	18	11	1	6	9	0	0	45	257	0	91	1
Family: Pseudocheylidae															
Anoplocheylus sp. 1	0	0	0	0	2	0	9	27	0	0	1	13	1	0	0
Family: Caeculidae															
Immature msp. 1	1	13	0	2	0	0	0	0	0	0	0	0	0	0	0
Microcaeculus sp. 1	0	0	0	0	0	0	0	2	0	0	0	3	1	0	2
Microcaeculus sp. 2	0	0	9	0	0	0	0	0	1	0	0	0	0	0	0
Family: Adamystidae															
Saxidromus sp. 1	0	3	0	0	7	0	0	0	0	0	0	0	1	0	0
Family: Anystidae															
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
msp. 1	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
Genus & Species:															
Anystis sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Family: Tetranychidae															
Bryobia praetiosa	0	0	0	0	0	1	0	2	0	0	0	0	1	0	0
Tetranychus urticae	87	70	0	0	0	1	0	2	0	0	0	4	0	0	0
Family: Linotetranidae															
Linotetranus sp. 1	0	2	0	0	0	4	0	18	0	0	0	6	0	0	0

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Sapling sites

Identification	Bainsvlei								Bainsvlei & Bloemdal		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Family: Raphignathidae															
msp. 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Cheyletidae															
Cheyletiella sp. 1	0	0	2	0	28	0	0	5	0	0	0	2	0	2	0

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

Chelyletus sp. 1	49	6	108	27	49	0	0	0	0	0	0	0	1	1	0
Family: Scutacaridae															
Imparipes sp. 1	6	10	0	7	4	3	0	0	0	0	0	0	2	32	0
Family: Tarsonemidae															
Hemitarsonemus sp. 1	48	79	2	15	7	13	38	1	0	0	3	1	8	63	12
Suborder: Prostigmata / Endeostigmata															
Family: Nanorchestidae															
Nanorchestes globosus	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
Nanorchestes sp.1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Speleorchestes meyeri	0	4	0	40	714	60	0	7	0	1	2	33	15	812	5
Order: Oribatida															
Immature msp. 1	1	0	0	2	0	0	0	0	0	0	0	3	5	0	0
Family: Euphthiracaridae															
Acrotritia sp. 1	0	0	0	0	0	0	20	0	0	0	9	85	5	3	10
Family: Brachychthoniidae															
Brachychthonius sp. 1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Family: Epilohmanniidae															
Epilohmannia sp.1	1	20	0	48	0	0	16	0	0	0	2	0	567	412	9
Family: Opiidae															
Oppiella sp. 1	89	87	4	44	16	31	21	118	392	0	0	0	3	0	0
Family: Galumnidae															
Galumna sp. 1	2	0	0	7	0	0	0	1	0	0	0	0	0	0	0
Family: Tectocepheidae															
Tectocepheus sp. 1	0	0	0	6	0	0	0	0	0	0	0	0	266	194	12
Family: Scutoverticidae															
Ethiovertex sp. 1	0	0	0	0	0	0	0	0	0	0	0	8	1	0	2
Family: Oribatulidae															
Immature msp. 1	0	2	0	0	1	3	3	3	0	0	309	16	438	96	8
Immature msp. 2	2	0	0	18	0	0	0	0	0	0	0	0	0	0	0
Oribatula sp. 1	0	47	0	1	0	5	2	55	2	1	241	120	1006	367	28
Oribatula sp. 2	0	0	1	0	0	0	0	0	0	0	0	3	2	0	0
Oribatula sp. 3	0	0	0	0	0	0	0	4	0	0	59	1	0	0	0

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Identification	**Sapling sites**														
	Bainsvlei								**Bainsvlei & Bloemdal**		**Petrusburg**				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Family: Chamobatidae															
Hypozetes sp. 1	0	0	0	1	1	0	0	0	0	0	0	0	5	0	0
Family: Protoribatidae															
Protoribates sp. 1	0	0	0	0	0	0	0	0	0	0	0	7	1	0	1
Order: Astigmata / Astigmatina															
Family: Acaridae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Tyrophagus putrescentiae	6	9	0	18	26	10	8	12	0	0	1077	75	2	0	16
Rhizoglyphus sp. 1	16	82	0	303	3927	880	0	0	0	0	0	0	1	0	0
Rhizoglyphus sp. 2	0	0	0	0	1	0	0	0	0	0	11	0	2	17	7
Rhizoglyphus sp. 3	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Caloglyphus sp. 1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Order: Pseudoscorpiones															
msp. 1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Class: Diplopoda															
msp. 1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0
msp. 2	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0
Class: Chilopoda															
Order: Geophilomorpha															
msp. 1	1	0	0	0	0	0	0	0	0	0	0	0	5	4	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	1	6	1
msp. 3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Class: Symphyla															
Order: Scolopendromorpha															
Family: Scolopendreliidae	0	0	0	0	0	0	11	0	0	0	3	7	3	5	0
Class: Malacostraca															
Order: Isopoda															
Family: Armadillidiidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	2	27	0
Family: Oniscidae															
msp. 1	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0
Class: Insecta															
Order: Collembola															
Family: Hypogastruridae															
Hypogastrura sp. 1	0	0	0	0	0	0	0	0	0	0	2	1	41	49	52

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Identification	Sapling sites														
	Bainsvlei								Bainsvlei & Bloemdal		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Hypogastrura sp. 2	15	256	0	3	33	77	2	25	4	3	0	216	300	6	140
Hypogastrura sp. 3	3	7	0	0	7	4	0	0	0	0	0	0	0	0	0
Hypogastrura sp. 4	26	27	5	2	0	0	0	0	0	0	0	0	0	0	0
Family: Brachystomellidae															
Brachystomella sp. 1	0	0	0	0	0	0	0	0	112	84	0	0	0	0	0
Family: Neanuridae															
msp. 1	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	5	1	0	0	0
Family: Isotomidae															
Folsomia sp. 1	171	27	0	2	4	2	0	0	0	0	0	209	8	89	825
Folsomides sp.1	1	7	1	0	181	3	38	26	29	41	0	0	0	1	0
Cryptopygus sp. 1	1402	1065	12	82	4	6	0	0	0	0	0	0	0	2	417
Cryptopygus sp. 2	120	69	6	6	0	0	208	0	1	12	106	179	0	0	0
Cryptopygus sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	1	8	13
Proisotoma sp. 1	31	0	4	2	0	0	0	0	0	0	0	0	0	0	0
Parasitoma sp. 1	6	0	0	0	0	0	0	0	0	0	0	1	5	0	0
Family: Entomobryidae															
Capbrya sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Capbrya sp. 2	3	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Entomobrya immature sp. 1	11	3	1	3	0	0	0	0	0	0	0	0	0	0	0
Entomobrya cf. *multifasciata*	26	7	4	17	165	10	0	0	1	1	6	1	0	1	0
Entomobrya sp. 1	2	0	0	0	4	6	0	0	0	0	0	0	1	0	209
Entomobrya sp. 2	0	3	7	0	2	0	0	0	0	0	0	0	0	0	0
Entomobrya sp. 3	52	47	0	1	0	0	0	0	0	0	0	0	0	0	0
Lepidocyrtus sp. 1	0	0	0	0	0	0	0	0	0	0	0	18	2	34	20
Seira sp. 1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
Seira sp. 2	5	3	0	0	23	4	19	2	0	0	0	0	0	0	0
Seira sp. 3	26	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Family: Neelidae															
Megalothorax sp. 1	0	0	0	0	0	0	4	0	0	0	1	0	0	0	0
Family: Sminthurididae															
Sphaeridia sp. 1	34	346	12	0	0	0	0	0	0	0	0	0	0	0	0
Sphaeridia sp. 2	23	7	0	4	7	4	1	19	0	0	0	0	1	91	51
Family: Bourletiellidae															
Bourletiella sp. 1	0	3	0	5	2	6	0	0	0	0	0	0	2	0	18
Bourletiella sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	37	1

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

A - Alfalfa Field 1 (2013, L1, L2 & L3), **B** - Alfalfa Field 2 (2013, L4, L5 & L6), **C** - Alfalfa Field 2 (2013, L8, L9 &L10), **D** - Alfalfa Control Fields (2013, CN, CS & CL), **E** – Maize fields (2013, NBT, RRBT, BTa & BTb), **F** – Maize Control Fields (2013, CN, CS & CL), **G** – Bainsvlei Onion Field (2013, U), **H** – Bainsvlei Onion Control (2013, CN, CS & CL), **I** – Maize fields (2012, BT & NBT), **J** – Maize Control Fields (2012, BT & NBT in Bainsvlei and Bloemdal), **K** – Petrusburg Onion Field (2013, U), **L** – Petrusburg Onion Control (2013, C1, C2 & C3), **M** – Butternut Field (2013, BN), **N** – Sunflower Field (2013, S1♀, S1♂, S2♀, S2♂, S3♀ & S3♂), **O** – Sunflower Control (2013, C1, C2 & C3).

Identification	Sapling sites														
	Bainsvlei								Bainsvlei &		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Order: Diplura															
Family: Japygidae															
msp. 1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Order: Orthoptera															
Family: Gryllidae															
Immature msp. 1	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0
Order: Dermaptera															
Family: Labiduridae															
msp. 1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0
msp. 2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Order: Isoptera															
Family: Termitidae															
msp. 1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	7
Family: Hodotermitidae															
msp. 1	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Order: Hemiptera															
Immature msp. 1	0	0	0	11	0	11	0	0	3	0	0	0	0	1	0
Immature msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Immature msp. 3	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
Immature msp. 4	2	3	0	0	0	0	0	0	0	0	0	0	0	1	0
Immature msp. 5	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0
Immature msp. 6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Immature msp. 7	1	0	0	4	0	4	0	0	0	0	0	0	0	0	0
Immature msp. 8	0	0	0	0	1	0	0	7	4	0	0	0	0	0	0
Immature msp. 9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Immature msp. 10	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Immature msp. 11	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Immature msp. 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Immature msp. 13	0	0	0	5	0	5	0	0	0	7	0	0	0	0	0
Immature msp. 14	2	0	0	2	0	1	0	1	0	0	0	0	0	0	0
Family: Reduviidae															
msp. 1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Family: Miridae															
msp. 1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
Family: Cydnidae															
msp. 1	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

A - Alfalfa Field 1 (2013, L1, L2 & L3), **B** - Alfalfa Field 2 (2013, L4, L5 & L6), **C** - Alfalfa Field 2 (2013, L8, L9 &L10), **D** - Alfalfa Control Fields (2013, CN, CS & CL), **E** – Maize fields (2013, NBT, RRBT, BTa & BTb), **F** – Maize Control Fields (2013, CN, CS & CL), **G** – Bainsvlei Onion Field (2013, U), **H** – Bainsvlei Onion Control (2013, CN, CS & CL), **I** – Maize fields (2012, BT & NBT), **J** – Maize Control Fields (2012, BT & NBT in Bainsvlei and Bloemdal), **K** – Petrusburg Onion Field (2013, U), **L** – Petrusburg Onion Control (2013, C1, C2 & C3), **M** – Butternut Field (2013, BN), **N** – Sunflower Field (2013, S1♀, S1♂, S2♀, S2♂, S3♀ & S3♂), **O** – Sunflower Control (2013, C1, C2 & C3).

Identification	Sapling sites														
	Bainsvlei								Bainsvlei &		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Family: Lygaeidae															
msp. 1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0
Family: Cicadellidae															
msp. 1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
msp. 2	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
msp. 3	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
msp. 1	1	4	0	0	0	0	0	0	6	4	0	0	0	0	0
msp. 2	4	4	0	0	0	0	0	0	3	8	0	0	0	0	0
Order: Thysanoptera															
Immature msp. 1	0	18	0	2	0	0	0	2	0	0	0	2	0	0	0
Immature msp. 2	39	14	18	7	6	6	4	1	0	0	1	0	2	2	1
Immature msp. 3	0	0	0	37	6	1	0	36	0	0	0	0	0	0	0
Immature msp. 4	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Immature msp. 5	1	7	0	255	0	255	0	0	0	0	0	1	1	0	5
Immature msp. 6	0	0	0	0	1	0	0	0	0	0	0	9	1	0	1
Immature msp. 7	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Immature msp. 8	3	4	2	1	0	1	0	0	0	0	0	0	0	0	0
Family: Phlaeothripidae															
msp. 1	13	2	0	2	10	2	0	0	8	11	0	2	0	1	0
msp. 2	0	0	0	1	3	0	0	1	1	0	0	0	0	0	0
msp. 3	4	0	0	2	0	1	0	1	0	1	0	7	0	0	7
msp. 4	1	1	0	1	0	1	0	0	0	0	0	1	0	0	3
msp. 5	1	0	0	0	1	0	0	0	0	0	0	1	0	2	1
Family: Aeolothripidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Family: Thripidae															
msp. 1	1	11	0	3	2	3	0	0	0	0	0	3	1	1	2
msp. 2	48	10	5	4	5	1	7	6	5	3	6	5	7	91	18
msp. 3	14	0	0	0	0	0	0	0	0	0	0	9	0	0	0
msp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
msp. 5	0	3	0	1	0	0	0	1	0	0	0	1	0	0	0
Order: Pscoptera															
Family: Lepidopsocidae															
msp. 1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Family: Trogiidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Family: Liposcelidae															
msp. 1	5	5	0	98	8	82	1	31	14	18	0	76	2	0	50

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

A - Alfalfa Field 1 (2013, L1, L2 & L3), **B** - Alfalfa Field 2 (2013, L4, L5 & L6), **C** - Alfalfa Field 2 (2013, L8, L9 &L10), **D** - Alfalfa Control Fields (2013, CN, CS & CL), **E** – Maize fields (2013, NBT, RRBT, BTa & BTb), **F** – Maize Control Fields (2013, CN, CS & CL), **G** – Bainsvlei Onion Field (2013, U), **H** – Bainsvlei Onion Control (2013, CN, CS & CL), **I** – Maize fields (2012, BT & NBT), **J** – Maize Control Fields (2012, BT & NBT in Bainsvlei and Bloemdal), **K** – Petrusburg Onion Field (2013, U), **L** – Petrusburg Onion Control (2013, C1, C2 & C3), **M** – Butternut Field (2013, BN), **N** – Sunflower Field (2013, S1♀, S1♂, S2♀, S2♂, S3♀ & S3♂), **O** – Sunflower Control (2013, C1, C2 & C3).

Identification	Sapling sites														
	Bainsvlei								Bainsvlei &		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Family: Elipsocidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Order: Coleoptera															
Immatures (Larvae)															
Scarabaeiform larvae msp. 1	6	4	0	1	0	1	0	0	1	2	0	0	0	0	1
Scarabaeiform larvae msp. 2	2	0	0	1	1	1	0	0	0	0	0	0	1	0	2
Scarabaeiform larvae msp. 3	50	207	7	10	7	4	0	28	11	6	0	1	0	0	0
Scarabaeiform larvae msp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scarabaeiform larvae msp. 5	7	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Scarabaeiform larvae msp. 6	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Scarabaeiform larvae msp. 7	1	2	0	0	1	0	0	0	1	0	0	0	0	0	0
Campodeiform larvae msp. 1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
Campodeiform larvae msp. 2	0	0	0	1	2	1	0	0	0	0	0	0	0	12	0
Campodeiform larvae msp. 3	0	0	0	2	1	2	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 1	3	0	0	5	40	5	4	0	0	0	0	2	0	0	0
Elateriform larvae msp. 2	65	56	0	14	88	8	30	7	2	3	1	5	37	4	3
Elateriform larvae msp. 3	1	0	0	2	7	2	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 4	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 5	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 6	3	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 7	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 8	17	3	2	0	0	0	5	0	3	5	5	1	7	44	3
Elateriform larvae msp. 9	0	0	0	0	5	0	0	0	0	0	0	0	0	1	0
Elateriform larvae msp. 10	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 11	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 12	3	10	0	0	0	0	0	0	0	0	0	0	1	0	0
Elateriform larvae msp. 13	1	0	0	2	0	0	0	2	0	0	0	0	1	0	3
Elateriform larvae msp. 14	0	1	0	0	2	0	5	0	0	0	0	0	1	0	0
Elateriform larvae msp. 15	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1
Elateriform larvae msp. 16	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0
Elateriform larvae msp. 17	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Elateriform larvae msp. 18	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Elateriform larvae msp. 19	0	0	0	1	0	0	0	1	8	16	0	0	0	0	0
Elateriform larvae msp. 20	7	0	0	3	0	1	1	2	0	0	0	0	0	2	0
Elateriform larvae msp. 21	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Elateriform larvae msp. 22	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
Elateriform larvae msp. 23	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Vermiform larvae msp. 1	25	15	0	0	0	0	0	0	0	0	0	0	0	2	0
Vermiform larvae msp. 2	0	3	0	1	0	0	0	1	0	0	0	0	0	0	1

A - Alfalfa Field 1 (2013, L1, L2 & L3), **B** - Alfalfa Field 2 (2013, L4, L5 & L6), **C** - Alfalfa Field 2 (2013, L8, L9 &L10), **D** - Alfalfa Control Fields (2013, CN, CS & CL), **E** – Maize fields (2013, NBT, RRBT, BTa & BTb), **F** – Maize Control Fields (2013, CN, CS & CL), **G** – Bainsvlei Onion Field (2013, U), **H** – Bainsvlei Onion Control (2013, CN, CS & CL), **I** – Maize fields (2012, BT & NBT), **J** – Maize Control Fields (2012, BT & NBT in Bainsvlei and Bloemdal), **K** – Petrusburg Onion Field (2013, U), **L** – Petrusburg Onion Control (2013, C1, C2 & C3), **M** – Butternut Field (2013, BN), **N** – Sunflower Field (2013, S1♀, S1♂, S2♀, S2♂, S3♀ & S3♂), **O** – Sunflower Control (2013, C1, C2 & C3).

Identification	Sapling sites		
	Bainsvlei	Bainsvlei &	Petrusburg

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Family: Carabidae															
Subfamily: Pterostichinae															
msp. 1	0	0	0	0	0	0	0	0	1	4	0	0	1	0	0
cf msp. 2	2	0	0	2	1	0	0	2	0	0	0	0	0	0	1
Harpalus sp. 1	0	1	0	0	4	0	0	1	0	0	0	0	1	0	0
Pterostichus sp. 1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2
Family: Histeridae															
msp. 1	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Family: Staphylinidae															
msp. 1	0	0	0	1	5	5	5	1	4	4	0	4	1	0	0
msp. 2	1	1	1	6	0	0	1	0	0	0	0	0	5	7	0
Family: Scarabaeidae															
msp. 1	17	22	4	9	0	0	0	0	11	9	0	0	0	0	0
msp. 2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Aphodius sp. 1	6	12	0	3	0	0	0	0	1	0	0	0	1	0	0
Aphodius sp. 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphodius sp. 3	0	0	0	0	0	7	3	2	0	1	0	1	4	0	1
Onthophagus sp. 1	4	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Rhyssemus sp. 1	2	0	0	0	4	3	0	0	1	0	0	2	0	0	0
Philonthus sp. 1	1	0	0	0	0	1	0	0	0	0	0	0	1	3	0
Philonthus sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Family: Elateridae															
Cardiotarsus acuminatus	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Family: Dermestidae															
Anthrenus sp. 1	0	0	0	0	0	0	2	0	0	0	3	1	0	0	0
Family: Anobiidae															
msp. 1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Family: Melyridae															
Astylus atromaculatus	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Family: Nitidulidae															
Brachypeplus sp. 1	0	0	0	0	2	0	0	0			0	0	0	0	0
Family: Silvanidae															
msp. 1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Family: Chryptophagidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Family: Latridiidae															
cf msp. 1	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

A - Alfalfa Field 1 (2013, L1, L2 & L3), **B** - Alfalfa Field 2 (2013, L4, L5 & L6), **C** - Alfalfa Field 2 (2013, L8, L9 &L10), **D** - Alfalfa Control Fields (2013, CN, CS & CL), **E** – Maize fields (2013, NBT, RRBT, BTa & BTb), **F** – Maize Control Fields (2013, CN, CS & CL), **G** – Bainsvlei Onion Field (2013, U), **H** – Bainsvlei Onion Control (2013, CN, CS & CL), **I** – Maize fields (2012, BT & NBT), **J** – Maize Control Fields (2012, BT & NBT in Bainsvlei and Bloemdal), **K** – Petrusburg Onion Field (2013, U), **L** – Petrusburg Onion Control (2013, C1, C2 & C3), **M** – Butternut Field (2013, BN), **N** – Sunflower Field (2013, S1♀, S1♂, S2♀, S2♂, S3♀ & S3♂), **O** – Sunflower Control (2013, C1, C2 & C3).

Identification	Sapling sites														
	Bainsvlei								Bainsvlei &		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Family: Mycetophagidae															
msp. 1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0
Family: Tenebrionidae															
msp. 1	0	0	0	0	0	0	0	0	1	11	0	0	0	0	0
Tribolium castaneum	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zophosis sp. 1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Family: Anthicidae															
msp. 1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
msp. 2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Anthicus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Anthicus sp. 2	0	0	1	1	7	1	0	0	0	0	0	0	0	0	0
Anthicus sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Formicomus sp. 1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Family: Chrysomelidae															
msp. 1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Family: Curculionidae															
msp. 1	0	1	0	1	0	1	0	2	1	0	0	0	0	0	0
msp. 2	0	9	0	2	0	0	0	3	0	0	0	0	0	0	0
Family: Scolytidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Order: Hymenoptera															
Exarate pupa msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Family: Braconidae															
msp. 1	0	2	0	0	0	0	0	0	8	0	0	0	0	0	0
msp. 2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Family: Mymaridae															
msp. 1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Family: Encyrtidae															
msp. 1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0
Family: Chalsididae															
msp. 1	1	0	0	0	0	1	0	0	2	0	0	0	0	0	0
Family: Platygastridae															
msp. 1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
msp. 2	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0
Family: Formicidae															
Immature msp. 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Immature msp. 2	2	0	0	1	0	1	0	0	0	0	0	0	0	0	0

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

A - Alfalfa Field 1 (2013, L1, L2 & L3), **B** - Alfalfa Field 2 (2013, L4, L5 & L6), **C** - Alfalfa Field 2 (2013, L8, L9 &L10), **D** - Alfalfa Control Fields (2013, CN, CS & CL), **E** – Maize fields (2013, NBT, RRBT, BTa & BTb), **F** – Maize Control Fields (2013, CN, CS & CL), **G** – Bainsvlei Onion Field (2013, U), **H** – Bainsvlei Onion Control (2013, CN, CS & CL), **I** – Maize fields (2012, BT & NBT), **J** – Maize Control Fields (2012, BT & NBT in Bainsvlei and Bloemdal), **K** – Petrusburg Onion Field (2013, U), **L** – Petrusburg Onion Control (2013, C1, C2 & C3), **M** – Butternut Field (2013, BN), **N** – Sunflower Field (2013, S1♀, S1♂, S2♀, S2♂, S3♀ & S3♂), **O** – Sunflower Control (2013, C1, C2 & C3).

Identification	Sapling sites														
	Bainsvlei								Bainsvlei &		Petrusburg				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
msp. 1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
msp. 2	5	5	0	1	0	7	0	0	0	0	0	0	0	0	0
msp. 3	61	80	0	89	7	96	0	66	88	48	0	1	0	0	0
msp. 4	24	41	1	16	0	0	0	0	0	0	0	0	1	0	15
msp. 5	2	12	0	0	0	71	0	0	0	0	0	0	0	0	0
msp. 6	0	26	0	7	65	0	0	0	0	0	0	0	0	0	0
msp. 7	0	7	0	0	0	0	0	0	0	0	1	1	0	0	0
msp. 8	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 9	0	0	0	1	0	22	0	1	0	0	0	0	0	0	1
msp. 10	11	11	0	22	0	0	0	0	0	0	0	0	0	0	10
msp. 11	0	1	0	0	19	1	0	187	0	0	0	0	1	0	0
msp. 12	16	10	0	188	0	0	0	0	0	0	0	0	0	0	0
msp. 13	0	0	0	0	0	0	0	0	0	0	0	0	1	18	0
msp. 14	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
msp. 15	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 16	0	0	0	0	5	19	0	0	0	0	0	0	0	0	0
msp. 17	0	1	0	19	0	0	0	0	0	0	0	0	0	0	0
msp. 18	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 19	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
msp. 20	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0
Order: Lepidoptera Immatures (Larvae)															
Obtect pupa msp. 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Obtect pupa msp. 2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0
Obtect pupa msp. 3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Eruciform larvae msp. 1	2	1	3	0	0	0	23	0	0	0	1	0	0	0	0
Eruciform larvae msp. 2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Eruciform larvae msp. 3	5	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Order: Diptera Immatures (Larvae)															
Vermiform larvae mps. 1	6	16	1	4	7	4	0	0	0	8	0	0	0	0	0
Vermiform larvae mps. 2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Vermiform larvae mps. 4	2	5	0	2	0	1	0	1	0	0	0	0	0	0	1
Vermiform larvae mps. 5	1	0	0	0	1	0	3	0	0	0	0	0	0	0	1
Vermiform larvae mps. 6	3	1	1	3	2	3	0	0	0	0	1	3	1	1	0
Vermiform larvae mps. 7	2	1	0	4	0	4	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 8	4	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Vermiform larvae mps. 9	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0

A - Alfalfa Field 1 (2013, L1, L2 & L3), **B** - Alfalfa Field 2 (2013, L4, L5 & L6), **C** - Alfalfa Field 2 (2013, L8, L9 &L10), **D** - Alfalfa Control Fields (2013, CN, CS & CL), **E** – Maize fields (2013, NBT, RRBT, BTa & BTb), **F** – Maize Control Fields (2013, CN, CS & CL), **G** – Bainsvlei Onion Field (2013, U), **H** – Bainsvlei Onion Control (2013, CN, CS & CL), **I** – Maize fields (2012, BT & NBT), **J** – Maize Control Fields (2012, BT & NBT in Bainsvlei and Bloemdal), **K** – Petrusburg Onion Field (2013, U), **L** – Petrusburg Onion Control (2013, C1, C2 & C3), **M** – Butternut Field (2013, BN), **N** – Sunflower Field (2013, S1♀, S1♂, S2♀, S2♂, S3♀ & S3♂), **O** – Sunflower Control (2013, C1, C2 & C3).

Identification	Sapling sites		
	Bainsvlei	Bainsvlei &	Petrusburg

Addendum 1: Species list of the avarage number of soil Arthropoda sampled in two localities within the Free State Province containing 15 sampling sites (2012 - 2013).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Vermiform larvae mps. 10	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 11	4	2	0	6	23	2	0	4	0	0	0	5	1	1	4
Vermiform larvae mps. 12	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Vermiform larvae mps. 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Vermiform larvae mps. 14	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Family: Psychodidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Family: Ceratopogonidae															
msp. 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Cheronomidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Family: Simuliidae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Family: Sciaridae															
msp. 1	0	0	0	0	1	3	0	2	0	1	0	1	2	0	1
msp. 2	0	1	0	3	0	0	0	0	0	0	0	0	1	4	1
msp. 3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Family: Phoridae															
msp. 1	0	0	1	0	0	0	0	0	0	0	0	1	1	1	1
Family: Sepsidae															
msp. 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Chloropidae															
msp. 1	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0
Family: Sphaeroceridae															
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
msp. 2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.

Identification	16-Apr-14												24-Apr-14												30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Kingdom: Animalia																																																
Phylum: Arthropoda																																																
Class: Arachnida																																																
Order: Araneae																																																
Family: Prodidomidae																																																
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Order: Acari																																																
Suborder: Mesostigmata																																																
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Parasitidae																																																
Pergamasus sp. 1	0	0	0	0	0	0	0	0	19	29	0	0	10	0	0	0	16	18	1	5	48	19	1	1	13	6	0	0	30	11	1	0	108	61	4	3	16	6	1	0	22	12	0	1	53	21	7	0
Family: Rhodacaridae																																																
Immature msp. 1	0	0	0	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gamasellopsis sp. 1	31	76	8	8	62	65	8	6	3	0	0	4	71	0	0	0	38	28	0	0	73	23	0	0	19	8	0	0	52	8	0	0	101	41	0	0	36	8	0	0	31	11	0	0	8	3	0	0
Gamasiphis sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Protogamasellus sp. 1	0	0	0	0	0	0	1	0	55	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Protogamasellus sp. 2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Phytoseiidae																																																
Typhlodromus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Ascidae																																																
Gamasellevans sp. 1	0	0	0	0	0	0	0	0	9	17	4	0	70	104	2	1	261	119	10	5	30	19	7	17	127	39	44	44	252	177	20	25	67	68	30	0	90	28	44	86	191	104	20	19	30	20	30	18
Family: Laelapidae																																																
Hypoaspis sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	3	0	0	1	2	0	0	0	0
Suborder: Prostigmata / Trombidoformes																																																
Immature msp. 1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Microtrombidiidae																																																
Immature msp. 1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.

Identification	16-Apr-14												24-Apr-14												30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Microtrombidium sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Family: Erythraeidae																																																
Erythraeus sp. 1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Family: Bdellidae																																																
msp. 1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Cunaxidae																																																
Cunaxa sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	1	0	1	0	1	1
Dactyloscheles sp. 1																																																
Family: Ragidiidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Family: Eupodidae																																																
Eupodes sp. 1	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	1	0	0	0	5	12	0	0	0	0	0	0	1	0	0	0	1	0
Eupodes sp. 2	7	0	9	13	11	28	3	6	8	20	1	6	12	37	15	15	74	0	23	92	13	9	18	0	35	106	15	4	16	12	0	7	0	18	20	0	23	2	10	27	19	43	1	8	3	19	9	2
Family: Tydeidae																																																
Brachytydeus sp. 1	0	0	0	0	0	0	0	0	21	9	2	0	24	13	0	0	12	0	0	30	0	0	0	0	0	52	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Caeculidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Family: Anystidae																																																
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Tetranychidae																																																
Tetranychus urticae	0	0	7	0	0	0	0	0	0	14	0	1	0	0	19	0	0	0	10	0	0	0	6	12	8	1	56	11	4	2	218	31	0	0	97	6	32	25	292	956	0	0	768	34	1	0	16	2

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.

Identification	16-Apr-14												24-Apr-14												30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Family: Linotetranidae																																																
Linotetranus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Family: Scutacaridae																																																
Scutacarus sp. 1	1	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	0	0	0	0	0	0	0	0
Imparipes sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Tarsonemidae																																																
Hemitarsonemus sp. 1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	19	34	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	11	5	0	0	0	0	0	0	0	0	0	0	0
Suborder: Prostigmata / Endeostigmata																																																
Family: Nanorchestidae																																																
Nanorchestes sp.1	0	0	0	0	2	4	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	84	0	0	0	0	0	0	0	0	0	0	0	0
Speleorchestes meyeri	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5	0	0	0	0	0	0	0	0	0	0	0	0
Order: Oribatida																																																
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	13	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Opiidae																																																
Oppiella sp. 1	0	0	1	15	0	0	6	13	2	7	1	2	0	0	1	8	0	0	0	0	7	0	9	11	0	0	6	67	0	0	6	0	3	0	42	59	0	0	3	24	0	0	1	6	6	8	15	0
Family: Oribatulidae																																																
Immature msp. 1	0	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Chamobatidae																																																
Hypozetes sp. 1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	6	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	1
Family: Protoribatidae																																																
Protoribates sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	7	0	0	11	11	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Order: Astigmata / Astigmatina																																																
Family: Acaridae																																																
Rhizoglyphus sp. 1	0	0	0	0	0	0	0	0	0	0	7	11	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhizoglyphu s sp. 2	0	0	0	0	0	0	13	2	0	0	0	0	1	0	1	12	3	0	4	5	0	0	1	1	2	0	2	0	1	0	12	0	0	1	6	0	3	0	32	51	0	0	9	0	0	0	0	0
Rhizoglyphus sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.

Identification	16-Apr-14												24-Apr-14												30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Class: Insecta																																																
Order: Collembola																																																
Family: Hypogastruridae																																																
Hypogastrura sp. 1	0	0	0	0	8	130	1	0	221	111	3	0	205	39	2	19	69	132	14	47	694	522	0	185	191	52	11	11	1405	162	1	0	1158	891	0	0	129	101	0	0	575	432	0	0	177	26	0	0
Hypogastrura sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	180	54	3	31	51	113	10	56	737	521	0	177	200	56	18	0	1111	162	0	2	1153	594	0	0	119	92	0	0	569	442	0	0	136	19	0	0
Xenylla sp. 1	7	0	1	7	6	0	1	5	1	0	6	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Family: Brachystomellidae																																																
Brachystomella sp. 1	8	24	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	163	0	0	0	0	0	0	0	0	0	0	0	293	359	0	0	1	8	0	0	10	0	92	0	140	4
Family: Tullbergidae																																																
Tullbergia sp. 1	0	0	0	5	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	31	0	0	5	4	0	0	0	0	0	0	4	49	0	0	2	6	0	0	0	0
Family: Isotomidae																																																
Isotoma sp. 1	3	0	0	0	5	76	0	0	34	66	2	0	51	12	1	6	265	67	2	1	368	260	0	20	55	35	0	0	408	325	0	0	918	1782	0	0	38	0	0	0	127	98	1	0	224	0	0	0
Parisotoma sp. 1	0	7	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	35	0	2	31	113	0	0	0	0	2	9	0	80	1	1	11	12	1	2	0	0	0	0
Family: Entomobryidae																																																
Capbrya sp. 1	2	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	5	3	0	0	1	3	0	0	0	3	1	0	48	88	21	10
Entomobrya cf. *multifasciata*	0	0	0	0	1	4	0	0	1	23	0	0	0	0	0	0	0	0	0	24	4	3	0	9	2	0	0	0	0	0	0	0	1	0	4	0	1	0	0	0	0	0	0	0	0	0	0	0
Entomobrya sp. 1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Entomobryoides sp. 1	0	0	0	0	1	0	0	0	0	0	0	0	0	6	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	42	0	0	0	0	0	0	0	0	0	0	0	0
Seira sp. 1	0	0	0	0	1	0	1	0	0	0	4	1	0	0	0	0	0	0	0	0	1	0	1	4	0	0	1	0	0	0	0	0	3	37	2	0	0	0	0	0	1	0	0	0	0	0	0	0
Willowsia sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	0	0	0	1	0	0	1	12	10	1	4	7	9	1	0	1	0	1	25	6	9	4	4	7	17	41	0	0	0
Family: Cyphoderidae																																																
msp. 1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Sminthurididae																																																
Sphaeridia sp. 1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	8	3	0	0	0	0	0	0	0	5	0	0	0	0
Order: Dermaptera																																																
Family: Labiduridae																																																
msp. 1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.

Identification	16-Apr-14												24-Apr-14												30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Order: Isoptera																																																
Family: Kalotermitidae																																																
msp. 1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Order: Hemiptera																																																
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0
Immature msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
Immature msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Immature msp. 4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Immature msp. 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Family: Miridae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Lygaeidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Cicadellidae																																																
msp. 1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 2																																																
msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 4	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	2	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Family: Aphididae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.

Identification	16-Apr-14												24-Apr-14												30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Order: Thysanoptera																																																
Immature msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Immature msp. 2																																																
Immature msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Immature msp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	1	0
Family: Phlaeothripidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0
msp. 4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Thripidae																																																
msp. 1	1	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Order: Pscoptera																																																
Family: Trogiidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Liposcelidae																																																
msp. 1	1	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	1	0	1	1	2	0	2	0	1	0	0	0	0	0	1	0	0	0	3	2
Order: Coleoptera																																																
Immatures (Larvae)																																																
Scarabaeiform larvae msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scarabaeiform larvae msp. 2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	7	1	0	0	0	0	0	0	3	0	0	0	5	23	0	0	7	3	0	0	2	0	1	0	7	1	0	0	12	0	1	0	0	0	0	1
Elateriform larvae msp. 3	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.																																																
Identification	16-Apr-14												24-Apr-14												30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Elateriform larvae msp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 5	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 7	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elateriform larvae msp. 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	21	0	0	0	0	0	0	0	0	0	0	7	0
Vermiform larvae msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Carabidae																																																
Subfamily: Pterostichinae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
cf msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Pterostichus sp. 1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Family: Histeridae																																																
Family: Staphylinidae																																																
msp. 1	1	0	0	0	1	4	1	0	3	3	1	0	1	0	1	0	2	0	0	0	4	1	0	0	2	0	0	0	3	1	1	0	4	3	1	0	1	3	1	0	1	0	0	0	1	2	0	1
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Scarabaeidae																																																
Aphodius sp. 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Aphodius sp. 2																																																
Aphodius sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Family: Dermestidae																																																
Anthrenus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Family: Anobiidae																																																
msp. 1	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Family: Nitidulidae																																																
Brachypeplus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Family: Silvanidae																																																
msp. 1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.

Identification	16-Apr-14												24-Apr-14												30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Family: Tenebrionidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Tribolium castaneum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zophosis sp. 1																																																
Family: Anthicidae																																																
Anthicus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Anthicus sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Family: Chrysomelidae																																																
msp. 1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Scolytidae																																																
msp. 1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Order: Neuroptera																																																
Immature msp. 1	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Coniopterygidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Order: Hymenoptera																																																
Family: Braconidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Family: Mymaridae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Family: Encyrtidae																																																
msp. 1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Chalsididae																																																
msp. 1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Platygastridae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	2	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.

Identification	16-Apr-14												24-Apr-14												30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Family: Formicidae																																																
msp. 1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	2	2	1	0	4	0	1	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	3	29	0	0
msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eruciform larvae msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Order: Diptera																																																
Immatures (Larvae)																																																
Coarctate pupa msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Vermiform larvae mps. 1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 4	1	0	0	0	1	0	19	0	26	18	1	0	8	2	0	0	15	0	1	0	108	8	0	0	6	0	0	0	9	2	19	9	113	1	6	1	1	1	3	0	3	4	5	0	5	0	4	0
Vermiform larvae mps. 5	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 6	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 7	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 8	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vermiform larvae mps. 10	1	0	3	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Ceratopogonidae																																																
msp. 1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0
Family: Cheronomidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Family: Scatopsidae																																																
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Addendum 2: Species list of soil Arthropoda sampled in two localities within the Free State Province containing 48 sampling sites collected over a period of 4 weeks in 2014

(Field and Litter A = Alfalfa, Field and Litter B = Grassland) ~ AA - Litter A in Field A, BA - Litter B in Field A, AB - Litter A in Field B, BB - Litter B in Field B.

Identification	16-Apr-14												24-Apr-14											
	Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Family: Sciaridae																								
msp. 1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Family: Phoridae																								
msp. 1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Agromyzidae																								
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	6	9	1	0	1	0	1	0	0	0	0	0	4	0	0	0
Family: Chloropidae																								
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Sphaeroceridae																								
msp. 1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Identification	30-Apr-14												7-May-14											
	Mic				Meso				Trap				Mic				Meso				Trap			
	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB	AA	BA	AB	BB
Family: Sciaridae																								
msp. 1	1	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
msp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Phoridae																								
msp. 1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
msp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Agromyzidae																								
msp. 1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
msp. 2	1	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Family: Chloropidae																								
msp. 1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Family: Sphaeroceridae																								
msp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0