MESOFAUNAL ASSEMBLAGES IN SOILS OF SELECTED CROPS UNDER DIVERSE CULTIVATION PRACTICES IN CENTRAL SOUTH AFRICA, WITH NOTES ON COLLEMBOLA OCCURRENCE AND INTERACTIONS

by

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"We know more about the movement of celestial bodies than about the soil underfoot"

~ Leonardo da Vinci (1452 – 1519)

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  'I can do all things through Christ who strengthens me'

  Phil. 4:13

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ABSTRACT

Soil is a complex medium, comprised of both biotic and abiotic components. Interactions between these components are responsible for the beneficial services provided within different ecosystems. The biotic component, which is also referred to as the active role players in the soil, is responsible for these services. The incidence of these organisms is influenced by the abiotic factors, which act as filtering mechanisms that select for certain species to occur within certain areas. Considering an ever-increasing human population, these services could prove beneficial, since it could improve crop yields at minimal cost without exploiting the soil resource. Many farmers are now changing their farming methods to more sustainable and conservation focused practices to try and reduce the disruption of soil community structures and to optimize the complexity and resilience of these communities. Disturbances lower the complexity of soil communities and therefore limit the services that could be provided. This study focused on the fluctuations in diversity of selected role players within the soil medium due to the presence of certain agricultural practices and environmental changes.

Sampling for this project was conducted at six localities in the Free State Province between 2011 and 2014. Three of the localities are located in the Nama Karoo Biome and the other three in the Grassland Biome. The farms Vaaldam, Koppieskraal, Thornberry and Klein Brittanje were selected due to the diversity of agricultural practices and management strategies applied. The rest of the localities were the Paradys Experimental Farm, which is the experimental farm of the University of the Free State, where a pesticide trial was conducted and the farm Eureka, which was exposed to pollutants from a goldmine tailings dam. The variation in events and the general environment posed the perfect opportunity to observe and evaluate fluctuations in the diversity of the selected faunal groups within the relevant soils.

Sampling was conducted in the porosphere of each plant. All the plants selected for sampling were in optimal condition and away from the edges of the field. Samples were marked and transported to the lab where the organisms were extracted by means of the Berlese-Tullgren funnel extraction method. Sorting and identification were subsequently completed from which a reference collection was compiled and stored in ethanol.

Fluctuations in the diversity of the selected fauna was observed throughout this study. Agricultural practices had a definite influence on the severity of these fluctuations. Mechanical and chemical disturbances usually had a reduction effect on the diversity at first, which was followed by an increase in the abundance of certain opportunistic species. In some cases, these increases were quite severe, since certain species would flourish in the absence of competition and predator pressure, especially in the case of introduced species. Incorporation of stubble into the soil should be carefully managed, as this could create problems such as compaction. In spite of a certain degree of compaction, it was still found that soils with a higher organic component were more resilient in the presence of disturbances. Stubble-burning influenced the vertical distribution of soil mesofauna due to the condensation effect of such an event. The influence of chemicals depend on the persistence of the chemical used, as well as the complexity of the community before exposure. In already compromised areas, the effect of chemicals were far more detrimental to the community structure than at a natural site where a single application was done. The effect of pollutants from a tailings dam reduced the diversity considerably and only a few species were present at these sites. For species to occur within this heavy metal polluted area, they must be able to either tolerate or avoid the pollutants.

It was clear that each locality with its specific influencing factors selected for certain species to be present. Fields that were minimally disturbed and where organic materials were incorporated into, the soils had a higher tolerance to disturbances. This was due to a more complex community structure within the soil, thus indicating that even in the presence of a disturbance, these soils could still provide services.

Keywords: Soil mesofauna diversity, tillage, stubble-burning, biocide application, pollution, faunal preferences

UITTREKSEL

Grond is ‘n komplekse medium wat uit beide biotiese en abiotiese komponente saamgestel is. Inteaksies tussen hierdie komponente is verantwoordelik vir die voordelige dienste wat in verskillende ekostelsels verskaf word. Die biotiese komponent, wat ook na verwys word as die aktiewe rolspelers in die grond, is verantwoordelik vir hierdie dienste. Die voorkoms van hierdie organismes word beïnvloed deur die abiotiese faktore wat dien as filtreeringsmeganismes wat verantwoordelik is vir die voorkoms van sekere spesies in sekere gebiede. Met verwysing na die groeiende menslike bevolking kan hierdie dienste as voordeel aangewend word omdat dit obrengste kan verhoog met minimale insetkostes, sonder om die grond uit te buit. Baie boere is in die proses om hul boerdery metodes te verander na ‘n meer volhoubare sisteem, met bewaring as uitgangspunt deurdat die versteuring in gondstrukture geminimaliseer word om optimale kompleksiteit en weerstand van grondgemeenskappe te verseker. Versteurings verlaag die kompleksiteit van grondgemeenskappe wat tot gevolg het dat die dienste wat hierdie gemeenskappe bied ingekort word. Hierdie studie fokus op die fluktuasies in diversiteit van geselekteerde rolspelers binne die grondmedium aan die hand van die teenwoordigheid van sekere landboupraktyke en omgewingsveranderlikes.

Grondmonsters vir hierdie projek was by ses lokaliteite in die Vrystaat tussen 2011 en 2014 versamel. Drie van hierdie lokaliteite is in die Nama-Karoo Bioom en die ander drie is in die Grasveld Bioom. Die plase Vaaldam, Koppieskraal, Thornberry en Klein Brittanje was geselekteer weens die diverse landboupraktyke en bestuursstrategieë wat daar gevolg is. Die ander lokaliteite was die Paradys Proefplaas van die Universiteit van die Vrystaat, waar ‘n proef met plaagdoders uitgevoer is en die plaas Eureka wat aan besoedeling van ‘n naby geleë goudmynslikdam blootgestel was. Die variasie in grondbestuurpraktyke en die algemene omgewing het die ideale geleentheid verskaf om diversiteitsfluktuasies van geselekteerde faunistiese groepe in verskillende gronde waar te neem en te evalueer.

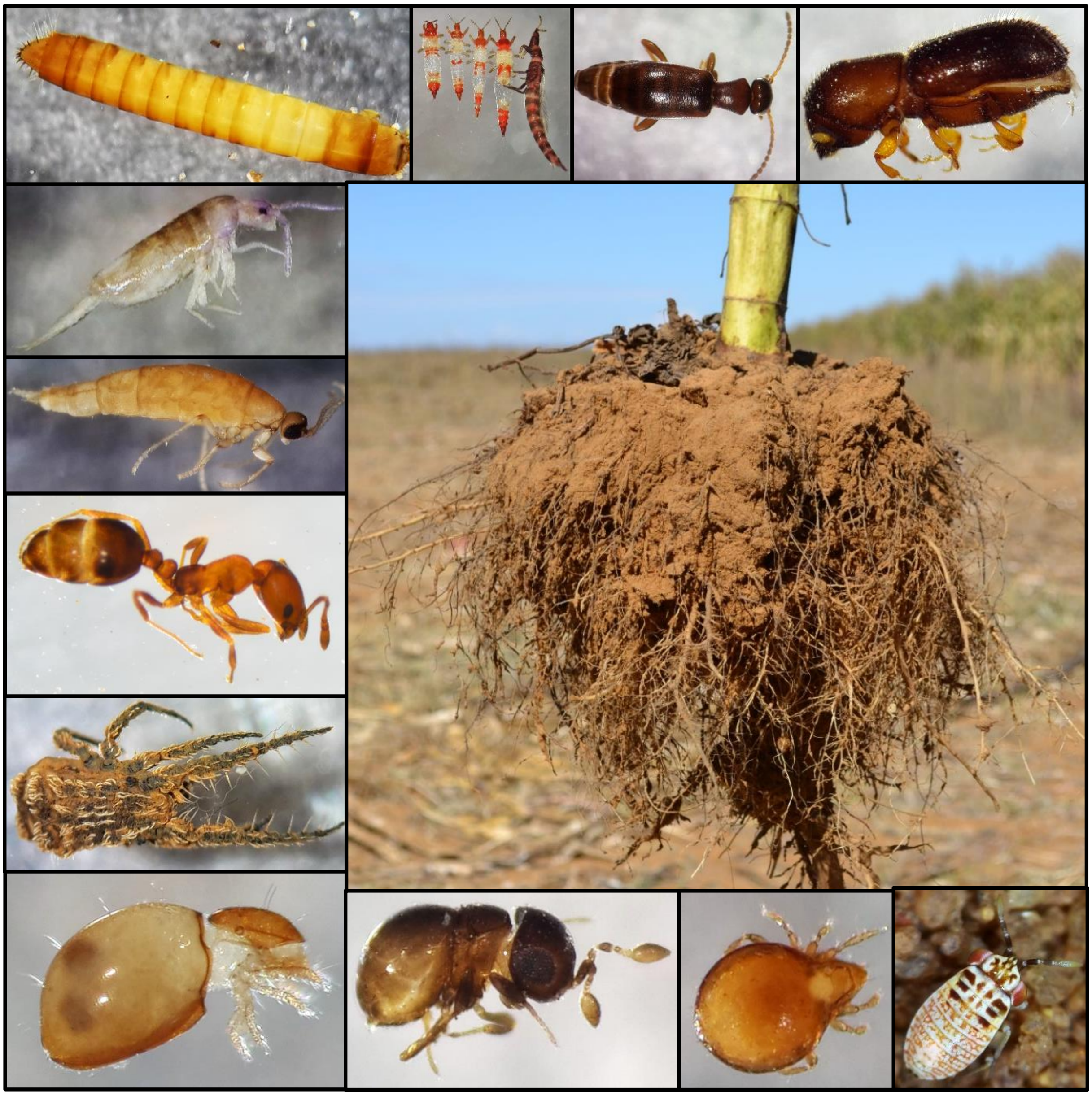
Al die grondmonsters was in die porosfeer van die plante geneem. Al die plante wat vir monsterneming geselekteer is, was in ‘n optimale toestand, en verwyder van die rante van die landerye. Die monsters was gemerk en tot by die laboratorium vervoer

waar die organismes deur middel van die Berlese-Tullgren ekstraksie metode ge-ekstraheer is. Sortering en identifisering was uitgevoer en ‘n verwyssingsversameling is saamgestel en in etanol gestoor.

Fluktuasies in die diversiteit van die geselekteerde fauna is reg deur die studie waargeneem. Landboupraktyke het ‘n defnitiewe invloed op die intensiteit van hierdie fluktuasies gehad. Meganiese en chemiese versteurings het gewoonlik ‘n aanvanlike afname in diversiteit getoon, gevolg deur ‘n toename. In sommige gevalle was hierdie toenames baie opvallend omdat sommige spesies floreer het in die afwesigheid van kompetisie en predatoriese druk, veral in die geval van indringer spesies. Die inkorporering van plantmateriaal in die grond moet baie omsigtig bestuur word omdat dit probleme, soos kompaksie, kan veroorsaak. Ten spyte van ‘n mate van kompaksie is daar tog gevind dat grond met ‘n hoër organiese samestelling meer weerstand teen verandering bied. Die brand van plantmateriaal in die lande het die vertikale verspreiding van grondmesofauna as gevolg van die kondensasie faktor beïnvloed. Die invloed van chemikalië word deur die volharding van die spesifieke chemikalië, asook die kompleksiteit van die grondgemeenskap voor bloodstellig bepaal. In gebiede wat reeds versteur was, het die effek van chemikalië ‘n groter invloed getoon as in die natuurlike veld waar slegs ‘n enkele bespuiting toegedien is. Die effek van besoedeling van ‘n slikdam het die diversiteit aansienlik verminder en slegs ‘n paar spesies was teenwoordig in die besoedelde gebiede. Hierdie spesies kon moontlik hier voorgekom het weens hul vermoë om swaarmetaalvergiftiging te vermy of te verdra.

Dit was diuidelik dat elke lokaliteit met sy spesifieke omstandighede selekteer vir die teenwoordigheid van sekere spesies. Landerye wat minimaal ontwrig was en waar organiese materiaal in die grond ingewerk was, het ‘n hoër toleransie vir versteuring getoon. Dit was as gevolg van ‘n meer komplekse gemeenskapstruktuur in hierdie grond wat tot gevolg gehad het dat selfs in die teenwoordigheid van versteurings, hierdie grond steeds ekostelsel dienste kon bied.

Chapter 1



OVERVIEW OF THE IMPORTANCE OF SOIL FAUNA IN AGRO-ECOSYSTEMS AND THE FACTORS THAT INFLUENCE THEIR DIVERSITY

With an ever growing human population and demand for food security, it is important to optimize crop yields in a sustainable manner. This has become one of the primary concerns and poses great challenges for agriculture, since it is only possible if soils are healthy. Soils with positive interactions between all its components, biotic and abiotic, can provide positive feedbacks such as healthy plants and even promote water and air quality (Cardoso *et al.* 2013). As such soils not only contribute to the quality of essential resources, but in a spatial sense also provide essential refuge due to the positive interactions between all components of the soil. Soil should therefore be monitored and if necessary managed over time (Beare *et al.*1995; Cardoso *et al.* 2013).

Soil is a complex medium, comprised of various biotic and abiotic components. The abiotic components are relatively well studied and can provide useful information regarding soil quality (Barrios 2007). The importance of the biotic component and its functioning has been recognised over the last 30 years, although ignorance to its conservation is clearly noted (Decaëns *et al*. 2006). It is very important when looking at soils and its functioning, to know that these components are all essential and part of a symbiotic cycle with multiple interactions between all of them, thereby providing beneficial ecosystem services which serve as indicators of soil health (Bardgett & van der Putten 2014; Fig 1.1). These services are largely dependent on organism occurrence, which in turn are influenced by a vast array of interactions between them and the numerous abiotic factors (Coleman *et al*. 2004).

To understand this interdependence between the different components of soil, it is important to acknowledge the processes involved and their role in nature (Cardoso *et al.* 2013). According to Scheu (2001), the soil medium and its activity have long and wrongfully been studied as a completely closed system. It has since been reassessed and the importance of the relationship between above- and below-ground communities accepted. These systems are interdependent, with above-ground communities relying on below-ground decomposition and mineralisation to function successfully, whilst they, in turn, influence the quality of organic matter and root exudates produced by plants through photosynthesis (Scheu 2001; Cardoso *et al.* 2013). In such a system, both above- and below-ground organisms are interconnected through plants which provide both refuge and dietary resources (Wardle *et al*. 2004). Modifications in below-ground communities and their resultant influence on the plant will therefore alter the above-ground community structure (Bardgett & van der Putten 2014). This is also true in the case of above-ground communities influencing plants which affects below-ground organisms. This process is known as the top-down and bottom-up effects. These modifications can be direct (root damage due to feeding) or indirect (microbial growth stimulation by grazing impacts). For example, nutrients such as nitrogen are also essential in above-ground arthropod development. Studies done on the response of aphid communities indicated that the presence of different soil organismal (earthworms, protozoa and collembolan species) activity within the rhizosphere led to an in increase in the above ground aphid numbers (Scheu 2001).

Probably one of the most vital functions of soil is the physical space, habitat and niche, which it provides for soil biota and their activities (Emmerling *et al.* 2002). The physical component is therefore vital and interlinked with both the biological and chemical components of soil. The quality or state of the physical component is usually exhibited by certain ’symptoms’ or the lack thereof. Poor water infiltration, aeration and poor workability is usually associated with soils of poor physical quality (Dexter 2004).

The chemical component of soil can be beneficial to soil processes as it has the capacity to bind and retain or provide elements. Another factor is soil pH as it has a direct correlation to nutrient availability and indirectly affects soil biota occurrence due to plant responses (Härdtle *et al.* 2004). Crop yield correlates positively with soil health, for all the processes within the soil medium, together with the above-ground processes conducted by the primary producers, contribute to the health of soils and vice versa (Cardoso *et al.* 2013).

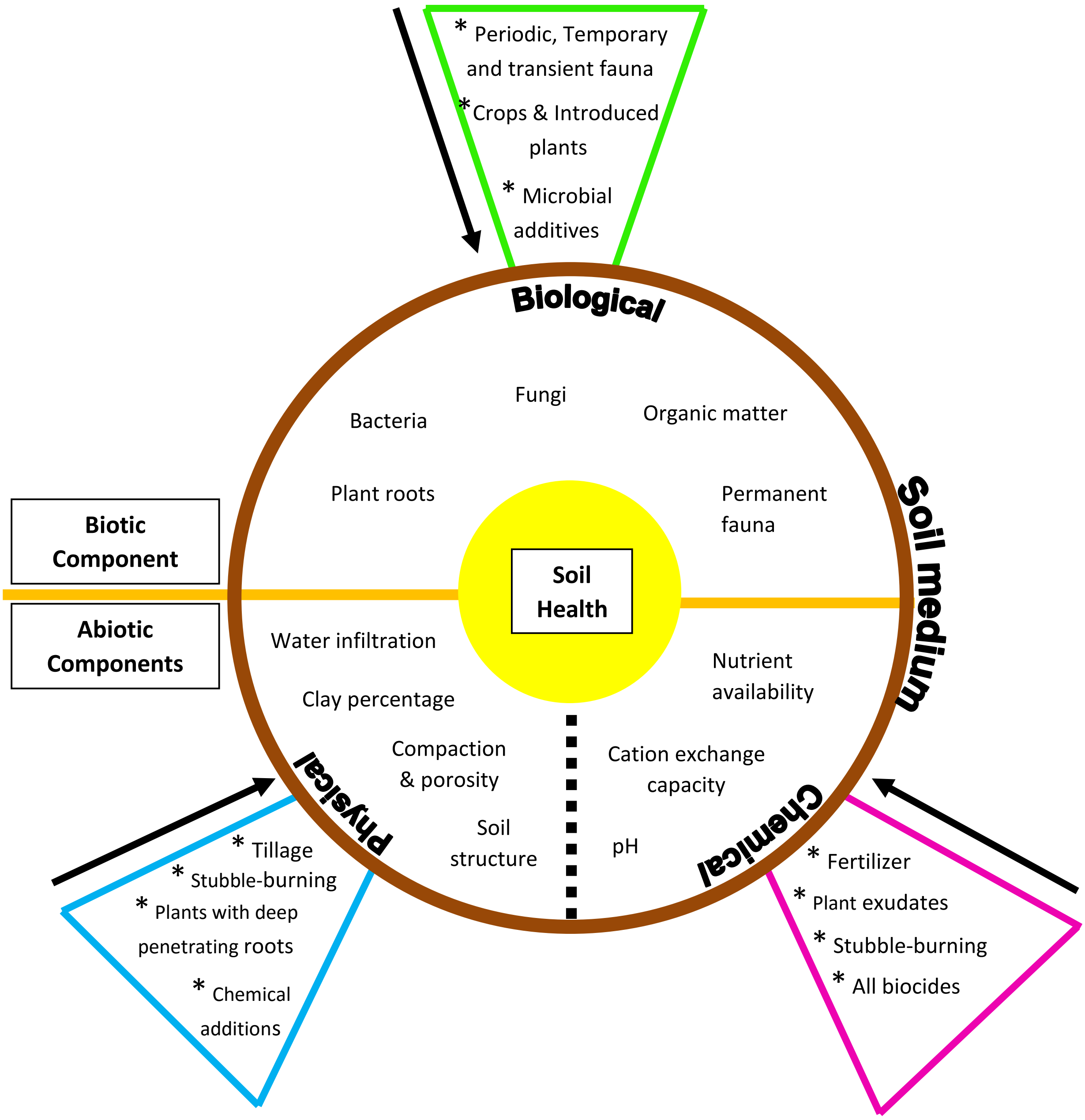


Fig.1.1: Different components of soil with the external and anthropogenic influences within the soil medium of an agro-ecosystem.

The management of agroecosystems, however, have an influence on all these components. Some are deemed more sustainable systems with the practice of less external influencing factors (such as no-tillage and organic farming), in contrast to the conventional tillage system, which is based on numerous human-driven disturbances. For a system to be considered as sustainable, a nutrient flux across all trophic levels must be present. This process is generally mediated by soil meso- and macrofauna, as well as the microbial community (Cardoso *et al.* 2013).

This dissertation focuses on the biological component – primarily on mesofauna, but also on certain macrofauna – and fluctuations observed in diversity and occurrence of particular species within an area in the presence of certain agricultural practices and disturbances. As many of these organisms presumably have the same ecological contribution in soil, it is important to evaluate the trophic structures within this diversity, rather than only examining species richness which could prove misleading. Due to the extent of soil biodiversity, it is impossible to cover the complete spectrum and therefore this dissertation will mostly focus on the mesofauna, within cultivated landscapes.

a) Biotic component of soil

The variation in soil biota is enormous and influence plant success directly (Moldenke *et al.* 2000). This variation in soil organisms is possible due to the heterogeneity within the soil medium and the presence or absence of stability in environmental conditions over time. These organisms vary in size and function and all play an active role in energy flow throughout the soil (Coleman *et al*. 2004), also altering its physio-chemical properties which have an effect on soil fertility as well as soil quality (Emmerling *et al.* 2002). There is, however, still a large gap in understanding some of these role players and what needs to be done to protect and enhance soil health (Coleman *et al*. 2004).

i)Spatial scales of biotic interactions

Soil can be divided into several sectors that are biologically relevant and central to its temporal and spatial heterogeneity. These sectors, referred to as spheres by Beare *et al.* (1995), are interlinked, although they are formed by distinct biological interactions across different scales. The biota responsible for the biological activity in soils are not randomly distributed throughout the soil, but are rather found in these biologically relevant spheres. Barrios (2007), refers to activity 'hot spots', but these are usually linked to carbon substrate availability. Beare *et al.* (1995) identify the spheres as the drilosphere, detritusphere, porosphere, aggregatusphere and rhizosphere (Fig. 1.2). The detritusphere constitutes the top part of the drilosphere and consists of decaying plant and animal matter. This region is important as it is the origin of soil organic matter and supports a specific biotic structure, is the primary arena of decomposition and is directly influenced by aboveground plant communities. The detritusphere has an influence on nutrient fluxes in the soil, with the severity of these fluxes depending on the biota and organic matter present (Vivanco & Austin 2008).

In the drilosphere, processes such as litter fragmentation, organic matter distribution and soil mixing takes place. These processes are mediated by macro-fauna such as earthworms and termites, forming matter rich sites which act as favourable niches, with an abundant food supply, for saprophagous fungi, mites and other non-arthropod soil fauna (Brown et al. 2000, Ettema & Wardle 2002).

The third sphere is the porosphere. This area supports organisms living within the water film and air filled pores such as protozoa, nematodes, arthropods and fungi (Haynes & Graham 2004). Mesofauna and macro-biota play an essential role in this sphere, where they move soil particles and form tunnels, macropores and even aggregates. These channels assist in water and nutrient movement throughout the soil, with some of the well-known role players – ants, termites and earthworms – enhancing the distribution process. The burrows created by the latter organisms not only enhance water filtration and nutrient distribution, but also increase soil porosity, root penetration and can even help the migration activities of smaller soil fauna in the presence of unfavourable conditions (Lavelle et al. 2006).

A variety of soil biota influence the soil aggregation sector (aggregatusphere). These aggregates, *i.e.* microaggregates (50-250µm) and macroaggregates (>250µm), are comprised of various physical and biological particles such as clay microstructures and organic matter (Beare *et al.* 1995; Cardoso *et al.* 2013). Aggregates are mainly formed as a result of bioturbation by the living component of soil. The properties of each aggregate can differ due to the difference in species that was involved in its formation, since the size of the species will determine the size of the aggregates formed. Some organisms, such as earthworms, have both a direct and indirect effect on the stability of the aggregates. They not only deposit mucus when forming these structures, but also stimulate microbial activity which enhances stability. Meso- and microfauna only have an indirect effect on soil aggregation due to stimulation or inhibition of certain biota in the soil (Beare *et al.* 1995). According to Coleman *et al.* (2004) and Cardoso *et al.* (2013), soil biota such as earthworms and other soil fauna, arbuscular mycorrhizal fungi, bacteria and plants produce organic substances which act as binding material. Furthermore it is important to know that all these factors involved in the aggregatusphere are influenced by the particular agroecosystems and its anthropogenic management activities (Beare *et al*. 1995). This, in turn, has implications, since aggregates not only supply stability to soil, but also play a role in the protection of carbon pools (Elliott 1985).

Finally, the rhizosphere. This is a very important region, for this is where the plant roots, more specifically the root hairs, interact directly with its environment and associated biota (Richardson *et al.* 2009). The rhizosphere is a spatiotemporally stochastic region with a variety of exudates produced by the plants which can stimulate microbial activity and influence nitrogen mineralization (Richardson *et al.* 2009). Microorganisms within the rhizosphere stimulate the germination and growth of arbuscular mycorrhizal fungi by removing inhibitors. These inhibitors can include self-inhibiting compounds or inhibitors in the soil medium (Coleman *et al.* 2004). This enables the fungi to extend its hyphae beyond the root’s epidermis, absorb phosphorus and provide it to the plant (Moldenke *et al.* 2000).

Soil fauna, such as Collembola play an interactive role in the rhizosphere. As this is the area where direct interaction between plants and their environment takes place, the presence of Collembola is important. These organisms can either have a direct influence on the productivity of the plant by feeding on the root hairs and living plant material or indirectly by grazing on fungi and excreting nutrients. The specific ecological function in this regard depends on collembolan species diversity, plant species affected, nutrient and soil water availability, and microbial interactions (Eisenhauer *et al.* 2011).

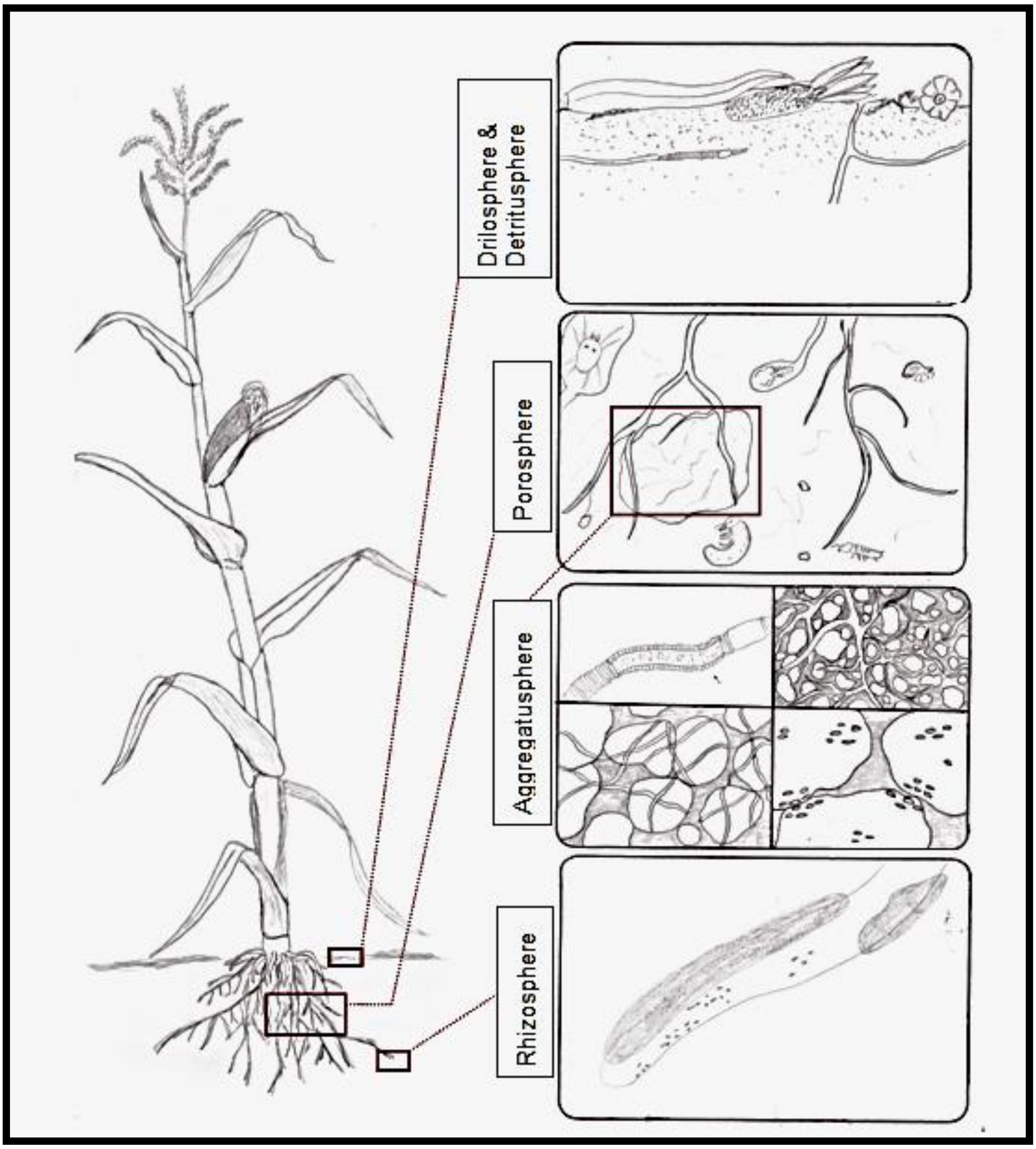


Fig 1.2: Biological relevant spheres of soil in an agricultural system. (Adapted from Beare *et al.* (1995) and Cardoso *et al.* (2013)).

According to Coleman *et al.* (2004), most of the carbon enter the rhizosphere as root exudates, shed cells and root hairs (root exfoliates). Carbon released into the rhizosphere has both positive and negative effects on plant growth. Some of the positive effects include an increase in water holding capacity, increased nutrient availability and the suppression of pathogens. Negative effects include the attraction of root feeding nematodes and induced microbial phytotoxin production. Root exudates are an integral part of the rhizosphere and its functioning and enhance soil aggregate formation. Nitrogen is one of the important nutrients needed for plant growth, but is not always available to plants. Root exudates in the rhizosphere can also stimulate nitrogen-fixing bacteria to provide the plant with this growth limiting nutrient (Moldenke *et al.* 2000, Coleman *et al.* 2004). Overall symbiotic relationships between soil biota within this sphere assists in the obtaining of nutrients and water by plants (Coleman *et al.* 2004).

Soil fauna is extremely diverse and to be able to study these organisms researchers either divide them into functional groups or make use of taxonomic groups (Barrios 2007). Another method of grouping these organisms into manageable study entities, would be to divide them according to their degree of presence in the soil, since some only reside in soils for certain stages and periods of their life cycle (Hasiotis & Bourke 2006; Djuuna 2013; Fig 1.3). An example would be the coccinellid beetle, *Hippodamia variegata*, which only hibernates in the soil during its adult stage. These organisms are referred to as transient species. There are temporary species which include species that completes one life stage of its life-cycle within the soil. These are usually larvae of Diptera and certain coleopterans which feed on decaying matter and plant roots respectively. Species known to complete their life-cycles within the soil, with the occasional emergence of adults are known as periodic species. Finally there are permanent species which complete their life-cycle within the soil without ever leaving this medium. Some collembolan species are known to be permanent residents of soils and their adaptations include the loss of pigmentation and the reduction of the furcula (Wallwork 1970; Coleman *et al.* 2004).

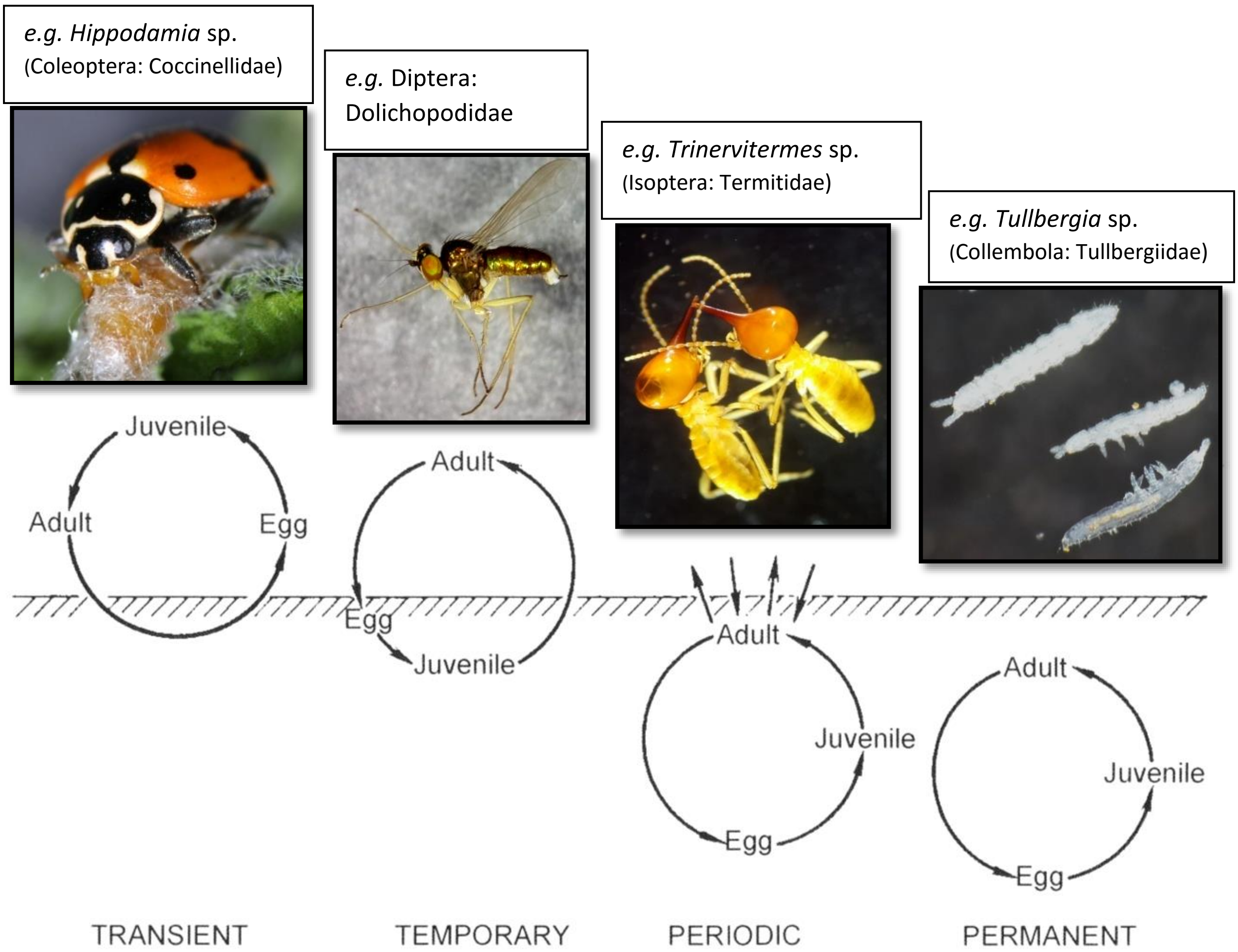


Fig 1.3: Classification of organisms collected from soils into groups according to their degree of presence in soil, illustrated by means of relevant South African insect groups. (Adapted from Coleman *et al.* 2004).

ii) Soil biota (excluding higher plants)

Soil organisms have traditionally been 'classified' on the basis of size, rather than ecological function, since there is still a large gap in the knowledge of these organisms (Fig 1.4). Size measurements are of the width of the organisms, for the length of soil biota can be misleading with mycelium of fungi extending up to a few metres in length (Swift *et al.* 1979). This classification consists of four groups: microflora and microfauna (*e.g.* bacteria, fungi and nematodes), mesofauna (*e.g.* mites and springtails and macrofauna (*e.g.* ants and termites) (Beare *et al*. 1995; Barrios 2007).

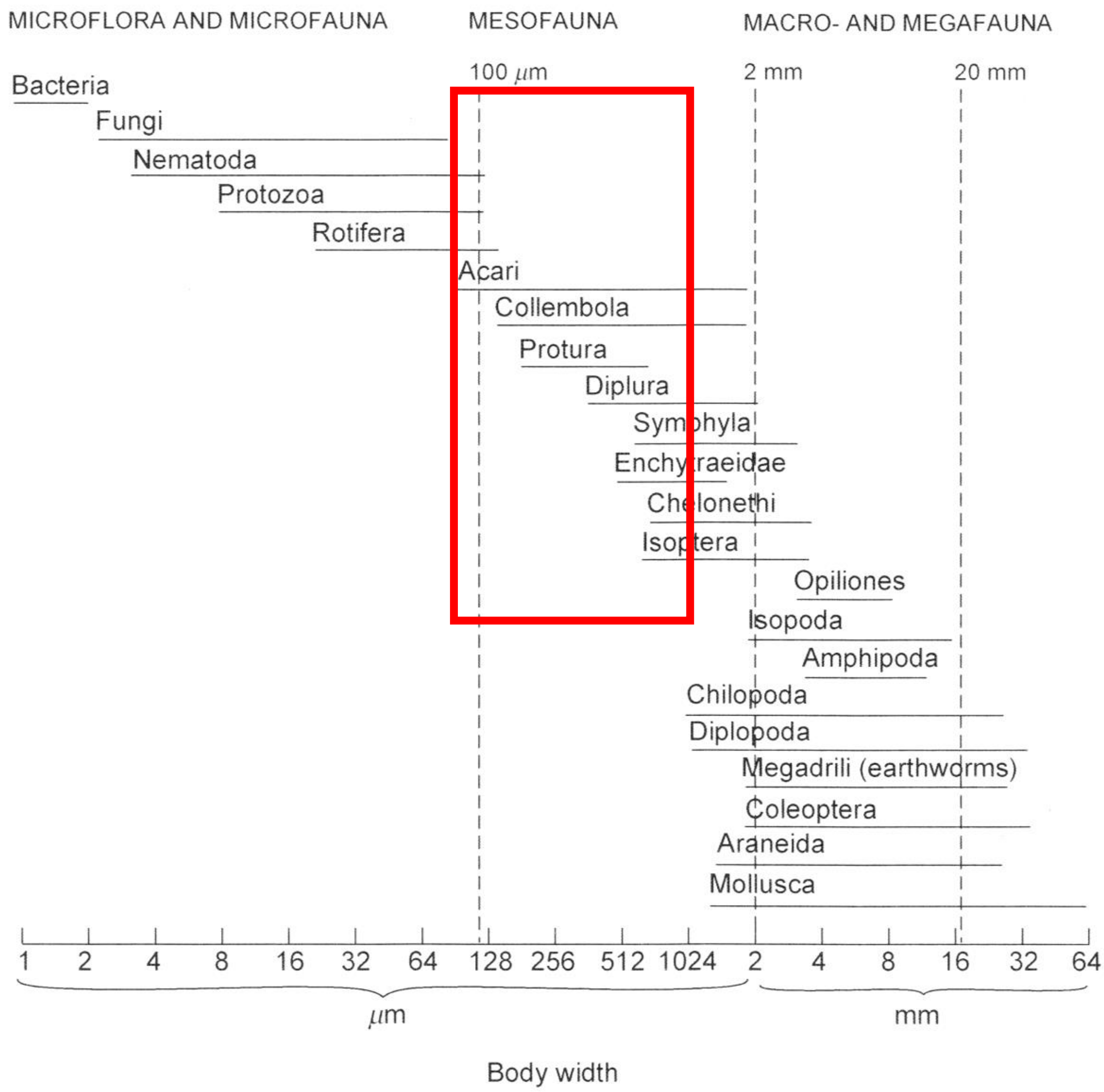


Fig 1.4: Size classification of soil organisms, in the context of decomposition, in terms of body width (Swift *et al.* 1979). Red rectangle depicts groups considered in this study.

All of these organisms form crucial components of ecosystems. This is due to their irreplaceable role in soil processes such as decomposition and nutrient cycling. Soil fauna, as well as fungal and bacterial activities, can even influence soil chemistry and the physical properties of soils (Cardoso *et al.* 2013). Soil fauna are also partly responsible for the spread of fungal and bacterial inocula throughout soil (Moldenke *et al.* 2000).

- **Bacteria**

Microbial populations are essential in soil processes. Bacteria are one of the most species rich domains and are most successful in the rhizosphere of soils, for it has the highest nutrient level of the different spheres (Coleman *et al*. 2004). Bacteria play a large role in the transformation of nitrogen in soil (Jackson *et al.* 2008). Although nitrogen fixation by bacteria is considered a relatively common process in soil, the bacteria responsible for this only occur under certain environmental conditions (Jackson *et al.* 2008).

- **Fungi**

Fungi is a versatile group and abundant in the soil. Due to its diversity and overwhelming abundance in soil, fungi play a major role in many soil processes. Certainly the most common and well-known process mediated by fungi would be decomposition. The success of fungi in soils can be attributed to its ability to overcome and thrive in the presence of physical and chemical constraints that might be encountered within the soil medium (Gonzáles-Chávez *et al.* 2004). This is possible due to its ability to readily distribute in soil, utilising available nutrients and spreading nutrients to depleted areas. Some fungi also help with sequestration and immobilisation of potentially harmful elements (Gonzáles-Chávez *et al.* 2004).

Cellulose fungi are quite common in crop agriculture, although there is still a lot that is not known on this topic (Gunathilake *et al.* 2013). One of the most important groups is the arbuscular mychorrhiza. They form structures within the roots of plants and send out hyphae into the surrounding soil, which is responsible for the uptake of nutrients, especially phosphate ions (Fig 1.5). This is a mutualistic relationship for they receive carbon from the plants whilst they are providing other mineral nutrients. The growth and germination of this fungus is stimulated by soil faunal feeding activity within the rhizosphere (Parkinson *et al.* 1979).

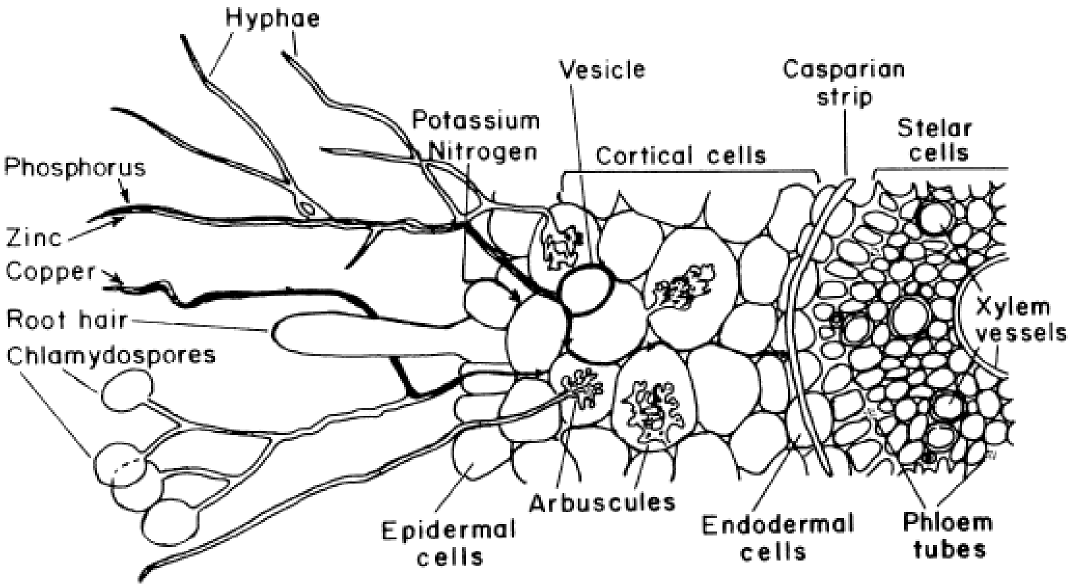


Fig 1.5: Endomychorrhizal hyphae extending from the epidermis of the plant root, into the rhizosphere, aiding in the uptake of nutrients. (Adapted from Moldenke *et al.* 2000).

- **Soil fauna**

Soil fauna are animals that complete at least one life stage in the soil. These organisms represent approximately 24% of the global diversity, with insects and arachnids as the best represented (Decaëns *et al*. 2006). The enormous diversity of soil organisms can be attributed to the availability of a variety of different niches and micro-habitats, spatial and temporal segregation, abundance of food sources and the relatively stable climatic conditions of soil (Decaëns *et al*. 2006). These factors do not only give rise to the large diversity, but also influence the complexity of the community structure and the distribution of these organisms throughout the soil (Parkinson *et al.* 1979; Barrios 2007).

A plant is considered as healthy if its physiological processes are functioning within an optimal range for that specific plant and therefore, when considering living soils, roughly the same principle can be applied to determine if soils are healthy or not (Ferris & Tuomisto 2015). The biological processes in soils are driven by its living/functional component and are known as ecosystem services. Ecosystem services contribute to the soil's resilience and its ability to sustain life. Both of these factors have economical benefits regarding mankind (Briones 2014).

Although it is impossible to add a specific economical value to each soil organism, it is important to know that they have an indirect economic value due to the ecosystem services that they provide, thus enhancing the state of their environment (Decaëns *et al*. 2006). This enhancement is due to various interactions between soil fauna (more specifically mesofauna), bacteria, fungi and even plants. This results in optimal nutrient cycling and healthier plants (Moldenke *et al*. 2000). Due to the diversity in organism size and dietary requirements, soil organisms can fulfil a number of advantageous activities including soil aeration, decomposition, water holding or draining capacity and even the spread of organic matter in soil (Briones 2014; Oke *et al.* 2007). In a crop agricultural setting the analysis of soil food webs, nutrient cycling and energy flow in the soil has proven insightful and has confirmed a relationship between trophic structure dynamics and agroecosystem stability (Barrios 2007).

Importantly, advantages of soil organisms also include the potential suppression of root pathogens (Briones 2014). These micro-organisms also provide the roots of plants with the needed nutrients (Emmerling *et al*. 2002). However, it proves difficult to ascribe ecological values to all soil biota and the significance of their diversity and interactions, for this takes place in an environment with many observational and experimental limitations (Barrios 2007). Although the diversity of these organisms is important in the functioning of soil, diversity as such can be meaningless if it is not brought into context with trophic structure and activity patterns (Freitas *et al.* 2012). This is because many of these organisms can mediate the same processes as a result of their feeding and general behaviour (Table 1.1). In spite of this similarity in broader function, a larger diversity is still more beneficial, since the higher the diversity, the larger their positive effect (*i.e*. more is better). However, there is still a lot of debate on the question regarding species richness and how many species would be necessary for an optimal thresh-hold (Barrios 2007). Nonetheless, the presence of certain species can be meaningful in different ways, since soil organisms often have environmental preferences and can therefore be used as indicators of such (Briones 2014; Decaëns *et al*. 2006).

Although there are many benefits to soil biota, it is important to keep in mind that not all soil fauna enhance plant growth. An example would be the effect of certain nematodes feeding on plant roots, resulting in a loss of nitrogen-fixing nodules on these roots, thus suppressing plant growth (Beare *et al*. 1995). Other influences that soil fauna have are size dependant. For instance, macrofauna can create burrows and change the pore sizes of soils, whereas mesofauna and microfauna are restricted to the already existing pores and have no influence in this regard. It is therefore important to keep in mind that the functions mentioned in Table 1.1 below, are provided at different spatial and temporal scales with variations in intensity (Coleman *et al.* 2004).

Table 1.1: Classification of soil fauna into groups according to size and general function of each group. (Adapted from Coleman *et al.* 2004.) Mesofauna, the focus of this study, highlighted in yellow.

Function	Size Classification		
	Micro-fauna (< 0.20mm)	Meso-fauna (0.2 – 2.0mm)	Macro-fauna (> 2.0mm)
Fragmentation of residues		x	x
Stimulation of microbial activity		x	x
Redistribution of organic matter/nutrients			x
Soil aggregation / biopore construction	x	x	x
Carbon sequestration			x
Nutrient cycling, mineralization/immobilization	x	x	
Humification		x	x
Fungal feeding	x	x	
Opening channels and galleries			x
Regulation of bacterial / fungal populations	x	x	
Mixing of organic and mineral particles			x

◊ **Microfauna**

This group consists of numerous very small animals that are restricted to the water film within the soil (Coleman *et al.* 2004). According to the size classification index compiled by Swift *et al.* (1979), this group is mainly represented by nematodes, protozoa and rotifers. Most soil protozoa are found in the top soil and due to the minute size of some species, they can infiltrate even the smallest pores within the soil medium (Coleman *et al.* 2004). Microfauna feed mainly on fungi and bacteria, even though predatory and parasitic species are also abundant. These organisms thus help with fungal and bacterial population management and excrete mineral nutrients (Beare *et al*. 1995). According to Coleman *et al.* (2004), studies suggested that the feeding activity of protozoa, together with the bacteria they feed on, enhance plant growth due to the production of plant-growth-promoting compounds within the rhizosphere. These organisms are not only beneficial in plant health, but can also be used as bio-indicators based on their sensitivity to environmental change (Németh-Katona 2008; Foissner 1999). Some rotifers and nematodes can survive unfavourable conditions in the form of cysts or undergoing anhydrobiosis (Coleman *et al.* 1999). Nematodes are a very large group and are represented in all trophic levels (Whalen & Sampedro 2010). Nematodes can be beneficial as bio-control agents, *e.g.* entomopathogenic nematodes can be used in the control of certain insect species (Nouh & Hussein 2014). Nematodes are successfully used as bio-indicators due to their sensitivity to disruptions in their environment (Pattison *et al.* 2004). The Tardigrada, finally, are organisms that are resilient as they can withstand various environmental disturbances ranging from dry periods to recovering after being frozen with liquid nitrogen (Møbjerg *et al.* 2011)

◊Mesofauna

The mesofauna are an essential part of the soil and are comprised of species from various orders with variable ecological importance (Culliney 2013; Barrios 2007). Mesofauna and microfauna share the inability to create their own space or burrows within the soil and are thus constricted to the already existing pores (Coleman *et al.* 2004). According to Culliney 2013, soil mesofauna through their feeding, directly affect mineralisation of nutrients by reducing these materials into minute fragments. This process increases the surface area for further microbial breakdown and in the process enhances nutrient availability. It has proven difficult to divide most of these organisms into specific functional groups, for they tend to shift between trophic levels when food sources are scarce or seasonal. Therefore it has been suggested that most of these organisms should be considered as omnivorous (Culliney 2013; Neher & Barbercheck 1999). These organisms also have an influence on the community structures of other soil biota whether it is due to growth stimulation as a result of their grazing activities or the dispersal of fungal spores (De Groot *et al.* 2016). According to Moldenke *et al.* (2000), grazing on microbes not only enhance plant productivity, but also prevent the microbes from obtaining the necessary nutrients to accumulate in their tissue. This could become a problem as these microbes form a layer around growing roots and can thus withhold nutrients from the plants.

Microarthropods can be numerous and are present in a wide range of soils. Due to their abundance, these organisms are regarded as significant contributors in the decomposition process in, for example, forests (McColl 1974). They are also an important part of food webs, as they feed on micro-flora and fauna and can fall prey to macrofauna, thus linking these groups. It is therefore important to examine soil as an ecosystem and include as many faunal groups as possible. The most abundant microarthropods are Collembola and Acari (Behan-Pelletier 2003).

Soil communities are thus dependent on each other. The opinion of Coleman *et al.* (2004) in this regard is supported by Santos *et al.* (1981), who found that nematophagous mites regulated the number of bacteriophagous nematodes. This resulted in higher numbers of active bacteria which increased decomposition rates. In another study, fungivorous mesofauna, which include certain Collembola and nematodes species, are beneficial to plant health in that they feed on phytopathogenic fungi (Schrader *et al.* 2013). This subsequently has a decreasing effect on plant root disease (Culliney 2013).

The **C**ollembola (springtails) are a very diverse group. They occupy various spheres within in the soil and have representatives at all trophic levels, although the majority seem to feed on fungi that are associated with decomposition (Coleman *et al.* 2004). The taxonomic classification of these organisms differs between authors and is often still under debate (*e.g.* Hopkin 1997), with the more recent molecular studies separating Collembola from the class Insecta (Sasaki *et al.* 2013; Nardi *et al.* 2003). Collembola will be treated as insects in this dissertation, under the classification suggested by Hopkin (1997), Fjellberg (1998), Triplehorn & Johnson (2005), and Fjellberg (2007), as the keys from this literature were used for identification. Collembola and their roles in soils will be discussed in Chapter 5.

Acari (mites) are probably the most abundant and species rich microarthropod group within soils and as such expose different feeding, reproduction strategies and methods of dispersal (Coleman *et al.* 2004). The majority of mites are free-living with Oribatida, Prostigmata, Mesostigmata en Astigmata most frequently sampled in soil (Krantz & Walter 2009). According to Coleman *et al.* (2004), the presence of mites can be an indirect benefit to plants, as some feed on plant pathogenic fungi and others are nematophagous which lower the number of phytophagous nematodes. This group of organisms should be assessed in the context of an ecosystem since their contributions are indirect, as is found in most of the other mesofaunal groups.

The **O**ribatida is a suborder of Sarcoptiformes (Krantz & Walter 2009). These organisms have a global distribution with fossil records dating back to the Devonian period. Oribatids are associated with decomposition and can be numerous under favourable conditions (Coleman *et al.* 2004). Although some oribatids can reproduce parthenogentically, most oribatids are considered “k strategists” as they have a slow reproductive rate and only have one or two generations per year (Coleman & Hendrix 2000; Coleman *et al.* 2004). The immature stages have proven difficult to identify, as their morphology can differ greatly from the adults. This level of polymorphism throughout the different life stages is unique to this mite group. Another characteristic that separate these mites from the other groups, is the presence of a sclerotized exoskeleton. These exoskeletons have high levels of calcium which is presumably due to calcium sequestration by feeding on fungal hypae that contain calcium crystals (Seastedt & Tate 1981).

Oribatids usually outnumber other mites, with the exception of the arctic tundra, grasslands and cultivated fields where prostimatid numbers are more prominent (Seastedt & Tate 1981). The decline in oribatid numbers in agroecosystems are ascribed to the precariousness of cultivation procedures, crop harvesting techniques, together with post-harvest treatments (De Groot *et al.* 2016; Wissuwa *et al.* 2013). All of these factors have an influence on the plant residue and fungal growth, thus resulting in changes in the food sources of the majority of oribatids (Wallwork 1983; Seastedt 1984). The influences of these organisms in the soil are mostly indirect due to their feeding on fungi and their ability to fragment plant residue (Coleman *et al.* 2004).

The **P**rostigmata and **E**ndeostigmata (Superorder: Acariformes) are also very old groups, containing fossils from the Devonian period (Dunlop 2010; Krantz & Walter 2009). This group is well represented in soil with species in all trophic levels. The majority of prostigmatid species are predaceous, with the occasional spike in the number of individuals of some mycophagous species. Species from the Eupodidae family are known to be opportunistic and together with other families such as Tarsonemidae and Tydeidae from this order, can reproduce rapidly after disturbances such as ploughing, burning and fertilizer applications in agricultural fields (Neher & Barbercheck 1999). Many of the smaller mites such as Nanorchestidae have stylet chelicerae with which they pierce fungal hyphae. Predatory species feed on arthropods, arthropod eggs or nematodes, depending on the size of the mites. Some of these mites have specific predation patterns with certain species or life stages feeding exclusively on selected prey. An example would be *Dolicothrombium* spp. also known as red velvet mites, which hatch after rain and feed specifically on termites. The effect of prostigmatid mites in the soil is presumed to be very small, but their exact effect is unknown and difficult to assess due to the small size of most species (Coleman *et al.* 2004).

Mesostigmata species richness is low in soils and most soil species are predatory or parasitic (Coleman *et al.* 2004). Species from the Uropodidae family can be polyphagous and can occur in large numbers in agroecosystems (Gerson *et al.* 2003). Studies have found that mesostigmatids, as in the case of prostigmatids, are important predators of arthropods, arthropod eggs and nematodes in agroecosystems (Koehler 1997; Jung *et al.* 2010). In thick litter layers Mesostigmata numbers are higher than that of prostigmatids. Some mesostigmatids are known to live in close association with other arthropods and certain genera are used as bioindicators of soil health (Minodora 2011; Coleman *et al.* 2004).

The **A**stigmata are the least common of the soil mites and only increase in agroecosystems after the soils have been enriched with manure or during post-harvest periods. These mites feed on microbes and their numbers increase in the presence of plant residues and moist conditions. These mites are also known pests in stored products (Walter *et al.* 1986).

Pseudoscorpionida are predators of various smaller arthropods, nematodes and enchytraeids within the soil. These organisms do not occur in high numbers in the soil and they prefer soils with a higher humidity (Witt & Dill 1996). Active searching is usually a more effective sampling method to use (Coleman *et al.* 2004).

Symphyla are omnivorous invertebrates that resemble centipedes. They are, however, easily distinguished from centipedes in that they lack fangs. These organisms occur in grasslands and cultivated fields and can become pests in greenhouse soils (Coleman *et al.* 2004).

Enchytraeidae (potworms) are one of the lesser known families within the mesofauna (Beare *et al*. 1995). They are small unpigmented worms from the Class Oligochaeta, which also contains the earthworms. These worms are globally distributed and commonly occur in moist soils. Enchytraeids are hermaphroditic even though they can also reproduce through fragmentation and parthenogenesis (Boros 2010). This increases their ability to distribute into new habitats (Coleman *et al.* 2004). This family has a direct influence on the biochemical cycle in soil due to its geophagic processing of soil and organic matter (Beare *et al*. 1995). They feed on small organic and mineral particles which are enriched with fungi and bacteria. This feeding strategy can influences decomposition, since fungi and bacteria could have inhibitory or enhancing effects on the decomposition process. It has been reported that they sometimes even feed on larger faecal pellets and the castings of other soil fauna (Coleman *et al.* 2004; Maraldo 2009). Their faeces then become part of the turnover pool of organic matter in soil and can help with the stabilization of soil structure (Coleman *et al.* 2004). Due to their movement through the soil, these organisms are also responsible for the distribution of nutrients and soil aeration as a result of pore size manipulation (Beare *et al*. 1995). Although these organisms seem to prefer more acidic soils with a higher organic component, environmental factors such as temperature and precipitation also have an influence (Lindberg 2003; Coleman *et al.* 2004). Enchytraeids show spatial and temporal heterogeneity, with their vertical distribution influenced by organic matter which can be altered by tillage in agroecosystems (Coleman *et al.* 2004).

Some of the more primitive organisms in soils are from the classes Protura and Diplura (Holm & Dippenaar-Schoeman 2010; Zborowski & Storey 2010). Proturans are also cosmopolitan in their distribution and associated with the rhizosphere of plants (Sterzyńska *et al.* 2012). Their trophic position is still unknown, although there is speculation that they are mycophagous. Diplura have been sampled more frequently than proturans in agroecosystems and are represented by two families, Japygidae and Campodeidae. Both of these families are predaceous, with Campodeidae also feeding on fungal mycelia and detritus. Microcoryphia and Pauropoda are less common in soils and information on pauropod ecology and biology are still incomplete (Coleman *et al.* 2004).

◊ **Macrofauna**

Soil ‘macrofauna’ are a very diverse group with a wide range of functions. These functions include the shredding of animal and plant residues (*e.g.* millipedes) and the vertical and horizontal distribution of the latter into the soil (*e.g.* termites and earthworms). These organisms do not only enrich the soil with organic matter, but also change the physical arrangement of soil particles, influencing pore sizes which in turn influence infiltration and emission processes (Beare *et al*. 1995; Barrios 2007). These organisms are responsible for more than just physical alterations to the soils composition and also play a role in community composition through predation (Decaëns *et al*. 2006). They also have an influence on mesofaunal communities since some of their immature stages are in the same size range as mesofauna, whilst their adults create burrows in which the smaller organisms live. Some of the organisms classified as 'macrofauna' serve as a link between above- and below-ground communities, since they function as temporary or transient species (Coleman *et al.* 2004).

Isopoda, Amphipoda and Diplopoda are saprophagous macroarthropods that play a part in the fragmentation of decaying plant material (Holm & Dippenaar-Schoeman 2010). Terrestrial diplopods are capable of detoxification, digestion (by means of bacterial enzymes) and the absorption of nutrients throughout the different parts of the digestive tract (Coleman *et al.* 2004; Zagrobelny *et al.* 2004). Millipedes are widely distributed and, although they occur in arid regions, are susceptible to desiccation due to the absence of a waxy layer on the epicuticle. Millipedes seem to play an important role in the calcium cycle as they tend to increase in calcium rich areas, avoiding foliage or vegetation with high phenols and rather feed on those with high calcium levels (Coleman *et al.* 2004).

Chilopoda are predators that make use of fangs to kill their prey and they occur in a wide range of habitats (Von Reumont *et al.* 2014. Their exoskeletons also lack a waxy layer which makes them vulnerable to desiccation (Sømme 1995). Other predator groups include the orders Scorpionida and Araneae (Deltshev & Curcic 2011). Spiders are part of Araneae which are known solitary hunters. Members from the Lycosidae (Wolf spiders) are commonly found in leaf litter and soil surfaces. This family is evident in agroecosystems (Kerzicnik *et al.* 2013).

Various members from the class Insecta form part of soil communities. These organisms form part of all four groups compiled in accordance to their presence in soils (Coleman *et al.* 2004; Fig 1.3). Coleoptera is a very large order that is represented within most trophic levels. These range from predaceous carabid and staphylinid adults (Holland & Reinolds 2003), to saprophytic tenebrionid adults and Elateridae larvae (wire worms) that can become pests of plants in agricultural fields. Beetles thus have, amongst others, an important influence in decomposition, regulation of prey community numbers and as agricultural pests (Barsics *et al*. 2013; Brygadyrenko & Nazimov 2015; Toscano *et al*. 2015). Many dipterans pupate in soils and have saprophytic larvae. These larvae are usually restricted to wetter conditions and can enhance decomposition rates (Frouz 1999). Some of the other insect orders that are also encountered in soils are Orthoptera (that lay eggs in the soil), Psocoptera (that feed on detritus, algae and fungi) and Hemiptera (that feed on plants roots and create an above- and belowground nutrient flux due to a temporary presence in soil) (Coleman *et al.* 2004).

Hymenoptera and Isoptera (termites) are insect orders that also form part of the macrofauna. These organisms are widely distributed and have a significant influence on soil structure as they make nests in the soil (Jouquet *et al*. 2006; Araújo *et al.* 2010). Formicidae (ants) is the hymenopteran family that have the largest influence on soil. Formicidae influence both the biotic and abiotic components of the soil (*i.e.*, amongst others, feeding on soil faunal groups and competing with other predators, and soil turnover and enriching soils with organic matter respectively) (Frouz & Jilkova 2008). Ants and termites are social insects with well-developed castes. Isoptera is represented by various termite families within soils (Wilson 1990). Termites have either protozoan or microbial symbionts that enable them to digest wood and cellulose (Radek 1999; Husseneder *et al*. 2005). This ability enables some species to become major pests responsible for large economic losses. The three feeding life styles of termites are wood-feeders, plant and humus feeders and fungus growers. These insects fulfil an important role as soil turnover agents in drier regions where earthworm numbers are limited (Coleman *et al.* 2004).

The effects of certain organisms such as earthworms, ants and termites are well studied and may be equally important in soil turnover (Beare *et al*. 1995). Ants and termites are known to modify their environment and play an important role as ecosystem engineers (Coleman *et al.* 2004; Barrios 2007). Some of these modifications increase water infiltration and nutrient dynamics, and eliminate soil crusting that influences plant emergence and root growth. Studies have indicated that perfectly timed applications of cow dung and straw increased soil porosity. Such applications are most sufficient just before rain and within the foraging period of termites who then work these materials into the topsoil layer (Barrios 2007).

Many studies have been done on earthworms and it is possible to divide the different species into functional groups (*e.g.* epigeic, endogeic and anecic species; Fig 1.6)

(Also see Sheehan *et al.* 2007 and Caro *et al.* 2013). In the case of earthworms, these functional groups have different effects and operate at different depths within the soil (Beare *et al*. 1995; Barrios 2007). Earthworms do not reflect an even spatial distribution and often occur in patches where the conditions are favourable (Coleman *et al.* 2004).

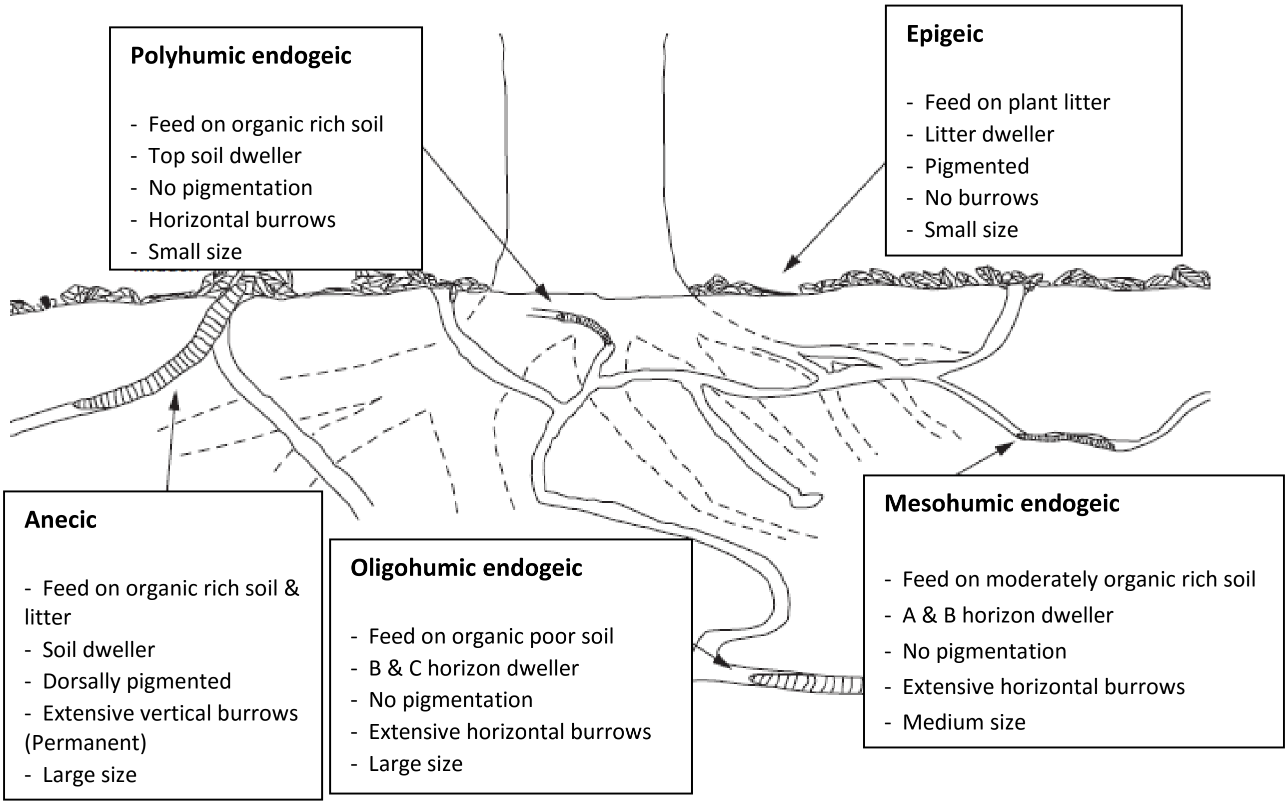


Fig 1.6: Summary of the functional groups of earthworms and their activity (Adapted from Coleman *et al.* 2004).

iii) Ecosystem services

All soil biota have ecological functions and although widely investigated there is still much that needs to be done. The indirect economical values of soil organisms depend on their ecological function, which in turn provides ecosystem services (Decaëns *et al*. 2006). Most species are divided into groups according to their trophic level. This, however, proves to be insufficient in a certain sense as all species' contributions are therefore deemed equal which is impossible due to the size variation and dietary plasticity expressed by various soil faunal species (Freitas *et al.* 2012).

Furthermore, it excludes the important activities of ecosystem engineers. The key soil ecosystem services provided by soil fauna include nutrient cycling, water permeability, decomposition and ecosystem engineering (Decaëns *et al*. 2006).

- **Decomposition**

The invertebrate decomposer community is a wide spread group that form the majority of faunal representatives in most terrestrial ecosystems (Coleman & Hendrix 2000). Decomposition is a process driven by an interdependent variety of biotic role players that are responsible for physical fragmentation, chemical degradation and increase of nutrient accessibility within an ecosystem. This is probably one of the most important ecosystem services provided by soil organisms. Depending on circumstances, *i.e*. litter fragment size and litter spread in the soil, this process is initiated by either macro-, micro- or mesofauna. The latter two groups are also responsible for an increase in decomposition rates due to the increase in surface area created from their shredding organic matter (Barrios 2007). In an agricultural context, the soil organic matter (SOM) that forms part of the decomposition process primarily consists of plant residue, supplemented by faunal excretions. This process is responsible for the breakdown and formation of long- and short-lived compounds that become part of the nutrient cycle where these elements are absorbed and utilized by the primary producers of the soil system. The decomposition rate depends on the type of plant residue, active biota, climatic conditions and the properties of the soil (Coleman *et al*. 2004).

- **Nutrient cycling**

Nutrient cycling is another important soil ecosystem service. This process can increase yields in agroecosystems, with, for instance, nitrogen-fixing bacteria and arbuscular mycorrhizal fungi providing the availability of nitrogen (N) and phosphorous (P) respectively. Although these nutrients can be added as fertilizers, in some parts of the world this is not possible and improved natural P management strategies have been proposed (Barrios 2007). Nutrient availability in soils is crucial, since nutrients are a limiting factor for biological activity in soil (Miller & Spoolman 2009). Nutrient supply directly influences plant productivity, which in turn influence the community structures of soil biota. Many studies have shown that soil organisms stimulate mineralization and the absorption of nutrients (*e.g.* Whalen 2014). From this it is obvious that in the presence of ample nutrients, plants will show optimal growth and nutrient uptake. Further, in the presence of a sufficient carbon-nitrogen ratio, plant growth is optimal and herbivore development is enhanced (Scheu 2001). According to Coleman and Hendrix (2000), agricultural soils with a complex faunal community, similar to that of naturally occurring faunal communities, lead to an increase in root biomass and an increase in N uptake which increased plant growth. According to the fertilisation manual of the Fertiliser Association of Southern Africa (2007), the accessibility of nutrients is influenced by the pH of soils, with certain nutrients more accessible at higher or lower pH values. Most of the plant nutrients can successfully be accessed between pH values of 5.5 and 7.5.

Microbes such as fungi, bacteria, protozoa and algae play essential roles in the sustainability of soil functioning and soil health. This is due to their immiscibility in decomposition, nutrient mineralization and soil respiration (Grant 2002; Larsen *et al.* 2015). In this regard the rate of nutrient mineralization will depend on the quantity and quality of organic matter (Cardoso *et al.* 2013), as well as the presence of certain functional biotic groups. Although these processes are driven by microfauna and -flora, their success is largely influenced by mesofaunal activities, such as microbe grazing (Coleman & Hendrix 2000).

- **Ecosystem engineering**

The concept of ecosystem engineering was proposed by Jones *et al.* (1994). The term ‘ecosystem engineer’ refers to an organism that alters its environment, thus affecting their immediate surroundings. In the case of soils, ecosystem engineers alter soil properties and resource availability to other soil biota. Such organisms therefore have an influence on the community structure of both fauna and flora within an area (Jouquet *et al.* 2006).

Termite nests harbour many different organisms that have evolved certain traits to share this space. These organisms range from ants, mites, beetles and even Collembola species (Coleman *et al.* 2004). If these survive in arid regions, they are not only regarded as ecosystem engineers, but also as keystone species within desert ecosystems (Coleman & Hendrix 2000). Earthworms are important as ecosystem engineers due to their burrowing and the production of castings which fertilize the soil. The burrowing activity of earthworms is responsible for variations in pore sizes throughout the soil. The orientation and depth of these burrows are species specific (Coleman *et al.* 2004). Except for the physical changes these organisms cause, they also influence the availability of nutrients and the structure of soil organic composition. A good example would be termites and earthworms that both alter the mineral and organic composition of soils, which increase the available food resources for other soil biota (Jones *et al.* 1994). This is a relevant process in soil ecosystems and provides a link between soil organisms which is different from direct trophic interactions (Wright & Jones 2006).

b) Abiotic component of soil

The functioning of soils is also regulated by various abiotic factors. These factors include physical, chemical and environmental conditions (Decaëns *et al*. 2006).

i) Soil types

Different soils have different properties due to variations in the soil formation process. These variations are directed by the available parent material, topography, climatic conditions, biota and the interactions of these factors over time (Coleman *et al*. 2004; Culliney 2013). The heterogeneity of physical and chemical properties present even at small scales within soil, is an important driver of complex biological communities (Barrios 2007).

- **Soil structure**

Primary succession is the first step in the formation of natural soil. This step depends on the physical weathering of rock and usually takes place over a long period of time. This leads to the formation of cracks and fractures, which serve as niches for various pioneer species and consequently enable secondary succession (Laliberté & Payette 2008). These pioneers – mostly lichens and mosses – help with the further breakdown of rocks while enriching and stabilising the substrate to sustain higher plants and invertebrates. Succession will take place, increasing the diversity of organisms and stabilising population numbers, therefore optimising the ecosystem services provided by the interactions between all the components within a specific space (Culliney 2013). The role of soil organisms in the modification of soil structure has been recognised by farmers long before their role in aggregation formation was conceptualised (Barrios 2007).

Soils can thus be seen as a compilation of various parts forming a multi-dimensional medium, with the physical soil particles as the primary building blocks (Decaëns *et al*. 2006). These individual soil particles give the soil its texture. Soil texture is stable over time and affect the water and gas balance within the soil medium (Cardoso *et al.* 2013). The texture of soil also influences the pore sizes in between particles, depending on the variation in particle sizes (Dexter 2004). According to Coleman *et al*. (2004), the term 'soil structure' refers to the spatial arrangement of soil particles, whether it is arranged as loose grains of sand or large irregular aggregates. These aggregates are formed by various binding agents such as organic compounds (polysaccharides and gums) and biological agents (plant roots and fungal hyphae), lending stability to the soil structure. The stability of these aggregates is influenced by the latter, as well as disruptions such as bioturbation and cultivation (Barrios 2007; Cardoso *et al.* 2013). Various studies have been done to determine the relationship between drying-wetting cycles, tillage and the formation of different types of soil aggregates (Coleman *et al*. 2004). Macro-aggregates are beneficial in that they are responsible for the stabilization of soils and the formation of macro-pores which influence the porosity and bulk density. Mechanical disturbances implemented for agricultural purposes, however, have a negative effect on these macro-aggregates (Cardoso *et al.* 2013), although wetting and drying cycles, which is influenced by irrigation, can result in the closer arrangement of soil particles (Coleman *et al.* 2004), which in a sense leads to aggregation resilience.

Aggregates can be divided into four types; plate-like, prism-like, block-like and spheroidal, which differ from clods, since clods form due to anthropogenic activities. When considering soil structure the porosity of soil is important, since pore cavity size can influence the community structure of its inhabitants and the permeability of water and air. The latter can become a problem in agro-ecosystems with regular disturbances. These pore sizes, in turn, are mainly caused by water in its liquid and gaseous phases (Coleman *et al*. 2004). According to Beare *et al*. (1995), pore sizes are also influenced by soil biota, which can therefore also reduce aggregate formation. Soils with deep penetrating macro-fauna have an increased porosity, water permeability and enhanced root growth when compared to soils that only have surface species.

- **Chemical composition**

Some chemicals occur naturally in soils due to certain abiotic and biotic factors such as primary production, nutrient cycling, cation-exchange capacity, SOM composition, etc. (Coleman *et al*. 2004). Nitrogen and phosphorus are key nutrients in cropping systems, since they can limit agricultural yields. Nitrogen is present in soils in many forms, of which some are not accessible by plants and need to be processed by the biological role players in soil. Examples of these forms include nitrates and organic nitrogen which is stored in the SOM. Phosphorus is also available in several chemical forms in soil, with the most common form being orthophosphates (Cardoso *et al.* 2013). Carbon sequestration is an important process conducted by plants. When the plants that act as a carbon sink die, the carbon will be returned to its previous state. Ants and termites influence carbon levels within the soil, since they carry carbon rich material into their burrows (Louw 2015). The addition of SOM to soil, not only enhances the soil structure, but has a key role in carbon sequestration and decreasing CO_2 emissions (Kabiri *et al.* 2015).

In the past, chemical components were used to determine levels of overall soil health by assessing the potentially available nutrients within the soils of cropping systems (Cardoso *et al.* 2013). This was proven as an inaccurate approach, since studies have indicated that certain elements can be present even though the biological component and its interactions were far less than those in the natural areas used as controls. Therefore chemical factors cannot solely be used as indicators of soil health or sustainable agriculture (Cardoso *et al.* 2013).

ii) Environmental influences

Community structures are subjective to all environmental factors throughout the different spatial domains. These factors can drive biodiversity or act as filters to select for certain species within an area (Decaëns *et al*. 2006).

- **Climate (humidity and temperature)**

Soils provide a stable environment with relatively small fluctuations in the temperature of the top 10-15m due to rain and the sun's radiation (Batjes & Bridges 1992; Márquez *et al.* 2016). This prevents the rapid freezing of water and thus the suppression of most life in the soil. The presence of water in a liquid form within the soil (as a water film or in soil pores) has a stabilising effect on the temperature in soil and therefore also influences the success of soil biota (Lehnert 2014). In soils water is also present in the form of vapour, which is of utmost importance to certain organisms that can gain or excrete water through their integument (Coleman *et al*. 2004). The presence of water in soil is influenced by both physical (*e.g.* permeability) and chemical (*e.g.* salinity) properties of the soil. As such water is a determining factor for microbial activity in the soil with the absence of water resulting in the loss of certain ecological functions. Microbial groups react differently to fluctuations in soil water content, *e.g.* in the presence of a lower water content, bacterial movement is restricted, whereas fungi are favoured and can expand their hyphae into the air-filled pores of the soil (Cardoso *et al.* 2013). Climatic conditions and the addition of SOM to soil, both living and dead, are thus important due to their influence on soil structure (Coleman *et al.* 2004).

Climate change will alter the determinants of soil faunal diversity and distribution. These factors include range shifts in plant distribution due to changes in rainfall patterns and soil temperatures, with the latter not only influencing the plants, but the soil fauna as well (Decaëns *et al*. 2006). It is, however, important to bear in mind that all soil ecosystems will react differently to the various climatic changes, for some are more susceptible to certain conditions than others (Decaëns *et al*. 2006). Soil processes are indirectly influenced by climatic conditions, as decomposition rates increase in warmer areas with a high humidity in contrast to the decrease in decomposition rates observed in dry and cold environments (Coleman & Hendrix 2000).

c) Agricultural practices

The influence of soil biota in agroecosystems is not merely a unidirectional event, but rather a complex web of both negative and positive feedbacks that affect yields. This is complicated even further with the implementation of agricultural practices and the effects of different crops (Barrios 2007). These external influences then alter soil community dynamics, which indirectly influence production sustainability and plant productivity due to the impact on soil fertility (Decaëns *et al*. 2006; Cardoso *et al.* 2013). As such these influences can have either a positive or negative effect on both soil fertility and soil quality (Emmerling *et al.* 2002). Anthropogenic manipulations also cause changes in organismal food web composition which contribute towards clarity regarding nutrient cycling under different conditions and in the presence of disturbances (Beare *et al*. 1992).

According to Oke *et al.* (2007) and Tsaifouli *et al.* (2015), factors such as physical disturbances of soils, the depletion of SOM and irresponsible management of fallow fields during off-seasons, all have a detrimental effect on soil microarthropod diversity. Fire and the use of pesticides can also decrease microarhropod numbers (Coleman *et al.* 2004; Fig 1.7; see fire studies by Gandar 1982; Hugo-Coetzee & Avenant 2011; Hutchins *et al*. 2011). The rehabilitation of such areas and re-establishment of soil fauna can be as easy as to discontinue the above-mentioned factors. However, in more severe cases such as mining the re-introduction of certain species is advantageous and often a requirement (Oke *et al.* 2007). The response and recovery period will, however, depend on the affected species, as well as the type and severity of the disturbance (Coleman *et al.* 2004).

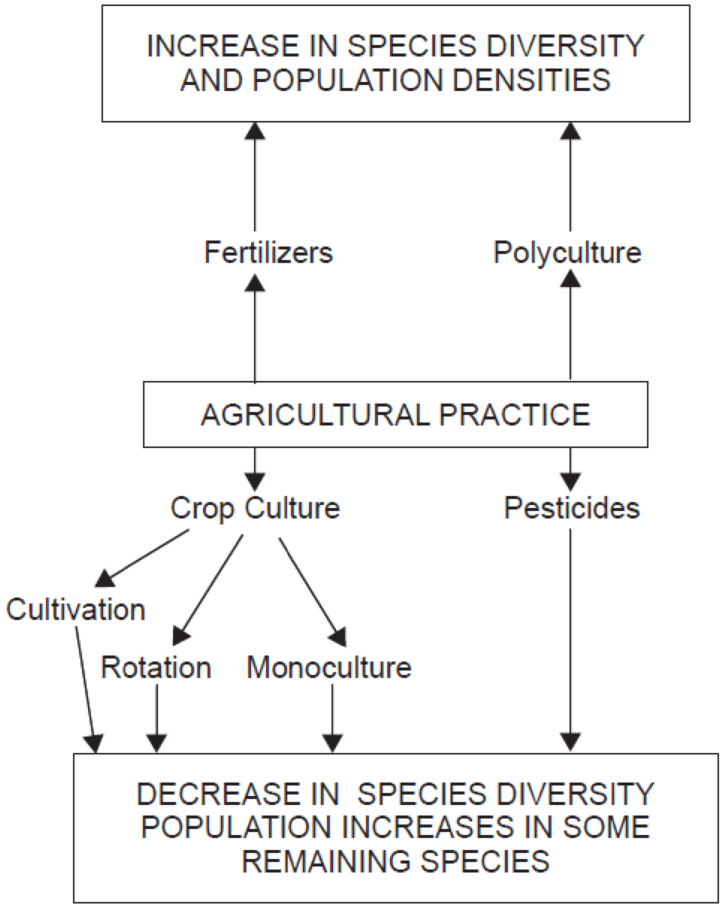


Fig 1.7: Influence of agricultural practices on soil microarthropod diversity (After Coleman *et al.* 2004).

i) Crop and natural vegetation

Plants are both directly and indirectly one of the strongest biological drivers of soil biota community structures (Bardgett 2005; Sylvain & Wall 2011). It has been reported that plants can alter the physical, chemical and biological components of soil (Karthikeyan & Kulakow 2003). These influences largely depend on the physical structure of the plant (shoot and leaf coverage; root system), litter quality and quantity, as well as nutrient uptake and excretion (Beare *et al*. 1995). According to Decaëns *et al*. (2006), anthropogenic influences, such as vegetation modification and the introduction of new plant species, will lead to changes in biotic interactions in soils. This is also true in cases where natural veldt is converted into agricultural fields. These alterations can have severe implications on the interaction between biota and can even influence abiotic factors, such as pH levels and water infiltration capability. Furthermore, decomposition is believed to be more efficient in areas with natural vegetation as opposed to areas with introduced plants. In a sense this also relates to the home-field advantage hypothesis, which proposes that litter decomposes at a faster rate in the area where the litter originates than it would in another area (Louw 2015).

Plants are primary producers and as such often act as a gateway for nutrients to other organisms. These nutrients (*e.g.* Fe, Ca, K, Mg, Na) are an essential part of soils. Different plant species will decompose at different rates and will provide specific nutrients to the soil during decomposition. This is true for all plants in both natural and agricultural environments (Beare *et al*. 1995). Plants have different defence mechanisms, one of which is the release of volatiles (Paré & Tumlinson 1999). This is referred to as herbivore-induced plant volatiles and has been applied in pest management. However, the severity of their effects is largely unknown in below-ground systems. The release of these volatiles is dependent on both above- and/or below-ground feeding. In some cases these volatiles can attract predators, thus adding to the complexity of the trophic structure (Schausberger *et al.* 2011), or act as cues to avoid already infested plants (Robert *et al.* 2012).

Variations in root architecture influence the quantity and spatial aspects of plant excretions, whereas roots of different plant species also expose differences in excretions as such. These exudates alter the rhizosphere community by means of inhibition and interaction between mycorrhizal symbionts and plant roots (Beare *et al*. 1995). In this regard it should be noted that agricultural development companies such as Soygro (Pty) Ltd., manufacture and produce microbiological inoculants for plants that are not harmful to the environment and promote sustainable agriculture (Soygro 2010). These inoculants, together with related products, enhance plant root mass and the function of the plant immune system. For example, Mieliepak®25 enhances root mass and is one of the products used in combination with Aktinos® (which protects against *Fusarium*) or Nemablok® (which protects against root nematodes) in the production of maize (Soygro 2010).

ii) Physical / mechanical disturbances

Soils were historically divided into groups according to the work effort that would be needed for cultivation purposes. Over time a more effective classification system was incorporated in terms of the percentage of sand, silt and clay present in the soil (Coleman *et al*. 2004). Mechanical perturbations can have both positive and negative effects in soils for it alters aggregate stability which in turn influences erosion, aeration, runoff and water infiltration (Cardoso *et al.* 2013).

- **Conventional tillage *vs* no-tillage systems**

When comparing the soil biota in conventional and no-till agro-ecosystems in Georgia, Beare *et al*. (1992) found that there was a distinct difference in community structure. The term 'conventional tillage' indicates the use of several invasive techniques such as ploughing and disking / disc-ploughing to incorporate the plant residue into the soil medium (Cardoso *et al.* 2013). Differences noticed in community structures are probably due to the burying of plant residue, as well as soil mixing, the latter implying disturbance of soil stratification. Soil mixing generally favours r-strategists and organisms with general feeding habits (Bardgett & Cook 1998; Briar *et al.* 2012). Although conventional-tillage areas are considered as a disturbed landscape, it was found that the microclimatic conditions below-ground showed less variation with nutrient pools spread out (Beare *et al.* 1992). These conditions have proved to promote bacterial production and more rapid nutrient turnover rates. On the other hand, with no-tillage systems the plant residues stay above-ground, while nutrient turnover in this case mainly relies on fungi. This process occurs at a slower rate and nutrient retention levels are higher (Beare *et al.* 1992).

Soil organic matter (SOM) is an important source of nutrients and refuge for soil organisms, but it can have negative effects in agriculture. These effects include an increase in pesticide usage, which not only harm non-target organisms at the application site, but can also infiltrate ground water and have a further detrimental effect (Cardoso *et al.* 2013). On the other hand, SOM can have a positive influence on the physical component of soils since it improves soil structure. This enhances characteristics such as porosity, aeration, water infiltration and water holding capacity

(Franzluebbers 2002; Lui *et al.* 2006). Differences in SOM stratification and the degree of disturbance thus results in variation in the physical, chemical and biological components of soils (Cardoso *et al.* 2013), with the latter indices driving microbial community structures and the food web they support (Beare *et al.* 1993). Therefore, converting from conventional practices to a no-till system will affect the biotic community structure and influence the efficiency of nutrient cycling. At first some nutrients will be inaccessible due to the incapability of plants to obtain it in the absence of competent microorganisms (Barrios 2007). In the long-term, however, soil biodiversity will increase with a reduction in production costs (Crittenden *et al.* 2015). One of the organisms that decreases during intensive land use is earthworms. This can be changed by converting to less intensive practices which will increase the number of earthworms and over time reflect an enhancement of soil properties (Coleman *et al.* 2004). On the other hand, Prostigmata mites have been shown to increase in the presence of cultivation practices (Beare *et al*. 1995).

The abandoned burrows of certain macrofauna can be beneficial in undisturbed soil, for it can be a rich source of mycorrhizal inocula and phosphorous to plants. This is not the case in ploughed soils. These channels can also aid in plant roots accessing nutrients and water sources in compact soils (Beare *et al*. 1995). It is, however, very important to keep in mind that compact soils have been reported to reflect a decrease in faunal species diversity due to a lack of mobility possibilities (Decaëns *et al*. 2006). Tillage is implemented to increase soil porosity, but this is only effective for a short duration, since negative long-term effects that include problems with water holding capability, reduced SOM and poor soil structure due to a decrease in stable aggregates are more obvious (Crittenden 2015).

- **Controlled stubble-burning**

Stubble-burning is a generally accepted practice in crop farming communities. In the short term this practice is preferred and can prove beneficial in preventing crop disease and improving time management ([1] Mrs E Badenhorst, personal communication). Some pests and diseases use plant residue as refuge and therefore it is important to manage these residues optimally, *i.e.* enhance SOM development, but prevent disease inocula (Bockus & Claassens 1992; Katan 2010). Ecologically

[1] Mrs E Badenhorst, 16 Parkdene, Kimberley, pers.comm, October 2015

the preferred management strategy would be to incorporate plant residue rapidly and maximally into the soil, but this is not always economically viable, since ploughing and working the stubble into the soil can be an expensive and time consuming venture ([1] Mrs E Badenhorst, personal communication).

Many ecosystems rely on fire to regulate some of its processes and thereby enhance functioning (Parr & Chown 2003; Van Wilgen 2009). Micro-arthropod abundance usually declines after fires (Hugo-Coetzee & Avenant 2001), with the recovery process and tempo still largely uninvestigated. However, it has been determined that the rate of recovery depends on the intensity of the fire (Zavala *et al.* 2014). Micro-arthropod communities recover faster after frequent, low intensity fires (as seen in grasslands), than they do in the presence of high intensity fires that occur less frequently (such as forest fires). Importantly, these observations were only made at the order level of organisms and thus do not reflect the influence on specific species. Another short-coming is that most studies only use samples taken several months after the fire has occurred. This poses a problem in that secondary effects cannot be separated from the effects of the fire itself (Malmström *et al.* 2008).

Fires are therefore a necessity in some ecosystems due to its integral role in maintenance and development (Van Wilgen *et al.* 2012). On a landscape scale it is also useful in the reduction of bush encroachment. Fires in grassland areas remove the detritus layer, thus clearing the soil surface and resulting in warmer and drier soil conditions (Santana *et al.* 2010). Invertebrates react differently to fire disturbances, with reaction similarity observed in certain trophic guilds due to the changes in plant community structure (Coleman & Hendrix 2000).

- **Field management**

Agricultural soils endure a wide range of disturbances that are associated with field management. This has an impact on the community structures of soil biota, since it modifies the primary factors responsible for soil biodiversity (Neher 1999). These modifications, such as habitat destruction and the use of biocides act as filters, in the process eliminating certain organisms by decreasing dietary resources (Neher & Barbercheck 1999). On a spatial scale, this can cause the local extinction of native

[1]Mrs E Badenhorst, 16 Parkdene, Kimberley, pers.comm, October 2015

species due to their lack of resilience after the disturbance or on account of increased pressure from competitors. When converting natural veldt to agricultural fields, the dynamics of soil communities often change dramatically on account of some organisms that are filtered out and better adapted organisms invade these open niches (Decaëns *et al*. 2006).

Compaction is one of most frequently encountered problems in agriculture which reduces soil porosity. This is usually caused by traffic of heavy agricultural equipment, livestock grazing within the fields and even water application by means of irrigation systems (Decaëns *et al*. 2006; [1] Mrs E Badenhorst, personal communication). The solutions to the problem is based on increasing porosity levels, thus management practices should be implemented to re-establish good physical qualities of soil (Hamza & Anderson 2005).

Practices to mitigate soil compaction have proved to be successful in arid, as well as semi-arid regions and can even be used in combination with one another (Hamza & Anderson 2005; Baumhardt *et al.* 2015). For long-term success the most common practice is the addition of SOM to the substrate to restore porosity. Another environmentally beneficial method is crop rotation which includes plants with deep penetrating root systems. In the short-term mechanical loosening of soils and a reduction of farm equipment traffic in an area can also be applied (Hamza & Anderson 2005). The presence of SOM in soils lowers the bulk density of the soil, which improves its structure due to the enhancement of aggregation factors. This, in turn, results in higher porosity which enables root, air and water permeability (Cardoso *et al.* 2013).

iii) Pests and Diseases

In general, most farmers regard soil fauna as pests. This is due to the few species that cause disease and yield loss. This could have been avoided with the promotion of diverse soil community structures that include members of all trophic levels (Neher 1999). With such complex communities all pests and diseases could have been managed through competition, predation and parasitism and the organisms would

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also contribute to plant health. Plants that grow in nutritionally depleted soils are weak and have a low tolerance to pest attack and disease infestation (Altieri & Nicholls 2003). Soil pests and diseases are difficult to control and therefore it has proven worthy of enhancing the use of biological control agents (Barrios 2007), and to enhance plant resistance (Soygro 2010).

Certain soil-dwelling species are pests of plants, but mesofauna in general are not considered to be pests. Most mesofuanal groups are mycophagous and some collembolans can protect plants from fungal infections (Coleman *et al.* 2004). In the UK, for example, *Coniothyrium minitans* is a mycoparasitic fungus which is used as a biological control agent against pathogenic fungi such as *Sclerotinia sclerotiorum* (Whipps *et al.* 2008). Due to dietary preferences there has been speculation on the contribution of mesofauna and certain other soil faunal groups with regard to their contribution to the dispersal of *C. minitans* in soils. These speculations have been tested in Europe and both the nematode *Acarus siro* and the collembolan *Folsomia candida* are shown to be responsible for transmitting *C. minitans* from infected to uninfected sclerotia, thereby contributing to the success of this mycoparasite being able to control fungal diseases caused by *S. sclerotiorum* (Williams *et al.* 1998).

According to Benckiser (1997), nematodes (microfauna), which are well-known pests of plants, can have significant economic impacts due to yield loss. In the past nematicides were used to manage this pest, but due to the contamination of ground water this was downscaled and the focus has shifted to the use of cultivars that show resistance to nematodes.

Tillage can have positive and negative attributes concerning pest control. Ploughing can disrupt slug populations by burying the eggs and exposing the slugs to larger predators above ground (Roger-Estrade *et al.* 2010; Douglas & Tooker 2012). In the absence of tillage, a general increase in the use of insecticides has become more common with the resultant, negative influence on predators (Wiebe & Gollehon 2006). Tillage reduces plant residue on the soil surface and therefore minimizes pests seeking refuge in stubble. Tillage is also an important method used to control plant disease with adverse effects due to the variation in practices and pathogens, *i.e.* tillage influence the severity of *Fusarium* blight (*Fusarium oxysporum*) on soybeans

(Joseph *et al.* 2016). If tillage is applied later in the season it could have negative effects on predator populations since this disturbs their overwintering sites. A decrease in parasitoid emergence has also been noted where tillage was applied (Roger-Estrade *et al.* 2010).

iv) Chemical applications

According to Decaëns *et al*. (2006), there were already more than 50 000 different pesticides used per year by 2006. These pesticides are destructive to agricultural and natural communities, with a far wider range of implications than observed in the area of application. This is largely due to bioaccumulation and the elimination of non-target organisms, such as pollinators and predators which act as bio-control agents.

The pH of soil is important since it influences microbial activity and correlates directly with nutrient availability (Cardoso *et al.* 2013). Earthworm numbers are lower in acidic soils which are soils with a low pH value (Coleman *et al.* 2004). To alter the pH in agricultural systems, farmers add lime. This has a neutralising effect on soils with a low pH level (Fertiliser Association of Southern Africa 2007).

Agricultural disturbances such as over-utilization/resource depletion and fertilizer applications can reduce the heterogeneity of microhabitats in soil which, in turn, have a negative effect on micro-fungal communities (Beare *et al*. 1995; Parfitt *et al*. 2010). A study by Römbke *et al*. (2009) showed that the use of pesticides reduced certain soil mesofaunal groups such as saprophytic Enchytraeidae. Gbarakoro and Zabbey (2013) conducted a study on the effect of the herbicides atriazine and gramoxone-bipyridilium on soil mesofauna. Their study was conducted on a fallow farmland and therefore they could conclude that the decline in mite numbers was due to herbicide levels and not a decrease in vegetation. Atriazine had the strongest effect of the two herbicides, albeit that mite numbers showed a more severe decrease when these herbicides were applied in combination.

As a result of the numerous non-targeted organisms affected by the use of biocides, certain regulations must be upheld. The misuse of biocides have an even further reaching negative effect on the environment and must be avoided at all costs. The use of pesticides in South Africa should adhere to Act No. 36 of 1947, which is the Act on Fertilisers, Farm Feeds, Agricultural Remedies and Stock Remedies (http://www.nda.agric.za/docs/Policy/PesticideManag.pdf).

d) Aim of the study

A healthy ecosystem is synonymous with a well-functioning food web which contain representatives of all trophic levels. To investigate this, I will aim to answer the following questions regarding fluctuations and variation in trophic structures and diversity of soil mesofaunal groups over different seasons.

- What organisms occur in natural veldt at different localities in the Free State?
- Is there similarity regarding organism occurrence in different agricultural soils of different crops in the Free State?
- What are the filtering effects of fire and mechanical disturbances on mesofaunal species assemblages?
- Do different biocides have an effect on mesofaunal occurrence?
- What is the impact of chemical pollution of mining activities on soil biotic diversity?
- Is there a difference in Collembola occurrence at selected sites in the Free State?

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Chapter 2

SAMPLING PROCEDURES AND DATA ANALYSIS

a) Sampling procedure

Similar sampling procedures were followed at all sampling sites. Sampling sites were selected to represent specific areas in the fields to investigate the effects of different influencing factors on mesofaunal occurrence. All of the plants at the sampling points where in optimal condition, with the exception of certain samples which are clearly pointed out in the data analysis.

i) Field sampling

Sampling was done in the porosphere of selected plants at the sampling points. According to Beare *et al.* (1995) and Barrios (2007), the porosphere is a biologically important area within the plant root zone and contain most of the mesofaunal activity. A trowel was used to collect soil up to a depth of ±15cm within the root masses of three plants at each sampling point. The soils collected from these plants were then lumped to form one sample with a mass of ±2kg. The plants used in this experiment were selected as representatives of the whole maize field and therefore were in the same condition as the rest of the plants in that specific field. All of the sampling points were selected ±25m away from the edges to eliminate variations experienced with edge effects. The samples were placed into a brown paper bag and transported to the lab in a cooler box (Fig. 2.1). This prevents the soil from drying out, overheating, and prevents condensation. All of the paper bags were clearly marked with sampling point numbers and date of sampling. All other observations were noted in a separate notebook.

Fig. 2.1: Sampling procedure. A-B: sampling of soil from the porosphere of the plants by means of a trowel; C: transporting samples in a cooler box.

Additional observations such as humidity percentages and relative compaction differences were noted at some fields. The humidity percentages were determined by means of a soil moisture meter (Model: PMS-714 of Lutron Electronic Enterprises). Compaction readings were determined by means of a Dickey-John compaction meter (Fig. 2.2). These readings could, however, not be obtained at all locations due to logistic problems. At Vaaldam farm, probes were inserted into the soil to determine soil temperatures and humidity. Available soil analyses done by agricultural consultants at the different localities have also been included in the study.

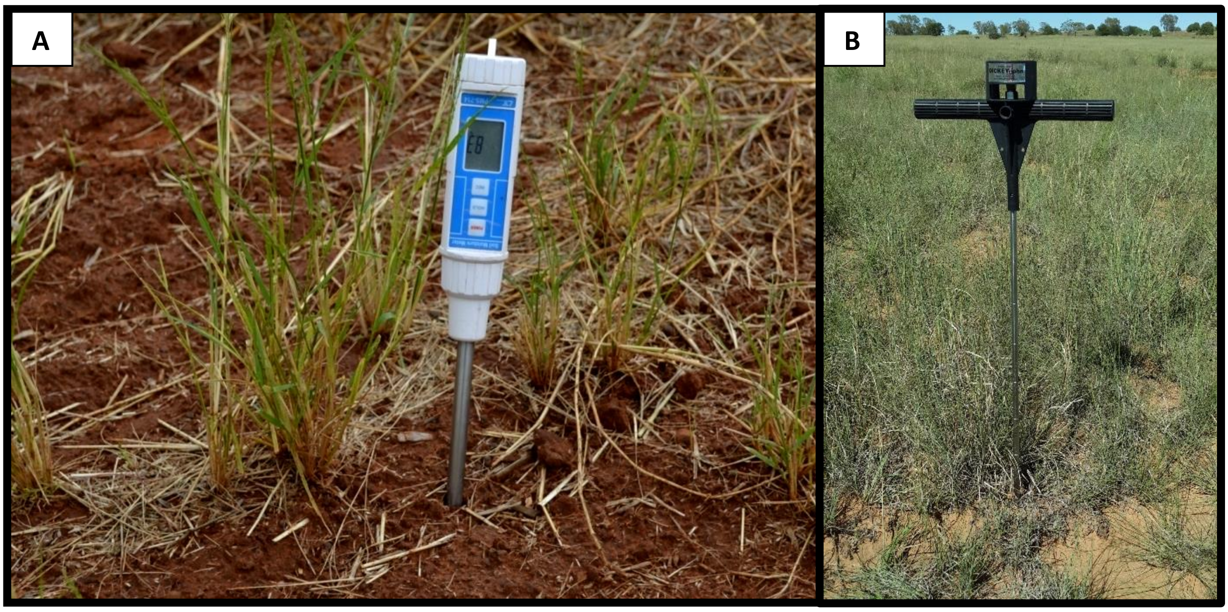

Fig. 2.2: Apparatus used to measure soil humidity and compaction. A: humidity meter; B: compaction meter.

ii) Laboratory extraction, sorting and identification

Due to the size difference and behavioural complexity of soil communities, together with their temporal and spatial distribution within this medium, it is impossible to extract all biotic role players by means of a single extraction method. Studies done on soil organisms are therefore focused on certain groups of organisms extracted by means of a specific method (Barrios 2007).

- **Laboratory extraction**

In this study all extractions were done by means of the Berlese-Tullgren funnel extraction method, the apparatus of which was set up at the University of the Free State (Fig. 2.3). This method is effective in extracting organisms in as specific size range (mesofauna and certain macrofauna) (see Sakchoowong *et al.* 2007; Smith *et al.* 2008; Bano & Roy 2016). Each extraction was done over a period of seven days within which the lights were never switched off.

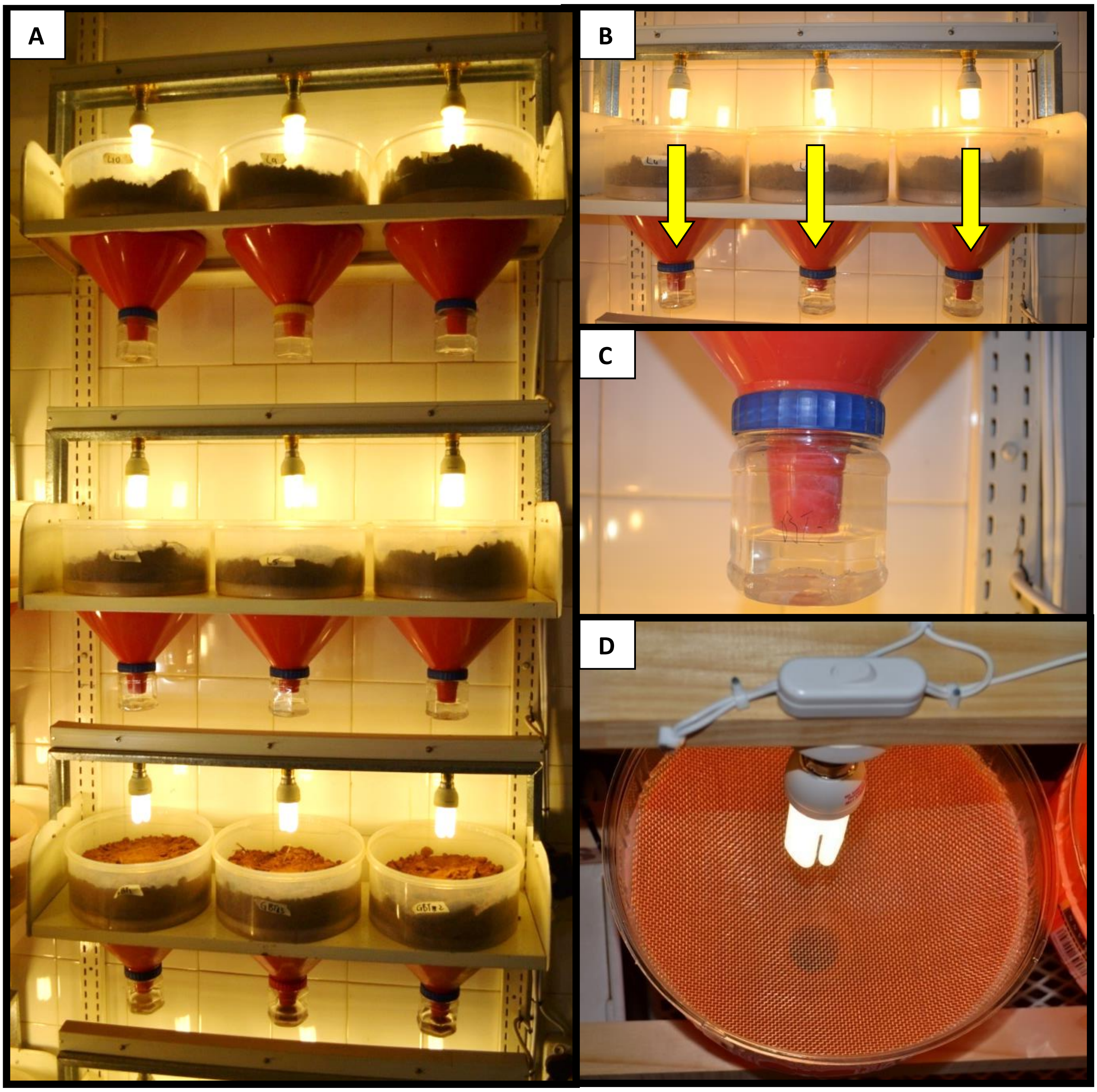

Fig. 2.3: Berlese-Tullgren funnel apparatus of the University of the Free State. A: strong frame supporting various funnels and an electrical system for the light bulbs; B: downward movement of organisms (yellow arrows) to avoid the heat and desiccation effect caused by the light source; C: plastic screw-on container half filled with a 70% ethanol solution; D: the grid placed into a funnel upon which a soil sample is placed.

- **Sorting during preliminary study (2011-2012)**

A preliminary study was done from 2011 to 2012. These samples were filtrated and the organisms were picked off the filtration paper with a needle or the fine hairs of a modified paint brush. The filtration paper was sprayed with ethanol every few minutes to prevent the specimens from desiccating (Fig. 2.4). This process allowed for the quantification and qualification of macro- and some of the larger sized mesofaunal groups. These results therefore only represent certain meso- and macro arthropods and was used as motivation to modify the sorting process of future studies. This modification would include organisms from a wider size range, which had previously been excluded.

- **Sorting during main study (2012-2014)**

During the primary study, samples were filtered through a micro sieve. The larger organisms that remained on the sieve were then washed into a small petri-dish with a 70% ethanol solution. This prevented the 'loss' of very small organisms due to blending with the white background of filtration paper and its rapid drying. The contents of each sample were therefore well represented with minimal loss of biological material. The sorting success was further enhanced by using differently coloured backgrounds to ensure maximum visibility. Organisms ranging in body length sizes between 100µm and 4cm were all well represented in the data (Fig. 2.5).

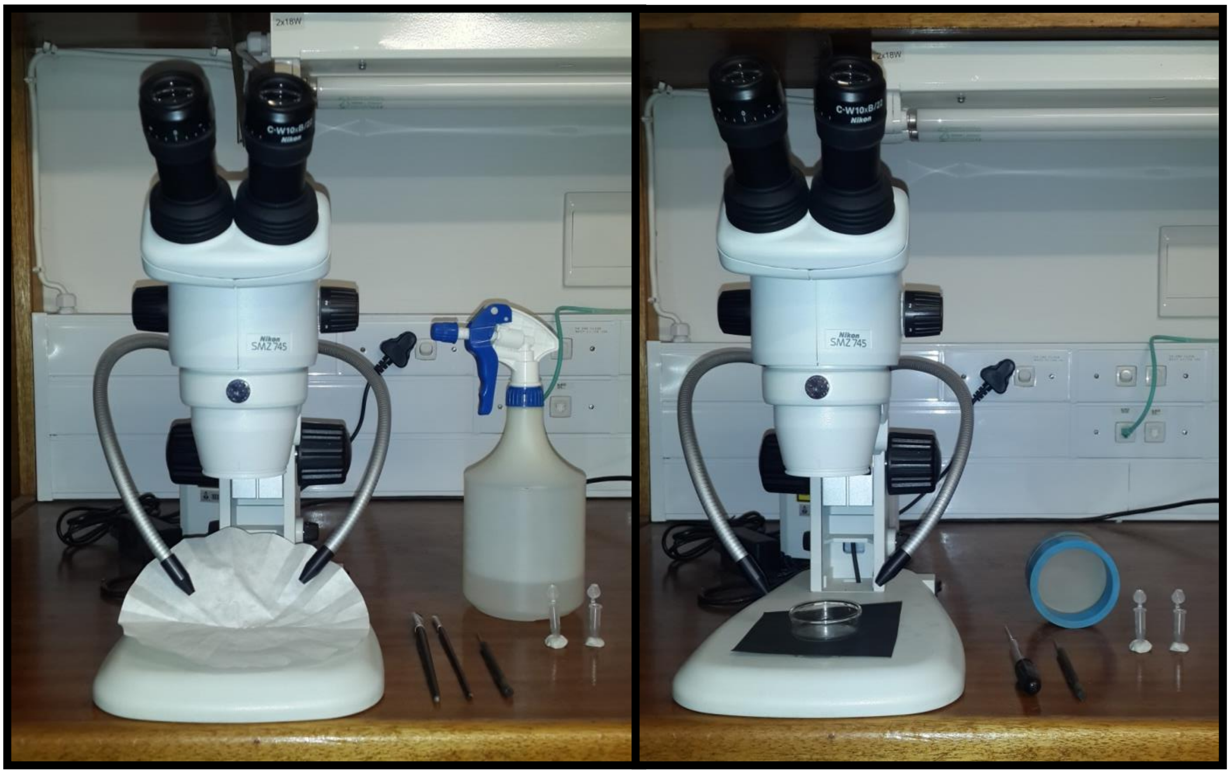

Fig. 2.4: Sorting apparatus. A: apparatus used during the preliminary study in 2011 and 2012; B: apparatus used during the primary study from 2012 to 2014.

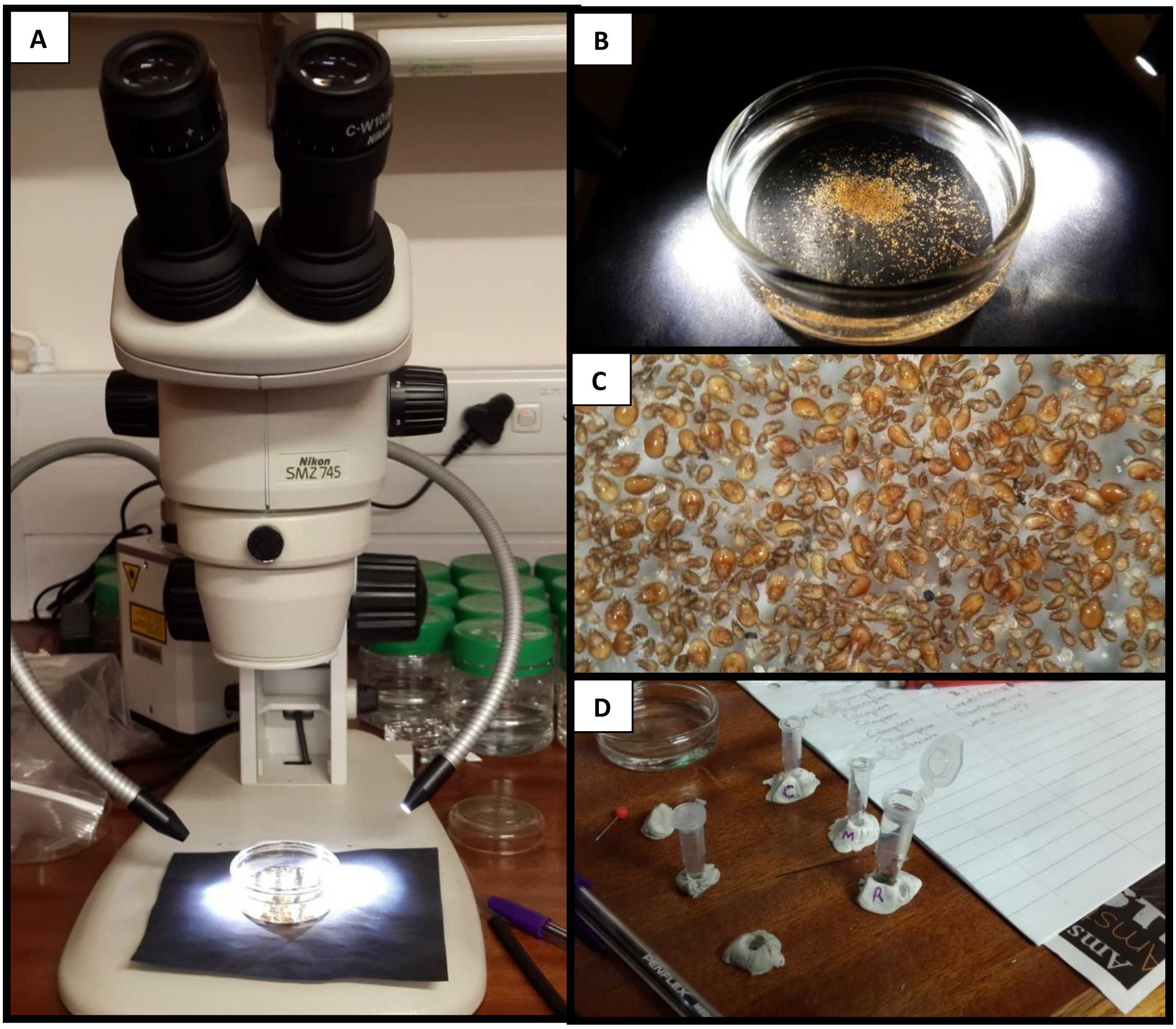

Fig. 2.5: Sorting of samples under a dissection microscope. A: Nikon dissection microscope used to sort samples; B: petri-dish with various sized mite species in 70% ethanol; C: magnification of the mites in the petri-dish in B; D: micro-tubes used to store the sorted material.

- **Identification**

Insects were identified up to family level by means of keys from Triplehorn & Johnson (2005) and Scholtz & Holm (2008), after which all organisms were quantified under a dissection microscope. A reference collection was compiled and stored in micro-tubes that contain a 70% ethanol solution. Microscope slides were prepared for collembolans which were then identified up to genus level by using keys from Fjellberg (1998 & 2007), as well as internet keys of Bellinger *et al.* (1996-2016). All collembolan slides were clearly marked with the sampling information and identification (Fig. 2.6), and send to the Muséum National d'Histoire Naturelle in Paris for verification, whilst mites and spiders were sent to experts for identification.

Fig. 2.6: Clockwise from top left: Collembola identification, slides and storage.

iii) Trophic level analysis

After identification, all organisms were divided into trophic levels according to literature and morphological analysis (Table 2.1; see Triplehorn & Johnson 2005; Scholtz & Holm 2008; Krantz & Walter 2009). The main trophic guilds of soil mesofauna are phytophages, mycophages, predators, saprophages, bacteriophages, ecto-parasites, parasitoids, algaephages and omnivores.

Table 2.1: Division of the main trophic guilds of soil faunal groups. See Addendum A for more information.

Phytophagous: Feeding on flora species ▲	Algaephagous: Feeding on algae ⬟
Saprophagous: Feeding on decaying material ■	Mycophagous: Feeding on fungi ⧗
Coprophagous: Feeding on dung ✦	Bacteriophagous: Feeding on bacteria ●
Omnivorous: Feeding on both fauna & flora ◎	Ecto-parasitic: Parasitize other organisms ⬭
Predaceous: Feeding on faunal species ⊕	Parasitoids: Feed on specific host ◇

b) Statistical analysis

Biodiversity is essential for the functioning of any ecosystem and it is therefore important to conserve it. Policy makers rely on diversity studies and focus on the protection of species richness and conservation of species unique to an area (Allen, Kon & Bar-Yam 2009). It is therefore important to make use of diversity, evenness and similarity or dissimilarity indices when evaluating the status of an area. According to Spellerberg and Fedor (2003), it is important to know what is meant by certain terms:

- Species richness – number of different species
- Species abundance – number of individuals representing the selected taxonomic groups
- Biodiversity – Index involving the relationship between species richness and species abundance

i) Shannon's diversity and evenness index

The Shannon's diversity and evenness index focuses on species richness and the distribution of the number of individuals between the represented species (Allen, Kon

& Bar-Yam 2009). This index is widely used in ecological and biodiversity studies (Spellerberg and Fedor 2003). The equation for diversity is:

$$H = \sum_{i=1}^{n} pi \ln pi$$

Where H = diversity as measured by the Shannon function;
pi = representation of the number of individuals of a taxonomic group as a proportion of the total number of individuals (proportion of total species abundance);
n = the number of species or taxonomic groups sampled in the specific sample (species richness)

The equation for evenness is:

$$E = H \div (\ln(n))$$

Where E = evenness of a sample (value between 0 and 1, with 1 indicating that individuals were evenly spread between the different taxa that was sampled)

Due to the similarities in feeding behaviour of soil mesofauna, trophic structures were incorporated into the diversity index calculated by means of species richness (number of different species) and species abundance (abundance of each species recorded for each sample). The diversity of each sample was presented in bar graphs with the trophic levels indicated as a proportion of the diversity of that sample and was calculated as follows:

$$\left(\frac{Number\ of\ individuals\ representing\ \ selected\ trophic\ level}{Total\ number\ of\ individuals}\right) \times 100 = Trophic\ level\ \%$$

$$\frac{(Trophic\ level\ \%) \times (H)}{100} = Index\ value$$

Index value = proportion of the diversity that is represented by the selected trophic level individuals

For purposes of this study, the Shannon's diversity and evenness index was calculated using Past3 (V3.08) and interpreted according to its user manual compiled by Hammer (2015).

ii) Bray-Curtis dissimilarity measure

This measure is commonly used by ecologists to determine the similarity or dissimilarity between samples. These resemblances are calculated as a value between 1 and 0, which is then multiplied by 100 to be expressed as a percentage (Clarke, Somerfield & Chapman 2006). The equation is as follows:

$$D_{12}^{B-C} = 100 \times \frac{\sum_i |y_{i1} - y_{i2}|}{\sum_i (y_{i1} + y_{i2})}$$

With y_{ij} = assemblage of data;
i = number of taxa;
j = number of samples

For purposes of this study, abundance data were used and transformed (Pretreatment: transform overall – square root) prior to analysis. The root transformation was applied to down-weigh the importance of the species that was highly abundant (see Clarke & Warwick 2002). The Bray-Curtis dissimilarity measure was calculated with Primer6 from which a dendrogram was drawn to indicate the similarity between samples as a result of hierarchical clustering, as indicated by Clarke & Warwick (2001). A similarity of 40% and more was regarded as meaningful, as is applied in most ecological studies (Quinn & Keough 2002).

c) References

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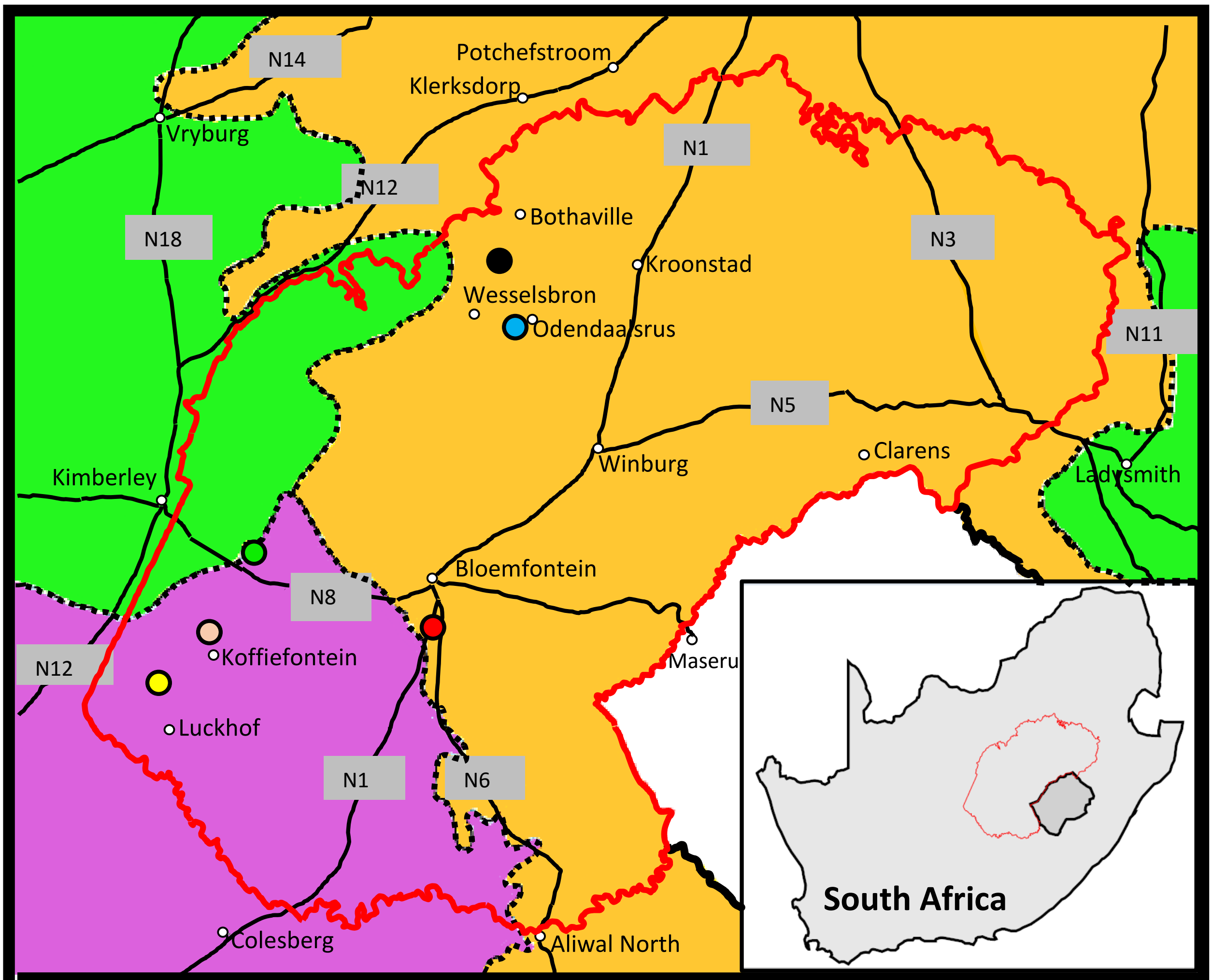
Chapter 3

STUDY SITES AND AGRICULTURAL APPLICATIONS

a) Site descriptions and relevant observations

South Africa is considered one of the major contributing countries to global biodiversity. Its variety in micro-climatic conditions enables this country to sustain such diversity throughout its seven biomes. South Africa's biome classification is based on dominating plant types in relation to rainfall and temperature (Oldeland *et al.* 2010, SANBI 2013). The effects of climate change are therefore far greater than just changes in temperature, for these changes will have serious implications on diversity. A moderate rise in temperature has been predicted to have noticeable effects where biomes such as the Grassland biome can become greatly reduced, with the Desert biome expanding into the Nama-Karoo biome (SANBI 2013). The Free State Province forms part of central South Africa and produces approximately 40% of the country's total maize production (Agriculture, Forestry & Fisheries 2014). This is possible due to adequate rainfall and the implementation of irrigation systems in the semi-arid regions (JADAFA 2014).

Sampling for this project was conducted at six localities in the Free State Province. Three of the localities are located in the Nama Karoo Biome and the other three in the Grassland Biome (Fig. 3.1). Four of the localities are farms, *i.e.* Vaaldam, Koppieskraal, Thornberry, Klein Brittanje, which were selected due to the diversity of agricultural practices and management strategies used by the farmers. The farm Paradys is the experimental farm of the University of the Free State which was used for a pesticide application trial. Lastly, the farm Eureka was exposed to goldmine pollutants which killed livestock. All these localities posed the perfect opportunity to examine and observe fluctuations in community structures of the selected faunal groups in the relevant soils.



Locality (Farms)	Coordinates	Biome	Rainfall per year
Vaaldam	29°28'51.1"S, 24°39'03.3"E	Nama Karoo	120 – 250mm
Koppieskraal	29°18'46.6"S, 24°58'54.6"E	Nama Karoo	120 – 250mm
Thornberry	28°57'36.0"S, 25°07'34.0"E	Nama Karoo	250 – 500mm
Paradys	29°13'35.9"S, 26°12'33.4"E	Grassland	250 – 500mm
Eureka	27°54'50.4"S, 26°39'21.3"E	Grassland	500 – 750mm
Klein Brittanje	27°39'40.2"S, 26°33'49.4"E	Grassland	500 – 750mm

Fig. 3.1: Sampling locations used in this study in the Free State Province of South Africa showing the associated biomes (Adapted from South African National Botanical Institute's website). Rainfall figures from Carruthers (2008).

i) <u>Locality 1:</u> Vaaldam farm

This farm is situated in the Koffiefontein district and consists of 210ha (21 X 10ha) of cultivated fields (Fig. 3.2). It forms part of the Oppermansgronde which is situated between Koffiefontein and Luckhof and forms part of a development program for upcoming farmers. Due to the inadequate rainfall of the Nama Karoo Biome, all of these fields are under centre-pivot irrigation systems which were set up in 2006. This farm has red sandy soil and these fields are all assembled on a minor slope. The natural veldt consists of dwarf-shrubs and grasses, which is typical vegetation of the Nama Karoo Biome.

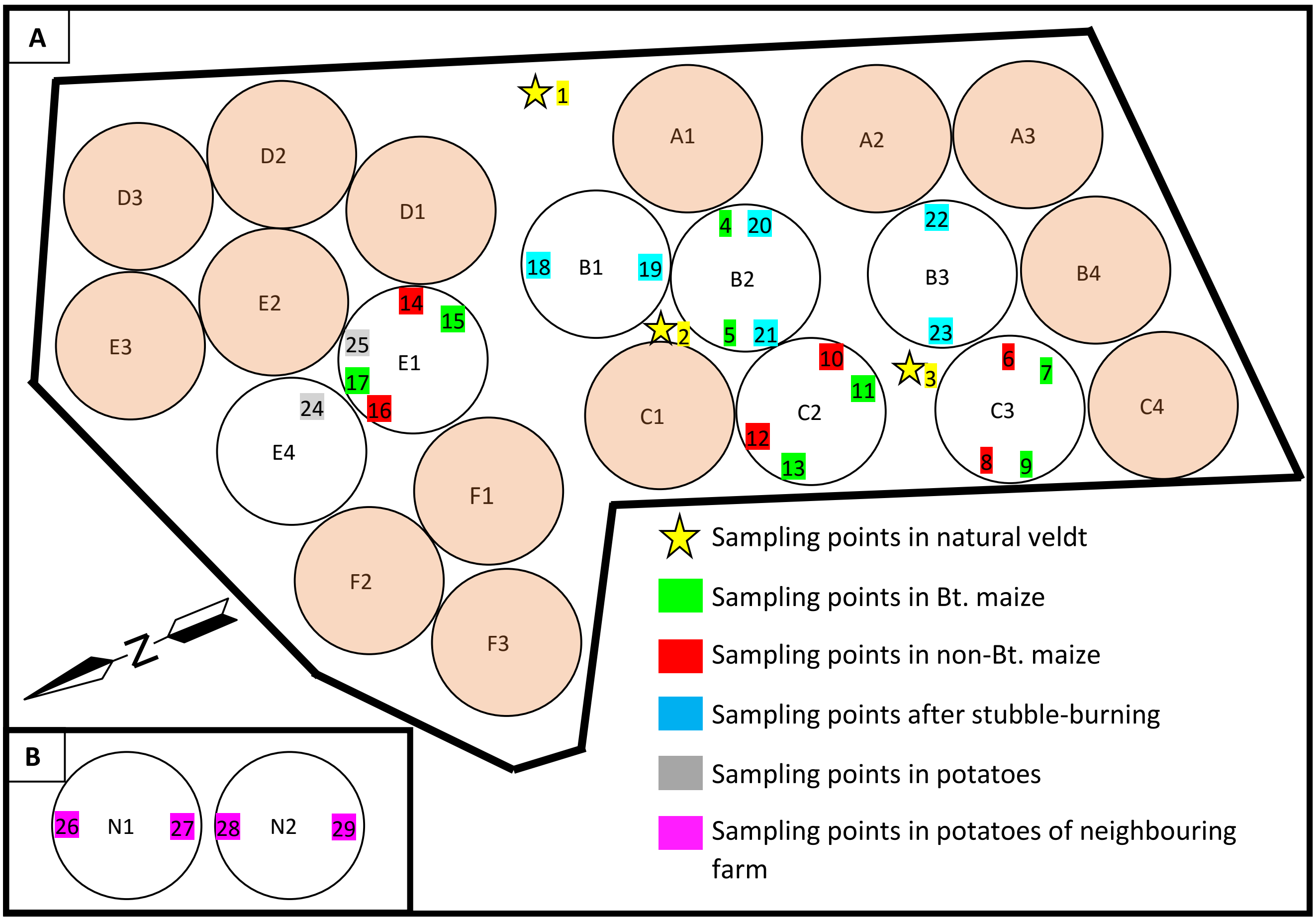


Fig. 3.2: Locality 1, situated between Koffiefontein and Luckhof. A: The layout of the agricultural fields at Vaaldam farm and its sampling points; B: Neighbouring farm's fields cultivated with potatoes. See Addendum B for species richness and abundance data.

During the sampling period, these fields were successfully cultivated with various crops. Cultivation and management strategies included the use of various mechanical, physical and chemical disturbances in combination with crop rotation and the absence of fallow periods. During a preliminary study in 2011, sampling was also conducted on a neighbouring farm's potato fields. At this farm pesticides were used more frequently. Three of the sampling points located at Vaaldam farm are within the natural veldt. These samples are control samples which monitor fluctuations in the mesofaunal numbers under natural conditions. These control areas are selected to be a representative of the surrounding Nama Karoo veldt. A crop rotation program was implemented at this locality and sampling was done in maize, cotton and potato fields (Fig. 3.3).

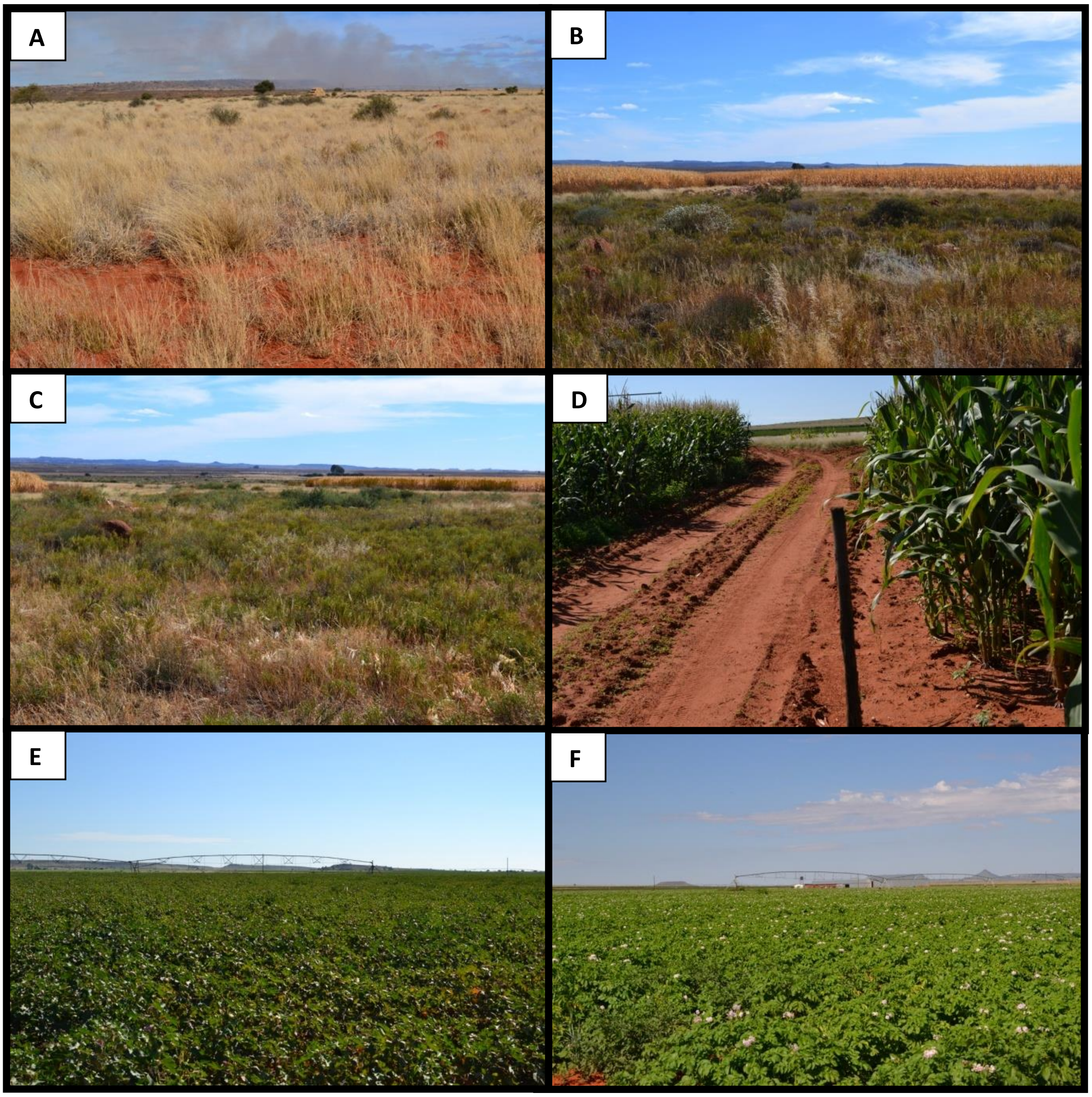


Fig. 3.3: Areas of sampling points 1-17 and 24-25. A-C: selected areas with a combination of grasses and dwarf-shrubs used for control samples; D: red sandy soil cultivated with maize; E: cotton field; F: Potato field.

Field B3 (Fig. 3.2), was overgrown with weeds in November 2012 and required additional herbicide application, as well as mechanical tools for eradication. Fields C2, C3 and E1 did not receive any biocides prior to planting and followed a similar program, with the exception of wet-ripping which was not applied at E1 (Fig. 3.4).

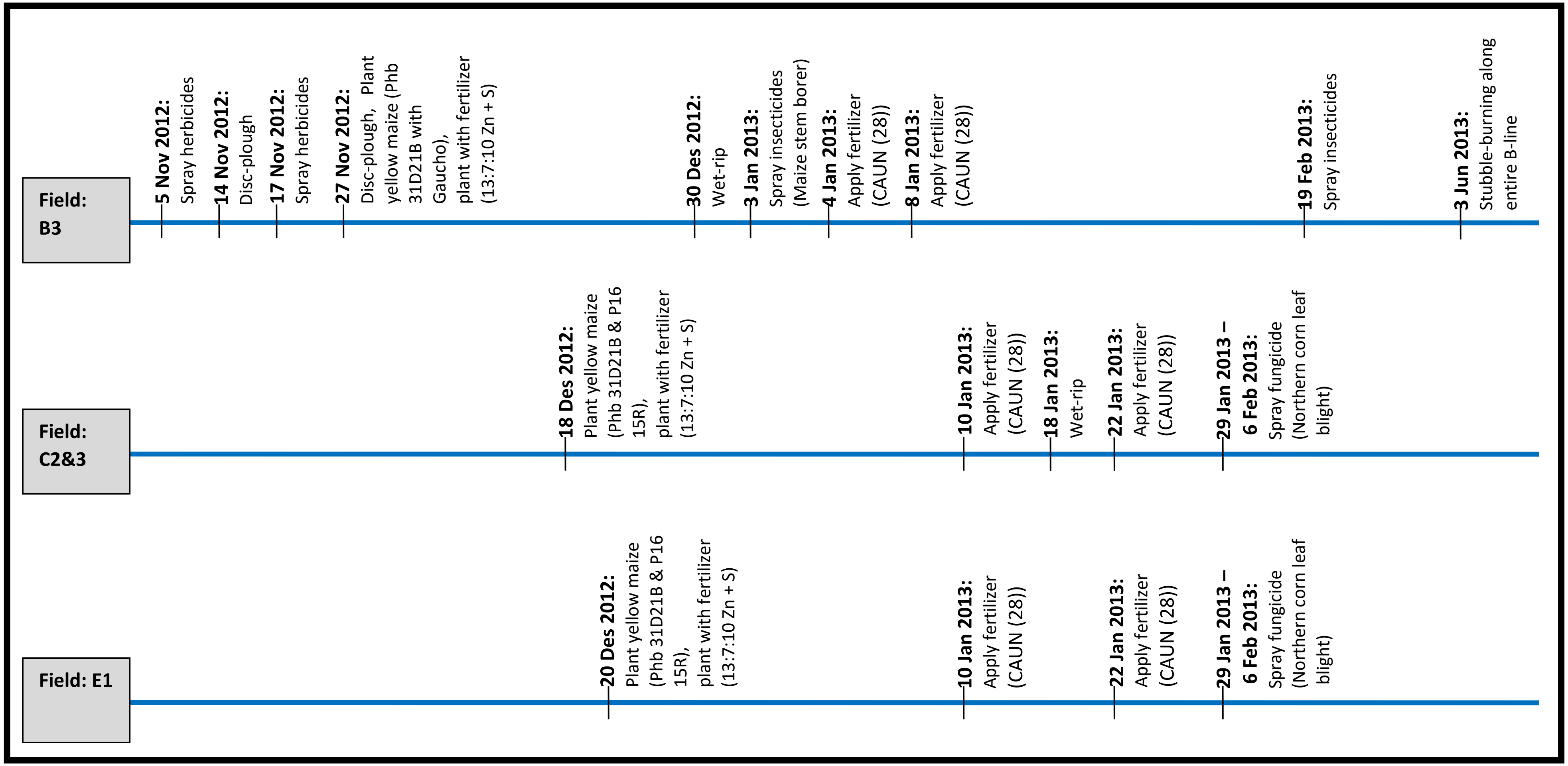


Fig. 3.4: Timeline portraying the different agricultural practices implemented over the course of the primary study which took place on the farm Vaaldam (November 2012 - March 2013).

Sampling was done before and after stubble-burning was applied. This process was influenced by stubble concentration and weed density. Fields with less weeds and dry, densely laid stubble burn more successfully than those in the presence of dense weed growth and sparsely distributed stubble. Stubble burn was hot but rapid and the centre pivot was on during the burning process to prevent damage to the apparatus (Fig. 3.5).

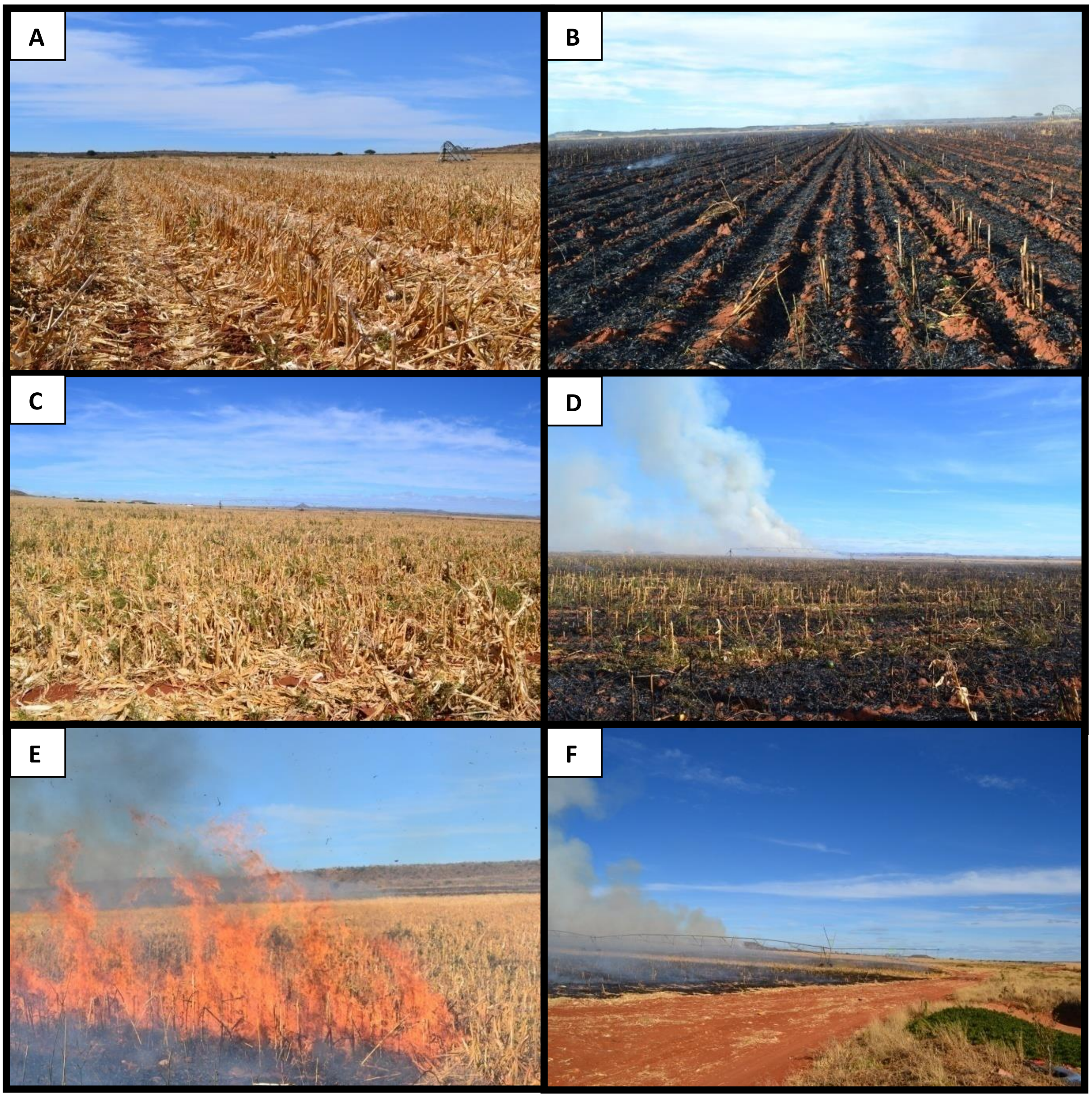


Fig. 3.5: Areas of sampling points 18-23. A & C: harvested maize fields, prepared for stubble burning; B & D: 90min after stubble-burning; E: stubble-burning; F: centre pivot spraying.

Soil analyses were done by various companies for the farmers, from which only certain values were selected (Table 3.1). Some of these chemical and physical properties of soils influence soil mesofauna occurrence, which could be direct (physical restrictions) or indirect (plant health influence resource availability), depending on the influencing factor (Neher & Barbercheck 1999). Soil analyses are not exact and the values should be within certain ranges. These ranges may differ between localities, as each locality has its own set of influencing factors which may suppress or enhance the effect of other factors. The pH values ranged between 4 and 7.2. The optimal pH range for nutrient absorption is between 5.5 and 6.8. Phosphor is essential for root development and P-values smaller than 15 would be considered as very low. Sulphur (S) enhances plant growth and a value between 8 and 12 is usually recommended. The sodium percentage (Na%) should not exceed 5% as this would affect the plants negatively. Magnesium percentages (Mg%) higher than 25-30% would be problematic at this locality, for this would suppress water infiltration and oxygen availability ([2] Mr N.J. van der Schyff, personal communication).

Table 3.1: Soil analyses at Vaaldam farm. The values were calculated by various external companies between 2009 and 2011. The values represent the mean average of an entire field and may vary slightly from the specific value of each sampling point.

Sampling point	Avg. Clay %	Colour*	pH	P	S	Na%	Mg%
4 & 20	-	D Ro-Br	5 – 6	15.7	5.65	1.3	26.5
5 & 21	-	D Ro-Br	5 – 6	9	9.98	1	25.1
6 & 7	11	D Ro-Br	4.5 – 6	16	5.92	1.35	25.6
8 & 9	11	D Ro-Br	4 – 5	26	5.75	1.4	24.3
10 & 11	11	D Ro-Br	5.5 – 6.5	45	6.09	1.3	23.7
12 & 13	11	D Ro-Br	5.5 – 6.5	5	4.93	1.4	26.7
14 & 15	12	D Ro-Br	5.5 – 6.5	19	14	1.3	26.8
16 & 17	12	D Ro-Br	5.5 – 6.5	19	14	1.3	26.8
18	-	D Ro-Br	5 – 6	14.5	5.57	1	24.3
19	-	D Ro-Br	6 – 7.2	2	9.44	1.1	29.2
22	-	D Ro-Br	5 – 6	22	6.86	1.45	27.7
23	-	D Ro-Br	5 – 6	6	9.6	1.65	26.6
24	15	D Ro-Br	5.5 – 6.5	19	23	1.2	21.5
25	12	D Ro-Br	5.5 – 6.5	19	14	1.3	26.8

* D Ro-Br = Dark reddish brown

Note that sampling point numbers are continues throughout the subsequent study sites.

[2] Mr N.J. van der Schyff, 2 Barnato str. Kimberley, pers.comm, October 2015

ii) Locality 2: Koppieskraal farm

Koppieskraal is situated in the Koffiefontein district, adjacent to the Riet River. Fields at this locality were also under centre-pivot irrigation systems. Even though these centre-pivot irrigation systems were only installed in 2004, these fields are much older than that and were previously cultivated by means of a flooding system. During the sampling period, the farmer applied various cultivation methods, excluding stubble-burning. The stubble was thus worked into the soil. The area surrounding the fields consisted of mostly grasses and dwarf-shrubs, although a thick strip of full-grown Eucalyptus trees are present on the one side of the field used in this study (Fig. 3.6). This field was cultivated with maize during the sampling period and has clay soil. Sampling point 33 is within the natural veldt next to an electric fence. A herbicide was applied at this sampling point in 2011 to clear the area underneath the fence.

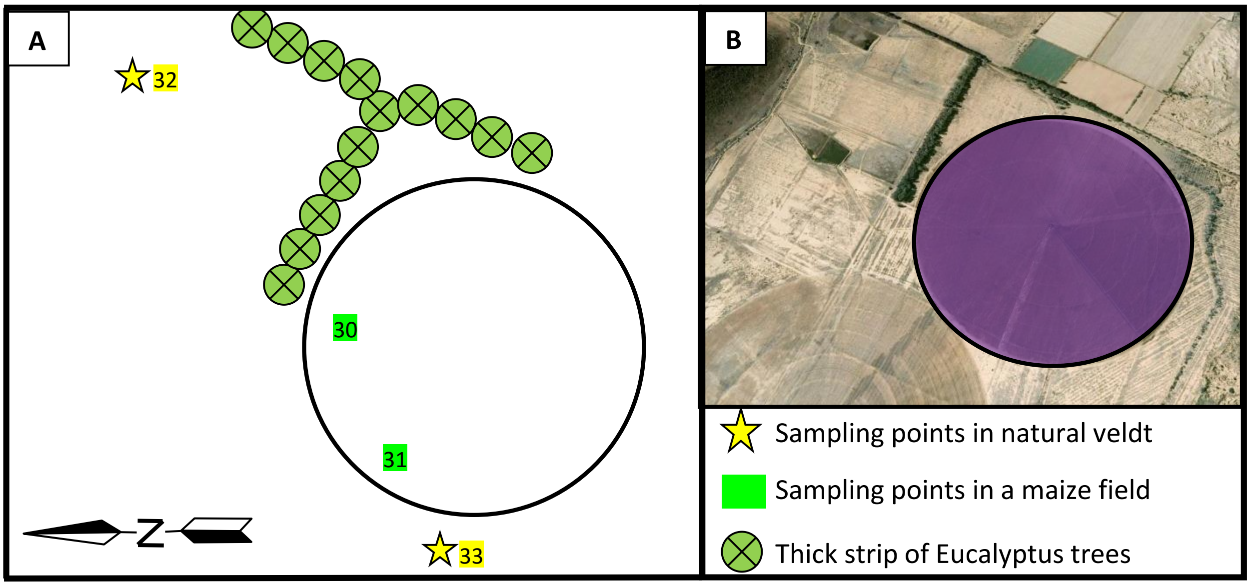


Fig. 3.6: Locality 2, near Koffiefontein. A: different sampling points in the field at Koppieskraal farm; B: selected field with its surrounding area containing trees, dwarf-shrubs and grasses. See Addendum B for species richness and abundance data.

After the maize was harvested, stubble was worked into the soil in preparation for the next planting season. This process can to a certain extent be very disruptive and creates large clods (Fig. 3.7 B). In fields with higher clay percentages, agricultural practices such as ploughing should only be performed under certain conditions to prevent the formation of large clods. These large clods are problematic for it requires a lot of water and intensive mechanical inputs to soften and even out a field that is in such a condition. These preparations are necessary to ensure a successful planting procedure ([3] Mr J.A. Badenhorst, personal communication).

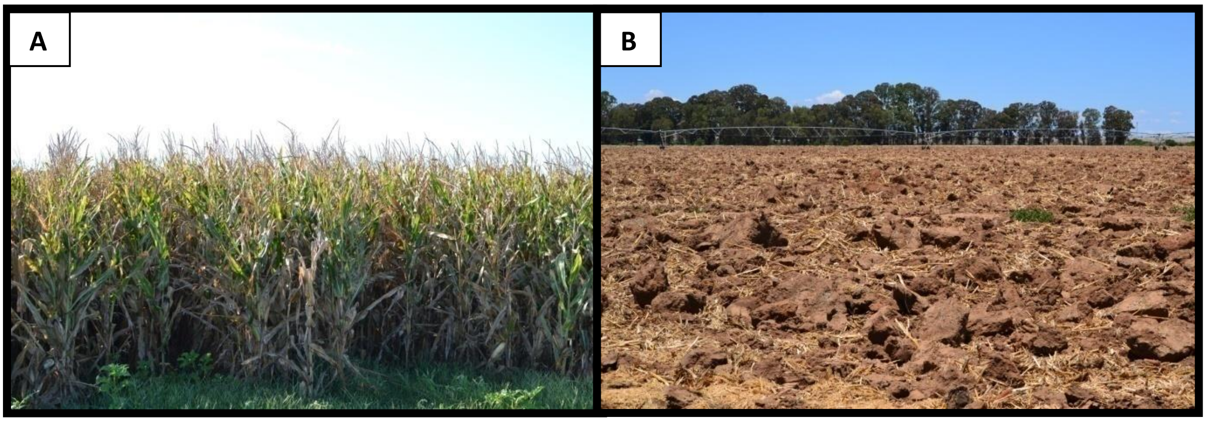


Fig. 3.7: Areas of sampling points 30-31. A: field cultivated with maize; B: stubble has been worked into the soil by means of ploughing.

In the soil mobility is important as it ensures survival from certain environmental factors (= vertical dispersal) and lower competition for food sources (= horizontal dispersal) (Krantz 2009). Within the soil, dispersal is mostly limited to walking/running, with the exception of organisms that occur in the water filaments and phoretic species. Climatic variation and soil humidity are two major influencing factors in vertical movement of soil fauna, since these organisms avoid extreme temperature changes and desiccation (Krantz 2009). Daily temperatures for this region over the course of this study varied between 7°C (winter months) and 38°C (summer months), with night temperatures reaching lows between -9°C (winter months) and 22°C (summer months) (Fig. 3.8). The soil's temperature is more stable and shows less variation as the depth increases (Table 3.2). Rainfall varied between 0mm and 43mm, with a mean average of 6.4mm over the sampling period (Fig. 3.8).

Table 3.2: Maximum and minimum temperatures recorded during the four days preceding each sampling date at sampling points 22-23. These values were calculated from data provided by Weather SA and the farmer who uses soil temperature probes.

	Above-ground		100mm below-ground		300mm below-ground	
Date	Max	Min	Max	Min	Max	Min
9 Jan 2013	36.7	14.7	31.5	18	26.5	22.5
18 Jan 2013	36.2	17.2	28	20.5	25	22
29 Jan 2013	36.3	13.1	31	20	26	22.5
6 Feb 2013	35.8	9.9	26	18.5	23	21.5

[3] Mr J.A. Badenhorst, 16 Parkdene, Kimberley, pers.comm, November 2015

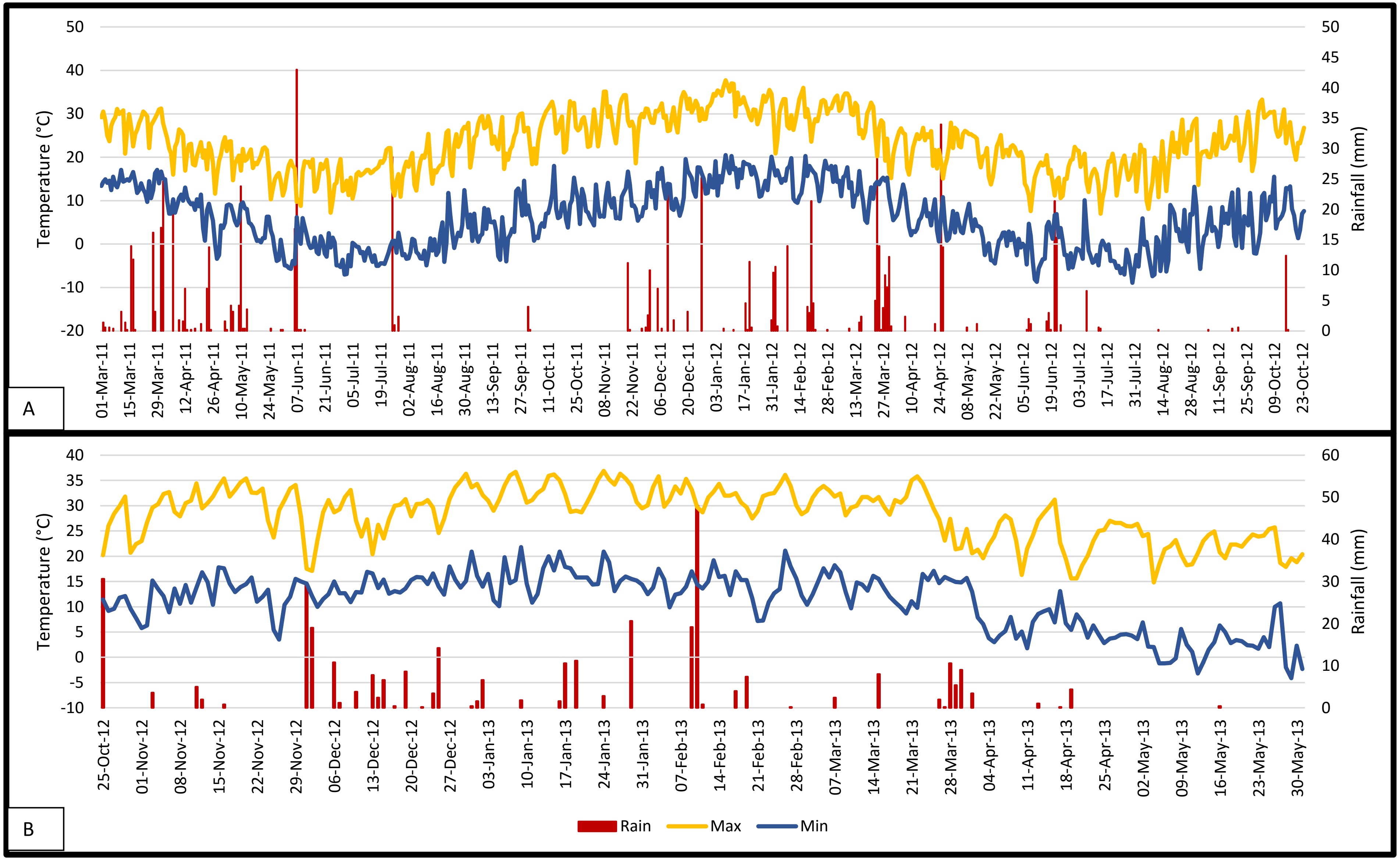


Fig. 3.8: Rainfall and temperature fluctuations during the study period, which were recorded for the Koffiefontein district by Weather SA. A: preliminary study, 2011 – 2012; B: primary study, 2012 – 2013.

iii) Locality 3: Thornberry farm

Thornberry is a privately owned farm, situated in the Jacobsdal district near Perdeberg, between Kimberley and Bloemfontein. The study sites of this locality are within the floodplains of the Modder River and variations in the physical properties of the soil occur between some sampling points due to the flow of the river (Fig. 3.9). Cultivation and management strategies of this farm include various mechanical disturbances and minimal chemical applications. This farmer rather enriches the soils with organic additives and products, supplied by companies such as Microbial Solutions (Pty) Ltd.

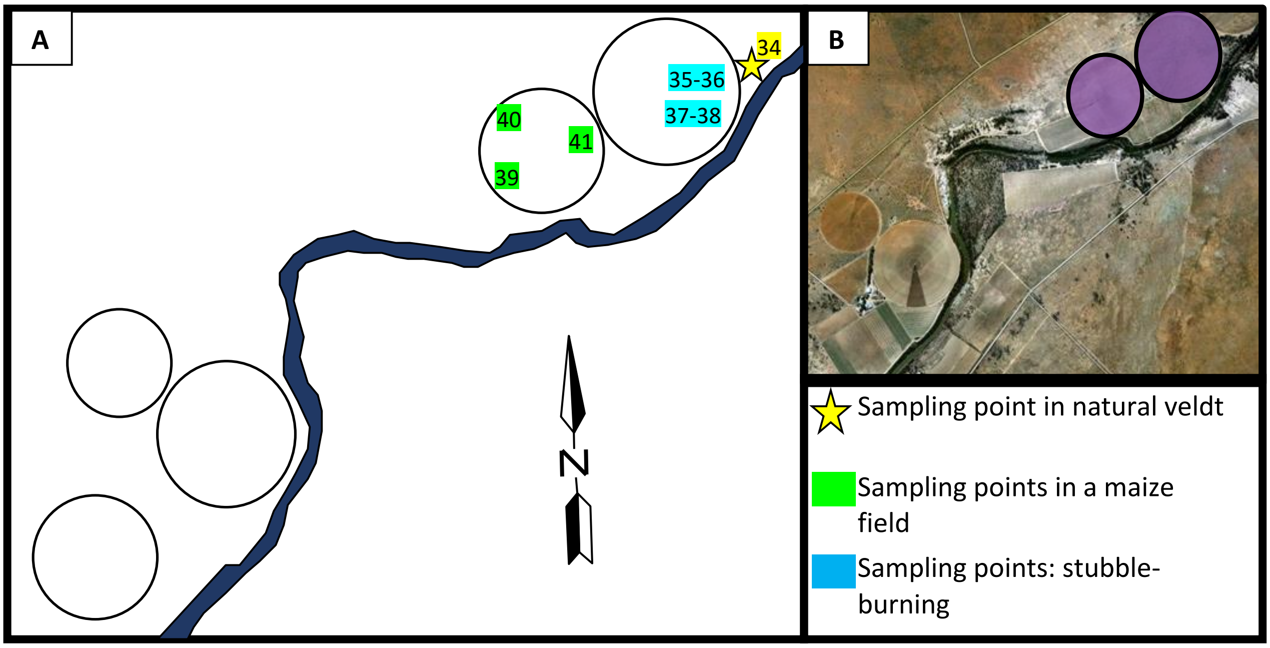


Fig. 3.9: Locality 3, near Perdeberg next to the Modder River. A: layout of the sampling points at Thornberry farm; B: the selected fields are indicated by the purple circles on a Google™ earth map. See Addendum B for species richness and abundance data.

Stubble-burning is not usually applied, but an exception on a section of one of the fields was made for this study (Fig. 3.10 D). Even though this farm still forms part of the Nama Karoo, it has a higher rainfall than the other two farms in the Koffiefontein district. The natural veldt therefore has less dwarf-shrubs and more grasses (Fig. 3.10 A). Although the rainfall is higher, cultivation is still only possible under irrigation systems (Fig. 3.10 B & C). Crop rotation is applied, with added fallow periods. During the fallow period, livestock roam the field and feed on the plant residues. The climatic conditions of this locality allows for the cultivation of various crop types such as pecan nuts, grapes, vegetables, cereals and seed.

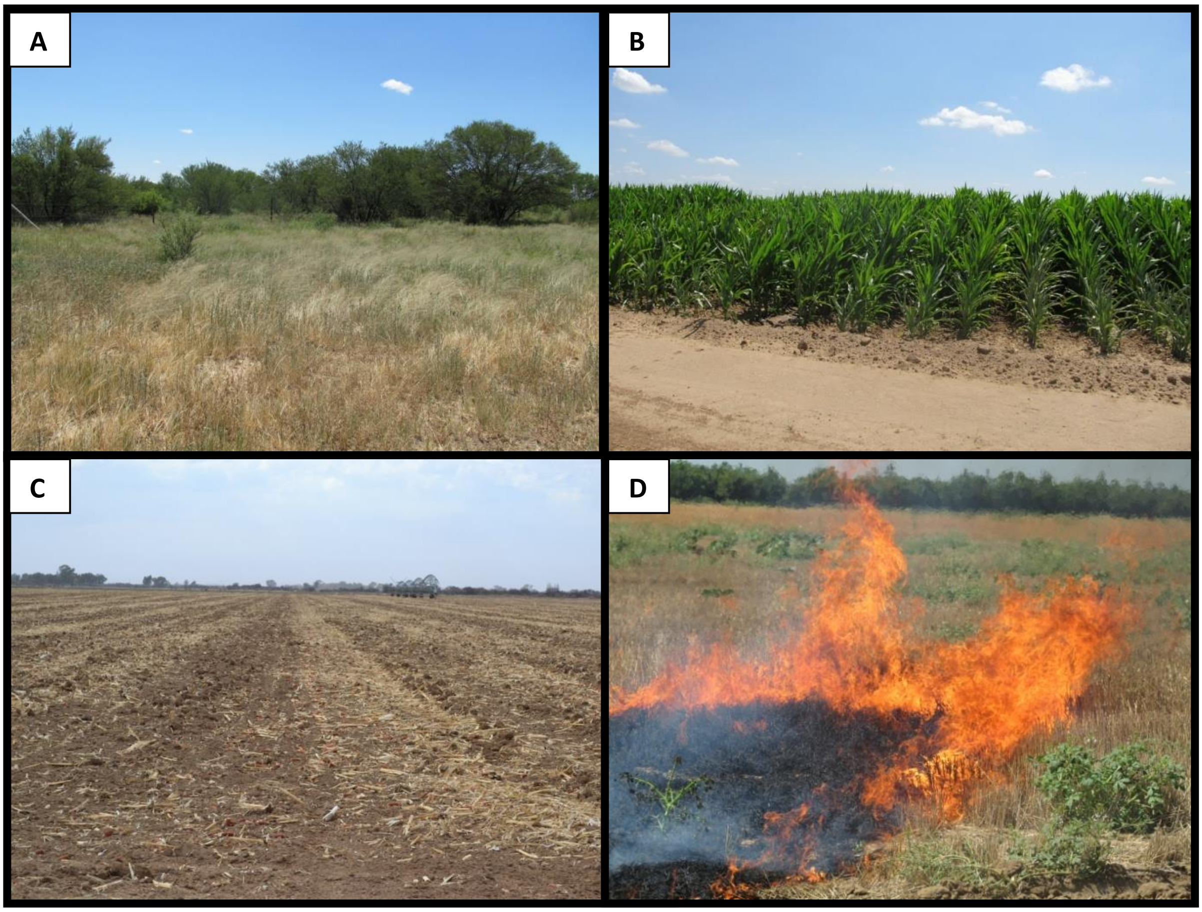


Fig. 3.10: Areas of sampling points 34-41. A: natural veldt as control sample; B: field cultivated with maize; C: fallow post-harvest maize field; D: stubble-burning application on wheat.

The pH values at this locality are within the optimal range (5.5-6.8). These soils contained sufficient P levels and high clay percentages. Sulphur values within the maize field are very high which collaborate with the green appearance of these plants, as sulphur deficiency symptoms include pale leaves and a purple colouration of the leaf stems. The Na% within the fields is high and at problematic levels, since it should preferably not exceed 5%. The Mg% is also very high, resulting in the 'tightening up' of soil (Table 3.3). The farmer applied a product to loosen the soil, but sampling was unfortunately discontinued prior to application. All of these factors are considered as indirect influences on mesofaunal diversity, as they influence plant growth.

Table 3.3: Soil analyses at Thornberry farm. These values were calculated in 2014 soils at each sampling point and some values may have varied slightly throughout the sampling period.

Sampling point	Avg. Clay %	Colour*	pH	P	S	Na%	Mg%
34	-	H Br	5.9	41	3	1	31
35	-	H Br	6.5	26	4	3	36
36	-	H Br	6.2	26	3	1	33
37 & 38	-	H Br	6.6	20	3	4	33
39 & 40	-	H Br	6.7	28	5	4	32
41	26	H Br	6.8	22	19	8	31
42	38	H Br	6.8	28	21	5	36
43	23	H Br	6.6	22	17	5	28

* H Br = Brown

Clay percentages for sampling points 39-41 were calculated at the University of the Free State. The principle of the bottle test was used to determine the clay percentage of each sample. The soil was mixed with water in a volumetric cylinder and after thorough mixing the cylinder was placed on a stable bench for the soil to settle (Fig. 3.11).

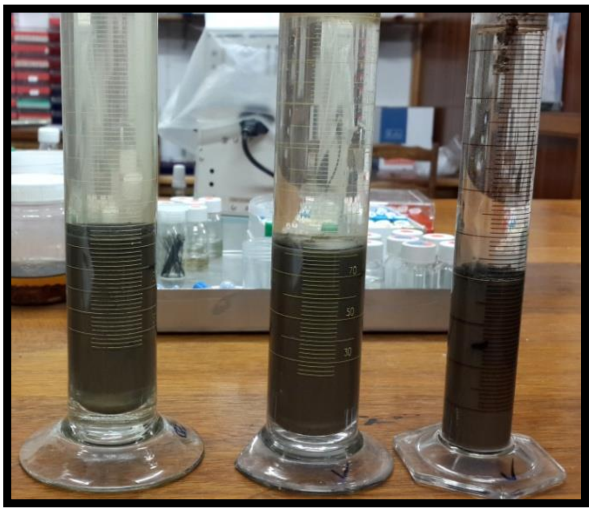


Fig. 3.11: Volumetric cylinders used in bottle test to determine clay concentration.

After 30 seconds the sand particles, which is heavier than the clay particles have settled and the volume of sand *vs* clay was noted per sample.

Once all the levels had formed and the water was clear, the volume of clay was measured. With these measurements the clay percentages were calculated by means of the following equation:

$$\frac{Volume\ of\ Clay}{Total\ Volume} \times 100 = Clay\ \%$$

During the study period, day time temperatures fluctuated between a minimum of 11.4°C in the winter months to a maximum temperature of 38.9°C in the summer. Night temperatures ranged between -6.2°C (minimum during winter) and 23°C (maximum during summer) (Fig. 3.12).

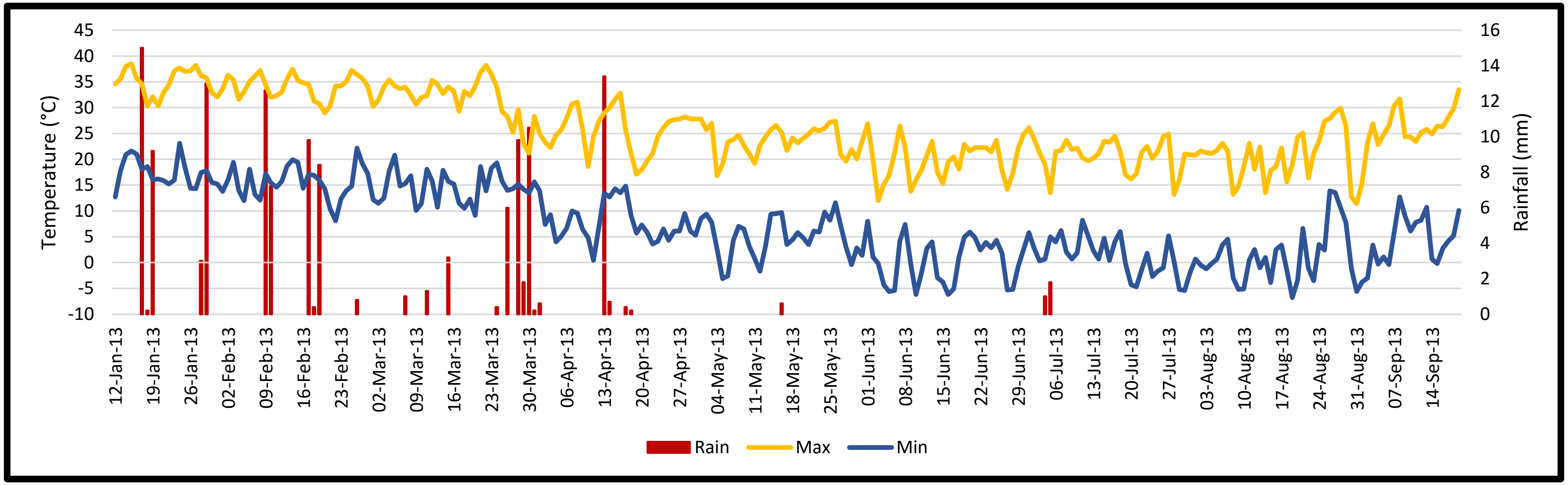


Fig. 3.12: Rainfall and temperature fluctuations during the study period, 2013, which was recorded for the Perdeberg area by Weather SA.

iv) <u>Locality 4:</u> Paradys farm

Paradys is the experimental farm of the University of the Free State and is situated just outside Bloemfontein on the N6 road to Reddersburg. This farm is in the Grassland Biome that has a higher rainfall than that of the Nama Karoo Biome. Its natural veldt therefore consists of grasses and although it has relatively the same annual rainfall as the farm near Perdeberg, the difference in seasonal temperatures is responsible for the dissimilarity in natural vegetation. The sampling site has red sandy soil and was used in a trial which compared the occurrence of selected faunal groups after the application of various biocides (Fig. 3.13). These biocides included an insecticide, three different herbicides and a fungicide.

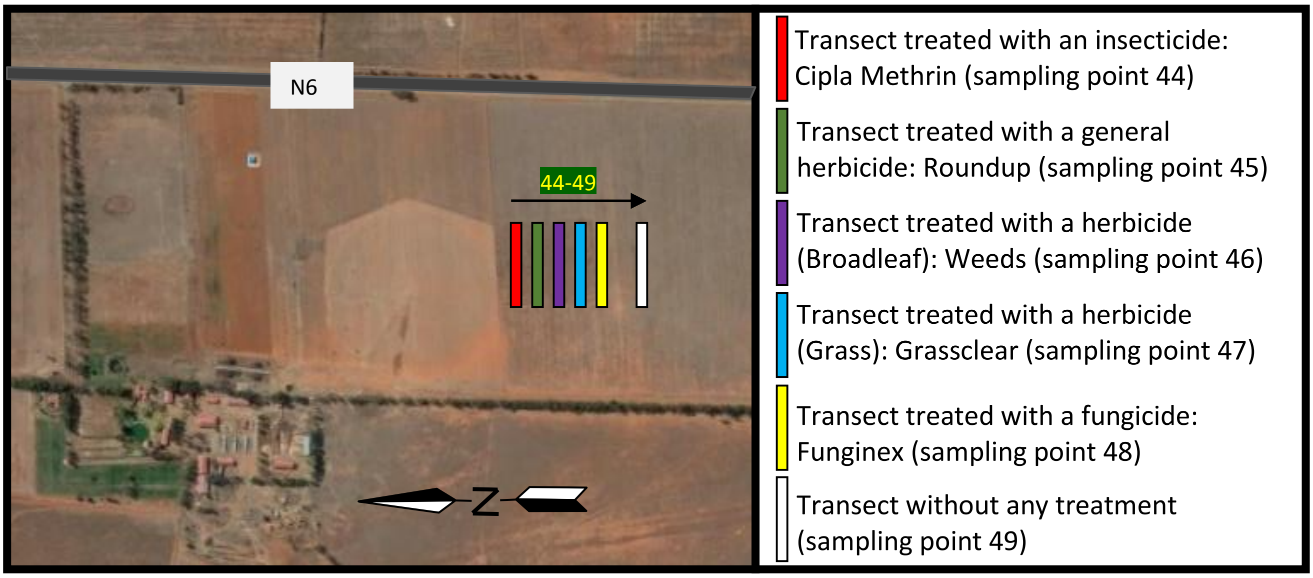


Fig. 3.13: Google™ maps layout of the sampling site at the Paradys experimental farm of the University of the Free State, which is divided into transects that each received different treatments. See Addendum B for species richness and abundance data.

The study was done over a period of five weeks, since the half-life of the selected biocides range between three and four weeks under normal circumstances. Each of these biocides has a different active ingredient and is used to eliminate or control a selected biotic group. Observations regarding some environmental factors are included in Figure 3.15 below. The biocides used in this study are commonly used and can be bought at any local garden centre, co-operative and even in some supermarkets. All of the biocides were applied with caution and all safety instructions were followed as indicated on the product label. Each biocide was made up according to the specific dosages indicated on its accompanying information sheet and applied on to the soil surface by means of a pressure sprayer. After each application, the pressure sprayer was washed with water to prevent contamination between the different transects.

Each of the application sites was clearly cornered off with four rods, which were each marked with coloured insulation tape (Fig. 3.14D).

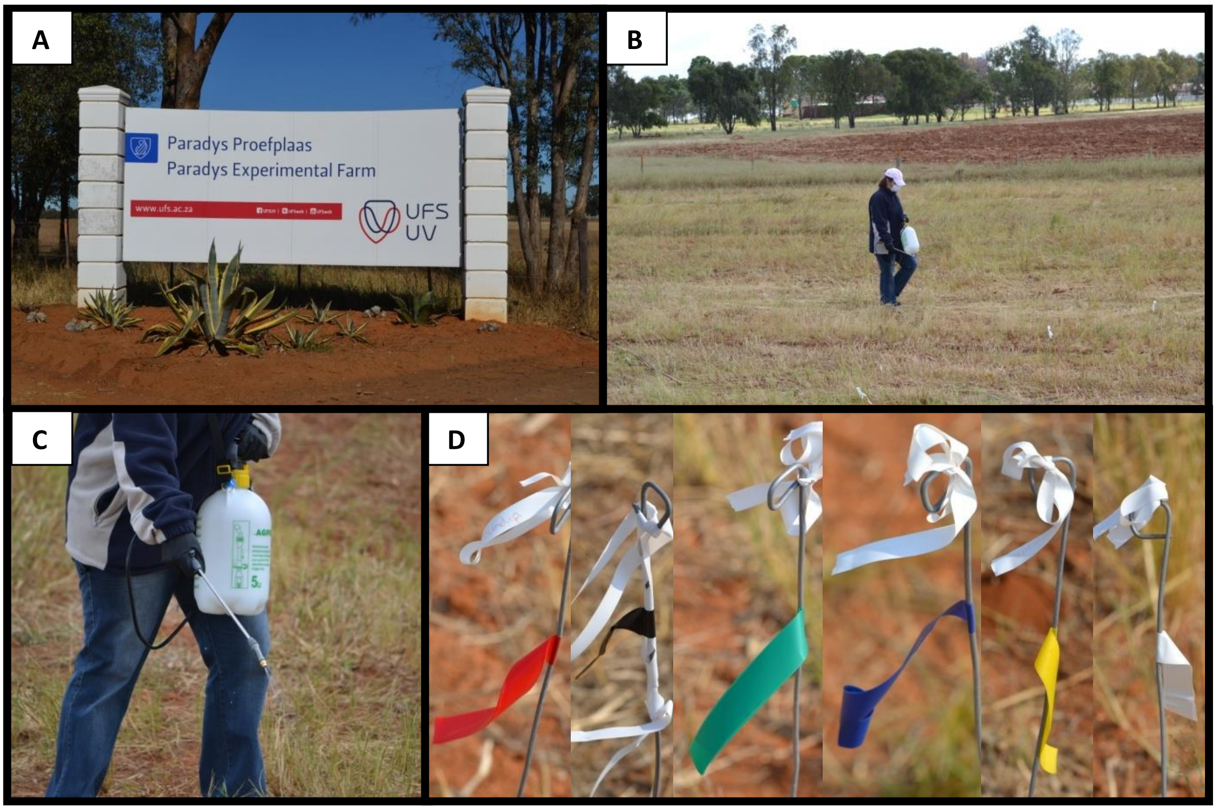


Fig. 3.14: Paradys, Experimental Farm of the University of the Free State. A: entrance to the farm; B-C: application of biocides in a grassland; D: multicoloured tagged rods used to mark the different areas of biocide application.

Sample number	44		45		46		47		48		49
15m	Insecticide		Herbicide (General)		Herbicide (Broadleaf)		Herbicide (Grass)		Fungicide		No Treatment
	2m	2m	2m	2m	2m	2m	2m	2m	2m	5m	2m

Sampling dates	Wind	Rain	Temp.	Average soil humidity (%)
Treatments: 16 April 2014	None	Cloudy	± 20	–
Sampling 1: 23 April 2014	Cold breeze	Previous night	± 15	9.8
Sampling 2: 30 April 2014	Gentle breeze	None	± 20	5
Sampling 3: 7 May 2014	Windy	None	± 20	3.6
Sampling 4: 14 May 2014	None	None	± 25	0.8
Sampling 5: 21 May 2014	Gentle breeze	Previous night	± 20	9.2

Active Ingredient:	
44:	Cypermethrin (Pyrethroid)
45:	Glyphosate (Glycine)
46:	MCPA (Phenoxy Compound)
47:	Haloxyfop-R Methyl Ester (pyridinyl-oxyphenoxy compund)
48:	Triforine (Triazole)

Fig. 3.15: Readings, layout and observations made on sampling dates at the six transects, each treated with a biocide containing a different active ingredient, sampled at Paradys Experimental Farm. The trial area was surrounded by natural grass veldt.

During this short study, daily temperatures varied between 12°C and 27.9°C. The nights were colder as this study was done during April and May 2013 with winter approaching. Night temperatures were measured from -1.5°C up to 13.3°C (Fig. 3.16). Soil humidity decreased over the course of the study as it did not rain often during the study period. External factors such as precipitation and temperature variations influence the soil environment, as well as the effectiveness and dispersal of biocides.

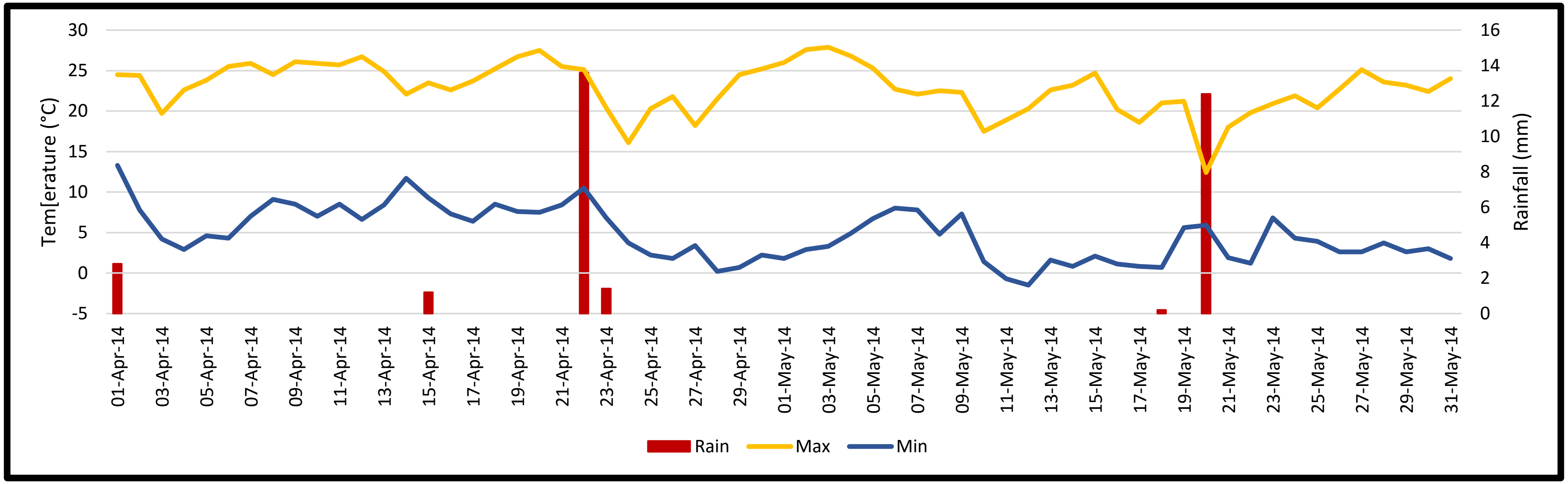


Fig. 3.16: Rainfall and temperature fluctuations during this study in 2014, which was recorded for the Paradys Experimental Farm of the University of the Free State by Weather SA.

v) <u>Locality 5:</u> Eureka farm

The farm Eureka is situated just outside of Odendaalsrus on the road to Wesselsbron. This area has many goldmines and the sampling sites at this farm are near one of the goldmine tailings dams (Fig. 3.17). A canal surrounds the dam to prevent the waste from spreading to nearby farms in the event of leakage (Fig. 3.18). This, however, has not been sufficient and this dam has spilled into the nearby veldt on two separate occasions. The first spillage occurred around the year 2000 and the second in 2006.

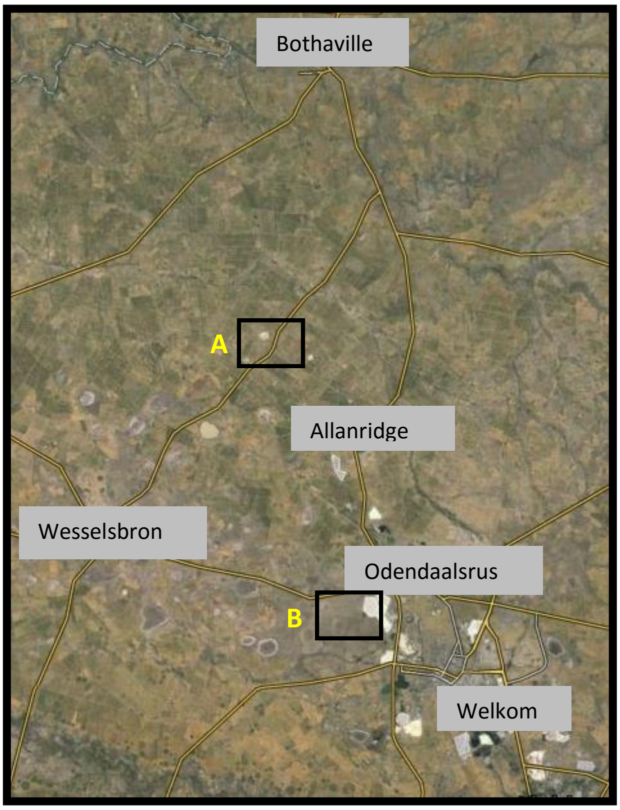


Fig. 3.17: North-West Free State. A: the farm Klein Brittanje; B: The farm Eureka .

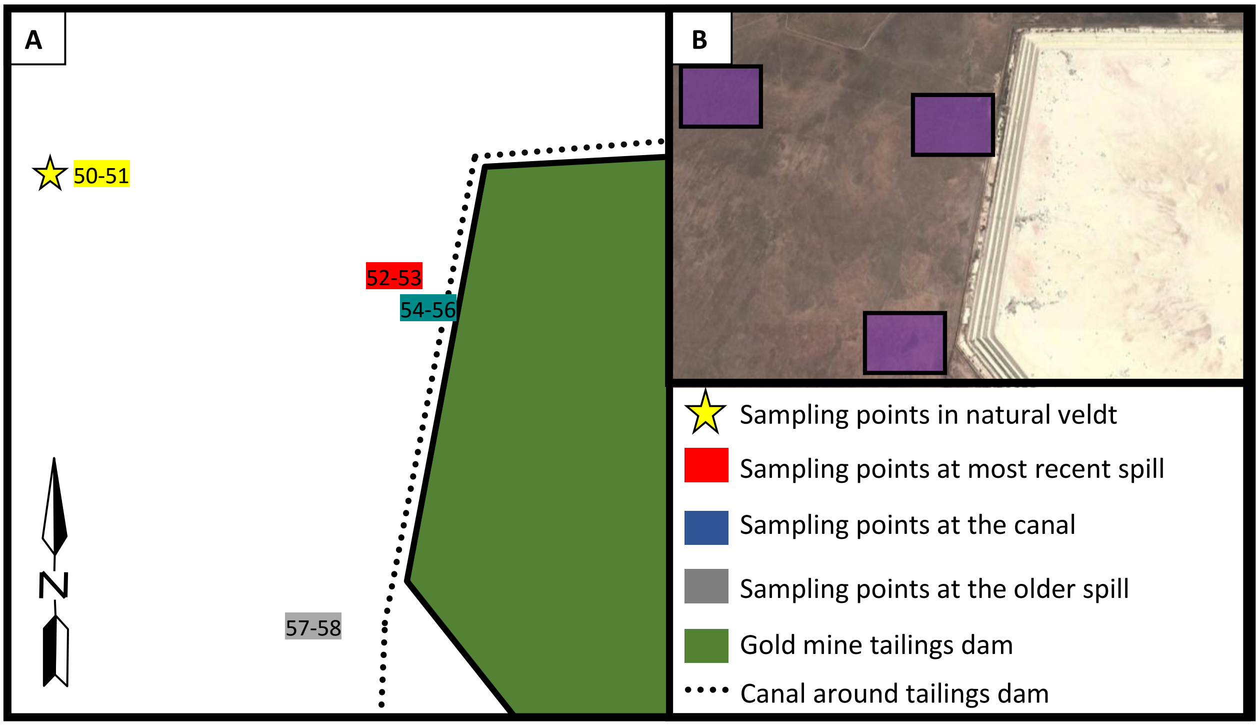


Fig. 3.18: Locality 5, situated next to a gold mine tailings dam. A: sampling points at the farm Eureka near Odendaalsrus; B: three main sites indicated in purple blocks on a Google™ earth map. See Addendum B for species richness and abundance data.

The natural veldt mostly consists of grasses. Sampling points 50 and 51 is in natural veldt with red sandy soil and these two sampling points are at a higher elevation than the rest of the sampling points. Over the course of this study, the condition of the veldt varied due to the changes in seasons and rain. Even in the presence of these natural changes, the detrimental influence of the tailings dam was still evident in the immediate vicinity (Fig. 3.19).

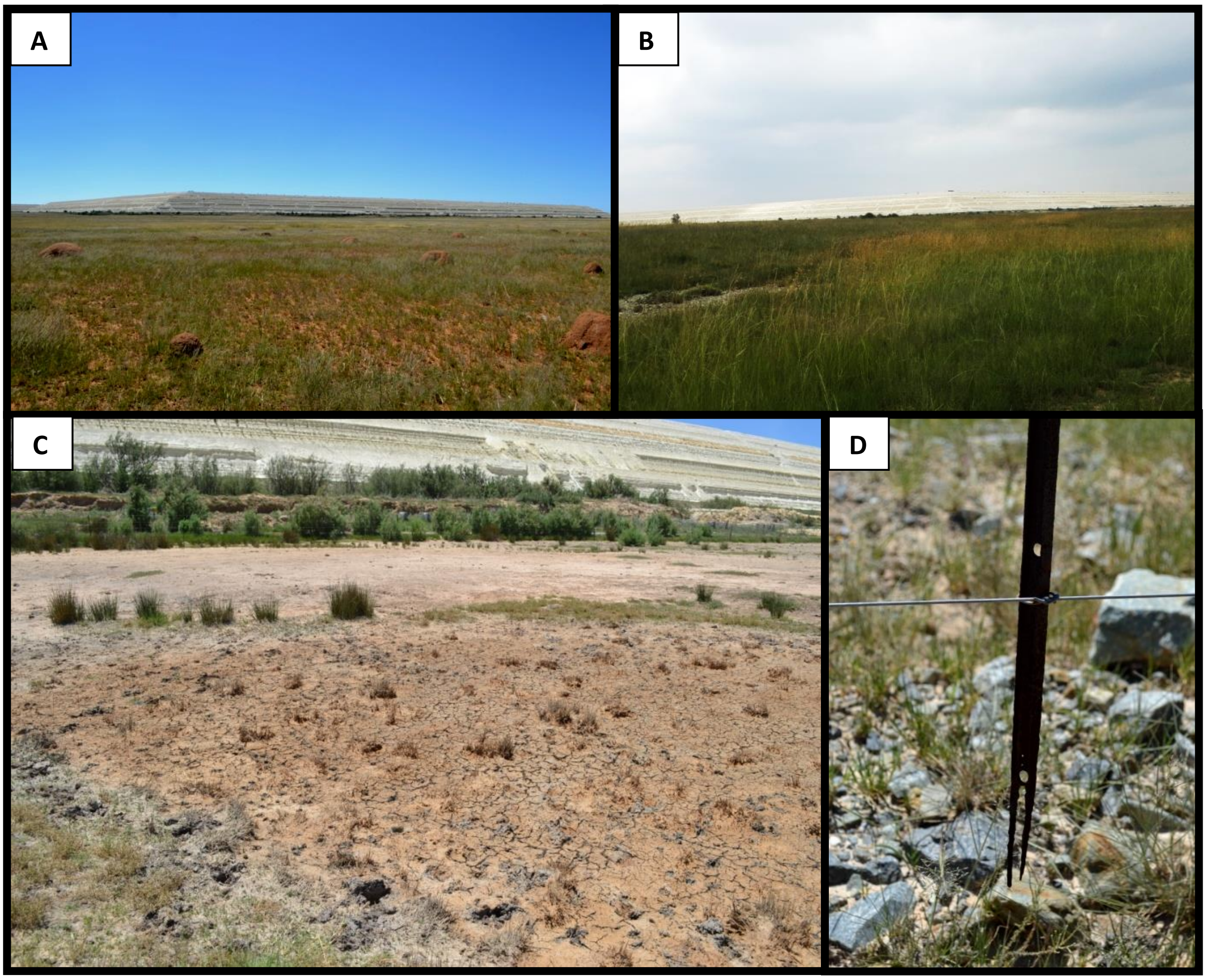


Fig. 3.19: Sampling points 50-53. A: natural veldt prior to the rainy season; B: natural veldt after the rainy season; C: loss of vegetation at leakage site; D: corrosive effect of tailings dam on iron rods used for fencing.

Sampling points 52 and 53 were in the area which was contaminated during 2006. Vegetation is sparse at this site and sampling was done at the two different plant types that were present namely a weed (sampling point 52) and bulrushes (sampling point 53). Sampling points 57 to 58 are situated in the area that was contaminated during a spill in 2000. Vegetative cover of this area is denser than that of the more recent spill. Sampling was done at bulrushes and grass (Fig. 3.20).

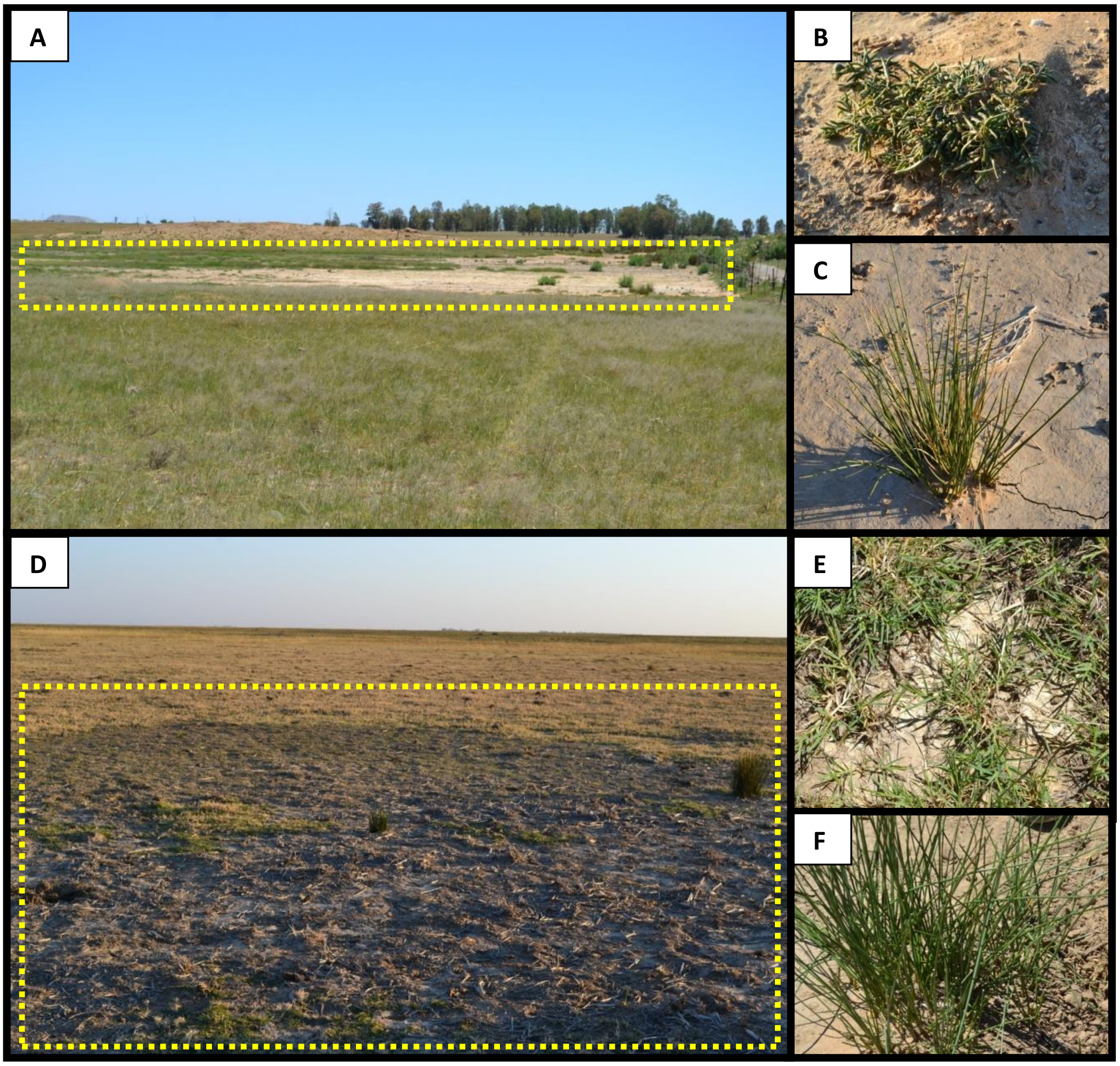


Fig. 3.20: Sampling points 52-53 & 57-58. A: area (in yellow block) contaminated in 2006; B: weeds growing in the contaminated area; C & F: bulrushes growing at both contaminated areas; D: area (in yellow block) contaminated in 2000; E: grass growing at the site that was contaminated during 2000.

Sampling points 54 to 56 are situated on the wall of the canal and just off the edge into the canal. Even though this area contains pollutants in both the water and soil, some vegetation types are still present. During the drier months the vegetation cover was sparse, but after the rain it increased in density and abundance. The vegetation at this site is represented by three plant types; reeds, bulrushes and grass (Fig. 3.21). At the back of the canal, a few Tamarisk trees were present, but no sampling was done here.

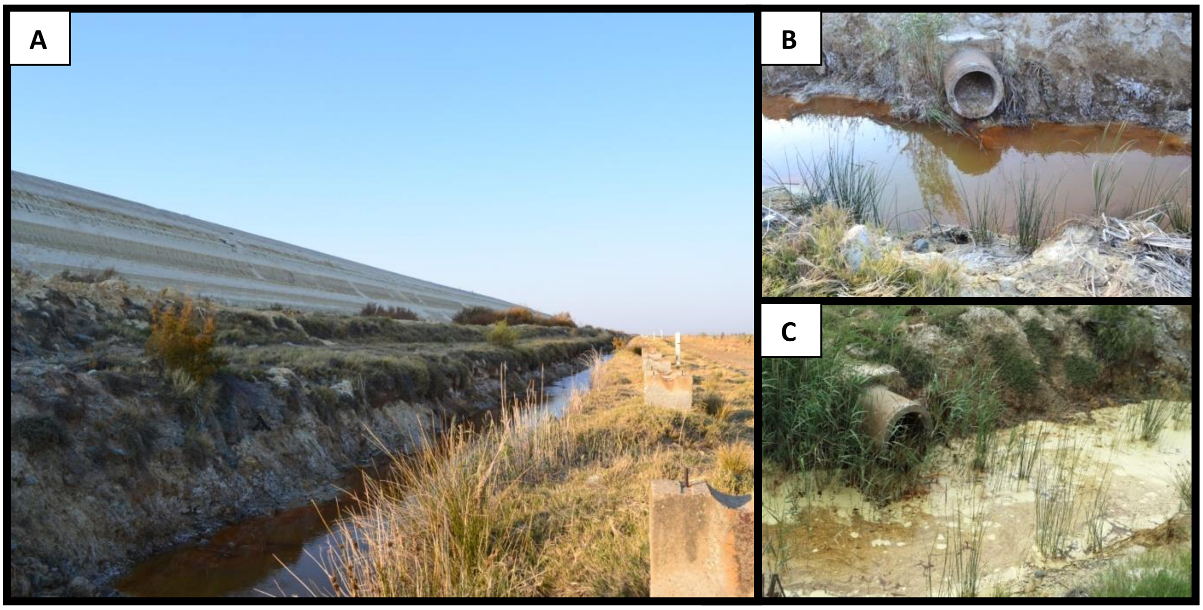


Fig. 3.21: Sampling points 54-56. A: the canal surrounding the gold mine tailings dam; B: water level and sparsely dispersed vegetation against the canal wall; C: definite increase in the vegetation cover during the wetter period.

Trace elements occur naturally in soils and although studies have been done on the occurrence of some these elements (see Hooda 2010 and Kabata-Pendias 2011), there is still data lacking of threshold levels and the influence of these elements in South African soils. This information can prove beneficial in the development of crop agriculture management strategies, since these elements can cause problems in the essential processes that provide our food sources and includes water systems and agricultural landscapes. Whilst such threshold levels are important in decision making, it is also essential to include other influencing factors such as pH, SOM levels, cation exchange capacity and clay percentages, since these factors influence the severity of the effects of some elements. The parent material of soils can be used as an indicator of expected levels, since the levels of certain trace elements seem to be related to specific rock types (Herselman 2007).

Many trace elements are essential to both fauna and flora in soils, even though some can have negative effects at high concentrations (Hooda 2010). These elevated concentrations are usually as a result of mining or agricultural pollution. Trace elements are not biodegradable and therefore accumulate in areas and their levels will only decrease as a result of displacement, *e.g.* leaching into ground water or spread by wind erosion (Herselman 2007).

Soil samples from the different sampling sites at the Eureka farm locality were analysed by the Geology Department of the University of the Free State, which provided information on elements in parts per million (Table 3.4). These elements have different effects on biota and were present at this locality due to mining activities nearby. The analysis revealed the presence of the following trace elements in the soils: Vanadium (V), Chromium (Cr), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Rubidium (RB), Strontium (Sr), Yttrium (Y), Zirconium (Zr), Niobium (Nb), Cadmium (Cd), Barium (Ba), Lead (Pb), Thorium (Th) and Uranium (U). Differences in ppm could be due to rain. Although Herselman (2007), mentions that some of these elements are essential for life, they can be harmful when higher concentrations accumulate in areas due to anthropogenic activities. According to Kabata-Pendias (2011), the presence of these elements are natural in soils. When the levels of these elements are higher than that of the natural occurrence levels, the soil would be considered as contaminated. If these levels reach the point where the health of biota and the environment is deteriorating, usually in the presence of anthropogenic activities, the soil is regarded as polluted. Threshold levels are determined according to the levels where the detrimental effects on biota and their functions are identified.

Over time these elements can, as mentioned, only be displaced by environmental factors such as wind removal and water leaching. This is evident in the table below, which shows the calculated values of the above mentioned elements on two dates. The total maximum threshold levels of selected elements in South African soils, as suggested by Herselman (2007), are: Cd = 3 mg/kg^{-1}, Cr = 350 mg/kg^{-1}, Ni = 150 mg/kg^{-1}, Pb = 100 mg/kg^{-1}, Zn = 200 mg/kg^{-1}, Cu = 120 mg/kg^{-1}. The recommended levels in agricultural soils are much lower, with a maximum permissible trace elements as follows; Cd = 2 mg/kg^{-1}, Cr = 80 mg/kg^{-1}, Ni = 50 mg/kg^{-1}, Pb = 56 mg/kg^{-1}, Zn = 185 mg/kg^{-1}, Cu = 100 mg/kg^{-1}, Co = 20 mg/kg^{-1} (see Herselman 2007).

Table 3.4: Chemical analysis (in parts per million) of soil collected from the different sampling points on the farm Eureka on two dates.

Sampling sites	Sampling Points	Sampling dates	Vanadium (V)	Chromium (Cr)	Cobalt (Co)	Nickel (Ni)	Copper (Cu)	Zinc (Zn)	Arsenic (As)	Rubidium (Rb)	Strontium (Sr)	Yttrium (Y)	Zirconium (Zr)	Niobium (Nb)	Cadmium (Cd)	Barium (Ba)	Lead (Pb)	Thorium (Th)	Uranium (U)
Older spill (2000)	**58**	25-Jun-13	43	57	11	28	7	36	5	41	31	12	286	3	<4	284	10	3	36
		03-Mar-14	56	142	19	47	23	74	22	55	40	15	269	4	4	344	29	8	72
	59	25-Jun-13	45	56	10	26	16	35	6	40	36	12	300	3	<4	281	9	<2	23
		03-Mar-14	44	61	16	29	9	54	7	40	34	13	290	3	5	288	13	4	43
Most recent spill (2006)	**52**	25-Jun-13	57	55	11	24	17	42	8	72	164	24	233	5	<4	621	14	5	<2
		03-Mar-14	40	85	10	21	17	35	9	38	146	12	289	3	<4	289	13	4	9
	53	25-Jun-13	70	90	16	42	9	62	12	83	83	20	205	5	6	448	19	6	9
		03-Mar-14	61	83	17	34	20	55	10	61	77	16	248	4	5	368	20	5	20
Control in natural veldt	**50**	25-Jun-13	39	47	3	9	17	18	9	19	15	5	315	2	<4	190	7	<2	<2
		03-Mar-14	39	47	5	11	9	15	10	27	17	8	379	3	<4	223	6	<2	<2
	51	25-Jun-13	49	45	7	14	18	21	4	20	28	8	308	2	<4	239	6	<2	<2
		03-Mar-14	41	48	4	12	17	16	7	24	17	7	329	2	<4	230	6	4	<2
On side of canal	**56**	25-Jun-13	51	384	10	36	16	40	109	16	47	11	204	<1	4	149	74	11	45
		03-Mar-14	40	312	15	35	14	41	58	12	46	10	225	<1	6	128	49	6	46
	55	25-Jun-13	38	293	21	43	12	55	57	11	39	10	228	<1	<4	115	45	6	49
		03-Mar-14	28	252	13	26	12	26	38	7	37	9	250	<1	<4	88	21	5	21
Inside canal	**54**	25-Jun-13	38	129	11	22	7	42	23	28	44	10	264	2	<4	225	23	5	54
		03-Mar-14	41	167	14	27	8	45	33	24	46	10	233	2	<4	207	23	3	57
SARM42 (measured)*			91	4233	29	127	23	39	<4	20	35	10	174	3	<4	265	10	2	<2
SARM42 (certificate)			92	4310	35	125	17	44	0	22	37	11	192	[8]	0	[250]	[10]	[5]	0

*SARM 42 – Certificate of analysis used as a control in soil analyses, establishing the accuracy of the measured data.

Day temperatures within the Odendaalsrus area fluctuated between 37°C (in summer) down to a mere 11.6°C (in winter) during the sampling period. The maximum night temperature during summer nights could reach 19.7°C, whilst during winter months temperatures dropped to a minimum of -4.8°C at the end of August 2013. No rain was recorded from 1 June to 19 October 2013. The rainy season began thereafter (Fig. 3.22). From 20 October 2013 to 26 March 2014, a total of 456.2mm of rain was recorded for this region. The rain water did not run off, but supplemented to the soil moisture level and the water table.

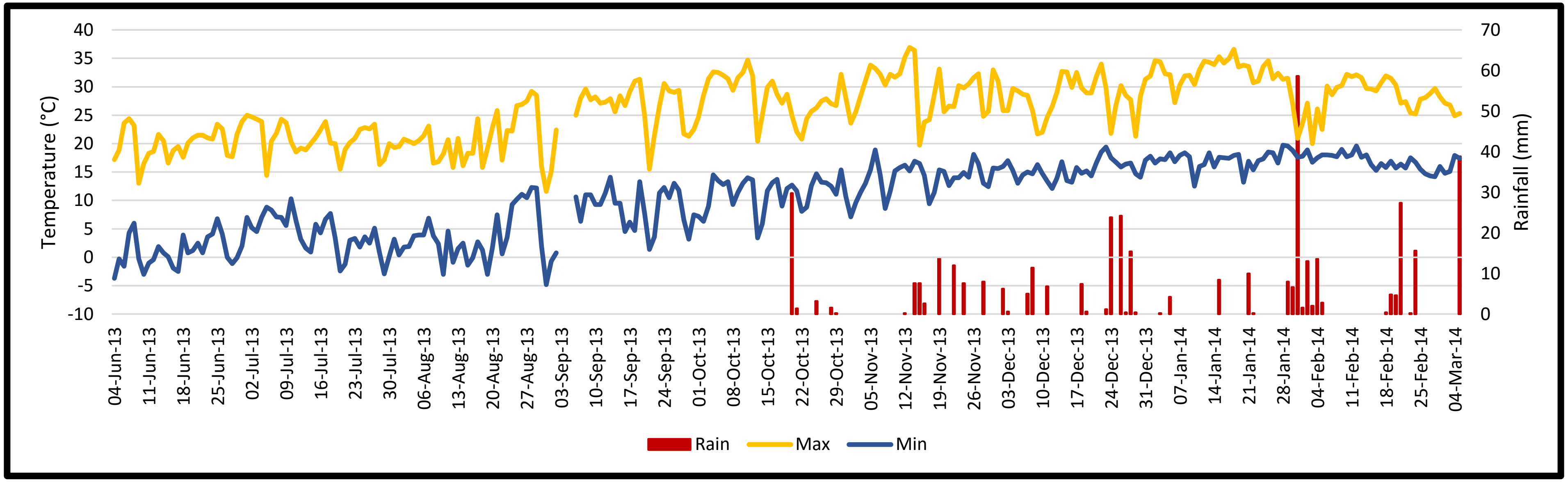


Fig. 2.22: Rainfall and temperature fluctuations for the Odendaalsrus district during the study period. (Recorded by Weather SA).

vi) Locality 6: Klein Brittanje farm

Klein Brittanje farm is divided into two halves by a railroad and the R719 road between Bothaville and Wesselsbron (Fig. 3.17). The fields at this farm have brown sandy soil and are cultivated with maize. These maize fields are spaced around a large pan, which is situated in the centre of the larger half of the farm (Fig. 3.23). Cultivation and management strategies included various mechanical and chemical practices. Stubble-burning has never been applied at this location and stubble is routinely worked into the soil.

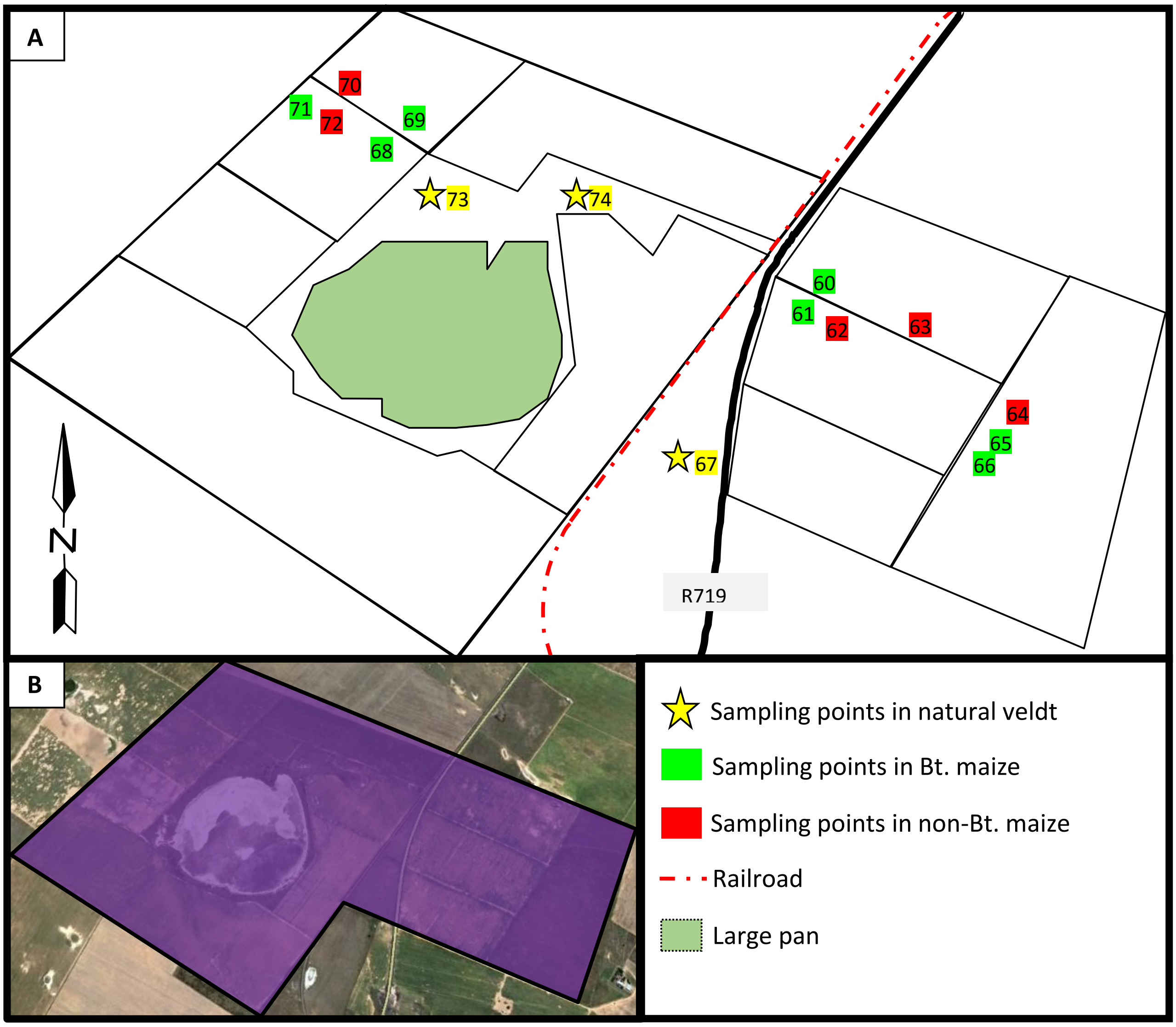


Fig. 3.23: Locality 6 at Klein Brittanje farm, which is situated between Wesselsbron and Bothaville. A: sampling points scattered throughout the different fields; B: layout of the fields surrounding a very large pan on a Google™ earth map. See Addendum B for species richness and abundance data.

Albeit that the previous farmer kept cattle on these fields during the winter months, management practices changed after the 2010-2011 planting season and no livestock was kept here during the sampling period of this study. A fallow-lay system was developed to rest selected fields and fields are only cultivated once every two years. This locality is in the Grassland Biome and the annual rainfall is high enough to support cultivation without irrigation during the rainy season (Fig. 3.24).

Sampling points 67, 73 and 74 are located in the natural veldt. Here specifically these sampling points were used as control areas to evaluate the fluctuations in mesofaunal assemblages relative to environmental changes. Some of the changes being that these fields form part of a fallow-lay system with sampling points 60, 63 and 68-70 fallow during the 2012-2013 planting season. No cover crops have ever been planted at this locality and soil improvements are mainly done by means of agricultural practices. The rest of the fields were cultivated with white maize.

Cultivation practices incorporated with maize production in this area include the application of lime, ammonia gas, herbicides and pesticides along with mechanical disturbances by means of a cultivation appliances. Some of these practices, however, can be excluded if the condition of the soil is good and the particular application would therefore be deemed unnecessary and costly. Sampling at this locality was conducted from 9 January 2012 to 12 May 2012 as part of a preliminary study and was continued from 4 February 2013 until 21 August 2013 as the main study period.

Sampling points 64 to 66 were in a field that was converted from natural veldt in 2011. This field therefore received a more intensive management approach during the preliminary study to control weeds and the re-growth of grasses. During the 2011-2012 planting season, Sumi-Alpha (a pyrethroid) and Kalash (a glyphosate) was used on all fields at sampling points 64 to 66 on 30 November 2011 and at sampling points 60 to 63 on 3 November 2011. Maize was planted from 25 to 30 November 2011 and harvested from the end of May 2012 up until June 2012. Thereafter these fields lay fallow until the next planting season. During the 2012-2013 planting season all active fields were planted from 29 to 30 November 2012.

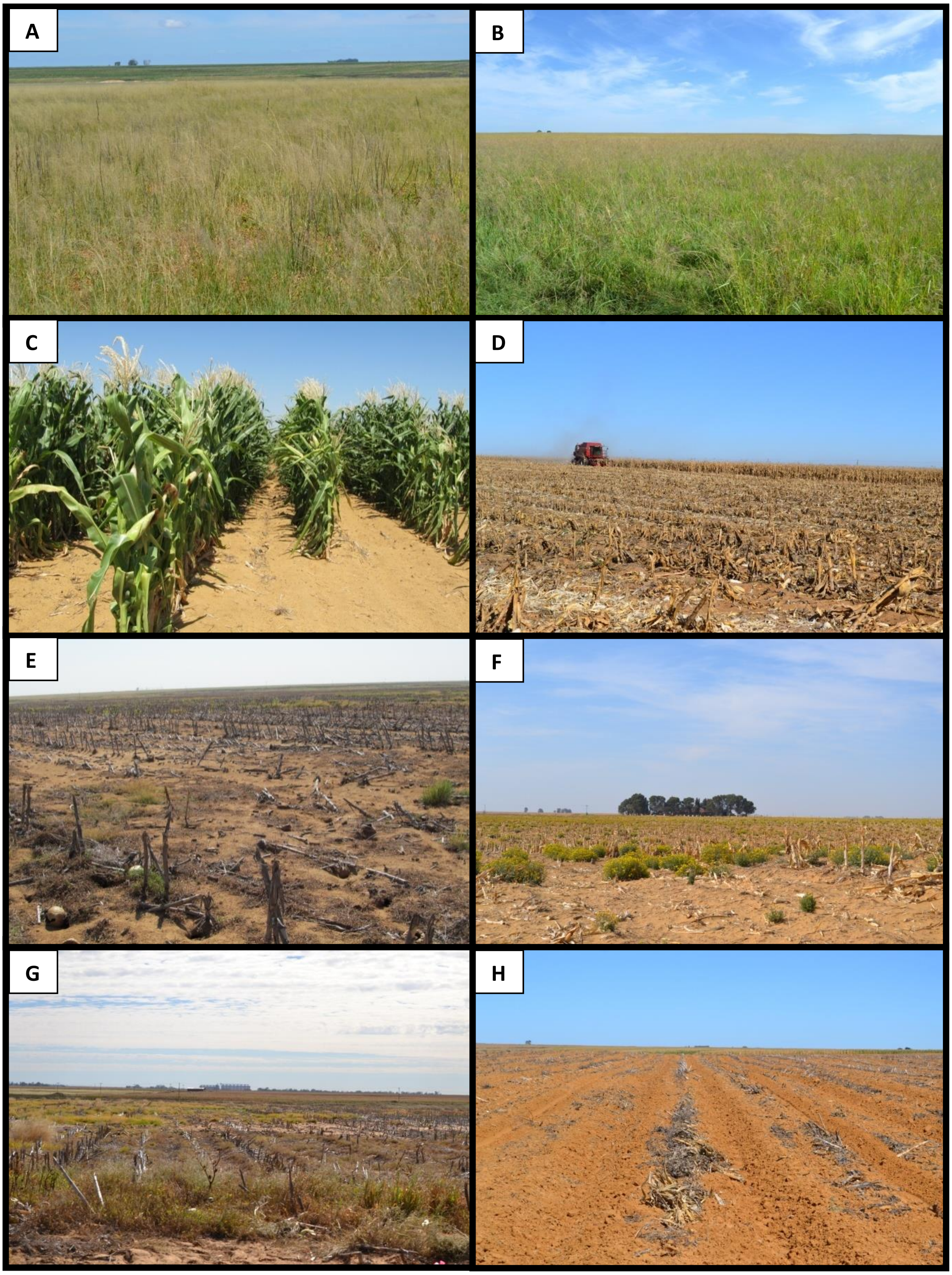


Fig. 3.24: Areas of sampling points 60-74 at Klein Brittanje farm. A-B: natural veldt used as control areas, which mostly consists of grasses; C: fully grown maize plants; D: harvesting process; E-G: post-harvest, fallow fields; H: field that was mechanically loosened by means of a ripper to enhance water penetration.

Karate Zeon (a pyrethroid), Callisto (a mesotrione) and Primagram (a mixture of S-metolachlor & atrazine) were sprayed in planted fields between 4 and 5 December 2012. The second round of chemical applications included Touchdown (a glyphosate) and Camix (a mesotrione) and was applied between 11 and 14 January 2013. Maize was harvested in June and sampling continued up until 21 August 2013. Rainfall at this location was lower than that stated for this area in the literature.

All the soil forms are Avalon, except for sampling points 64 and 74 which are Westleigh and sampling point 66 which is Longlands ([4] Mr G.F.R Nel, personal communication). Most of the sampling points have a low pH value, indicating that the soil is relatively acidic. The P values within the fields are all at a desired level, whereas the value at sampling point 67 in the natural veldt is very low. Some of the values could not be obtained, as the sampling points within the natural veldt was only partially analysed. The S levels are sufficient for most of the sampling points, but are very low at sampling points 68, 71 and 72 which are all located within the same field. A Na% that is higher than 5 is deemed problematic. This locality, therefore, has several sampling points that are situated in areas that have a high Na%. Mg% is also high throughout the sampling points, but is still within the acceptable range (see summary in Table 3.5). Plant water availability differed between the different sampling points as a result of the variable soil depth of these areas.

During the summer months, daily temperatures were over 30°C with the highest at 36.9°C and during winter temperatures were much lower with the lowest daily temperature measured at 6.4°C. Maximum night temperatures peaked at 20°C during the summer, whilst the minimum during the winter months was -6.6°C. This area is part of a summer rainfall region and received 507.6mm during 2012, which is just within the expected range for this region (Fig. 3.25).

[4] Mr G.F.R. Nel, Golden Fleece, Bothaville, pers.comm, November 2015

Table 3.5: Soil analyses of Klein Brittanje farm. These values were calculated between 2011 and 2014.

Sampling point	Average clay %	Colour*	pH	P	S	Na%	Mg%	Plant available water (mm)
60	10 - 15	H Br	5	34	33	6	27	220 - 240
61	10 - 15	H Br	4.6	35	22	2	26	180 - 200
62	10 - 15	H Br	4.6	35	22	2	26	180 - 200
63	5 - 10	H Br	5	24	33	6	27	80 - 100
64	5 - 10	Gl Br	4.3	29	44	2	22	80 - 100
65	10 - 15	Gl Br	4.4	29	44	2	22	80 - 100
66	10 - 15	Gl Br	4.3	29	44	2	22	80 - 100
67	16 - 20	H Br	4.9 - 5	< 10	-	-	-	-
68	10 - 15	Gl Br	4-4.5	>35	0-3	3-5	15-20	160 - 180
69	10 - 15	Gl Br	5-5.5	>35	7-11	3-5	25-30	160 - 180
70	10 - 15	Gl Br	5.5-6.5	>35	>15	3-5	25-30	180 - 200
71	10 - 15	Gl Br	4-4.5	>35	0-3	0-3	20-25	160 - 180
72	10 - 15	Gl Br	4-4.5	>35	0-3	0-3	20-25	160 - 180
73	10 - 15	H Br	4-4.5	-	-	-	-	180 - 200
74	15 - 20	H Br	4-4.5	-	-	-	-	220 - 240

* H Br = Brown   * Gl Br = Yellow Brown

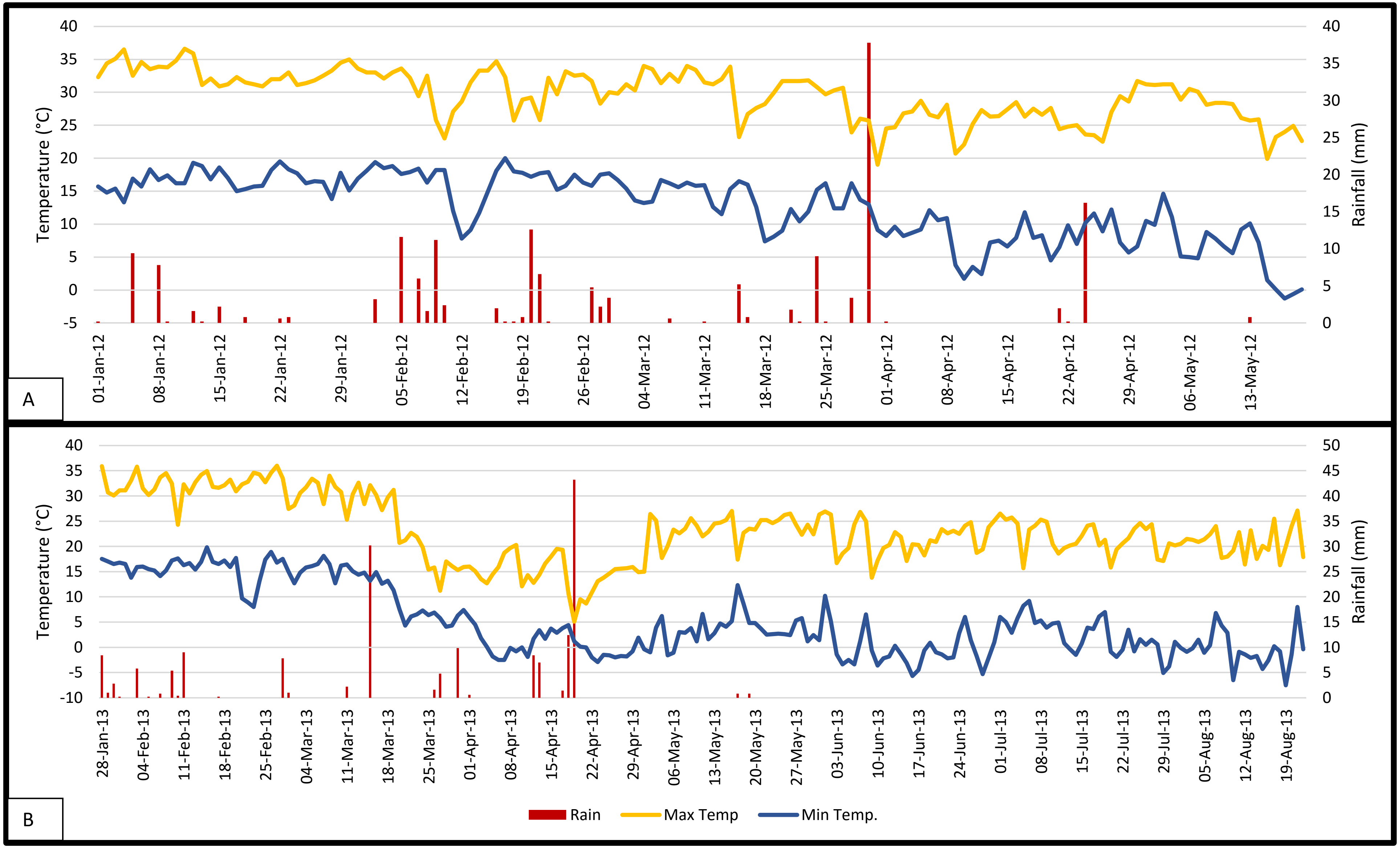


Fig. 3.25: Rainfall and temperature fluctuations during the study period at Klein Brittanje farm, which was recorded for the Bothaville district by Weather SA. A: preliminary study, 2011 – 2012; B: primary study, 2012 – 2013.

vii) Agricultural disturbances

As part of cultivation programs farmers have to apply certain disruptive practices. Most of these disturbances form part of soil preparation and protection, plant protection or are applied to enhance plant growth (Table 3.6). All of these applications are carefully planned with the help of agricultural professionals, not only to ensure sustainable farming, but rather a combination of sustainability and cost efficiency.

Table 3.6: Mechanical, physical and chemical disturbances applied during the production of various crops throughout the sampling sites ([2] J.A. Badenhorst, [1] E. Badenhorst & [4] G.F.R. Nel, personal communication).

Disturbance	**Equipment / Application**	**Effect of application**
Disc-ploughing (Penetration ±20 cm)	 G.F.R. Nel	Reduces the size of plant residue and mixes it into the top soil
Wet-rip (Penetration ±45cm)		Enhances root penetration and growth by reducing density of soil
Hoeing	 G.F.R. Nel	Removes weeds that grow between maize rows
Rip – Fallow fields (Penetration ±1.m)	 E. Badenhorst	Enhances water infiltration into the soil, thereby building up water supply for next crop

[1] Mrs E Badenhorst, 16 Parkdene, Kimberley, pers.comm, October 2015
[3] Mr J.A. Badenhorst, 16 Parkdene, Kimberley, pers.comm, November 2015
[4] Mr G.F.R. Nel, Golden Fleece, Bothaville, pers.comm, November 2015

Table 3.6 continued: Mechanical, physical and chemical disturbances applied during the production of various crops throughout the sampling sites

Stubble-burning		Reduces plant residue and manages certain pests and diseases
Maize harvesting		Can lead to stumping. Reduced traffic in fields will lessen soil densification and rather use track drive tractors to reduce soil compaction
Potato harvesting	 E. Badenhorst	Disrupts top soil to expose potatoes and indirectly adds plant residue in topsoil, can lead to stumping by tractors and trailers
Rod-weeder	 G.F.R. Nel	Reduces weed growth, prepares seed bed
Fertilizer – Gas	 G.F.R. Nel	Enhances plant growth

Table 3.6 continued: Mechanical, physical and chemical disturbances applied during the production of various crops throughout the sampling sites

Fertilizer – Organic		Enhances plant growth
Fertilizer – Inorganic (usually applied with planting)	 G.F.R. Nel	Enhances plant growth
Herbicides	 G.F.R. Nel	Reduces weeds and re-growth of previous crops
Insecticides	 G.F.R. Nel	Manages pest populations
Lime application	 G.F.R. Nel	Balances pH and prevents chemical compaction

b) References

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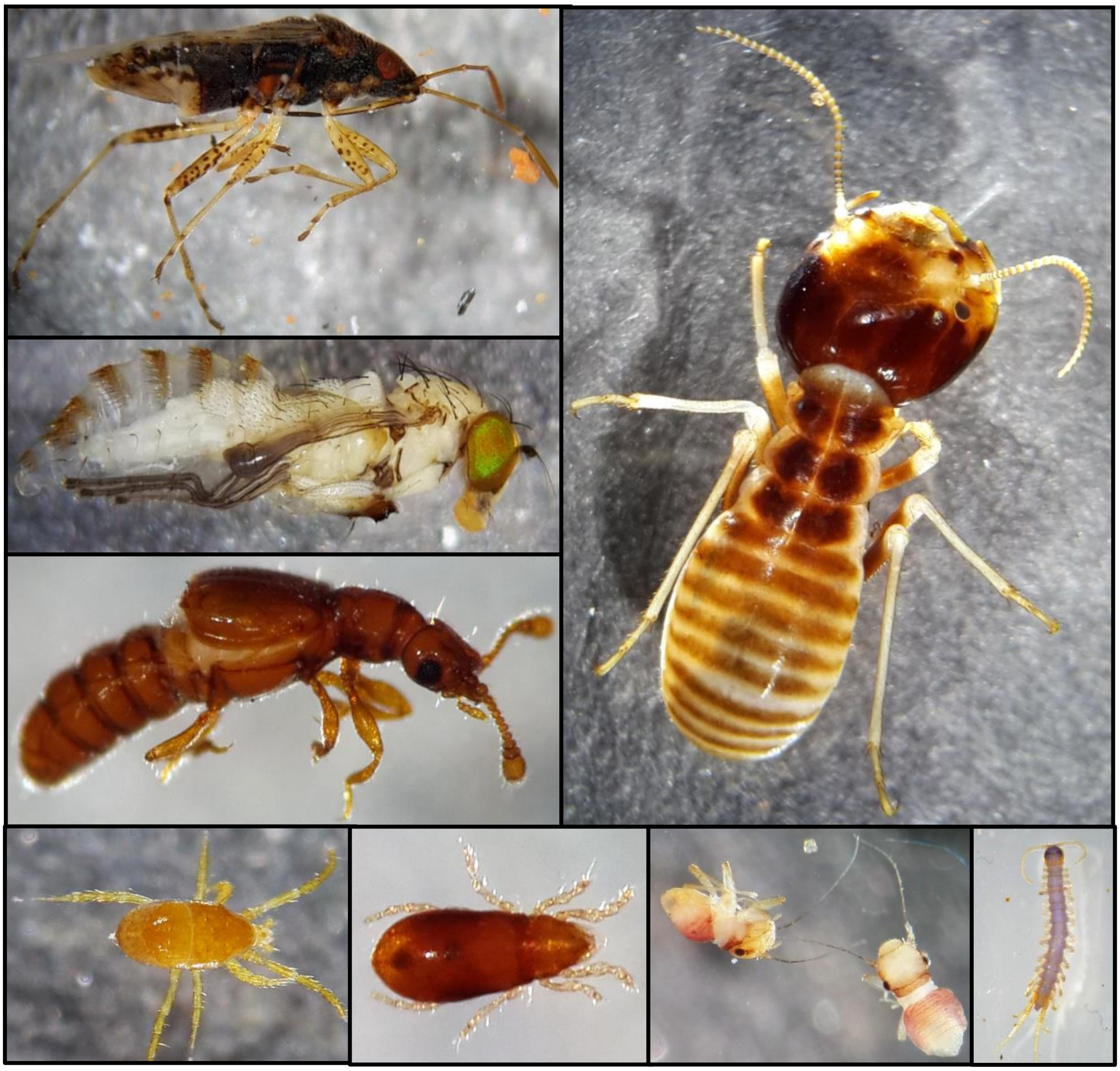
Chapter 4



SOIL MESOFAUNA RECORDED IN DIFFERENT AGRICULTURAL LANDSCAPES

Soil is a multidimensional medium, providing stability and diverse habitats to biota. These organisms have undergone certain adaptations to inhabit and benefit from this habitat. As such soil biota have become an essential part of soils and mediate many of its processes (Neher & Barbercheck 1999). Soils with a relatively constant plant cover tend to sustain a more diverse biotic community, where plants, both as primary producers and soil cover, provide nutrition and protect these organisms from extreme changes in temperature and desiccation. In return, soil fauna enrich the circumstances of the plant by improving soil aeration, water infiltration and nutrient availability (Chakraborty *et al.* 2012, Wall 2012).

Soil fauna are very diverse and represented in all trophic levels by a wide range of taxa. Variations in the life strategies of these taxa, in combination with fluctuating environmental conditions and disturbance intensity, are responsible for the seemingly inconsistency in faunal responses to different agricultural practices (Kladivko 2001). Benckiser (1997) supports the fact that agricultural practices are not solely responsible for changes in mesofaunal diversity, since their vertical distribution largely depends on climatic conditions, even though species distribution is definitely influenced by agricultural practices (Villani & Wright 1990).

Agroecosystems are largely maintained by anthropogenic driven factors such as mechanical disturbances (tillage), soil additives (fertilizer & biocides) and the addition/removal of plant material (Neher & Barbercheck 1999, Wardle *et al.* 1999). These processes can limit the functionality of soil biota in that they disrupt food webs and prolong the recovery period due to the frequency of these applications (Benckiser 1997).

Recently farmers and scientists have been working towards incorporating soil fauna into their management strategies since they are regarded as beneficial role players during production (Wardle *et al.* 1999, Roger-Estrade *et al.* 2010). Many farmers are therefore moving from conventional farming systems to more conservation focused programs such as reduced tillage and even no-tillage systems. The latter systems are argued to therefore require the ecological input of soil taxa to regulate soil function processes (Benckiser 1997, Kladivko 2001).

The complexity of soil food web structures are influenced by many factors which are important during the assessment of the implications of agricultural practices and environmental influences on soil fauna. This approach was deemed more effective than relying only on species richness, since many species can occupy the same trophic level or functional group and could therefore collectively merely provide a limited range of services (Beare *et al.* 1995). All graphs provided in this study analyse food web structures in the soil therefore indicate the fluctuations in trophic groups as a proportion of the diversity of each sample. It is important to note that the scale of the y-axis of the different graphs differ. The study sites were fully described in Chapter 3.

a) Environmental influences

The occurrence and abundance of soil mesofauna is greatly influenced by the physical component of the soil since associated organisms mostly depend on existing pore spaces in the soil medium (Coleman *et al.* 2004). Other factors that influence the abundance of these organisms include soil moisture and to a lesser degree soil temperature and pH (Benckiser 1997, A'Bear *et al.* 2013).

i) Soil organismal diversity in the porosphere of different plants

Soil faunal diversity is higher in the porosphere of plants than in the rest of the soil. This could be ascribed to the increase in both food sources and soil humidity in this region as a result of root exudates and fungal growth. Furthermore, crop rotation is common practice in many agricultural systems and, over and above the porosphere as such, different crops have different effects on soil mesofaunal diversity (Benckiser 1997).

- Cotton (Preliminary study)

Sampling points 18 and 19 are located within a cotton field at the farm Vaaldam (Fig. 3.2). The preparation of these fields only involved disc-ploughing and the wheat stubble was therefore incorporated into the soil. According to Benckiser (1997), systems that make use of wheat, as part of crop rotation programs, have a higher number of collembolans present. This fact is supported at this site where more than 80% of the individuals from these samples were Collembola, which, in turn, were the majority of mycophagous species sampled. Over the course of the sampling period, collembolan numbers increased at a more rapid rate than that of the other groups present. This led to a decrease in both richness and evenness at both the Bt. and non-Bt. cotton plant sites (Fig. 4.1).

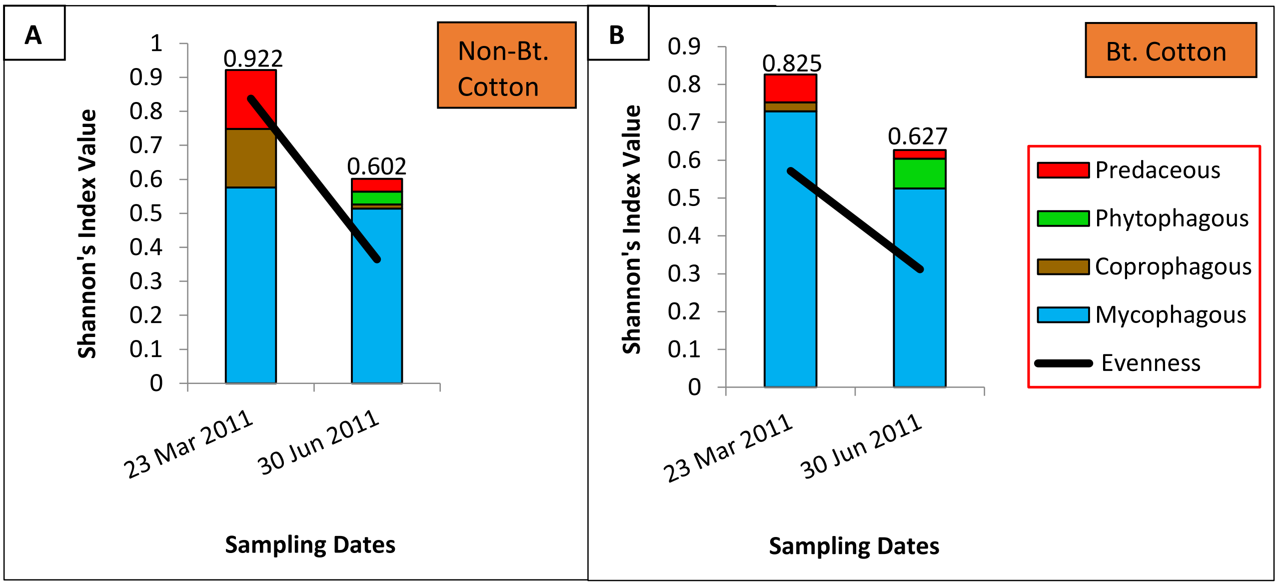


Fig 4.1: The represented trophic levels incorporated as a proportion of the soil faunal diversity (value indicated on top of each bar) within a cotton field on the farm Vaaldam in 2011. A: sampling point 18 with non-Bt cotton; B: sampling point 19 with Bt. cotton.

- Potatoes (Preliminary study)

Sampling was conducted in various fields at the farm Vaaldam, cultivated with potatoes (Fig. 3.2). Potatoes are tuber crops and therefore have a different root system than maize and cotton. According to Benckiser (1997) Collembola numbers in fields that are cultivated with tuber crops are less than those cultivated with cereal crops. This is true for these samples, since collembolans were present in lower numbers when compared to those sampled within the cotton and natural veldt. Sampling points 24-25 (Fig. 4.2 B, A) were located in fields that received minimal pesticide application. The diversity at sampling points 28-29 (Fig. 4.2 E, F) was higher than that of 26-27 (Fig. 4.2 C, D), since the latter was planted later and the plants were thus smaller, disturbance in the fields more recent and resilience not complete.

Even though these samples showed much variation between one another and no meaningful trends within trophic levels were present, it was clear that the higher pesticide usage at sampling points 26-29 (Fig. 4.2 C-F) had a definite negative effect on the diversity in these fields (Fig. 4.2), which is supported by Pimentel & Edwards (1982). The diversity values of 0 observed within the fields where biocides were used more extensively, was due to a reduction in species richness and the abundance of all species that were present was very low (Fig. 4.2 C-F). Due to the short comings of the preliminary study (see Chapter 2), these were used as motivation for a more focused study on the influence of certain biocides on soil fauna species assemblages (Chapter 5).

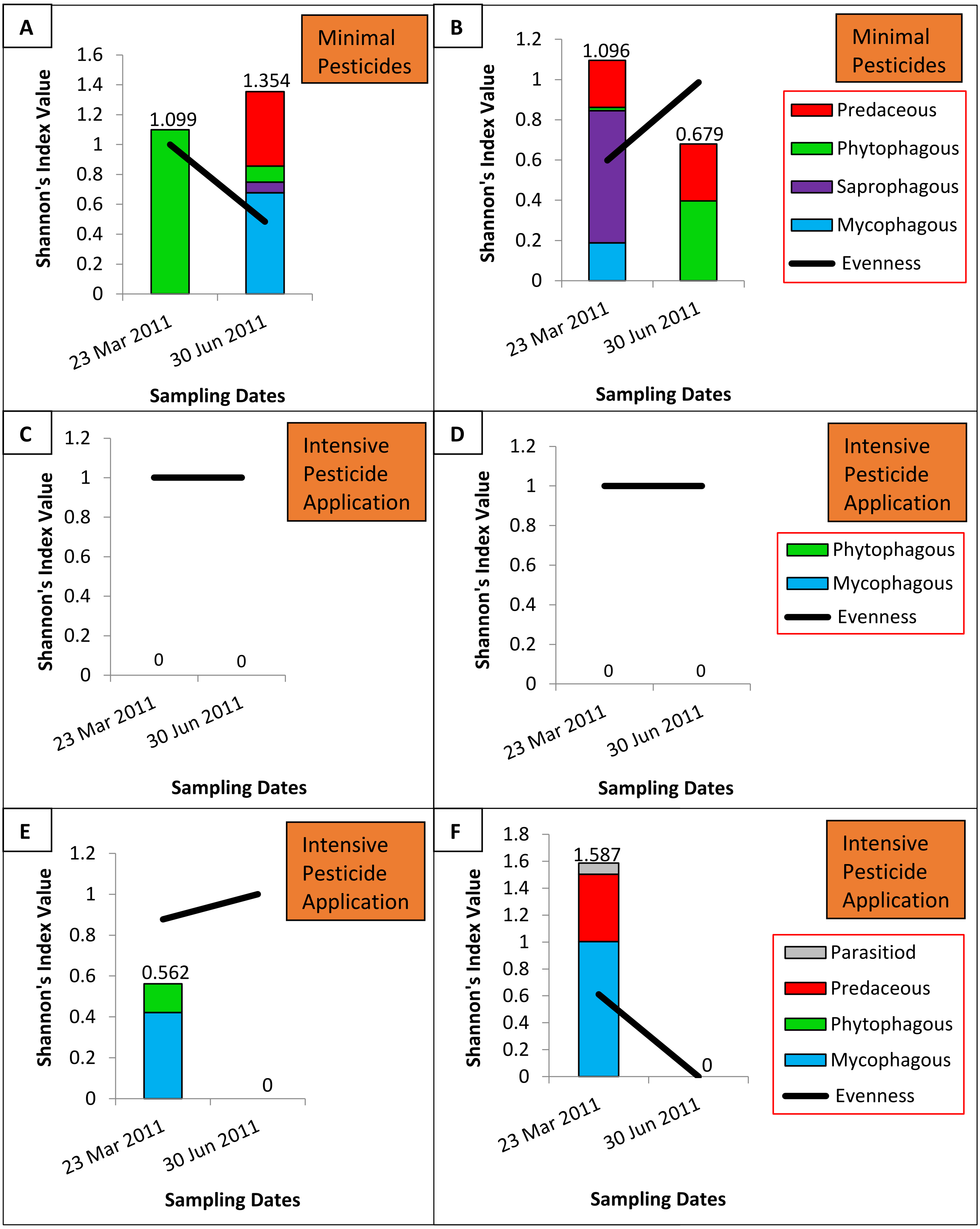


Fig 4.2: The represented trophic levels incorporated as a proportion of the soil faunal diversity (value indicated on top of each bar) within potato fields on the farm Vaaldam and the neighbouring farm in 2011. A: sampling point 25; B: sampling point 24; C, sampling point 26; D: sampling point 27; E: sampling point 28; F: sampling point 29. A–B: minimal pesticide application; C–F: more intensive pesticides application.

- Natural veldt (Preliminary study)

During the preliminary study at the farm Vaaldam, a single sampling point was selected within the natural veldt for the first two sampling dates (Fig. 4.3 A). These samples were collected at sampling point 2, but were deemed too close to the agricultural fields since this area (a combination of dwarf-shrubs and grasses) was covered in post-harvest crop residue. Sampling point 1 (mainly grasses) was subsequently added to ensure a more accurate outcome in monitoring soil fauna fluctuations due to environmental factors and determining the presence of naturally occurring species within the area (Fig. 4.3 B).

The decrease in diversity noted in November 2011 was as a result of a dry spell in the three proceeding months. The diversity was increased substantially during the January samples since it had rained approximately 75mm between these sampling dates (Fig. 3.8), and species richness usually increase with the increase in humidity (Waagner *et al.* 2011).

Sampling point 1 (Fig. 4.3 B) showed a higher diversity than sampling point 2 (Fig. 4.3 A) during the majority of the sampling dates. This is expected since sampling point 1 is further away from the agricultural fields and therefore less disrupted. Sampling point 1 was expected to have a relatively high diversity because it is within a grassland area which, according to Wall (2012), has a more stable plant cover, which, in turn, provides more sustainable conditions for the establishment of complex communities. Although sampling point 2 had a relatively lower diversity than that of sampling point 1, both sampling points tend to follow the same trend in diversity fluctuations throughout the wetter part of the sampling period (summer rainfall area). These sampling points, however, reacted differently during the drier season (spring), which could be attributed to the higher species richness in grasslands, since it provides a better cover than those of the dwarf-shrubs at sampling point 2. The trophic levels were represented by relatively even proportions which is expected in the absence of disturbance, together with a high species richness and relatively small fluctuations in the number of individuals as indicated by the difference between maximum and minimum abundance recorded (see Addendum B). The presence of the predatory mites, *Microcaeculus* spp. (Caeculidae), at these sampling points indicate the absence of disturbance as these mites are sensitive to mechanical disturbance due to their low mobility (Krantz & Walter 2009).

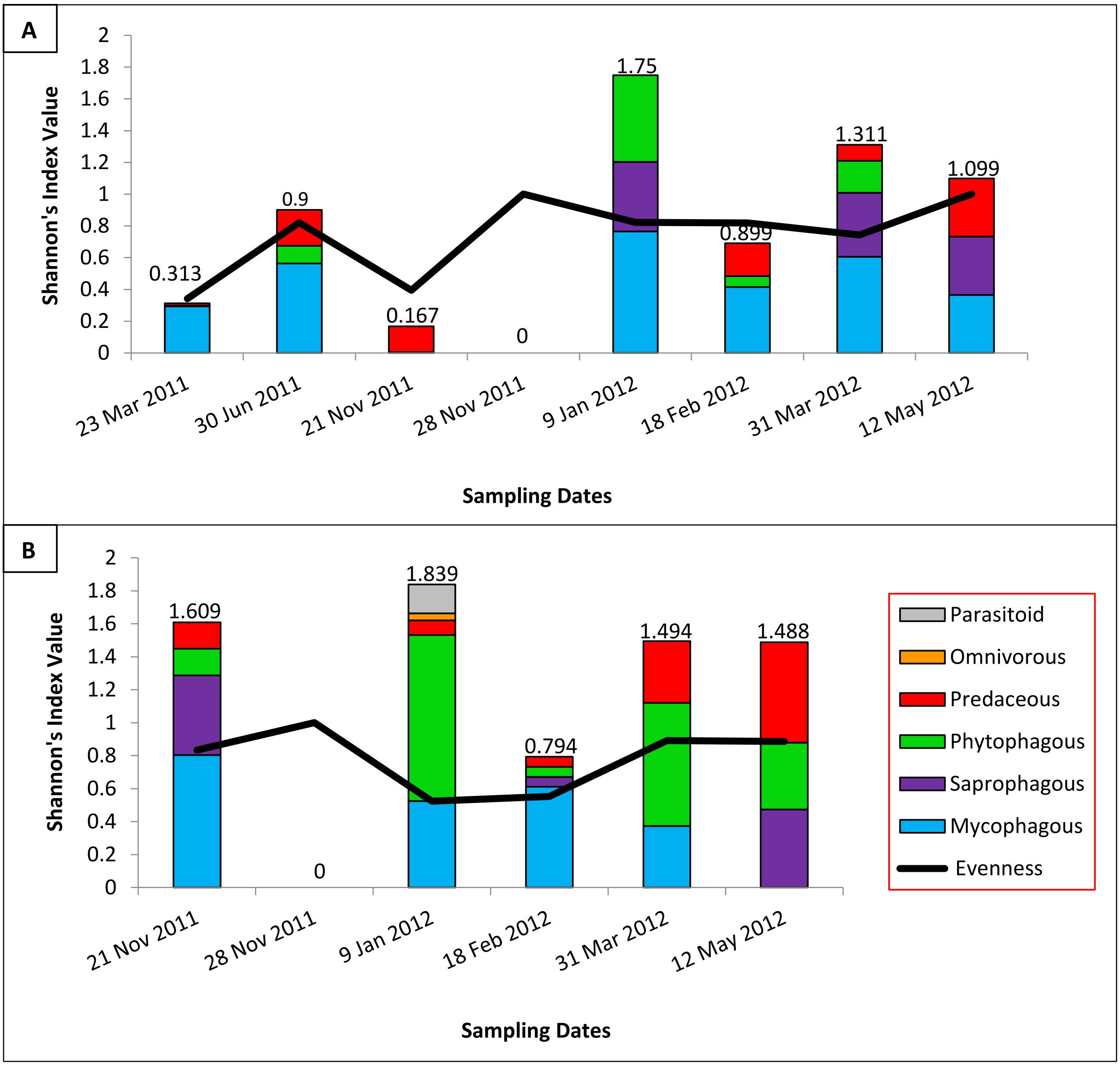


Fig 4.3: The represented trophic levels incorporated as a proportion of the soil faunal diversity (value indicated on top of each bar) within the natural veldt the farm Vaaldam in 2012. A: sampling point 2; B: sampling point 1. (See Addendum A for faunistic detail.)

ii) Variation in soil organism diversity at different clay percentages

In agricultural fields it is important to determine the physical texture of the soil since this would aid in the selection of an appropriate management program. Furthermore, by determining the percentage levels of sand, silt and clay the management programs can be tweaked to benefit a specific field.

During 2013, sampling was conducted in a maize field on the farm Thornberry. This field is situated in the flood plain of the Modder River (Fig. 3.9). The flow and shift over time of this river had, however, influenced the composition of the soil of the flood plain, leading to much variation within a very small area. Due to this phenomenon, it was possible to evaluate the variation in diversity of soil organisms at different clay percentages within the same field. This was ideal as these sampling points would therefore be within the same field of the same crop and receive exactly the same agricultural management treatments.

The clay component of soil influences the structure of soil, since soil with a higher clay content shows a higher compactness which influences organism incidence. This is due to the limited pore space within which soil mesofauna occur (Coleman *et al*. 2004). The services provided by soils are therefore influenced by the physical properties of the soil due to its indirect influence on the biotic component of the soil (Bennet *et al.* 2010).

A difference in diversity was noticed at the different sampling points of this survey, with sampling point 39 (26% clay), showing the lowest diversity values throughout most of the survey (Fig. 4.4 A). This is ascribed to the high abundance of a few species of mites that occurred at this sampling point. Individuals of Oribatulidae (*Oribatula* (*Zygoribatula*) spp.), as well as Tydeidae species dominated and due to their high numbers, lowered the diversity value (Fig. 4.4 A; Addendum B), probably on account of competition for space and nutrition.

Sampling point 40 (38% clay) showed the highest diversity at the beginning of the sampling period (Fig. 4.4 B). This was due to high species richness and even though certain species were present in high numbers, it did not influence the diversity values during the first three samples. In a more detailed analysis, however, this changed, with Tydeidae mite species increasing as the study progressed and the collembolan *Entomobrya* cf. *multifasciata* (Entomobryidae) (Fig. 4.5), increasing during the May and June samples, all of which lowered the diversity values (Fig. 4.4 B).

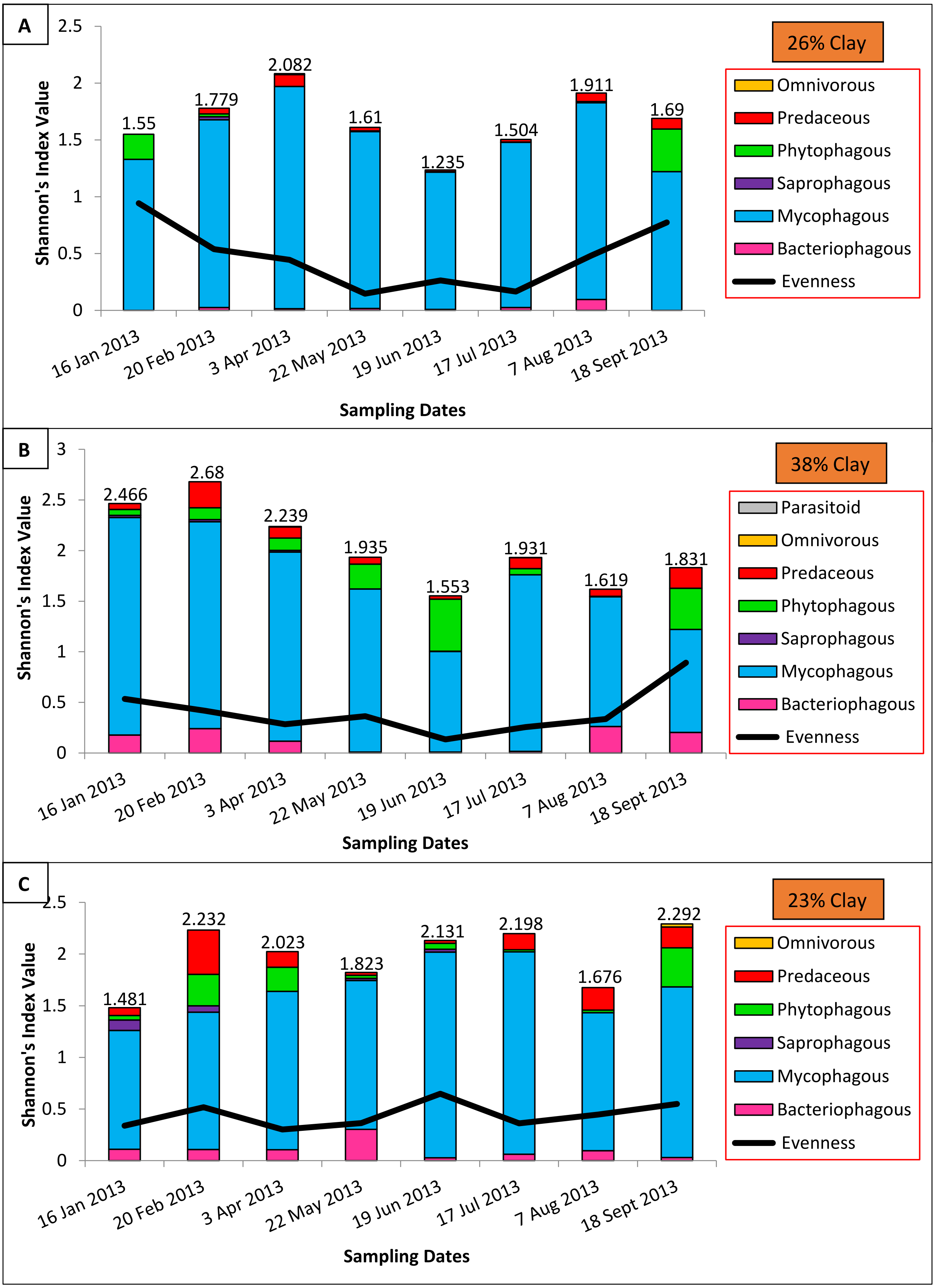


Fig 4.4: The represented trophic levels incorporated as a proportion of the soil faunal diversity (value indicated on top of each bar) at three sampling points within a maize field on the farm Thornberry in 2013. A: sampling point 39 (clay content 26%); B: sampling point 40 (clay content 38%); C: sampling point 41 (clay content 23%). (See Addendum A for faunistic detail.)

As this site become drier, the spike in the number of individuals declined (Fig. 4.5 B) and diversity values increased again. This sampling point had the highest clay content and it could therefore be deduced that its bulk density was the highest, thus influencing the size of the organisms that occur at this site, with the collembolan family Neelidae (*Megalothorax* sp. 1) (Fig. 4.6), only sampled here during February and April 2013.

Fig. 4.5: Dorsal view of *Entomobrya* cf. *multifasciata* sampled on the farm Thornberry during 2013.

Fig. 4.6: Lateral view of Neelidae (*Megalothorax* sp. 1) sampled on the farm Thornberry during 2013.

Sampling point 41 had the lowest clay percentage, *i.e.* 23%. The diversity of this sampling point did not fluctuate as much as those of the other sampling points (Fig. 4.4 C). The seemingly 'more stable' humidity level that probably attributed to this could be due to the fact that this sampling point was drier throughout the complete sampling period (Fig. 4.7 C). The presence of bacteriophages were noted throughout this study and their consistency within the diversity index could be due to a 'more stable' humidity level, since bacteria are dependent on a certain degree of water availability within the soil medium.

It was noted throughout this survey that the number of individuals spiked after there was a spike in the humidity levels (Fig. 4.7). The interphase between these spikes, however, differed between the different sampling points, which could be due to the different life strategies of the various species that occupy the soils of these sampling points. Faunal incidence relative to humidity was more erratic at sampling point 41 and did not seem to follow any pattern, probably because of specific species preferences, such as pore space and water retention, that differ from those of the other two sampling points.

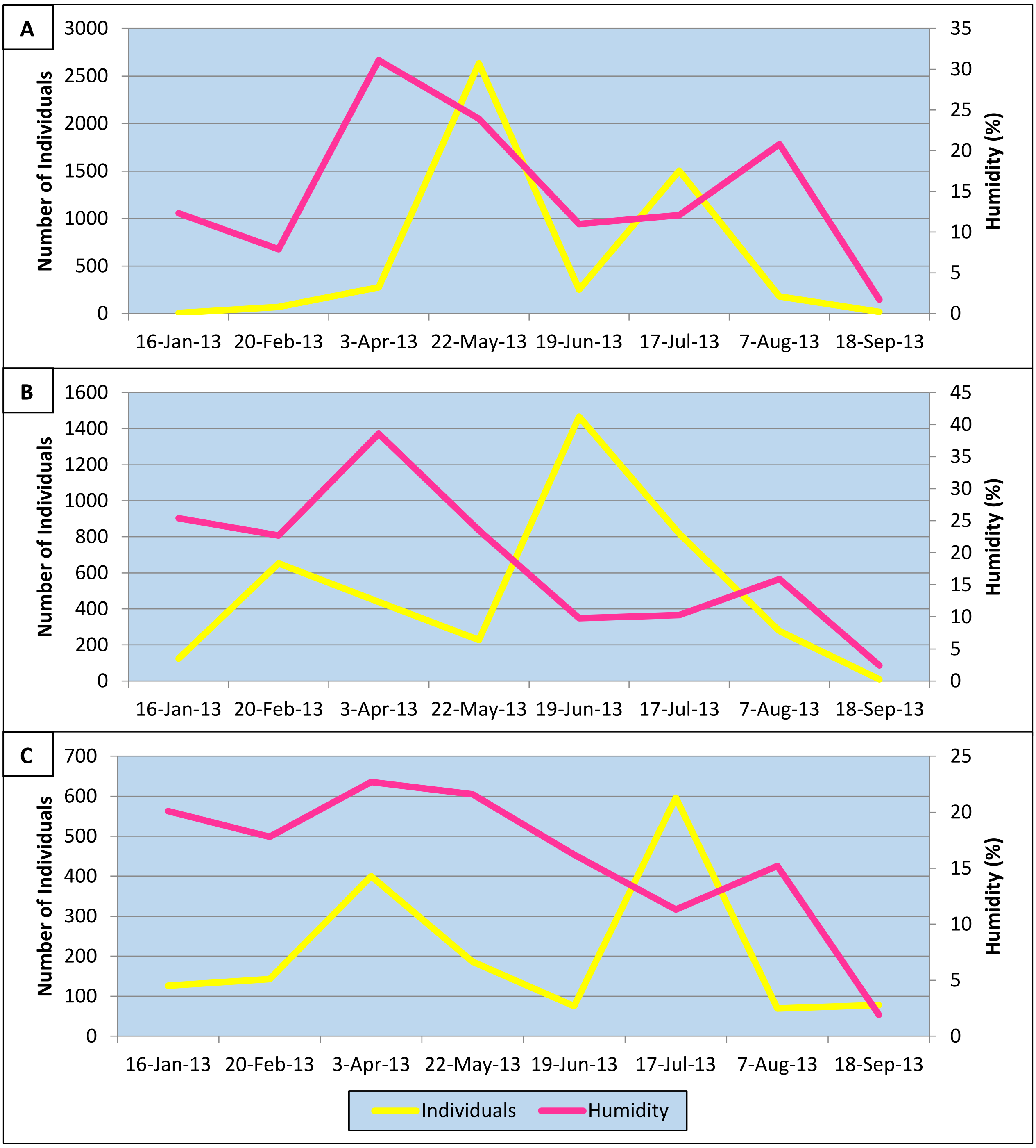


Fig 4.7: Fluctuations in humidity and number of individuals that were sampled at three sampling points within a maize field on the farm Thornberry in 2013. A: sampling point 39 (clay content 26%); B: sampling point 40 (clay content 38%); C: sampling point 41 (clay content 23%). (See Addendum A for faunistic detail.)

Similarity indices between all samples from the different sampling points within this maize field were tested. In ecology, a similarity larger than 40% is regarded as meaningful and therefore focus was only on these clusters (Quinn & Keough 2002) (Fig. 4.8).

Although the clay percentages of sampling points 40 and 41's differed with ±15%, these two sites were clustered together most of the time as indicated by the orange blocks (Fig. 4.8). This could simply be due to the clear cut variation in soil type and structure which was created by the river over time, and although these sampling points were expected to differ, they were actually occupied by the same species as they are in the same proximity of the field.

Sampling point 39 was on the opposite side of the maize field (Fig. 3.9), and formed a cluster on its own which is indicated by the green block (Fig. 4.8). The clay percentage difference between sampling points 39 and 41 was a lot less than the difference between sampling point 40 and any of the other two. Therefore, these two sampling points were expected to cluster at least once, which was the case, as indicated by the purple block.

The cluster indicated in the blue block was in July. Over all the sampling points within the maize field (Fig. 4.4), including the sample from the natural veldt (Fig. 4.9), an increase in diversity was noted from June to July. This was due to an increase in temperatures the five days preceding sampling in July.

Throughout this study it seemed that the clay percentage had a larger effect on soil mesofaunal occurrence and species richness than abundance (also see Addendum B). Physical and chemical components, *i.e.* pore size and water retention, of the soil in this field selected for certain species.

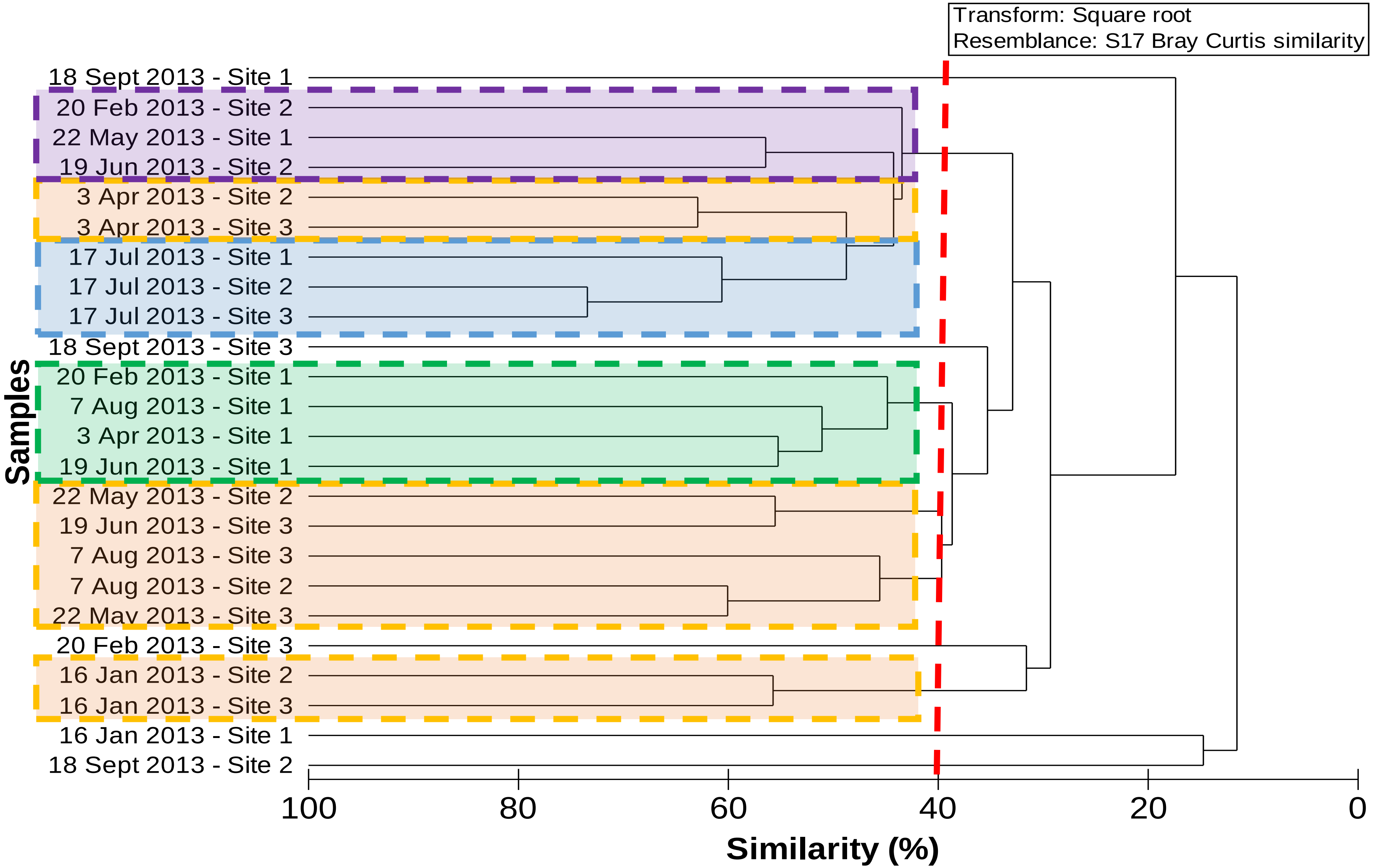


Fig 4.8: Similarity between the different samples using a cluster diagram; Site 1 = Sampling point 39 (26% clay), Site 2 = Sampling point 40 (38% clay), Site 3 = Sampling point 41 (23% clay).

Only a single sampling point (sampling point 34, Fig. 3.9) was selected in the natural veldt at this locality, for the objective was to focus on the clay percentages within an agricultural field and not the effect of agricultural practices. Sampling point 34 was located within a grassy area and showed relative stability in its diversity values. Although the influence of temperature is regarded as lesser than that of rain, it was responsible for the increases in diversity in July and September 2013 (Fig. 4.9), as minimal rain was observed during the winter months. This is as a result of the higher minimum temperatures (avg. temp. ± 3.5°C) measured on the days prior to sampling in the respective months (Fig. 3.12). Minimum temperatures in the five days prior to sampling in June (avg. ± –3.5°C) and August (avg. ± 0.4°C) was much lower, thus suggesting that the temperatures prior to the sampling dates influenced the diversity of these samples.

This opinion is supported by Krab *et al.* (2010), who stated that soil microarthropod communities are controlled by means of bottom up factors, since the distribution and abundance of these organisms are determined by the soil *per se* (thus temperature and humidity) and food quality. This was seen as the influencing factors at sampling point 34, with the higher temperatures in the grassveldt creating a viable food source and an increase in organismal activity, which led to an increase in organism diversity.

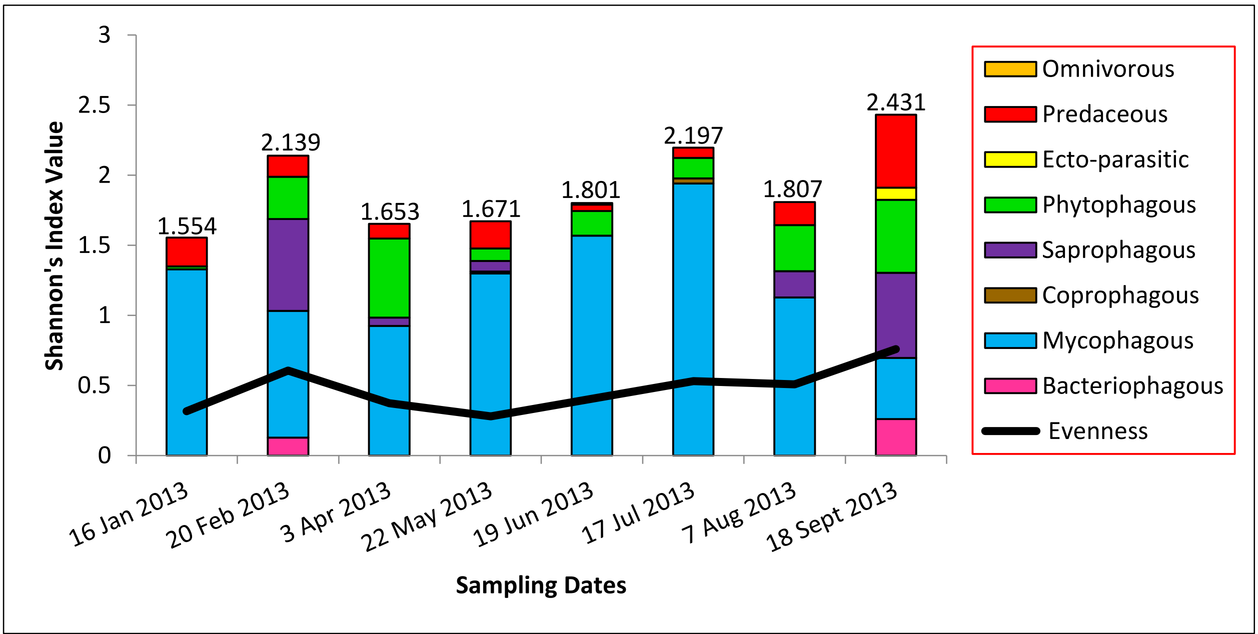


Fig 4.9: The represented trophic levels incorporated as a proportion of the soil faunal diversity (value indicated on top of each bar) within the natural veldt, sampling point 34, at the farm Thornberry during 2013. (See Addendum A for faunistic detail.)

iii) Diversity differences within a maize field on a topographical gradient

Gradients in agricultural fields can be problematic since it complicates effective application of water and other soil additives. During the preliminary study fluctuations in organism diversity were studied in a field with a gradual north-eastern slope at the farm Vaaldam. This field was cultivated with Bt. maize with a non-Bt. section through the middle of the field (Fig. 3.2). Sampling points 14-15 were at the top of the slope and 16-17 at the bottom. Prior to the first sampling date, 28 November 2011, a herbicide was sprayed to eliminate the regrowth of the previous potato crop. The effect of this herbicide was noted throughout the whole field with no mesofauna sampled on this date (Fig. 4.10) (see also Chapter 5 for biocide experiment). Organism diversity showed an increase in January, although the diversity was still low, with individuals only representing certain trophic levels. The organisms sampled in January were general phytophages (Hemiptera) and predators (Araneae) which are all more mobile than periodic and permanent soil faunal groups and therefore could have moved into this area.

During this survey, it was noted that the trophic levels of the samples within the Bt. maize field were similar at the top and bottom of the slope, with a slight difference in the bar boxes of the specific trophic levels. This indicated that the slope had an influence on food source species abundance and not the presence or absence of certain food source species of corresponding dates. Even though sampling point 15 showed a diversity of 0 on 9 January 2012, a phytophage (Gelechiidae m.sp. 1) was present in this sample. The same for sampling point 17, which was only represented by a *Hypogastrura* species (Hypogastruridae), which is mycophagous and thus corresponds with the sample from 12 May 2012 at sampling point 15 (Fig. 4.10 A & B). The latter is most likely and introduced species, which could explain their presence in areas that is unfavourable to the native species.

As previously mentioned, the non-Bt. maize at the bottom (sampling point 16) and top (sampling point 14), of the slope followed the same trends and filled the same trophic levels, with the only exception in the presence of saprophages throughout sampling

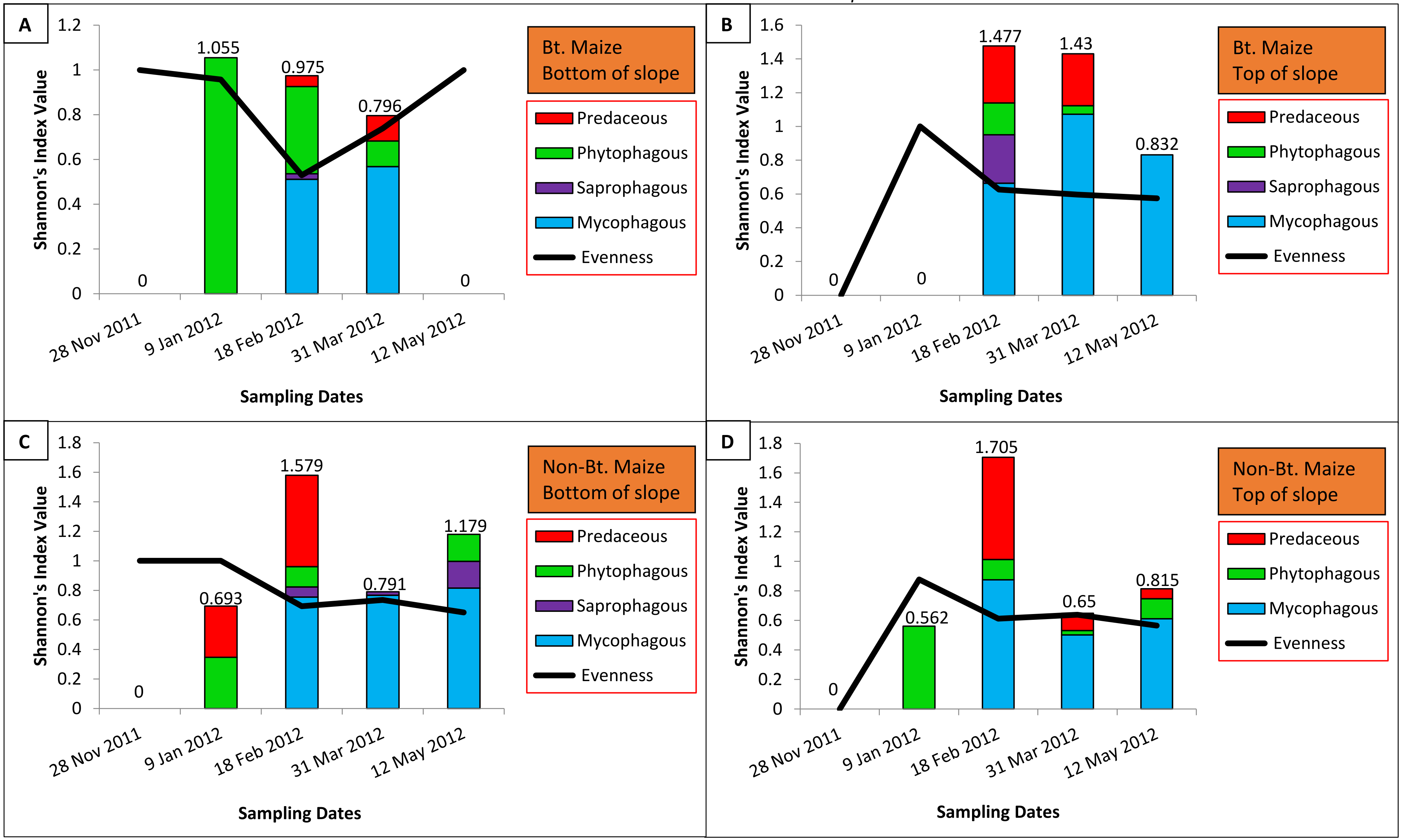


Figure 4.10: Sampling points 14-16 sampled within a maize field during a preliminary study at the farm Vaaldam. A: sampling point 17 in Bt. maize; B: sampling point 15 in Bt. maize; C: sampling point 16 in non-Bt. maize; D: sampling point 14 in non-Bt. maize. Sampling points 14 – 15 are at a higher elevation than 16 – 17. (See Addendum A for faunistic detail.)

point 16 (Fig. 4.10 C & D). Sampling point 16 was the lowest in altitude and was waterlogged, leading to possible drowning effects and more dead plant material.

During the primary study in 2013, sampling was conducted at the same sampling points to evaluate the effect of the slope with the inclusion of the smaller organisms that, due to extraction shortcomings, were not extracted during the preliminary study. This study, however, took place over a shorter time period and no pesticides or herbicides were used during this sampling period. The diversity in general was much higher than that of the preliminary study, which was expected, since a wider variety of organisms could be included due to the expansion of the size range sampled. The species richness together with the minimum and maximum abundances for 2013 are indicated in Addendum B.

During this sampling period, a general trend was noticed throughout the field whereby the dominant trophic levels were mycophages and predators (Fig. 4.11). The richness increase noticed at three of the sampling points on 18 January 2013 could be as a result of fertilizer application on 10 January (Fig. 3.4), whereby the food resources for some fauna were increased. The decrease in diversity noticed in the 6 February sample was caused by the application of a fungicide, which was sprayed between 29 January and 6 February to control northern corn leaf blight (see Chapter 5), and affected the whole field.

The overall effect of the gradient was minimal during both surveys, since this was not a very steep gradient and all crop applications were managed to ensure maximum effectiveness and minimal runoff. This was possible due to the technology of center-pivot irrigation systems which can be programmed for more precise application delivery. Although the effect of Bt. and non-Bt. maize on soil faunal diversity was not the objective, it seemed that there were only slight differences and that the faunal diversity fluctuated similarly in the presence of external factors.

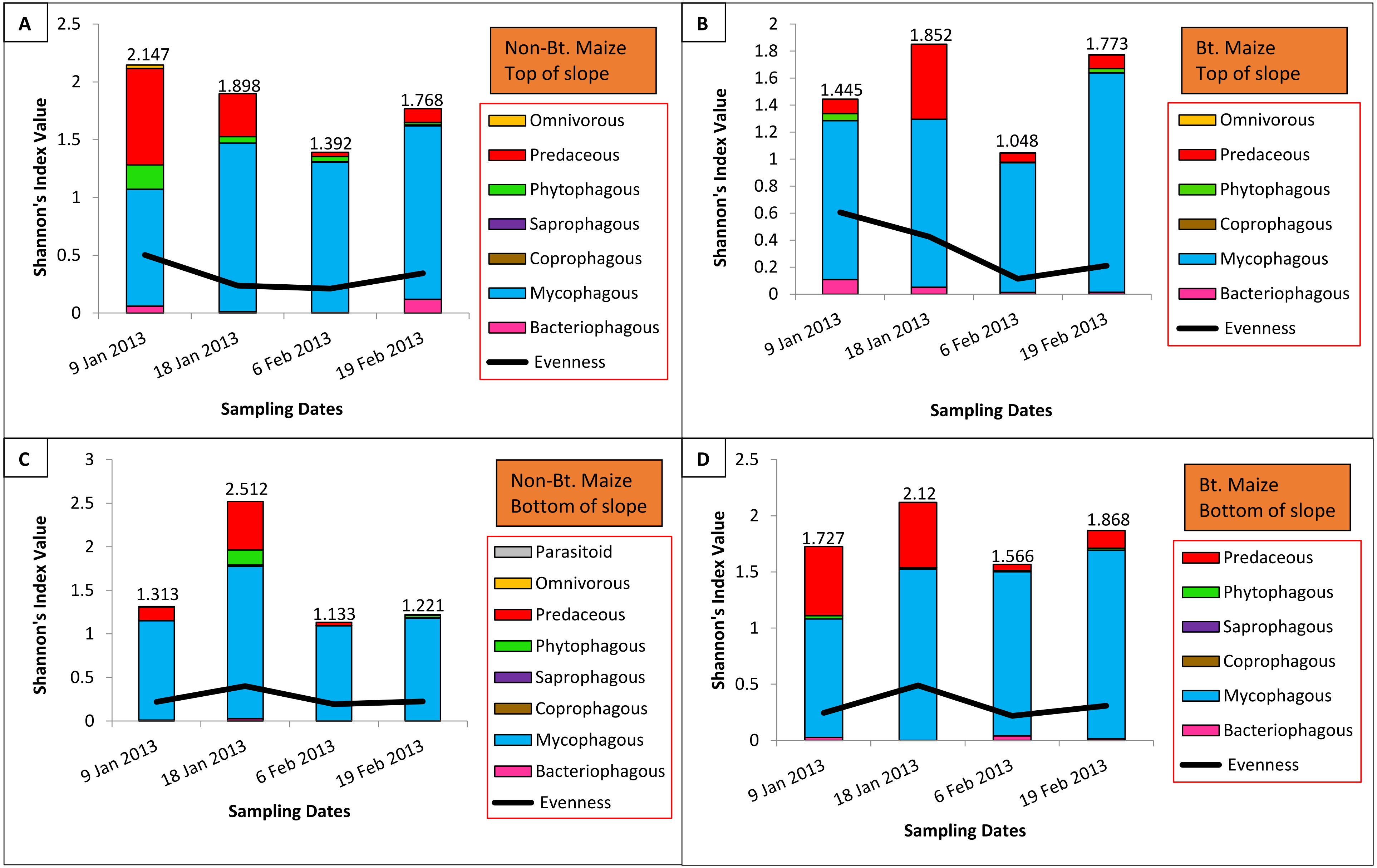


Figure 4.11: Sampling points 14-16 sampled within a maize field during 2013 at the farm Vaaldam. A: sampling point 14 in non-Bt. maize; B: sampling point 15 in Bt. maize; C: sampling point 17 in non-Bt. maize; D: sampling point 16 in Bt. maize. Sampling points 14 – 15 is at a higher elevation than 16 – 17. (See Addendum A for faunistic detail.)

b) Anthropogenic influences

Soil organisms show different responses to different changes in their environment. These include changes in the physical part of their environment which can be brought on by agricultural practices (Kladivko 2001). Although some reactions are more general, such as mesofaunal diversity decreasing post-harvest (Benckiser 1997).

i) <u>Influence of controlled stubble-burning and its alternative on mesofaunal diversity</u>

Fires have various influences in nature and are considered to be an important abiotic factor that influences the functioning and shaping of terrestrial ecosystems. Naturally occurring fires are of benefit because they can stimulate the growth of certain vegetation types (*i.e.* in fynbos biome) and clear old or dead plant material for new plants to grow (*i.e.* in grassland and savanna biomes)(see Chapter 1 and van Wilgen 2009). The effect of fires is, however, dependent on the intensity of the fire, type of ecosystem affected and the season. These factors should all be taken into account when controlled fires are used in management programs. This practice has been successfully implemented in South African National Parks to eliminate invasive plants and enhance plant biomass (van As *et al*. 2012).

At Koppieskraal farm, stubble-burning was not applied during the preliminary study in 2011-2012, but the stubble was rather worked into the soil as humus supplement to a heavy clay soil. As previously mentioned, management strategies should be selected carefully, since all environments differ and react differently to disturbances. By incorporating stubble into the soil, the diversity stayed relatively stable in this field, even though the field was ploughed before sampling on 21 November 2011 (Fig. 4.12 A & B), this event was a major disturbance (Fig. 3.7 B). On 12 May 2012, six months after the disturbance, the four trophic levels that were present before the disturbance, were all represented again, indicating that a six month period was required at this locality for resilience and recovery in mesofaunal diversity to be observed.

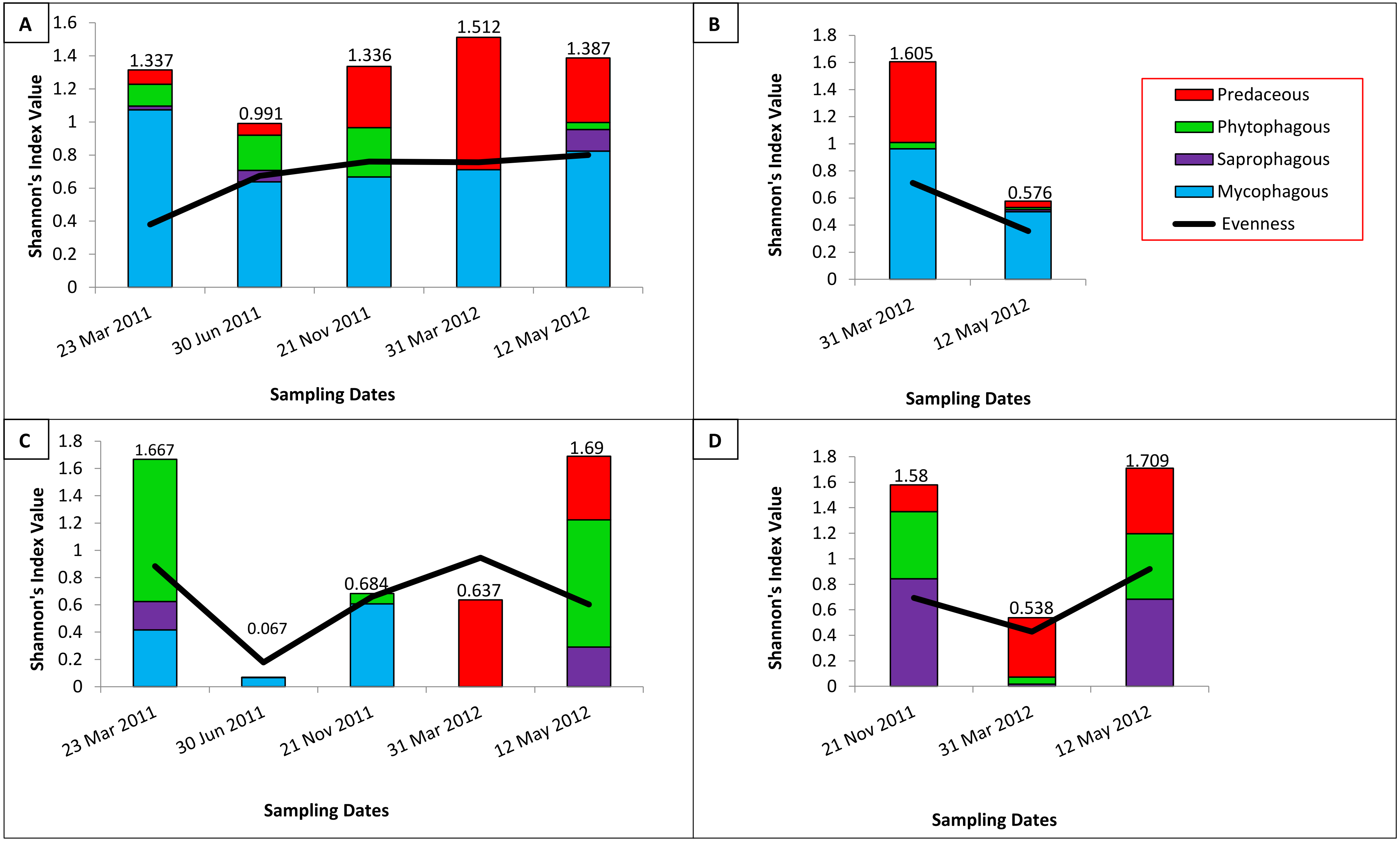


Figure 4.12: Sampling points 31-33 sampled at Koppieskraal farm during a preliminary study during 2011-2012. A: sampling point 31 within a maize field; B: sampling point 30 within a maize field; C: sampling point 33 in natural veldt; D: sampling point 32 in natural veldt. (See Addendum A for faunistic detail.)

A control, sampling point 33 (Fig. 3.6), for the preliminary study at Koppieskraal farm was selected, but due to the effect of herbicide application at this sampling point, a second control was added, *i.e.* sampling point 32 (Fig. 4.12 C & D). Between 23 March and 30 June 2011, a herbicide was sprayed at sampling point 33. This led to an increase in dead plant material and a decrease in general mesofaunal diversity accompanied by a spike in the abundance of Hypogastruridae individuals (See also Chapter 5 – Biocide trial). The areas surrounding crop fields are always influenced by ongoing agricultural practices, even though it is not always that visible. After the field was ploughed in 2011, it was left fallow and agricultural activity was halted. This allowed the soil fauna communities time to re-establish and on 12 May 2012, the diversities of the two different control sites – sampling points 32 & 33 – both had a diversity of 1.7 which consisted of the same trophic levels (Fig. 4.12 C & D).

In agricultural fields controlled stubble-burning is used to manage certain pests, reduce preparation costs and reduce plant stubble which can become too thick and therefore limit the effectiveness of no-till planting. This reduction of plant material could have both a negative and positive effect on soil and its inhabitants. According to van As *et al*. (2012), the negative effects would include the loss of top soil by wind and water erosion, an increase in temperature fluctuation due to the loss of an insulating layer, increased water loss and loss of possible food sources. On the other hand, nitrifying bacteria are stimulated and at the farm Thornberry, bacteriophages from the mite family Nanorchestidae showed an increase a month after stubble-burning was applied (Fig. 4.13 A & B) and counteract the nitrogen loss as a result of the fire, thus increasing the available nitrogen in the soil. The ashes that remain on top of the soil contain calcium, magnesium, phosphorus, potassium and sodium. Presumably carbon levels would also increase. In agricultural landscapes, chemical elements after stubble-burn are usually incorporated into the soil with water applications or tillage. As controlled stubble-burning forms part of soil preparation, these now fallow areas are usually cultivated again and are thus less affected by extreme temperature fluctuations, as well as wind and water erosion. However, if these fields are left fallow, implications such as an increase in soil compaction due to strong winds and water loss do occur. The implications of this is a reduction in the diversity of soil organisms, as seen on 3 April 2013 at sampling points 37 and 38 (Fig. 4.13 A & B).

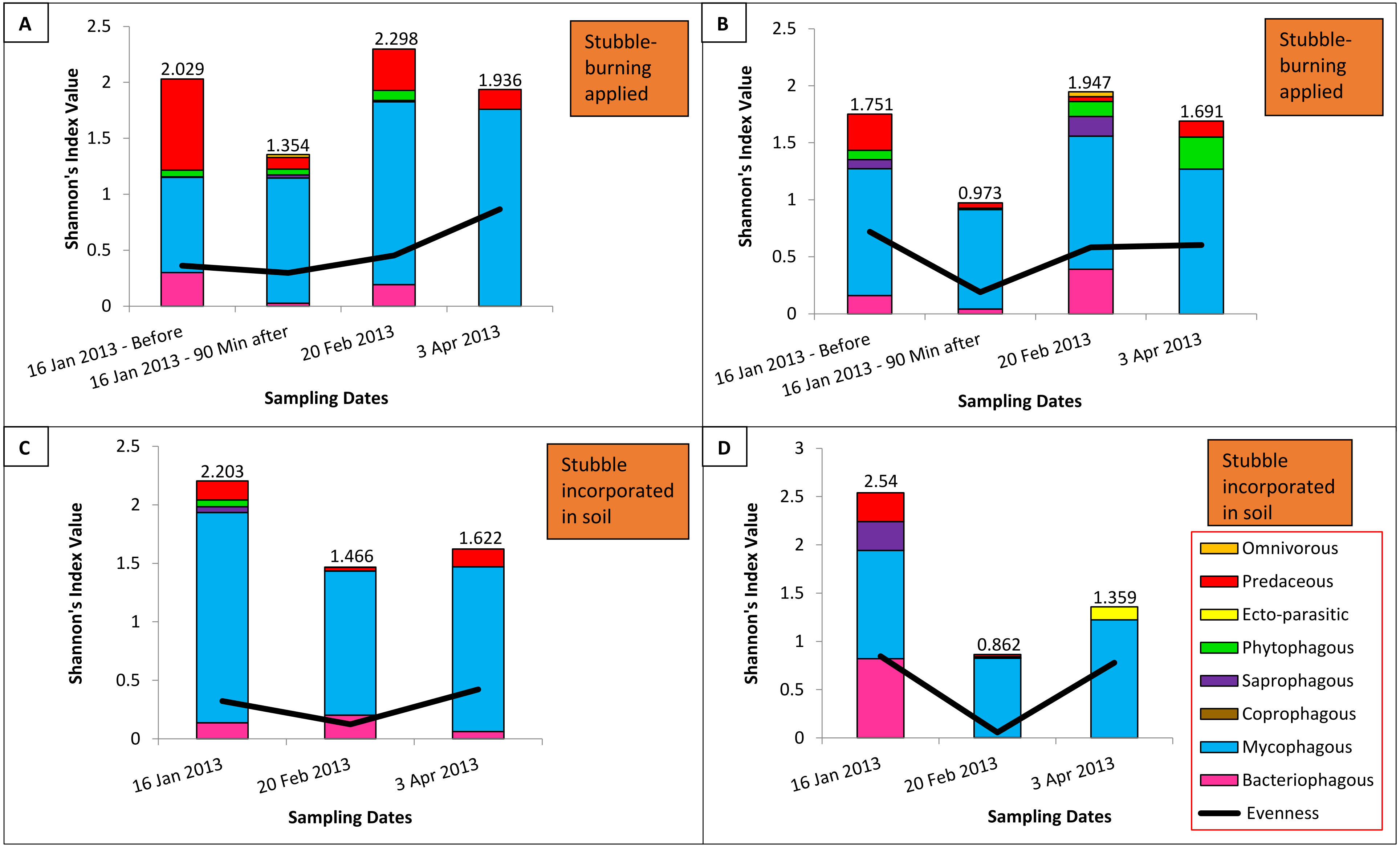


Figure 4.13: Sampling points 35 – 38 at the farm Thornberry where stubble-burning was applied in 2013. A – B: sampling points 37 and 38 where stubble-burning was applied ('Before' indicating sampling done prior to stubble-burning and '90 Min after' indicate sampling done 90 minutes after stubble-burning was applied); C: sampling point 35 with stubble worked into the soil; D: sampling point 36 with stubble worked into the soil. (See Addendum A for faunistic detail.)

At sampling points 35 and 36 the wheat stubble was incorporated into the soil, thus adding soil organic matter (Fig. 4.13 C & D). Samples from 16 January 2013 showed relatively similar diversity values with a definite decrease in diversity values on 20 February 2013. This was due to the organismal flux which occurred due to the increase of food sources. The evenness was therefore much lower, with mycophagous Tydeidae mites (*Pronematus* sp.), reaching a total of 4103 individuals at sampling point 35 and 5809 individuals at sampling point 36. The diversity value at sampling point 36 was higher than that of 35, due to the higher number of individuals sampled across different species, as opposed to only a single species, *e.g.*: *Nanorchestes* spp. (Nanorchestidae). Bacteriophages accounted for another 1260 individuals, thus resulting in a more even spread of individuals across the represented species and within the trophic structure. Other organisms that was present in high numbers (>200) include Oribatulidae (*Oribatula* (*Zygoribatula*) spp.), Tarsonemidae (*Hemitarsonemus* sp.) and Scutacaridae (*Scutacarus* sp.) mites (Addendum A).

Although species richness and abundance of most species increased between 16 January and 20 February 2013, the above mentioned mite species were responsible for the lower diversity and evenness values. This was due to a surge in their abundance which completely dominated these samples (Fig. 4.14; also see Addendum B).

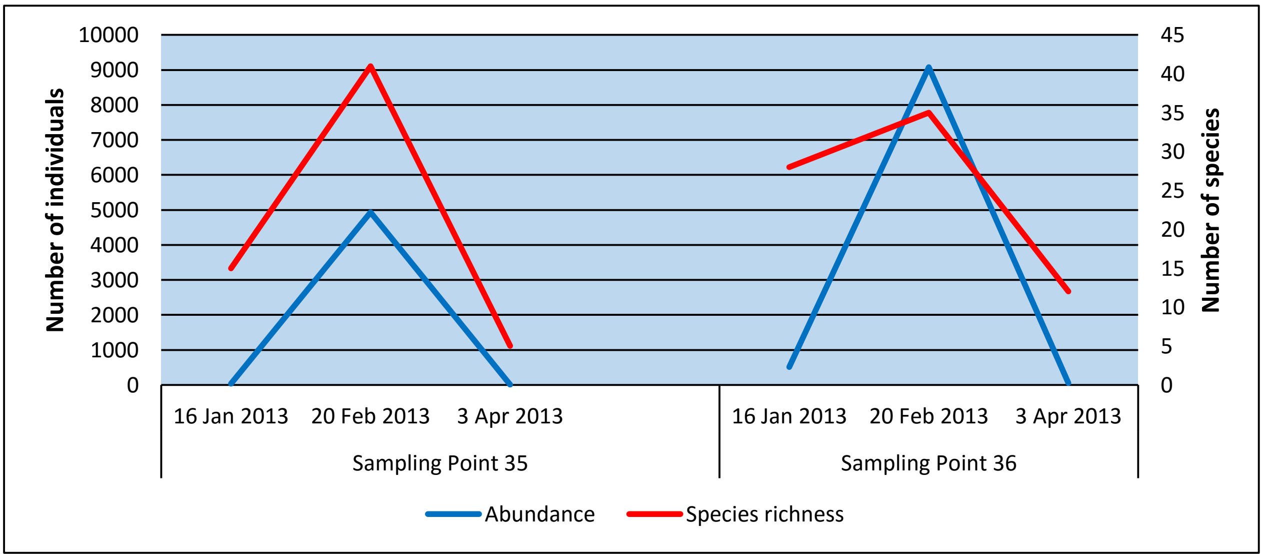


Figure 4.14: Sampling points 35 and 36, indicating a spike in both species richness and abundance in February 2013 at the farm Thornberry.

Controlled stubble-burning can have adverse effects on soil faunal diversity, as was observed at the farm Vaaldam (Fig. 4.15). These effects are due to the variations in stubble quantity and quality, rate and intensity of the fire and the presence of weeds or living plant material. Soil humidity is another factor that can influence the diversity of the organisms sampled, which, according to Krab *et al.* (2010), play a role in the vertical distribution of soil faunal groups. It was noticed that the soil humidity increased after stubble-burning was applied, presumably due to condensation and organismal vertical movement as a result of habitat change. It was therefore possible that the increase in diversity recorded at sampling points 18 and 20, which were very dry before stubble-burning was applied, could be ascribed to the increase in humidity and therefore the vertical migration of soil fauna. This was confirmed with the sampling of collembolans, such as *Mesaphorura* spp., which according to Benckiser (1997), occur at a depth of 15-20cm in the absence of agricultural disturbances (Addendum A).

The rest of the sampling points at Vaaldam farm showed a decrease in diversity 90min after stubble-burning was applied. This could be due to a ‘hot’ fire, as opposed to above, or the loss of plant material. Sampling point 19 showed a higher proportion of saprophages, which could be due to the higher density of stubble present at this sampling point. Sampling points 21 to 23 showed the presence of phytophages, even after stubble-burning was applied, which was probably due to the weeds that were growing in these two fields. The presence of the weeds influenced the efficiency of the stubble-burning process, since the fields with more weeds burnt out more rapidly than the fields with less weeds. Furthermore, the fields with less weeds had a higher quantity and quality of stubble, thus creating a slower and hotter fire.

After a seven week period with no agricultural activities, a final sampling was conducted. All of the sampling points showed an increase in diversity even though the fields were left fallow during this period. Control samples were taken at sampling points 1 to 3 within the natural veldt to compare the influence of normal environmental factors on faunal occurrence (Fig. 4.16). Diversity decreased in the natural veldt as a result of a decrease in temperature, since these samples were taken during winter.

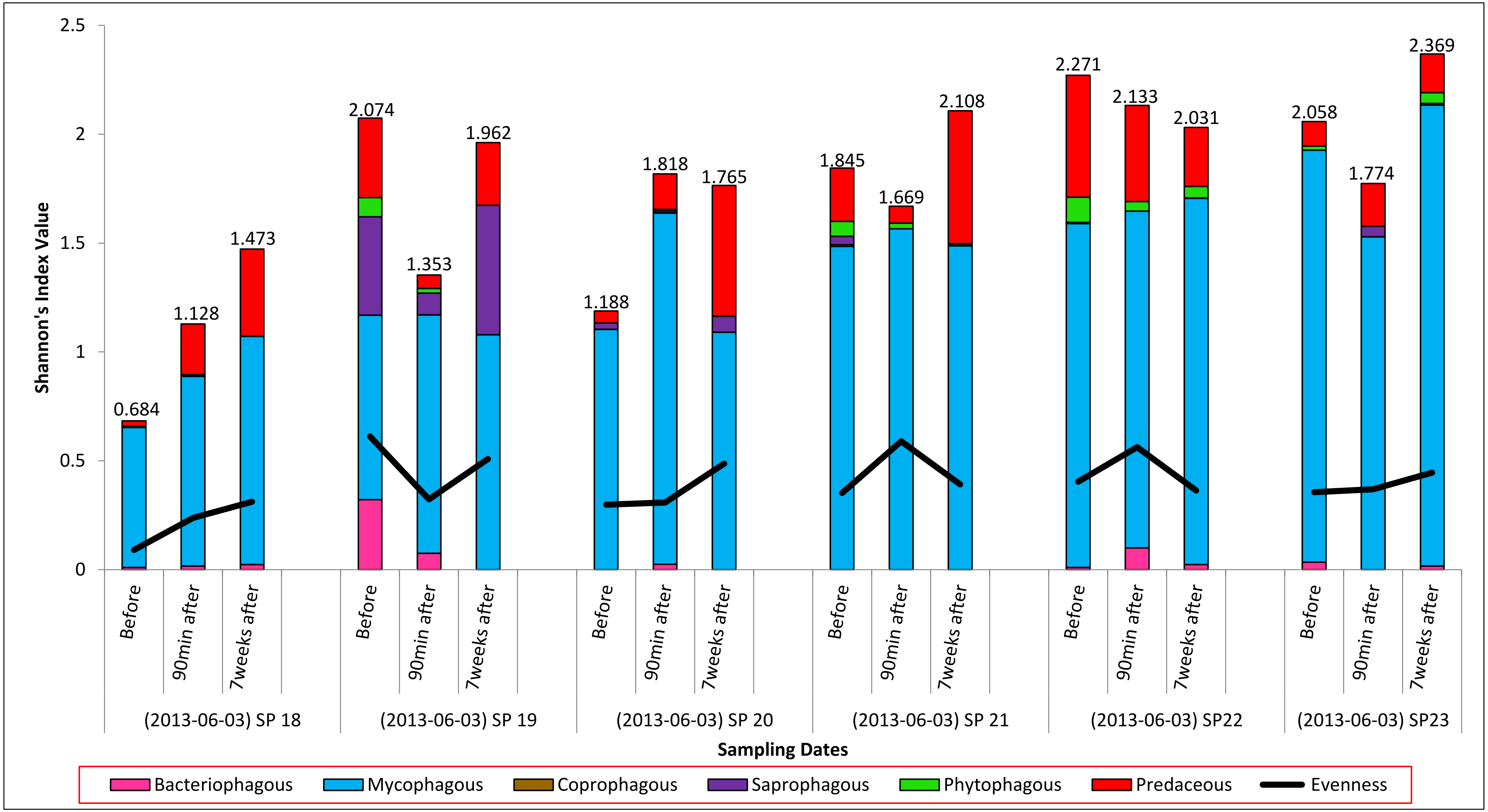


Figure 4.15: Sampling points 18 – 23 on the farm Vaaldam where stubble-burning was applied in 2013. Samples were taken prior to stubble-burning, 90min after stubble-burning and 7weeks after stubble-burning. (See Addendum A for faunistic detail.)

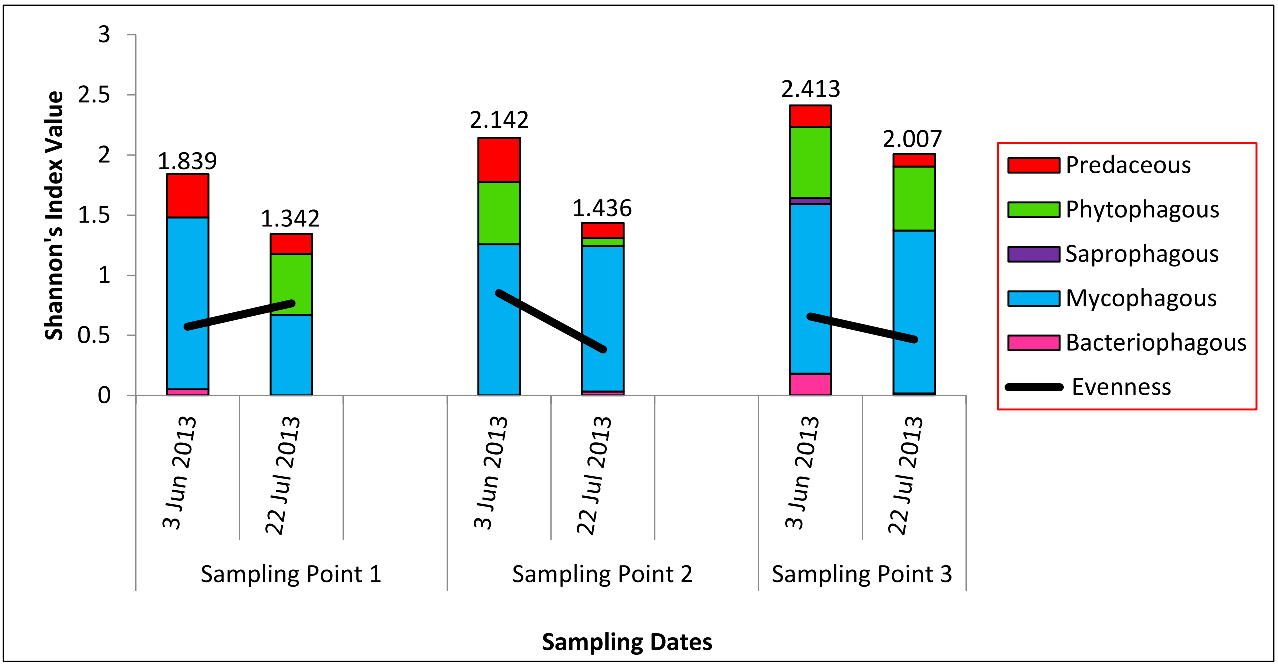


Figure 4.16: Sampling points 1 – 3 in the natural veldt on the farm Vaaldam were stubble-burning was applied in 2013. (See Addendum A for faunistic detail.)

Stubble-burning therefore had different effects throughout this study. The effects varied due to seasonality, the type of plant material that was burnt and location. These factors, according to Malmström *et al.* (2008), makes it difficult to predict the effect of fires on soil organisms, since the abiotic components of soils influence the penetration of heat based on the fact that transportation of heat into soil is regulated by factors such as texture and moisture. However, the diversity seemed to be generally higher at both Vaaldam and Thornberry after 5-7 weeks passed, than prior to stubble-burning and the natural veldt. At Thornberry the diversity stabilised after 11 weeks to approximately the same as prior to stubble-burning.

Where wheat stubble was incorporated into the soil an increase in organisms were noted. However, by leaving these fields fallow, the compaction increased at the farm Thornberry. Unfortunately, the effect of compaction could not be evaluated at the farm Koppieskraal, since this field was already ploughed before sampling. In the stubble-burning trial, soil compaction even increased at the farm Thornberry due to strong winds during the summer months and the lack of cover. The maize stubble that was burnt at the farm Vaaldam during winter burnt slower and less effectively than the wheat at Thornberry farm. This is because maize stems are thicker than that of wheat and wheat stubble covers the field more densely, allowing for a more successful fire.

ii) <u>Effect of mechanical disturbance in fields</u>

With the choice of a management programme, it is important to evaluate the soil beforehand and select management tactics accordingly, since incorrect agricultural practices can have a detrimental effect on the soil. According to Arnhold *et al.* (2015), agricultural malpractices can lead to serious soil degradation and low water infiltration, which in turn will lower crop yields. Other effects of tillage that directly or indirectly influence mesofaunal numbers is the decline in soil moisture, influence of the C/N ratio and an increase in soil temperature (Zhang *et al.* 2015).

During the preliminary study at the farm Vaaldam, stubble-burning was applied prior to disc-ploughing (Fig. 4.17 A, Table 3.6). Even with less mechanical disturbance, larger soil faunal individuals were still reduced and the interference affected the vertical distribution of smaller organisms. These smaller organisms include various mites and collembolan species amongst others. Due to the extraction exclusion of very small organisms during the preliminary study in 2011, the agricultural disturbances reduced the diversity with every disturbance until the diversity was 0 on the Shannon's Index. Eventually only individuals of the family Tullbergiidae were observed.

Farmers have become more aware of the importance of biodiversity in their soils and are trying to convert their programs to more conservation orientated practices where the ecological function of soil faunal groups replace certain agricultural practices, *e.g.* the promotion of biological control agents instead of biocides (Roger-Estrade *et al.* 2010). During the primary study in 2012, stubble-burning at this field was discontinued and the stubble and weeds were worked into the soil by means of a disc-plough (Fig. 4.17 B, Table 3.6). This field had a lot of weeds growing amongst the stubble, giving rise to the presence of phytophages. After tillage is applied in a field certain organisms tend to increase rapidly due to their short life cycle, dispersal ability or opportunistic feeding behaviour (Neher & Barbercheck 1999). This was indicated by the presence of omnivores within 20 minutes after tilling was applied on 14 November 2012 (Fig. 4.17 B). According to Neher & Barbercheck (1999) and

Kladivko (2001), tillage seems to have diverse effects due to the diversity in functional groups in the soil. However, after tillage applied, bacteria will increase.

On 27 November 2012, the regrowth of weeds was too severe for planting and this field had to be disc-ploughed again. A shift in the proportion of each trophic level was observed, as well as an increase in the bacteriophages, in accordance to the opinion of Neher & Barbercheck (1999) and Kladivko (2001). This was observed both times during November 2012 but the effect was better illustrated during the second ploughing period (Fig. 4.17 C). Predator presence increased after disc-ploughing exposed the soil organisms and showed a proportional decrease as time passed, allowing prey species to find shelter (Fig. 4.17).

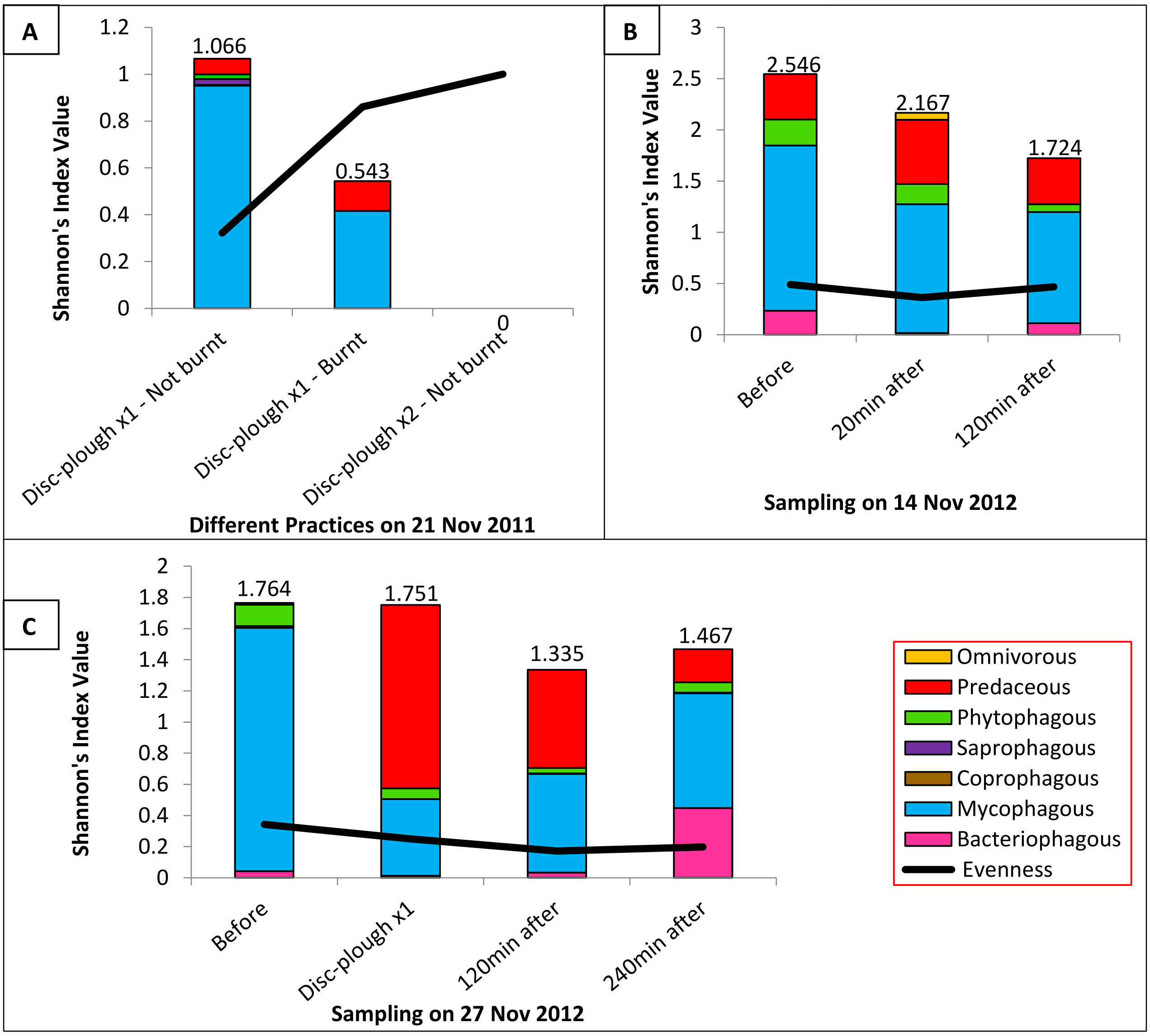


Figure 4.17: Fluctuations in diversity as a result of disc-ploughing at the farm Vaaldam. A: field preparations during preliminary study in 2011 that included stubble-burning; B – C: field preparations in the absence of stubble-burning during the primary study in 2012. (See Addendum A for faunistic detail.)

iii) Mesofaunal diversity relative to conversion of natural veldt to maize field

During the preliminary study, sampling points 64 to 66 (Fig. 3.23) showed much variation within the trophic composition (Fig. 4.18). These sampling points are located in a field that was converted from grassland to an agricultural field in the same season. RoundupReady-Bt. maize was the first crop planted in this field. The preparation of this field was quite extensive as explained in Chapter 3.

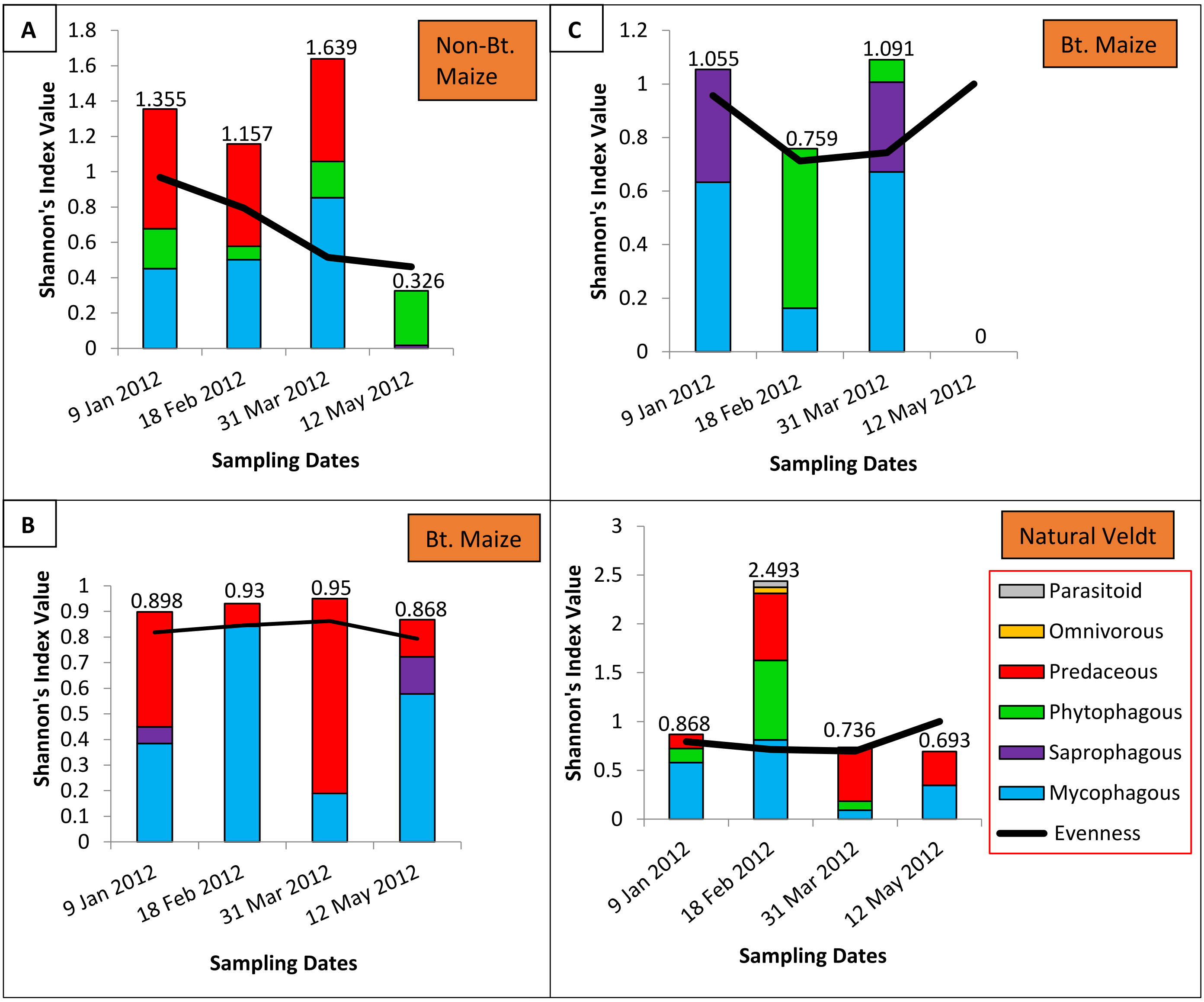


Figure 4.18: Sampling points 64 – 66 on the farm Klein Brittanje during the preliminary study in 2012, where natural veldt was converted into a maize field. A: sampling point 64 within non-Bt. maize; B: sampling point 65 within Bt. maize; C: sampling point 66 within Bt. maize; D: sampling point 73 within the natural veldt. (See Addendum A for faunistic detail.)

In 2012 sampling points 64 and 66 showed similar trends in diversity values, although the trophic composition differed considerably. Sampling point 64 was represented by mycophages, predators and phytophages, with sampling point 66 represented by mycophages, phytophages and saprophages (Fig. 4.18 A & C). Sampling point 65 was situated in between the other two sampling points and was represented by mycophages, phytophages and saprophages (Fig. 4.18 B). The diversity values of the latter, however, remained relatively constant. These fields were situated along a gradual slope, which could have been responsible for the variation between the samples. Tillage reduced the diversity of trophic level representatives, for the natural veldt sustained representatives from each trophic level (Fig. 4.18 D).

As part of the soil preparation prior to the primary study, biocides were used. The effect of these biocides, however, could not be determined since no sampling was conducted prior to spraying and sampling only commenced in February 2013. Fluctuations in the diversity values occurred throughout the study period within all three sampling points. Sampling point 65 and 66 more or less followed the same trend throughout the sampling period, with sampling point 64 only showing a large difference in index value and trophic composition in February and June 2013 (Fig. 4.19). This could be due to the fact that sampling point 64 was situated in a slight depression in the landscape.

Although these fields showed a difference during 2012, when they were first planted, they seem to follow the same trends as the other fields at this location during 2013. It therefore seems that the change from a natural veldt community to a field community of soil organisms was less than one year. This is observed in the fluctuations that are similar to those of the other fields, as oppose to that of the natural veldt portrayed in Fig. 4.23.

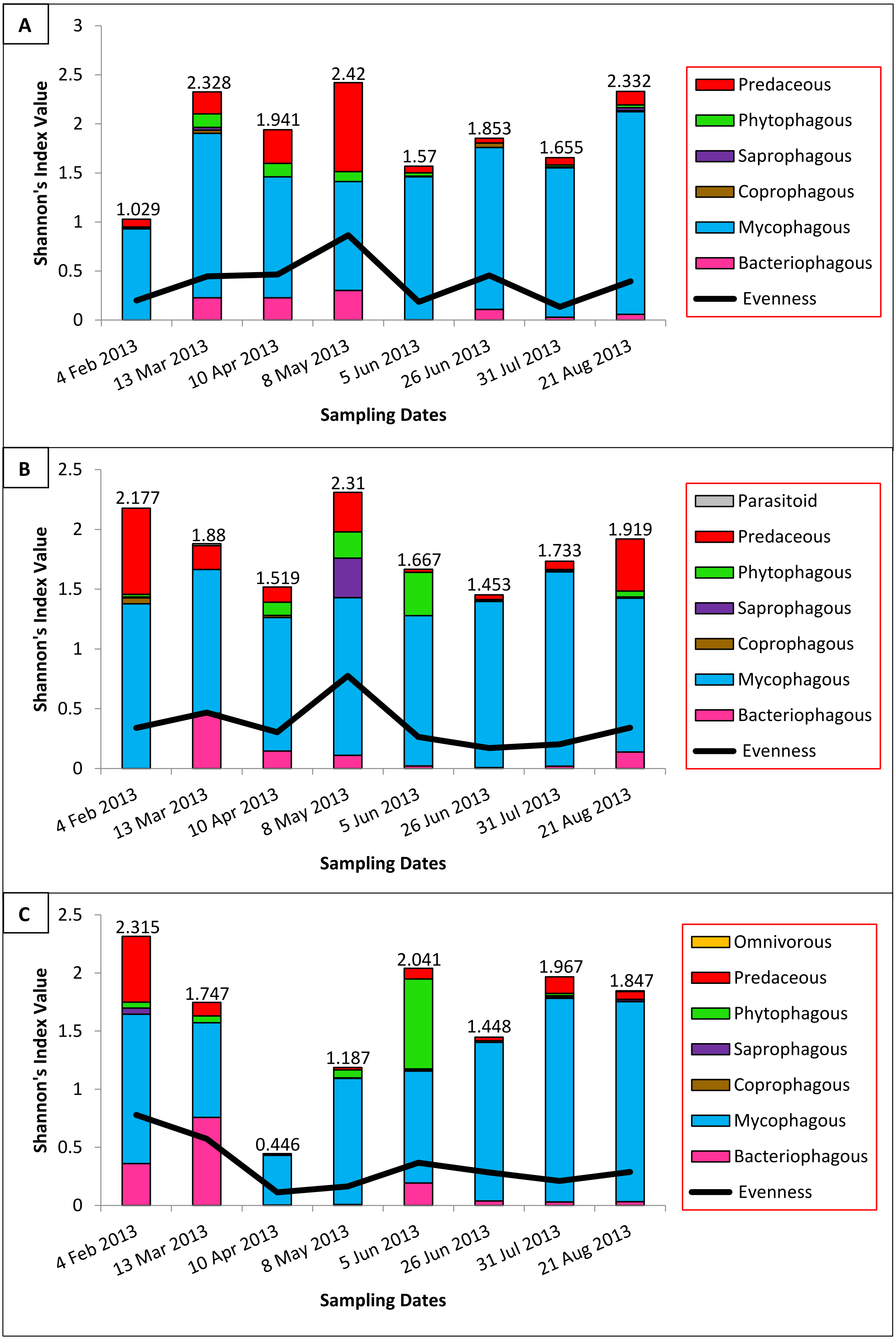


Figure 4.19: Sampling points 64 – 66 on the farm Klein Brittanje during the primary study in 2013, where natural veldt was converted into a maize field. A: sampling point 64 within non-Bt. maize; B: sampling point 65 within Bt. maize; C: sampling point 66 within Bt. maize. (See Addendum A for faunistic detail.)

iv) <u>Soil organism diversity in fallow fields *vs*. planted fields</u>

Fallow lay systems are used in agricultural landscapes to enable the soil to recover and in the process retain moisture, especially in dryland cultivation systems such as the farm Klein Brittanje. This survey was conducted to examine the soil organisms that occur in fallow fields. This information should prove meaningful in the understanding of soil organism populations and the effect of post-harvest rest periods on these organisms. Various trophic levels were present throughout the fallow and planted fields on Klein Brittanje farm (Fig. 3.23). The diversity values within both fallow and cultivated fields were also relatively high and did not fluctuate as much as those of the irrigated fields at the farm Vaaldam. This could be due to hystereris, *i.e.* memory of past circumstances and events used in the response to current conditions (Wall 2012), that implies that the environment would recover and try to restore its status to what it was before the disturbance. This process is more successful in an agricultural system that has rest periods or a fallow lay system. These soils also tend to have a higher resilience, since their diversity is more stable without dramatic organism turnover and succession.

As previously mentioned, the diversity throughout sampling points 60 to 63 stayed relatively constant with minimal fluctuations (Fig 4.20). The diversity within all four sampling points at 4 February 2013 was high with an H-value of > 2.4, after which it decreased. Sampling points 60 and 63 (Fig. 4.20 A & B) were fallow during 2013 and showed an increase in the diversity value during April, whereas sampling points 61 and 62 in the cultivated fields (Fig. 4.20 C & D) showed an increase in April and increased even further in May. These increases were due to an increase in larvae in the soil, in preparation for the onset of winter. The rest of the fluctuations are not as severe and are due to fluctuations in collembolan and mite numbers. Although all trophic levels were represented at all of these sampling points, a more regular presence of bacteriophages was noted at sampling points 61 to 62, indicating the presence of bacterial activity which is usually a sign that mechanical disturbances had occurred. This is expected since these sampling points are within the cultivated fields (Fig. 4.20 C & D).

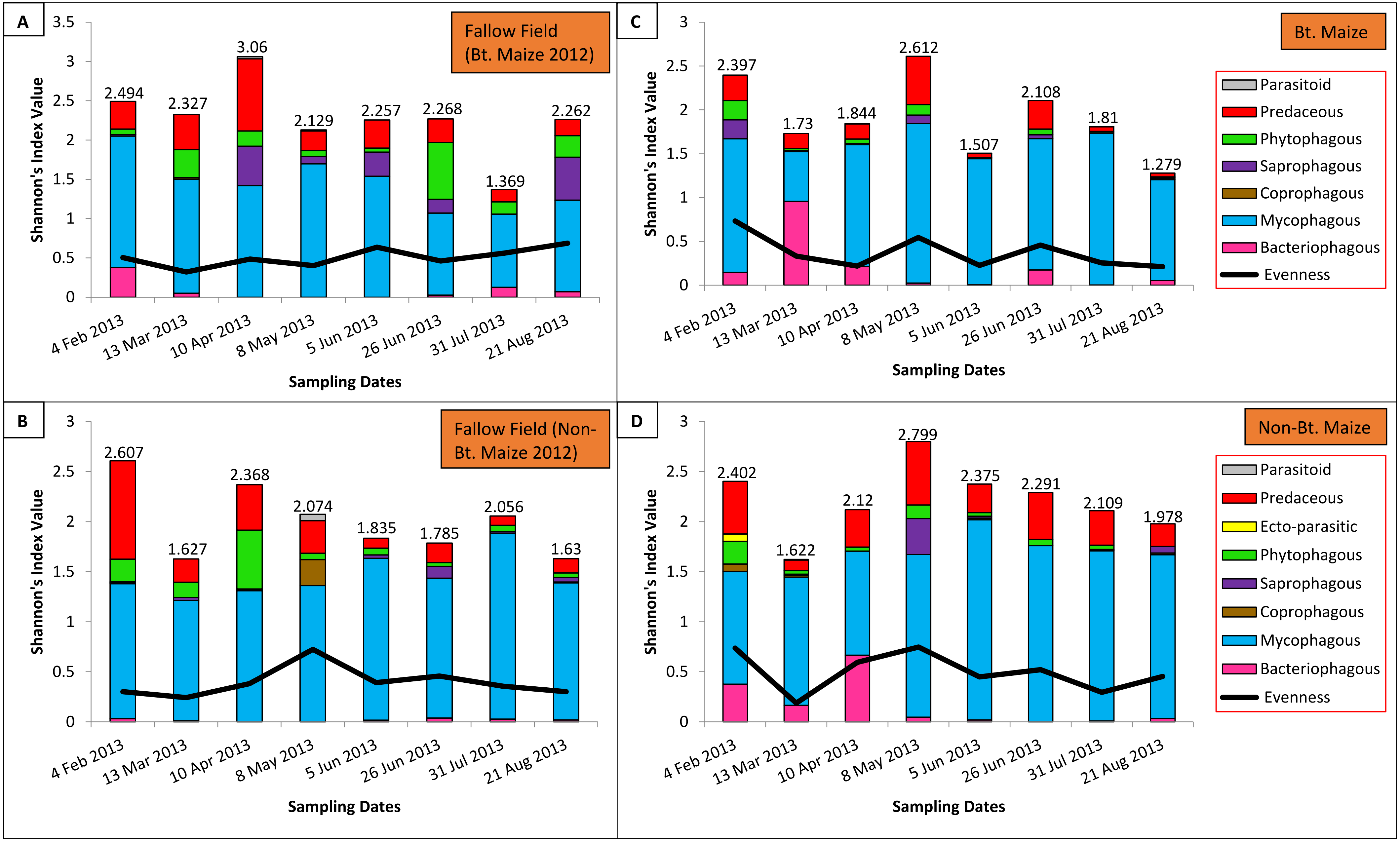


Fig 4.20: Sampling points 60 – 63 on the farm Klein Brittanje during the primary study in 2013. A: sampling point 60, fallow field after cultivation with Bt. maize in 2012; B: sampling point 63, fallow field after cultivation with non-Bt. maize in 2012; C: sampling point 61, field cultivated with Bt. maize; D: sampling point 62, field cultivated with non-Bt. maize. (See Addendum A for faunistic detail.)

Sampling points 71 and 72 showed a high proportion of phytophages, similar to the fallow fields adjacent to it. These sampling points showed a relatively stable trend in diversity values (Fig. 4.21), although the trophic proportions of these two sampling points differed from that of the other cultivated fields (Fig. 4.20) and they had a lower species richness (Addendum B).

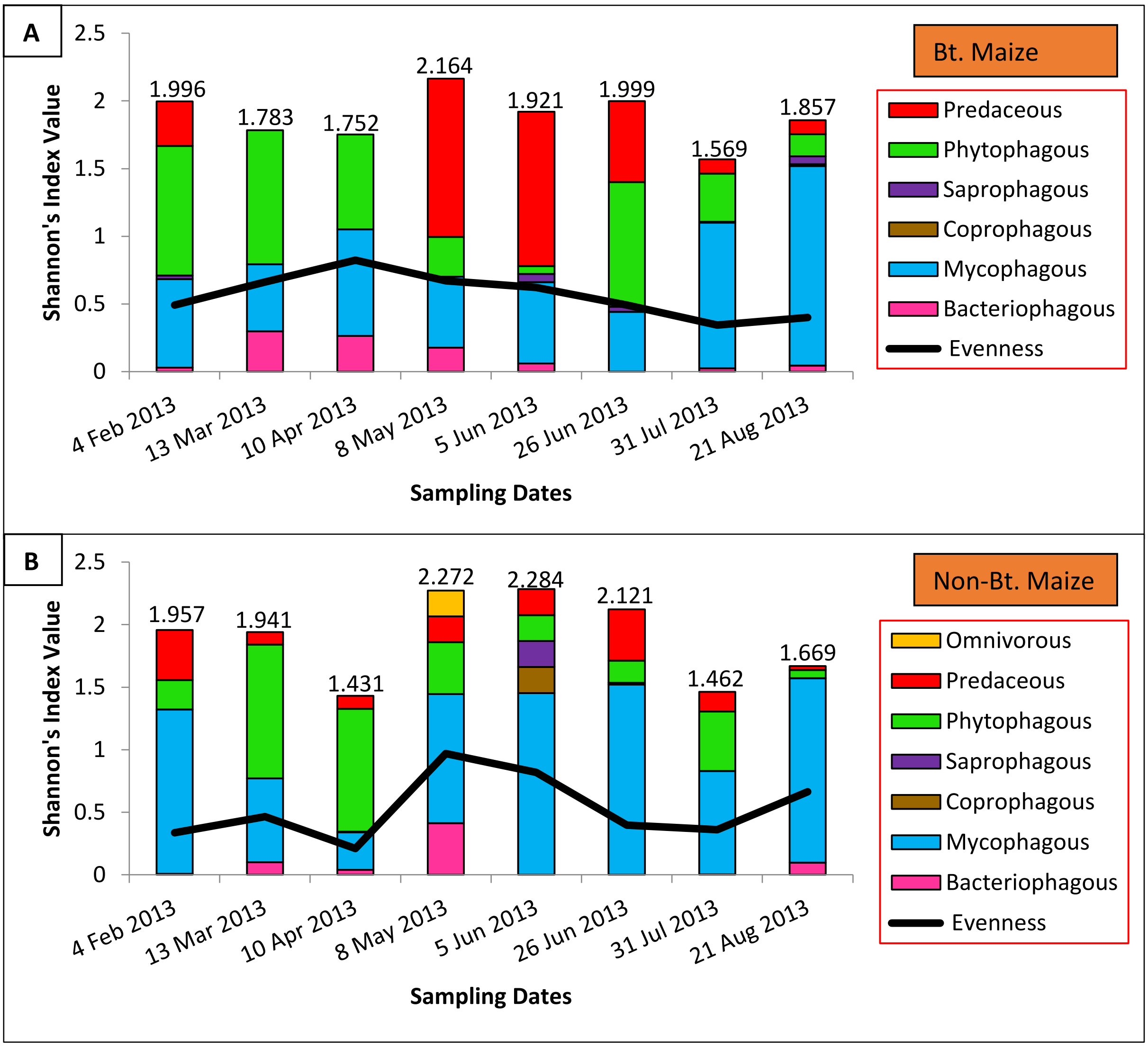


Fig 4.21: Sampling points 71 – 72 on the farm Klein Brittanje during the primary study in 2013. A: sampling point 72, field cultivated with Bt. maize; B: sampling point 71, field cultivated with non-Bt. maize. (See Addendum A for faunistic detail.)

Sampling points 68 to 70 on Klein Brittanje farm were left fallow during 2013. The diversity in these fields fluctuated greatly, with no apparent pattern of diversity values. These fields, however, show the presence of bacteriophages that correspond with the fact that they were mechanically ripped to encourage water infiltration and retention.

The relatively high phytophage presence within these fields is due to the regrowth of maize (Fig. 4.22).

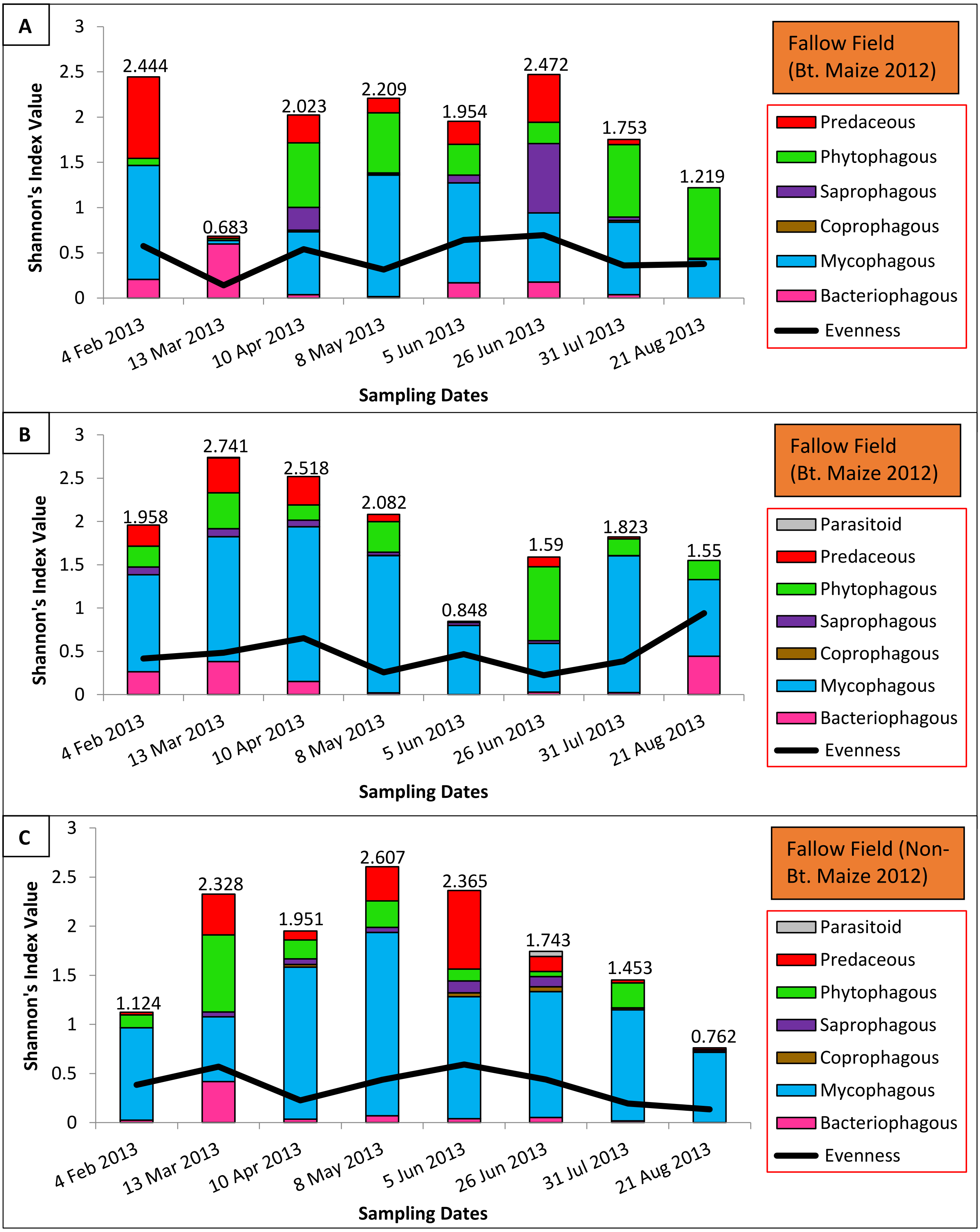


Fig 4.22: Sampling points 68 – 70 at the farm Klein Brittanje during the primary study in 2013. A: sampling point 68, a fallow field after cultivation with Bt. maize in 2012; B: sampling point 69, a fallow field after cultivation with Bt. maize in 2012; C: sampling point 70, a fallow field after cultivation with non-Bt. maize in 2012. (See Addendum A for faunistic detail.)

Sampling points 67, 73 and 74 were within natural veldt at Klein Brittanje farm. These sampling points showed relatively constant diversity values with representatives from all trophic levels (Fig. 4.23).

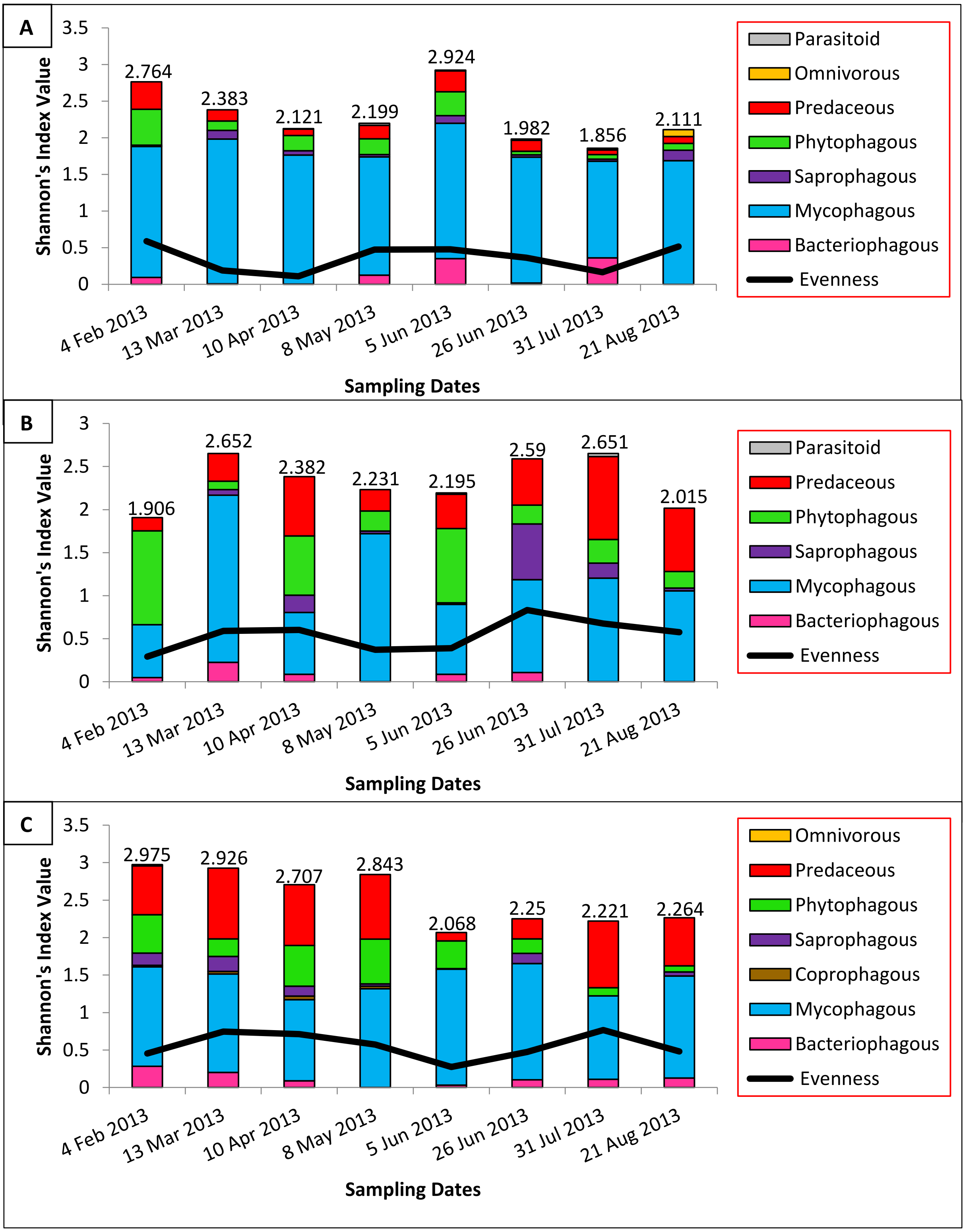


Fig 4.23: Sampling points 67, 73 and 74 in the natural veldt on the farm Klein Brittanje during the primary study in 2013. A: sampling point 67; B: sampling point 73; C: sampling point 74. (See Addendum A for faunistic detail.)

Sampling points 73 and 74 had similar vegetation and even though the humidity was higher at sampling point 73 throughout this study, these two sampling sites showed the same trends in diversity values and trophic representation (Fig. 4.23 B & C). Sampling point 67 had a higher humidity and a much thicker grass cover throughout the duration of this study. This give rise to the larger proportion of mycophages that was present (Fig. 4.23 A).

c) Conclusion

Soils are inhabited by a wide range of taxa, which are adapted to be successful under the particular conditions provided by the soil. These organisms provide many ecosystem services that can benefit both the biotic and abiotic components of the soil. Since their mobility is limited, these organisms are, however, sensitive to change, because they cannot readily migrate to more favourable conditions.

Agricultural fields are in a state of 'chronic disturbance', especially in irrigated systems that are cultivated twice per annum. Such systems showed large fluctuations during this study, indicating a large species turnover rate and the presence of active ecological succession. The dry land fields at Klein Brittanje showed more constant diversity values, since these fields are part of a management program that include fallow periods that allow recovery. The diversity values at Klein Brittanje were much higher than those at the farm Vaaldam, which indicate a more stable and successful soil community. It is also indicated by the species richness which was higher at Klein Brittanje than that of Vaaldam and with the smaller fluctuations between the minimum and maximum abundance recorded at Klein Brittanje (Addendum B).

Furthermore, it was found that certain soil mesofauna are definitely present in higher numbers in fields with specific plants. This was noted with the higher diversity presence of collembolans within the cotton field and natural veldt, as opposed to potato fields. The diversity values between samples collected from fields with grasses also differed from those from fields with dwarf-shrubs. This is due to the higher plant cover provided by grasses and their input into the humus layer surrounding each plant. This increases food sources and help maintain moisture levels, two important factors that influence soil faunal diversity.

Physical factors such as clay percentage also influence soil organisms since clay soils have reduced pore spaces (the habitat of soil mesofauna) – and retain more moisture which can lead to waterlogging. This study at Thornberry farm, found that the close proximity of sampling points showed relatively slight changes in species assemblages in spite of different clay percentages and allowed for some species to still be present at both the highest and lowest levels. In a system with a more gradual transition from a low to high clay percentage the difference in soil faunal communities would probably be larger. Coupled to this is that soil faunal numbers are also influenced by soil moisture and a spike in soil moisture usually correlated with a spike in the number of individuals that were present. This was also true for changes in temperature, where an increase in the diversity values was observed within the natural veldt at Thornberry farm, when the temperatures rose in the five days prior to sampling during the colder months. This was probably due to an increase in plant growth activity, together with an increase in organism activity.

The effect of a slope in an irrigated agricultural field at the Vaaldam farm was minimal, since the management of this field was adapted to address this problem and reduce water logging and erosion. Therefore, only small effects were noted with the drier parts having less organisms than the wetter parts, with a noteworthy increase in saprophages in the wetter parts. Biocides and fertiliser were, however, applied throughout this study which could have affected these results. Separate studies should be conducted to evaluate the role of such applications at different localities.

Stubble-burning at both the farms, Thornberry and Vaaldam, initially reduced diversity values, but these values increased again within a month after stubble-burning was applied. This was due to the addition of trace elements and the stimulation of nitrogen fixing bacteria within these fields on account of the burning process which could increase both plant growth and the trophic levels represented, in particular bacteriophages. The condensation effect after a fire also influences the organisms present. This is probably due to the small size and limited mobility of these organisms that were automatically transferred towards the surface as the moisture moved upwards. This is suggested by the presence of collembolans, specifically *Mesaphorura* spp., that are usually found much deeper in the soil. When the stubble was incorporated into the soil, food source in the soil were increased, leading to a spike in organism numbers. Over time these numbers even out, giving rise to a higher evenness value, and create a more stable environment that is not dominated by a single group or species. Soils with a higher organic compound tend to be more resilient in the presence of disturbance. However, it is important to manage fallow fields because in the absence of plant cover these fields can develop problems such as increased compaction which was noted at the farm Thornberry.

The immediate effects of tillage include the reduction of larger organisms and the general disruption of the vertical distribution of soil organisms. Again at the farm Klein Brittanje, an increase in bacteriophages was noted since bacterial activity is known to increase after tillage. The conversion from natural veldt to a maize field, required extensive practices which led to the variations recorded within the trophic levels and diversity values. However, a year later, these fields contained trophic levels similar to the other older fields and reflected more stable diversity values than in the previous year.

Throughout this study it was noted that all soil communities tend to restore its diversity and establish a more stable trophic structure after disturbances were discontinued and external influencing factors were removed from the system. The resilience and recovery rate of these communities, however, depended on the severity of the disturbances as well as environmental factors (climate, vegetation, soil type).

d) References

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Chapter 5

POLLUTANTS, PESTICIDES AND AGRICULTURE – THE EFFECTS ON SOIL FAUNAL GROUPS AND HOW COLLEMBOLA FITS IN

Soils are inhabited by a wide variety of biota with representatives from all three domains; Bacteria, Archaea and Eukaryota. This enables soils to form many combinations of food webs and interactions that lead to complex community structures, providing various ecological services (Scheunemann *et al.* 2015). This complexity and thus optimal functioning of soil communities are threatened by anthropogenic influences, such as biocide applications, mining activities and agricultural practices (van Straalen & van Rijn 1998, Parker 2010).

Soils endure multiple disturbances, which include environmental as well as human-driven disturbances (Wall 2012). Due to the presence of mechanical disturbances during agricultural soil preparation, human selection has taken place and organisms which are more tolerant to such conditions, have become dominant. This is problematic since this reduction in species richness leads to a reduction in diversity of these soil organisms and causes genetic vulnerability (Neher & Barbercheck 1999, Brussaard *et al.* 2010). Unfortunately anthropogenic influences are not limited to physical disruptions, but include pollution and the use of biocides and chemical fertilizers, which further reduce species richness. These applications not only reduce diversity, but also interrupt ecological succession within the applied area. The application of biocides has become an essential part of farming and although recovery will take place, the system may retain traces of certain disturbances (Neher & Barbercheck 1999).

In crop agriculture various chemicals are used for a variety of purposes. These chemicals do not all have the same effect on soil biodiversity and the influences can be direct or indirect, depending on the type of chemical and the organisms present (Wall 2012). Collembola is one of the soil faunal groups that contribute to the ecological function of soil ecosystems. Although these organisms have been ignored for quite some time in South African soils, their role in these soils are presumed to be important (Janion *et al.* 2011). Furthermore, some collembolan species, such as *Folsomia candida*, have also been beneficial as test organisms and are widely used in experiments on the effects of pesticides and pollutants in the environment (Fountain & Hopkin 2005). Several case studies are presented below to demonstrate the role of collembolans in the soil.

a) Grassland pollution by a gold mine tailings dam

Mining plays a major role in the South African economy, but there are some negative effects (Wright *et al.* 2014). These mines produce large volumes of mine waste which is stored in tailing dams. Under poor management these dams can become a major problem which usually leads to environmental contamination on account of seepage or leakage (Rösner & van Schalkwyk 2000). This is of concern since these draining contaminants are acidic and contain high levels of heavy metals (McCarthy 2011, Rembuluwani *et al.* 2014).

In the case of seepage from tailing dams, appropriate measures must be taken. Rösner and van Schalkwyk (2000), suggested the liming of contaminated soil in order to prevent heavy metals from contaminating the ground water and to ensure future land use and re-cultivation. Unfortunately, the rehabilitation procedures applied at the location of this case study could not be obtained.

Although trace elements occur naturally in soils all over the world, they can be harmful to the environment if natural levels are exceeded. Tailing dams contain high levels of such elements and compounds. Contamination by these elements can be through wind and water distribution and are responsible for cardiovascular, respiratory and neurological problems in humans that live in close proximity to the tailing dams (Wright *et al.* 2014). Some elements can even affect plant growth, *e.g.* Cobalt (Co), nickel (Ni) and sink (Zn) are phytotoxic elements and result in the loss of plant cover which poses complications for rehabilitation processes (Rösner & van Schalkwyk 2000).

The importance of soil communities and the need to protect these organisms has been recognised and pursued in many countries, with seemingly promising outcomes in countries such as Germany, Australia and Switzerland (Dunger & Voigtländer 2005). Some of the strategies applied include the development of assessment criteria that can use soil organisms to evaluate soil quality. Collembolan species have proven to be an advantage for such assessments, since they have, amongst others, shown a clear successional pattern during a 46 year study at an abandoned mine in Germany (Dunger & Voigtländer 2005). Collembola have also been beneficial in experiments with heavy metal contamination and extreme environment evaluation, since certain Collembola prove to be more tolerant than others, a factor which could be used in bio-indication (Fountain & Hopkin 2004).

i) <u>Sampling points at spillage sites</u>

The soil of this locality, Eureka farm (Fig. 3.18), was analysed and a list of heavy metals that were detected is provided in Chapter 3 (Table 3.4). Soil samples from 26 June 2013 and 3 March 2014 were used in the analysis. The fluctuations in these values were can be ascribed to environmental factors such as wind, rain and according to Rösner (1999) elements like Ni and Zn have a high mobility in soils. This locality is in a summer rainfall area and it rained during the sampling period, which influenced the levels of these elements on account of leaching (Fig. 5.1).

According to Cortet *et al.* (1999), studies at sites with heavy metal pollution showed reduced respiration and nitrification rates in the soils. This reduction is due to the decrease in faunal activity (Filser *et al.* 1995) and would render the re-establishment of plants challenging.

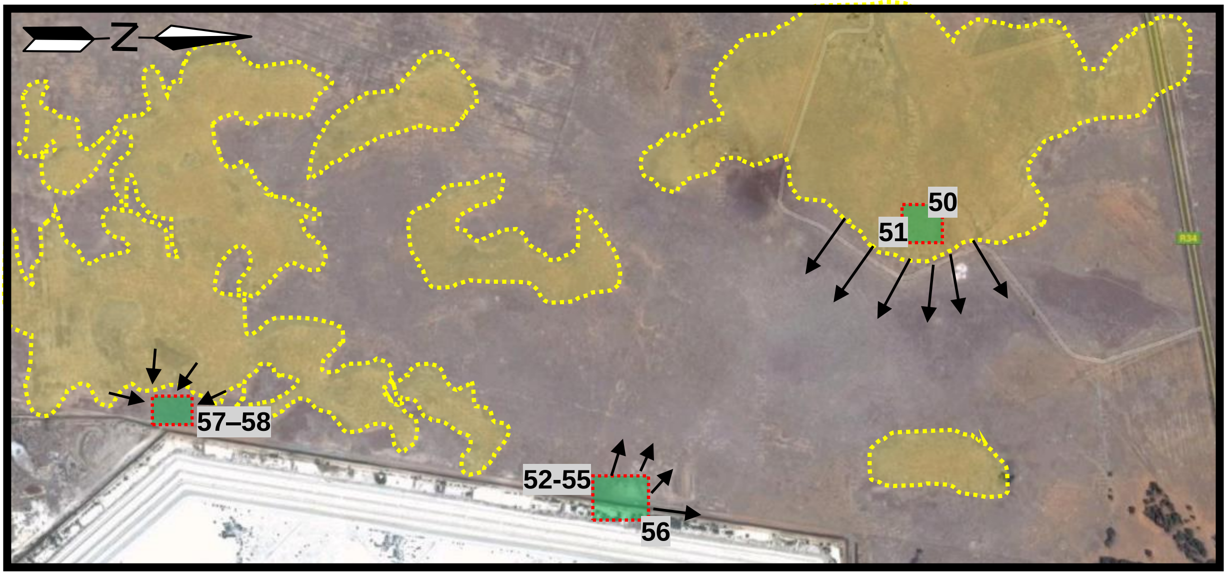


Fig. 5.1: Google™ image of sampling points (red boxes) on the farm Eureka near Odendaalsrus. The areas circled in yellow indicate elevated areas, responsible for leaching effects (black arrows), in turn responsible for differences in pollutant levels after rain (see Chapter 3, Table 3.4).

On 3 March 2014, most of the samples showed a decrease in diversity. This was due to an overall reduction in both species richness and abundance. The only sampling points that showed no decrease in diversity on 3 March was sampling point 57 (Fig. 5.2 A) and sampling point 50 (Fig. 5.4 A), which were both sampled amongst grasses in a grassland. This could be due to the plant cover and/or the influence of a larger root mass, acting as sanctuary against the changing environment.

Phytophagous organisms seemed to be represented in most samples throughout the sampling period with the only exception at sampling point 53 (with bulrushes) on 3 March 2014 (Fig. 5.2 D). The phytophages were represented by various groups, with a high number of individuals sampled from the Hemiptera (Coccoidea: cf. Pseudococcidae nymphs & Aphididae nymphs) on 8 June 2013 (Addendum A). Oribatida was poorly represented with only a few Oppiidae individuals recorded. On 11 January 2014 at sampling point 52, Drosophilidae sp. 1 was recorded (Addendum A). According to Hopkin (1997), certain species in this family are resistant to certain pollutants which could explain their presence at this sampling point. Sampling on 11 January 2014 at sampling point 57 (Fig. 5.2 A), showed a decrease in diversity and evenness due to the presence of 117 collembolan individuals of the same species (*i.e.* Bourletiellidae sp. 2).

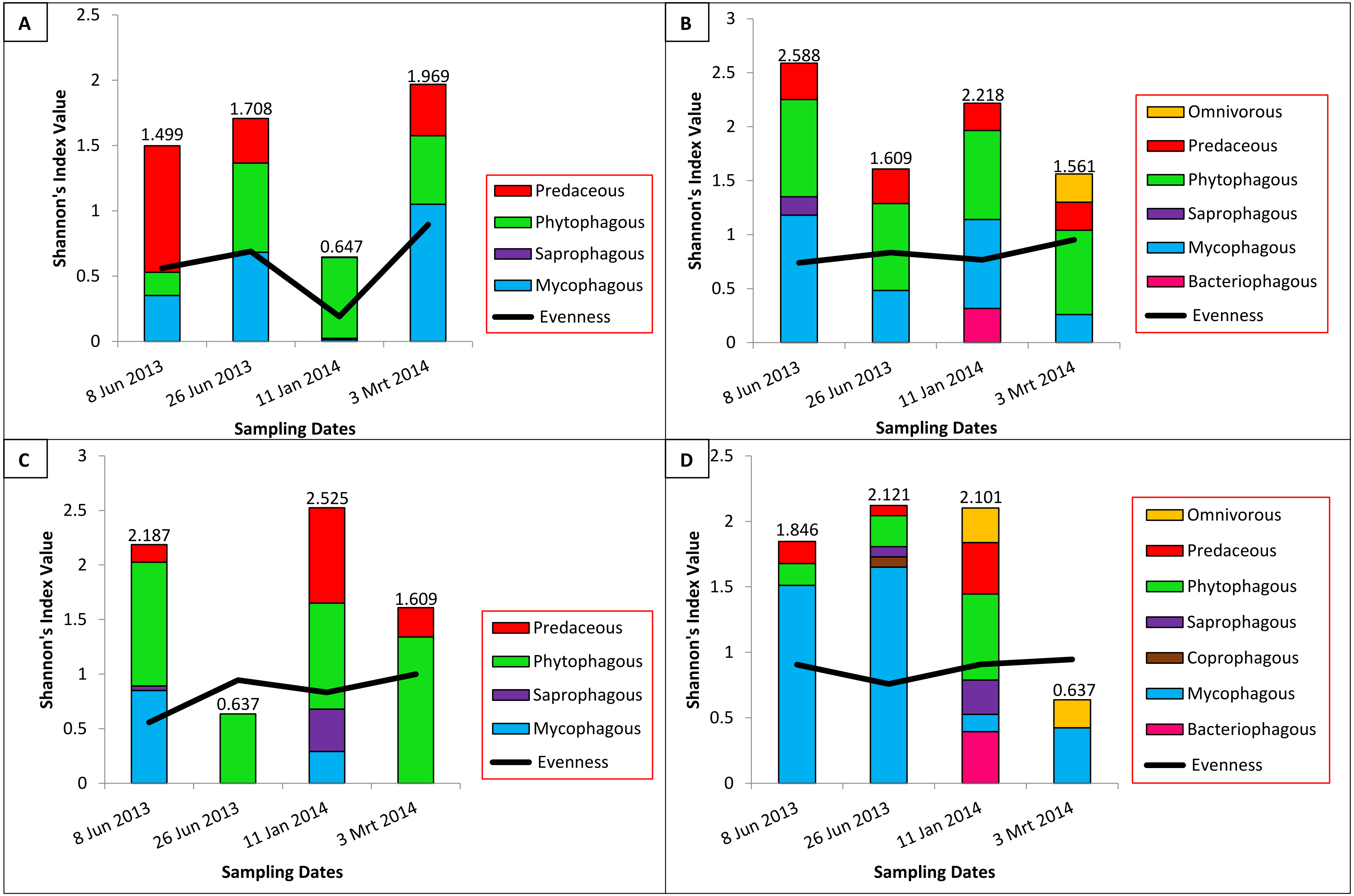


Fig. 5.2: Sampling points within contaminated veldt at the farm Eureka near Odendaalsrus. A: sampling point 57 amongst grass (Spillage in 2000); B: sampling point 58 amongst bulrushes (Spillage in 2000); C: sampling point 52 amongst weeds (Spillage in 2006); D: sampling point 53 amongst bulrushes (Spillage in 2006). (See Addendum A for faunistic detail.)

Sampling points 54 and 55 were represented by different trophic levels, even though they were only 2m apart from one another on the same mine dump canal wall (Fig. 5.3 A & B). This could be due to the differences in plant porospheres sampled, humidity and/or heavy metal levels (Chapter 3, Table 3.4). The diversity at sampling point 56 increased over the first three sampling dates, which could be due to the decreasing water level in the canal. The diversity decreased again after heavy rains (Fig. 5.3 C). After the rain (Fig. 3.22), the presence of the heavy metals also increased in the canal (Table 3.4).

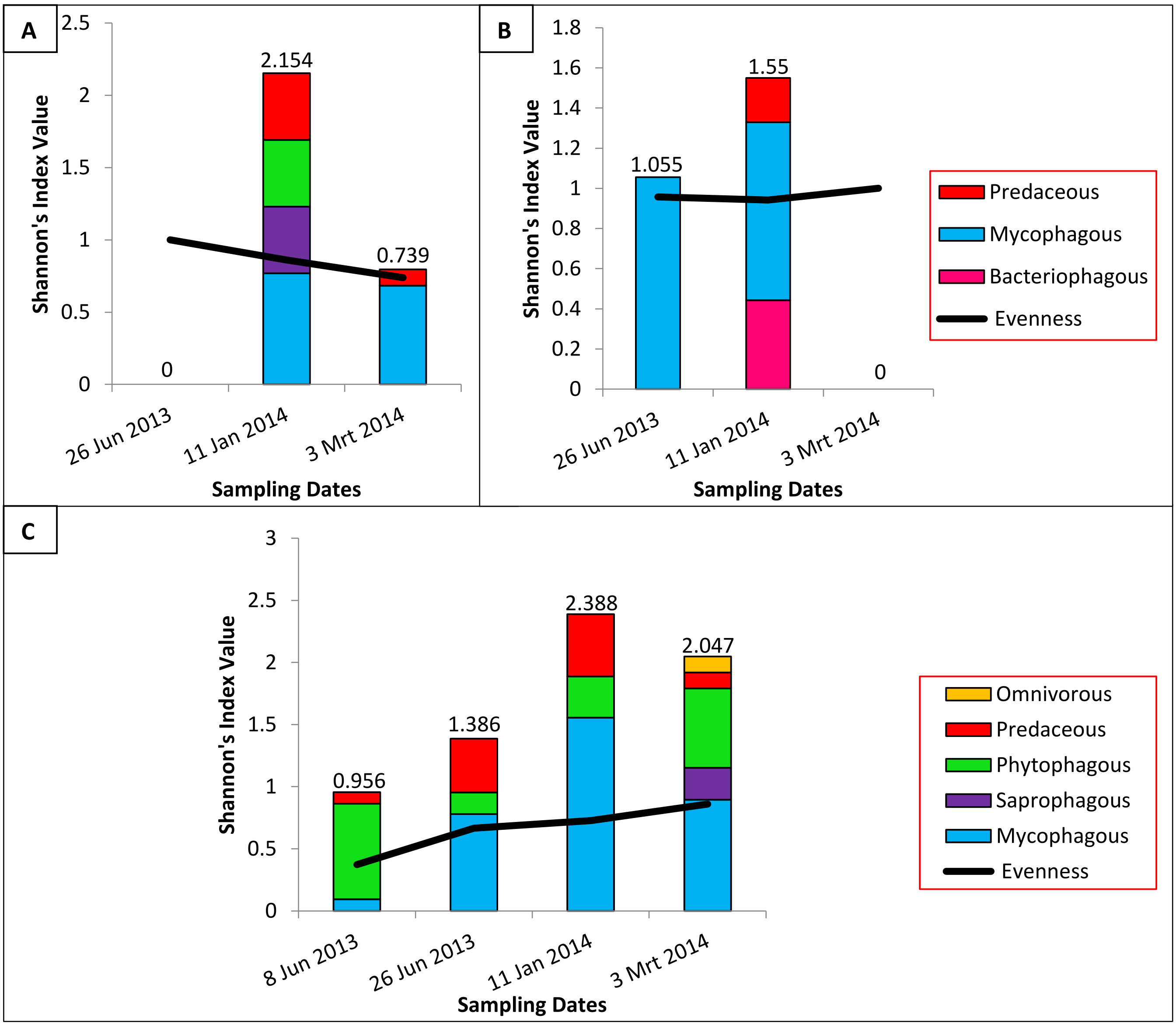


Fig. 5.3: Sampling points 54 – 56, situated on the wall of a canal which surrounds a gold mine tailings dam on the farm Eureka near Odendaalsrus. A: sampling point 55 amongst grass on the wall of the canal; B: sampling 54 at reeds on the wall of the canal; C: sampling point 56, amongst bulrushes growing on the inner side of the canal wall. (See Addendum A for faunistic detail.)

ii) Sampling points as control (away from spillage sites)

Sampling points 50 and 51 were selected to serve as control samples, but after the chemical analysis of these soils, it was noted that the pollutants were also present here (Chapter 3, Table 3.4). This was due to wind dispersal of the chemicals, but a control of sorts was provided in that levels were lower than that of the actual spillage sites. These sampling points were represented by a higher number of individuals and the diversity seemed more stable (Fig. 5.4).

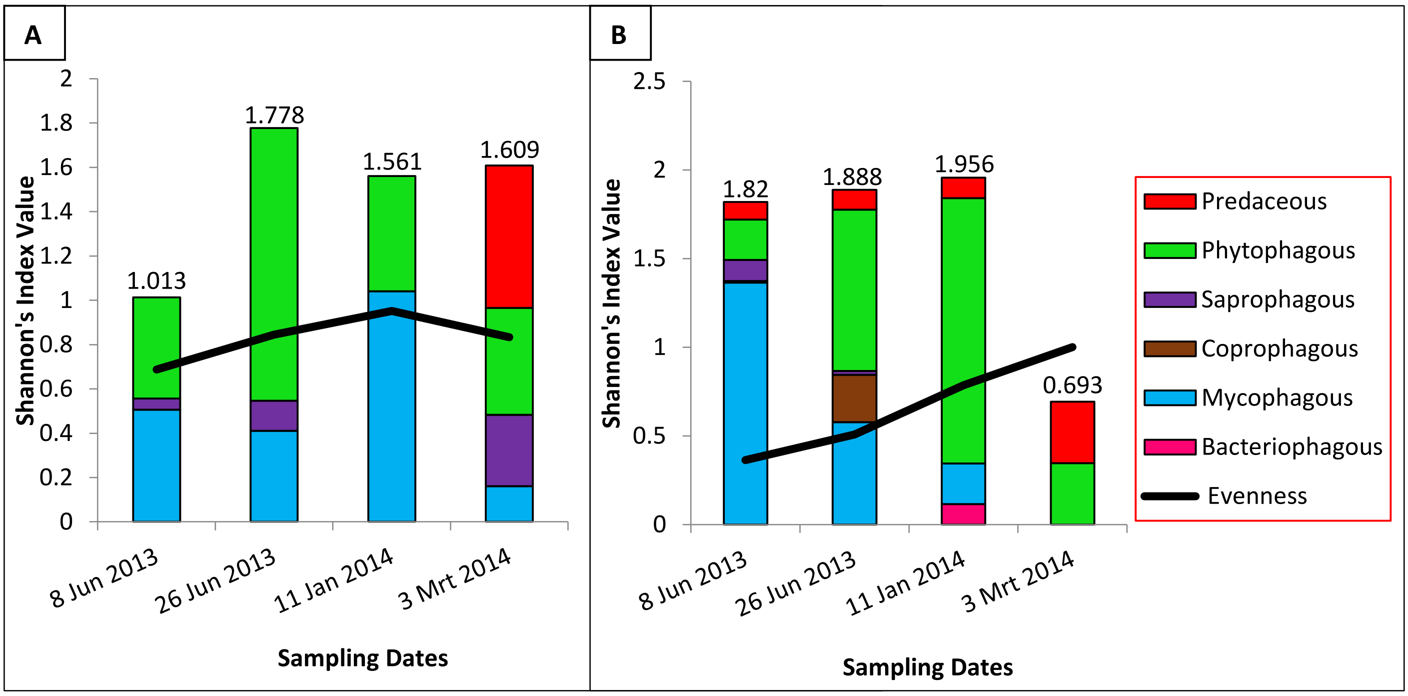


Fig. 5.4: Samples within the veldt that was not contaminated by waste dam spillage on the farm Eureka. A: sampling point 50 amongst grass; B: sampling point 51 at a dwarf-shrub. (See Addendum A for faunistic detail.)

The increase noted in the diversity on 11 January 2014, was due to an overall increase in the presence of various hexapod larvae. Throughout this study the trophic proportions were relatively evenly spread between the represented trophic levels and was seldom only dominated by a certain trophic level. This could be an indication that the organisms that occur at these sampling points are better adapted to this environment and even though they are present in low numbers, most trophic levels were still represented.

b) Pesticides in a grassland

Farmers are under pressure to supply the growing demand for food, which results in the intensification of land use (Scheunemann *et al.* 2015). To achieve optimal yields, farmers rely on chemical inputs to enhance plant growth and protect plants from ailments and phytophagous pests. These additives, however, do not only influence the target organisms, but in many cases also have a negative effect on other parts of the environment and its inhabitants. An example is the disruption of communication between alfalfa plants and their associated N-fixing bacteria in the presence of specific insecticides. This has proven problematic, with reduced plant growth as a result of inefficient N-fixation (Wall 2012). In a study done by Beare *et al*. (1992), the effect of biocides on non-target organisms of a food-web was illustrated with significant differences noted in the numbers of certain organisms after their predators or prey items were eliminated by a specific biocide.

Soil fauna fulfill important functions in the soil from which humanity can benefit. One of the important uses of soil fauna are as indicators of disturbances and pollution. The indicator reaction of these organisms can be due to indirect or direct effects (Cortet *et al.* 1999). Predatory mites from the cohort Gamasina are affected more severely by pesticide applications than other organisms. Herbicides, on the other hand, can cause different effects, since they are not only responsible for the loss of plant cover, but glyphosates can influence the production of plant defense compounds, increase detrimental bacteria and decrease beneficial fungi and biological control agents (Wall 2012).

Evaluations in the environment are more challenging than laboratory studies, since in the laboratory all conditions can be controlled. Furthermore, on site studies have proven more realistic with a higher validity, since it takes the whole ecosystem into account (Cortet *et al.* 1999). It is important to know the history of a site before any conclusions can be drawn from soil organismal reactions. This is largely because areas that have had previous exposures and disturbances could react differently since their resilience is compromised to the possible exclusion of certain species even before testing has begun (Cortet *et al.* 1999). These findings were supported by Wall (2012), who stated that biocide applications showed adverse effects due to the variation in organisms present in the affected ecosystem and that effects are short-lived in the presence of only one application.

The increase in diversity on 30 April 2014 at Paradys farm, could be due to rain and warmer nocturnal temperatures (Fig. 5.5). Sampling point 48 (Fig. 3.13), a fungicide application, showed a high number of mycophages which mainly comprised the collembolan family Brachystomellidae (*Brachystomella* sp. 1) (Addendum A), explaining the lower diversity at the fungicide application sampling point on 30 April 2014. The decrease in diversity on 7 May 2014 (Fig. 5.5 A-D, F), was due to an increase in abundance, but a decrease in species richness in most of these samples. The only exception was at the fungicide sampling point where species richness increased, therefore giving rise to the small increase in diversity (Fig. 5.5 E).

During the middle weeks (30 April 2014 – 14 May 2014), the proportion of the diversity represented by predators were higher than during the first and last sampling dates (Fig. 5.5). This is due to the opportunistic feeding behavior of predators which increased at this location due to an increase in the number of individuals, and since individuals with a shorter life cycle increased rapidly after the chemical disturbance. These numbers, however, only increased up until 7 May 2014, with the evenness decreasing over time as the number of individuals increased and then decreased over the next sampling periods. This was evident in the increase in evenness and richness on 14 May 2014. On 21 May 2014, all organisms were represented by relatively low numbers, except for *Brachystomella* sp. 1 (Addendum A), which still showed more abundance than the other organisms and caused a reduction in evenness and diversity in the samples. The collembolan numbers that fluctuated was mainly due to rainfall, since these numbers were the highest at the sampling dates with higher humidity readings after the rain (Chapter 3). Bacteriophages was represented by individuals from the mite family Nanorchestidae (Addendum A).

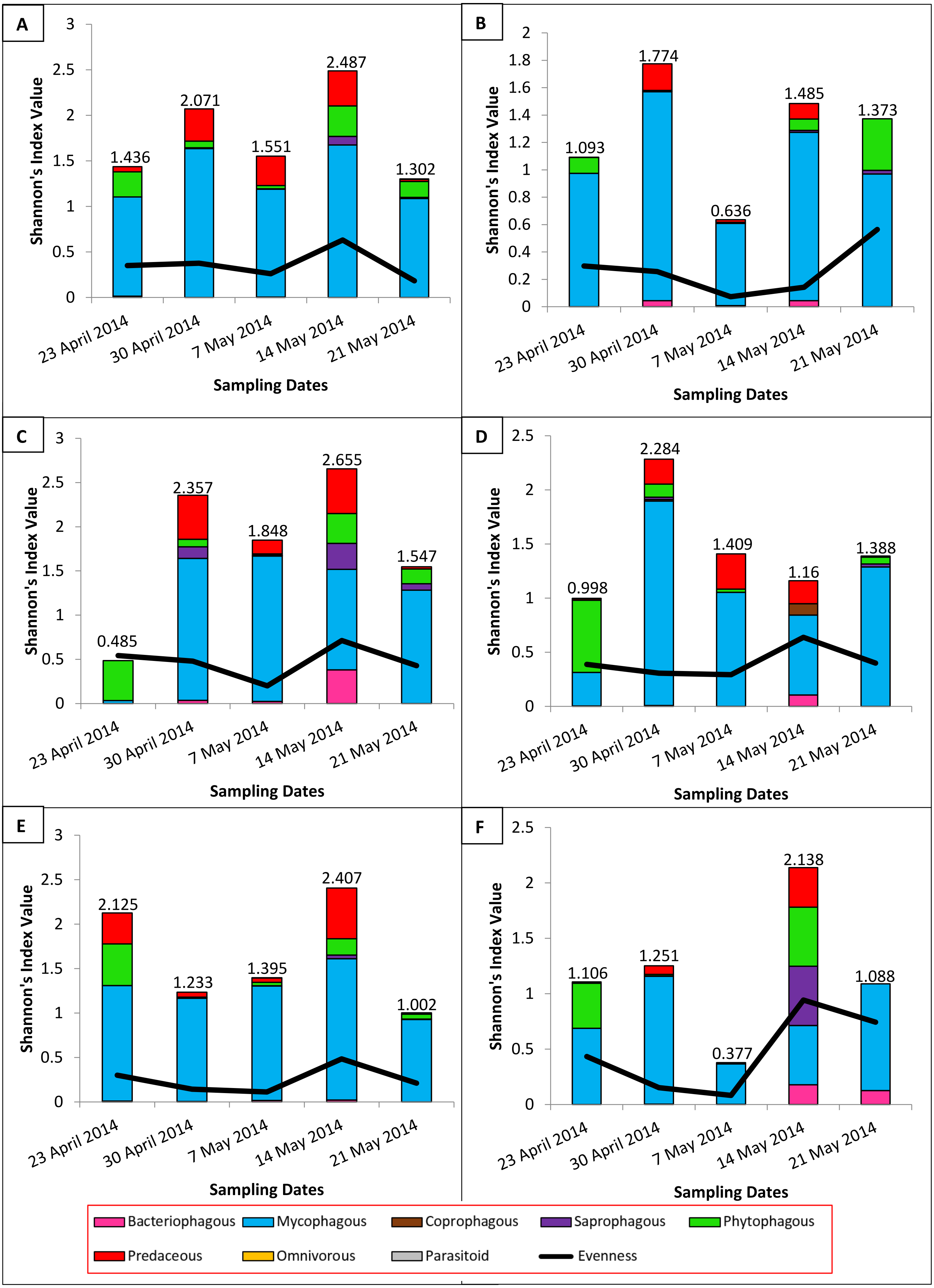


Fig. 5.5: Application of biocides in a grassland at the Experimental Farm of the University of the Free State during 2014. A: Insecticide – sampling point 44; B: Control (no biocide) – sampling point 49; C: Broadleaf herbicide – sampling point 46; D: Broad spectrum herbicide – sampling point 45; E: Fungicide – sampling point 48; F: Grass herbicide – sampling point 47. (See Addendum A for faunistic detail.)

The organisms recorded during this study did not react to the biocides as severely as the fields in Chapter 4. This was probably due to the fact that those fields were regularly disturbed, both chemically and mechanically, which reduced their species richness and resilience level. Pimentel and Edwards (1982) state that the negative effects of biocide usage were only detected after a number of applications were conducted. Another factor that should be kept in mind is that the biocides used in this experiment were just the normal garden variety biocides and not the most persistent chemicals available on the market. The study area did not have a high clay percentage and it rained during the study, which also influenced the persistence of these biocides. According to Pimentel and Edwards (1982), both the toxicity and persistence of each biocide will affect the recovery period of a site and should be kept in mind when choosing a biocide.

c) Collembolan incidence and observations

Collembola are one of the largest groups of soil biota and are important role players in food web structures, since this group has representatives at all trophic levels (Sabias *et al*. 2011). The role of Collembola in soils reaches even further, since individuals of this group can be used as bio-indicators and in the study of ecotoxicology (Janion *et al.* 2011). The term soil biocoenosis was coined by Karl Mobius in 1877 (Dunger & Voigtländer 2005) and is used to describe the living organisms that occupy the same habitat and their interactions. The soil biocoenosis of South Africa is, however, poorly studied, with many role players and their respective activities still unknown (Janion-Scheepers *et al.* 2015).

According to Hopkin (1997), the majority of collembolans in terrestrial ecosystems are mycophages and saprophages. Sabias *et al.* (2011) suggested that by feeding on microbes and plant material these organisms can influence decomposition and the nutrient availability to plants. In agricultural systems collembolans can either be beneficial, as their feeding can influence mycorrhizal growth or even lower the extent of some fungal diseases, or detrimental, as pests of forage crops such as alfalfa (Hopkin 1997).

i) Koffiefontein District (Vaaldam farm & Koppieskraal farm)

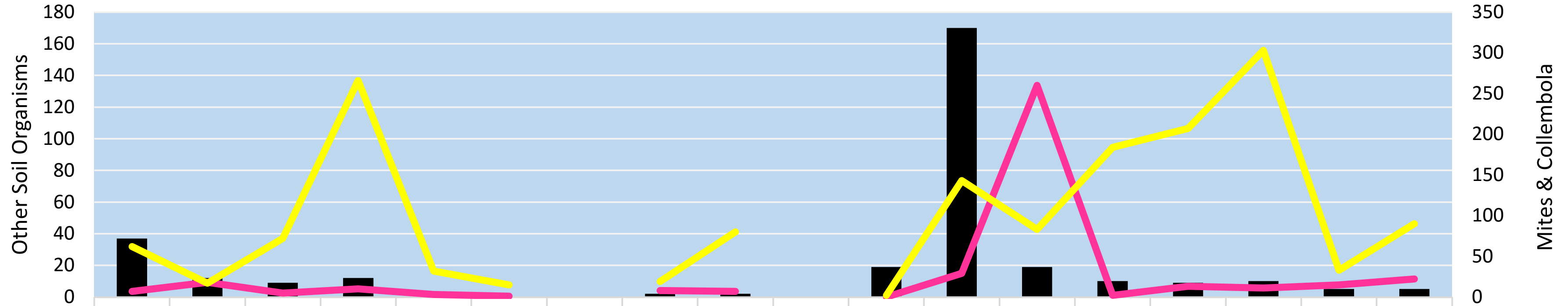
The farms Koppieskraal and Vaaldam are located in the same district and the natural veldt is typical of the Nama Karoo Biome (Fig. 3.3 A-C). Although there has been conflicting opinions regarding plants and their influence on mesofaunal diversity, Sabias *et al.* (2011) concluded that plant species richness did in fact influence the diversity of Collembola, since a higher plant species richness during their study did show an increase in collembolan numbers. Wardle *et al.* (1999) added that soil organisms will have a higher diversity amongst weeds than amongst certain crops, since the biomass of maize, for example, is lower than that of weeds and plant biomass, specifically root biomass, influences soil organism presence. According to van As *et al*. (2012), abiotic factors such as climatic condition and soil type would influence the diversity of plant species that occupy a specific area, which then determine the fauna that will be present.

The farm Vaaldam is located in a drier part of South Africa, with red sandy soils (see Chapter 3). Therefore it would be expected to have less diversity with a higher number of specialized species in the natural veldt and due to the 'chronic' disturbance in the cultivated fields, a filtering effect would be expected. This would select for species with a high reproduction rate and resistance to chemical disturbances. The Collembola from sampling point 1 (Fig. 3.2), at this locality was represented by low numbers with small variation (Fig. 5.6 A). *Proisotoma* spp. (Isotomidae), were the most abundant at this sampling point. Other collembolans sampled at this sampling point included *Mesaphorura* sp. 1 (Tullbergiidae), *Sphaeridia* sp. 1 (Sminthurididae) and *Hypogastrura* sp. 1 (Hypogastruridae) (Addendum A; Fig. 5.8). *Hypogastrura* sp.1 is a more opportunistic species and due to the minimal disturbance in this grassy area, was only represented by 8 individuals. Sampling point 2 was only sampled twice and since these sampling dates were during winter, only a low number of individuals was sampled and consisted of *Proisotoma* spp. (Isotomidae) and *Hypogastrura* sp. 1 (Hypogastruridae) (Addendum A). These low numbers at both sampling points are ascribed to a low humidity since this farm falls within the Nama Karoo Biome which receives relatively low rainfall.

Sampling point 3 differed from sampling points 1 and 2 in that this sampling point fluctuated more severely, with a spike in collembolan numbers during the January 2013 sampling dates. This large fluctuation was again as a result of *Hypogastrura* sp. 1 (Hypogastruridae), but on 18 January 2013 a large number (55 individuals) of *Sphaeridia* sp. 1 (Sminthurididae) was also recorded (Addendum A). This sampling point contained the same collembolan species as the other two samples in the natural veldt, with the exception of higher numbers of individuals and the presence of *Entomobrya* cf. *multifasciata* (Entomobryidae) that were relatively constant throughout the study period. This difference could be due to an increase of humidity or agricultural chemicals as a result of drift - fertilizer applications in the surrounding fields (Fig. 3.4), or due to the variation in plant species, with this site having the highest number of different dwarf-shrub species between the three sampling points in the natural veldt (Fig. 3.3 C). In general the collembolan numbers seemed to follow the same trend as the other soil organismal numbers, although it seemed as if the fluctuations in collembolan numbers were observed a month after that of the other soil organisms at these sampling points (Fig. 5.6 A).

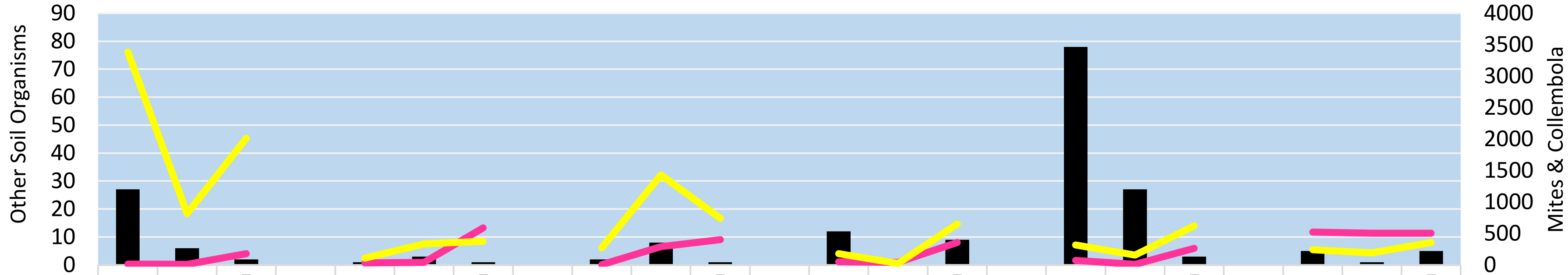
During the stubble-burning study, Collembola generally declined 90 min after stubble-burning was applied and then increased again over time during the following 7 weeks (Fig. 5.6 B). During this period, no mechanical or chemical disturbances were applied in these fields. *Mesaphorura yosii* and *Tullbergia* sp. 2 (Tullbergiidae) and *Proisotoma* spp. (Isotomidae) showed large increases in numbers after 7 weeks had passed. A few *Hypogastrura* sp. 1 (Hypogastruridae) specimens were only present in one of the fields before stubble-burning was applied and their numbers were greatly reduced 90 min after the stubble-burning event. After the 7 week period, the *Hypogastrura* sp. 1 (Hypogastruridae) numbers only increased again at sampling point 23. Only a few *Entomobrya* cf. *multifasciata* (Entomobryidae) were sampled and these specimens were mostly recorded before stubble-burning was applied and 7 weeks thereafter. This is because this collembolan species is larger than the other collembolans and are epiedaphic (surface-dwelling), and could probably not evade the effects of the fire as well as the euedaphic (soil-dwelling) species. However, they seemed to return over time, since they are phytophagous and weed growth was present in some fields.

A



	Sampling point 1						Sampling point 2		Sampling point 3							
	9 Jan 2013	18 Jan 2013	6 Feb 2013	19 Feb 2013	3 Jun 2013	22 Jul 2013	3 Jun 2013	22 Jul 2013	14 Nov 2012	9 Jan 2013	18 Jan 2013	29 Jan 2013	6 Feb 2013	19 Feb 2013	3 Jun 2013	22 Jul 2013
Other Soil Organisms	37	12	9	12	2	0	2	2	19	170	19	10	9	10	5	5
Collembola	7	18	5	10	3	1	8	7	0	29	260	2	13	11	15	22
Mites	62	17	72	266	32	15	19	80	2	143	83	184	207	303	33	90

B



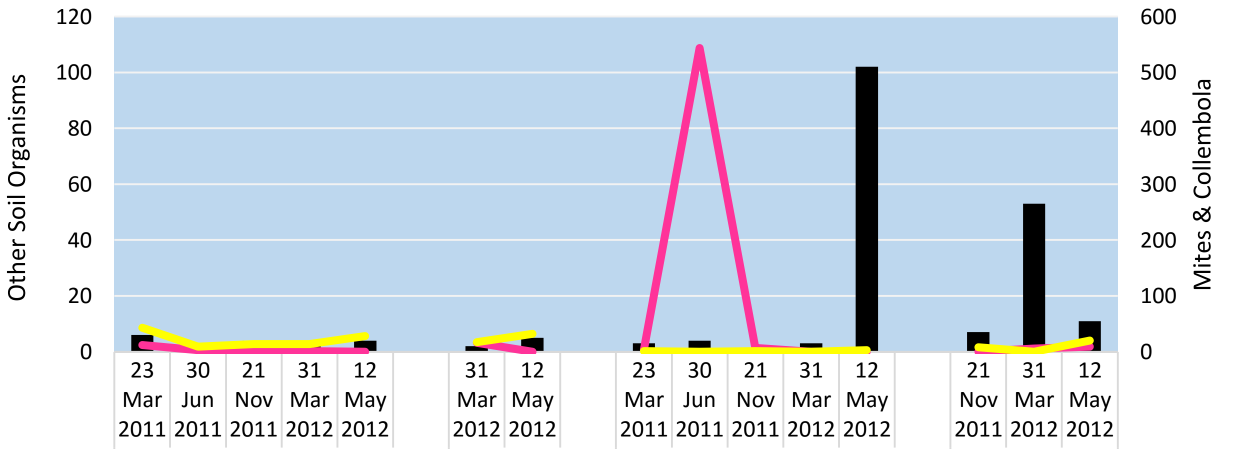
	Sampling point 18			Sampling point 19			Sampling point 20			Sampling point 21			Sampling point 22			Sampling point 23		
	Before	90min After	7 Weeks After	Before	90min After	7 Weeks After	Before	90min After	7 Weeks After	Before	90min After	7 Weeks After	Before	90min After	7 Weeks After	Before	90min After	7 Weeks After
Other Soil Organisms	27	6	2	1	3	1	2	8	1	12	0	9	78	27	3	5	1	5
Collembola	16	10	180	33	39	589	7	289	400	48	42	355	74	9	263	522	502	505
Mites	3382	814	2015	108	335	377	274	1432	742	181	23	652	318	157	620	241	192	355

Other Soil Organisms | Collembola | Mites

Fig. 5.6: Fluctuations in soil faunal numbers at the farm Vaaldam during 2013. A: sampling points 1–3 in the natural veldt (control samples); B: sampling points 18–23 in the agricultural fields which form part of the stubble-burning study. (See Addendum A for faunistic detail.) (Note: y-axis not on the same scale)

According to Benckiser (1997), *Mesaphorura* spp. are expected to occur relatively deep in the soil (15-20cm). Their presence higher up in the soil could be due to the condensation effect, with moisture levels moving upwards through the soil during a fire and in the process ’pulling’ these organisms up as well. Another reason for this presence of these organisms is that they are saprophagous and may have moved upward to benefit from the root masses of all the burnt plants, thereby also aiding in the decomposition process.

One of the responses mentioned by Pimentel and Edwards (1982) in their study, was that there is an increase of collembolan numbers and a coinciding decrease in other soil arthropods. This was also noted at the farm Koppieskraal (Fig. 3.6) in Chapter 4 (Fig. 4.12) and here (Fig. 5.7) and is considered a case of ecological trade-off, where the most likely introduced *Hypogastrura* sp.1 (Hypogastruridae) inhabits an area unsuitable for endemic species (see Leinaas *et al.* 2015). This farm has clay soils which could have influenced the persistence of herbicides, thereby increasing their effect even more. Alternatively, the increase in collembolan numbers could also be due to genetic mutations by this species, since many insects and mites have evolved a level of resistance to certain biocides (Pimentel & Edwards 1982).



	Sampling point 31					Sampling point 30		Sampling point 33					Sampling point 32		
	23 Mar 2011	30 Jun 2011	21 Nov 2011	31 Mar 2012	12 May 2012	31 Mar 2012	12 May 2012	23 Mar 2011	30 Jun 2011	21 Nov 2011	31 Mar 2012	12 May 2012	21 Nov 2011	31 Mar 2012	12 May 2012
Other Soil Organisms	6	2	4	2	4	2	5	3	4	1	3	102	7	53	11
Collembola	12	3	0	1	0	16	0	4	544	7	0	0	0	6	9
Mites	43	9	14	14	28	17	32	1	0	1	0	3	8	0	20

Fig. 5.7: Fluctuations in soil faunal numbers at the farm Koppieskraal during 2011-2012. (See Addendum A for faunistic detail.)

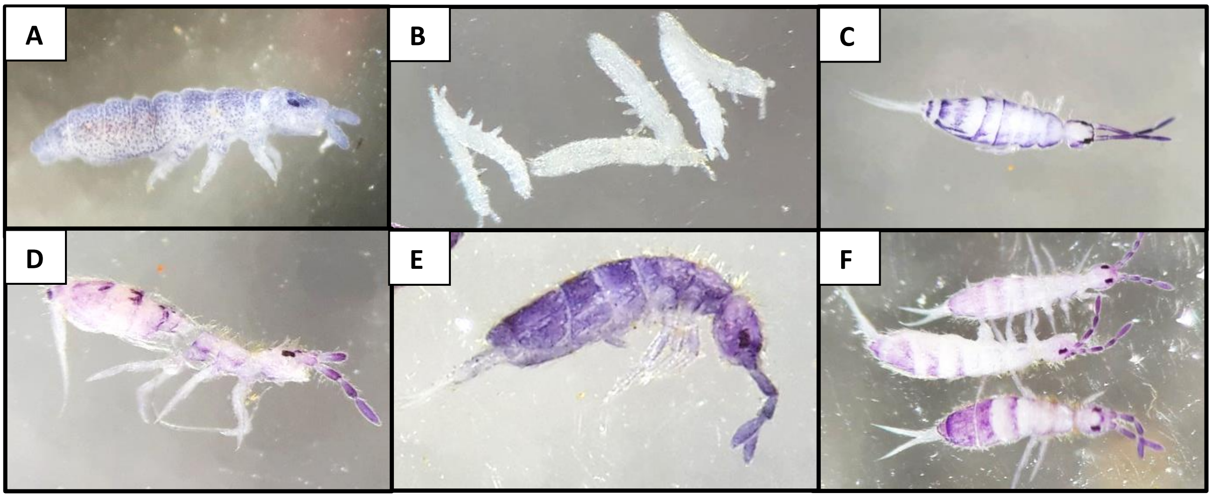


Fig. 5.8: Collembola representatives from the farm Vaaldam. A: *Hypogastrura* sp. (Hypogastruridae); B: *Mesaphorura* sp. (Tullbergiidae); C: *Entomobrya* cf. *multifasciata* (Entomobryidae); D: *Capbrya* sp. (Entomobryidae); E: Entomobryidae sp.; F: *Capbrya* sp. (Entomobryidae).

ii) <u>Jacobsdal District (Thornberry farm)</u>

This locality has a higher rainfall than that of the two previously mentioned farms and is situated next to a river (Fig. 3.9). Sampling point 39 (26% Clay) showed relatively low collembolan presence with the highest number of individuals sampled in July 2013 (Fig. 5.10 A). This spike was due to an increase in *Proisotoma* sp. 4 (Isotomidae) individuals. Other collembolans at this sampling point include *Hypogastrura* spp. (Hypogastruridae), *Entomobrya* cf. *multifasciata* (Entomobryidae) and a single individual of *Lepidocyrtus* sp. 3 (Entomobryidae) (Addendum A; Fig. 5.9). In general the fluctuations in collembolan numbers were not as severe as those of mites. This could be due to the low numbers of collembolans compared to the high numbers of mites in this field. Although collembolan numbers were relatively low, it seemed that the collembolans and mites tend to fluctuate in the same manner in the agricultural field. This was, however, not the case for sampling point 34 in the natural veldt, where there was no correlation. This could be due to food source abundance in the field, as well as a higher humidity.

At sampling point 40 all organisms followed the same trend, indicating that this sampling point did not have a filtering effect that selects for certain organisms and that it could, in effect, sustain organisms throughout different taxa (Fig. 5.10 A). The fluctuations at this sampling point were mainly due to *Entomobrya* cf. *multifasciata* (Entomobryidae) numbers. Other collembolans sampled at this sampling point include *Hypogastrura* spp. (Hypogastruridae), *Proisotoma* sp. 4 (Isotomidae), *Folsomides parvulus* (Isotomidae), *Lepidocyrtus* sp. 3 (Entomobryidae) and a minute collembolan, *i.e. Megalothorax* sp. 1 (Neelidae). *Megalothorax* sp. 1 (Neelidae) was only sampled during February and April 2013. Although there is very little known about their activities in soil, they seem to be mycophagous.

Sampling point 41 had the lowest clay percentage, with *Entomobrya* cf. *multifasciata* (Entomobryidae) responsible for the increase noted on 3 April 2013. *Hypogastrura* sp. 3 (Hypogastruridae) numbers increased on 17 July 2013. *Proisotoma* sp. 4 (Isotomidae) and *Folsomides parvulus* (Isotomidae) were also present at this sampling point, but *Proisotoma* sp. 4 (Isotomidae) was not nearly as well represented as in the other two sampling points within this field. The temporal incidence of *Folsomides parvulus* (Isotomidae) was the highest at this sampling point, although the number of individuals never exceeded 5.

Sampling point 34 is situated in natural veldt that experienced no disturbances. This is evident, since minimal fluctuations in the number of individuals of all the sampled fauna were observed (Fig. 5.10 A). Both mite and collembolan numbers were relatively low compared to that of the maize field. This is probably as a result of humidity differences, since the humidity was much higher in the agricultural field. Another influencing factor is that at this site microbial products and organic fertilizer was used instead of inorganic products. This would provide higher quality and quantity of food sources in the fields, which could therefore sustain a higher diversity of individuals. The natural veldt community structure is therefore more stable and would theoretically endure climatic changes, such as droughts, better. The natural veldt also differed from the agricultural field, showing that here *Brachystomella* sp. 2 (Brachystomellidae) and *Proisotoma* sp. 4 (Isotomidae) are the best represented over the course of this study.

Entomobrya cf. *multifasciata* (Entomobryidae), *Capbrya* sp. 2 (Entomobryidae) and Bourletiellidae sp. 3 was also present in a few samples. The spike in collembolan numbers on 3 April was due to large numbers of *Brachystomella* sp. 2 (Brachystomellidae), *Proisotoma* sp. 4 (Isotomidae) and Bourletiellidae sp. 3.

The effect of disturbances on soil faunal groups, in this study, can differ due to variability of the plant material burnt as a management tactic, the soil type and the kinds of weeds present. All of these would also influence the intensity of the fire and how it would affect the soil fauna. Climatic conditions can also influence the effect that controlled fires have and some organisms react differently to the same influencing factors applied during different seasons. A reduction in mites and other organisms was observed 90 min after stubble-burning was applied, which is supported by a study done by Malström (2008). However, collembolan numbers showed a slight increase (Fig. 5.10 B). This was due to a rising number of *Hypogastrura* sp. 3 (Hypogastruridae), which is probably due to opportunism and as a result being able to reproduce in an area without competition or predation pressure or move into an open or disrupted niche.

Sampling was also conducted at two sampling points were the wheat stubble was incorporated into the soil (Fig. 5.10 B). At these sites a clear increase in faunal numbers was observed. This is ascribed to the increase in food sources within the soil. At these two sampling points it was again noted that the collembolan numbers reacted in the same manner as elsewhere during the survey, whereas the mite numbers were lower in the presence of a higher number of collembolans and other organisms. The dominant collembolan here was *Hypogastrura* spp. (Hypogastruridae), with the rest of the collembolan numbers consisting of *Entomobrya* cf. *multifasciata* (Entomobryidae), *Proisotoma* sp. 4 (Isotomidae) and *Folsomides parvulus* (Isotomidae) (Addendum A). These collembolans were expected, as they are all mycophagous and euedaphic, with the exception of *Entomobrya* cf. *multifasciata* (Entomobryidae) which is phytophagous and epiedaphic, and therefore fulfil an essential role in decomposition (mentioned above, but also see Larsen *et al.* 2004 and Debeljak *et al*. 2007 for further explanation). The spikes in collembolan numbers on 20 Feb 2013, was due to the presence of large numbers of *Hypogastrura* spp. (Hypogastruridae), which are probably all introduced species and therefore opportunistic.

Soil fauna diversity decreased on 3 April 2013 since the compaction of the soil were much higher due to strong winds in the absence of plant cover at these sampling points. This created a filtering effect, selecting for smaller organisms and excluding *Entomobrya* cf. *multifasciata* (Entomobryidae) from the sample. At this locality, the farmer enriched the soil with organic additives and decrease invasive practices. This enhanced species abundance, especially that of the mites which exceeded collembolan abundance due to the restriction of collembolan mobility in clay soil. These mites are smaller and move more freely within the small soil pores.

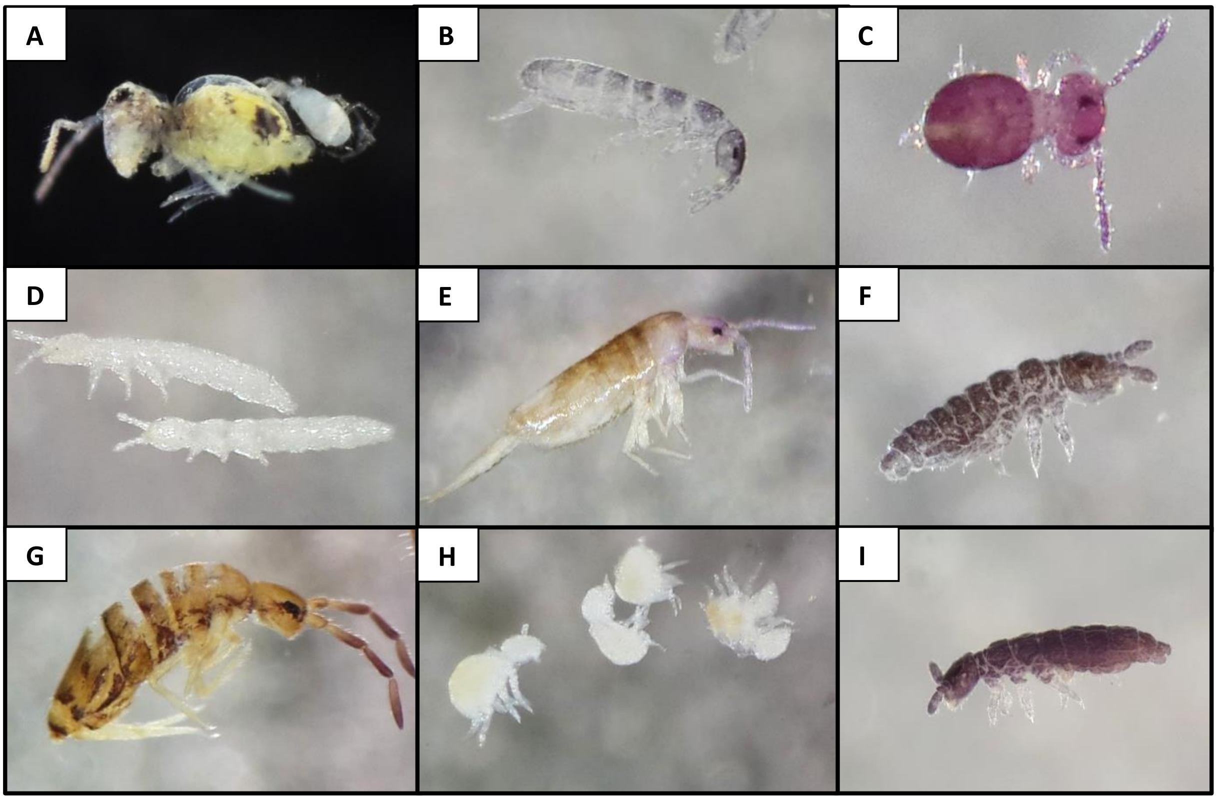
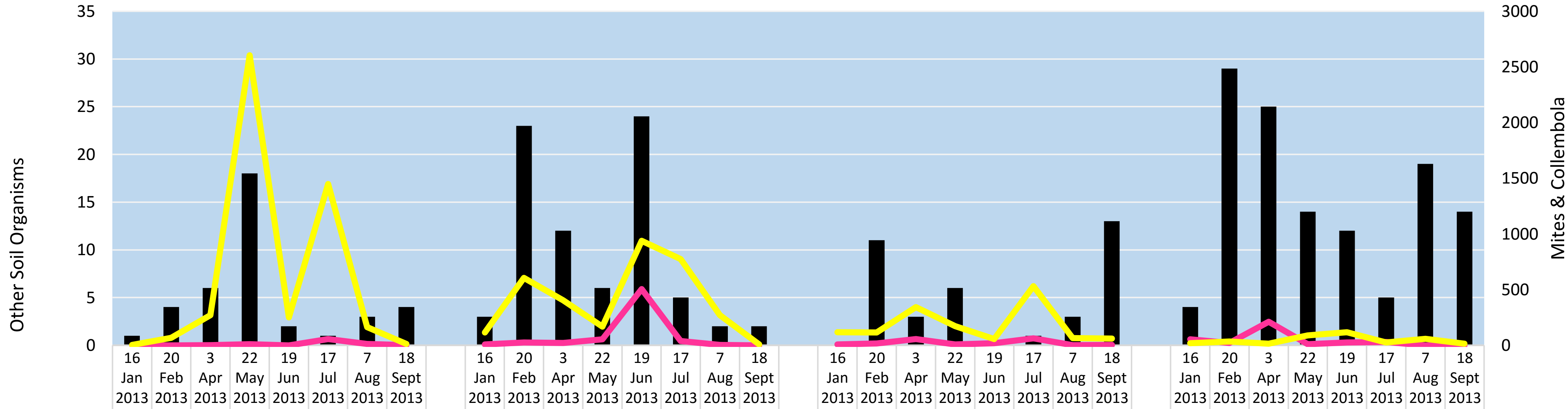


Fig. 5.9: Collembola representatives from the farm Thornberry. A: Bourletiellidae with a parasitic mite; B: *Proisotoma* sp. (Isotomidae); C: *Sphaeridia* sp. (Sminthurididae); D: *Folsomides* sp. (Isotomidae); E: *Lepidocyrtus* sp. (Entomobryidae); F: *Hypogastrura* sp. (Hypogastruridae); G: *Capbrya* sp. (Entomobryidae); H: *Megalothorax* sp. (Neelidae); I: *Hypogastrura* sp. (Hypogastruridae).

A



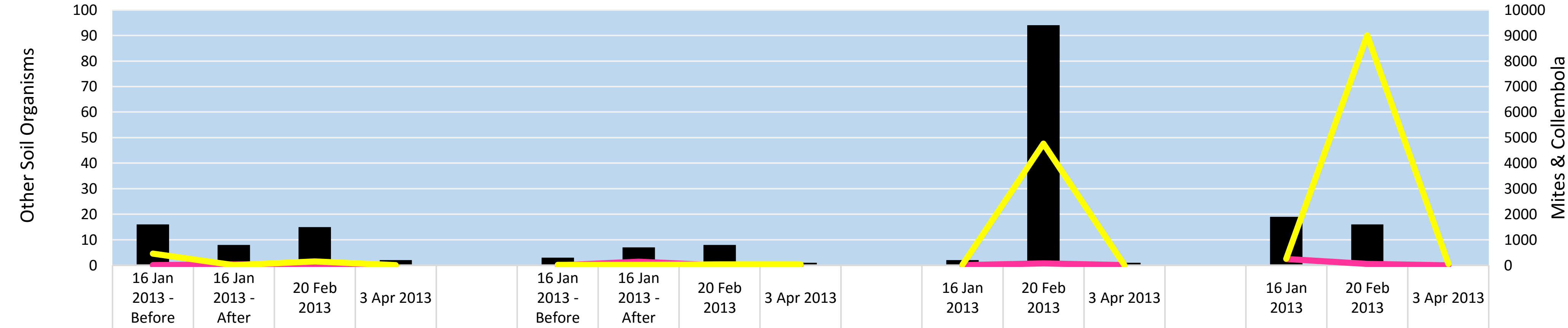
Sampling point 39	16 Jan 2013	20 Feb 2013	3 Apr 2013	22 May 2013	19 Jun 2013	17 Jul 2013	7 Aug 2013	18 Sept 2013
Other Soil Organisms	1	4	6	18	2	1	3	4
Collembola	2	0	1	11	0	55	12	2
Mites	4	66	271	2605	251	1448	164	12

Sampling point 40	16 Jan 2013	20 Feb 2013	3 Apr 2013	22 May 2013	19 Jun 2013	17 Jul 2013	7 Aug 2013	18 Sept 2013
Other Soil Organisms	3	23	12	6	24	5	2	2
Collembola	7	25	22	54	504	38	4	0
Mites	114	606	405	167	938	774	271	7

Sampling point 41	16 Jan 2013	20 Feb 2013	3 Apr 2013	22 May 2013	19 Jun 2013	17 Jul 2013	7 Aug 2013	18 Sept 2013
Other Soil Organisms	0	11	3	6	0	1	3	13
Collembola	8	16	55	7	18	63	1	4
Mites	118	116	342	173	57	532	65	61

Sampling point 34	16 Jan 2013	20 Feb 2013	3 Apr 2013	22 May 2013	19 Jun 2013	17 Jul 2013	7 Aug 2013	18 Sept 2013
Other Soil Organisms	4	29	25	14	12	5	19	14
Collembola	53	22	212	10	26	29	0	0
Mites	19	34	15	88	117	26	58	14

B



Stubble-burning applied	16 Jan 2013 - Before	16 Jan 2013 - After	20 Feb 2013	3 Apr 2013
Other Soil Organisms	16	8	15	2
Collembola	5	39	14	2
Mites	458	5	151	8

Stubble-burning applied	16 Jan 2013 - Before	16 Jan 2013 - After	20 Feb 2013	3 Apr 2013
Other Soil Organisms	3	7	8	1
Collembola	0	139	2	16
Mites	19	19	35	32

No stubble-burning	16 Jan 2013	20 Feb 2013	3 Apr 2013
Other Soil Organisms	2	94	1
Collembola	4	78	3
Mites	28	4761	6

No stubble-burning	16 Jan 2013	20 Feb 2013	3 Apr 2013
Other Soil Organisms	19	16	0
Collembola	243	56	2
Mites	252	9005	51

Other Soil Organisms — Collembola — Mites

Fig. 5.10: Fluctuations in soil faunal numbers at the farm Thornberry, 2013. A: sampling points 39 – 41 within a maize field and 34 within the natural veldt; B: sampling points within a wheat field where stubble-burning was applied on one part and tillage on the rest. (See Addendum A for faunistic detail.)

iii) Bloemfontein District (Paradys Experimental Farm)

Due to the general prominence of Collembola and Acari in soils, these organisms tend to be helpful in the evaluation of soil ecotoxicology. Studies have been done on many collembolan species from different families and it has been suggested that the effects on reproduction during ecotoxicological studies were more valuable than the actual mortality rate (Cortet *et al.* 1999). The trial below was an ecotoxicological trial of sorts, albeit that only the ecological succession rate was analyzed.

Sampling point 44 (Fig. 3.13) was in a transect sprayed with an insecticide (Fig. 5.12). The effect was clear, since the number of other organisms were initially relatively low and increased after the effect of the insecticide had worn off. Mite numbers fluctuated and the number of collembolan individuals increased as the mites decreased at the end of the study period. The majority of collembolan individuals sampled at this sampling point was predominantly *Brachystomella* sp. 1 (Brachystomellidae) and *Sphaeridia* sp. 1 (Sminthurididae), with low numbers of *Isotomodes* sp. 1 (Isotomidae), *Proisotoma* sp. 3 (Isotomidae), *Capbrya* sp. 1 (Entomobryidae) and *Tullbergia* sp. 1 (Tullbergiidae) (Addendum A; Fig. 5.11).

The effect of the herbicides was more complicated and no general trend could be determined. The broad-spectrum herbicides seemed to have an umbrella effect with most organisms occurring in low numbers. A slight increase in collembolan individuals was, however, noted on 30 April 2014 and a bigger increase on 21 May 2014 (Fig. 5.12). These two spikes were due to the relatively high number of *Xenylla* sp. 1 (Hypogastruridae) individuals at these dates. This application seems to have a large meaningful effect, since even mite numbers were relatively low. Although the number of individuals was low at this transect, Collembola was still represented by *Sphaeridia* sp. 1 (Sminthurididae), *Brachystomella* sp. 1 (Brachystomellidae), *Proisotoma* sp. 3 (Isotomidae), *Isotomodes* sp. 1 (Isotomidae), *Xenylla* sp. 1 (Hypogastruridae), *Tullbergia* sp. 1 (Tullbergiidae), *Pseudosinella* sp. 1 (Entomobryidae) and *Seira* sp. 1 (Entomobryidae) (Addendum A; Fig. 5.11). Collembolan species richness was therefore not too low, with the presence of more collembolan species at this sampling point, explaining the relatively high diversity values of the pesticide trial in Fig. 5.5.

The grass herbicide had the second most disruptive effect, as no pattern can be observed in the fluctuations, thus indicating a more unstable community. Mite and collembolan numbers were higher than in the presence of the other herbicides (Fig. 5.12). This could be due to the increase in organic matter as meaningful grass die-back was experienced. The spike in Collembola was due to an increase in *Brachystomella* sp. 1 (Brachystomellidae). The rest of the collembolans recorded were *Sphaeridia* sp. 1 (Sminthurididae), *Proisotoma* sp. 3 (Isotomidae), *Isotomodes* sp. 1 (Isotomidae), *Tullbergia* sp. 1 (Tullbergiidae), *Seira* sp. 1 (Entomobryidae) and *Capbrya* sp. 1 (Entomobryidae). With the grass die-back, *Sphaeridia* sp. 1 (Sminthurididae), which is a phytophage, was no longer present.

Fluctuations within the mesofauna community at the broadleaf herbicide application did not show the same severity as with the grass herbicide. This could be due to the grassland site that did not contain many broadleaf plants. Collembola and mites increased when the number of individuals of the other organisms decreased (Fig. 5.12). Collembola was represented by relatively low individual counts of *Sphaeridia* sp. 1 (Sminthurididae), *Brachystomella* sp. 1 (Brachystomellidae), *Proisotoma* sp. 3 (Isotomidae), *Isotomodes* sp. 1 (Isotomidae), *Xenylla* sp. 1 (Hypogastruridae), *Tullbergia* sp. 1 (Tullbergiidae), *Capbrya* sp. 1 (Entomobryidae) and *Seira* sp. 1 (Entomobryidae) (Addendum A). Interestingly *Xenylla* sp. 1 (Hypogastruridae) showed tolerance to both the broadleaf and grass herbicide applications, although Haque *et al.* (2011), observed a reduction in the abundance of a *Xenylla* sp. in the presence of certain herbicides.

At the fungicide transect *Brachystomella* sp. 1 (Brachystomellidae) was dominant and contributed most of the sampled collembolans. *Proisotoma* sp. 3 (Isotomidae), *Isotomodes* sp. 1 (Isotomidae), *Sphaeridia* sp. 1 (Sminthurididae), *Tullbergia* sp. 1 (Tullbergiidae), *Pseudosinella* sp. 1 (Entomobryidae) and *Seira* sp. 1 (Entomobryidae)

(Addendum A) were also recorded. Overall a general increase and decrease in organismal numbers was noted over time. At the control in the natural veldt, the mite count was the highest and the most collembolans were also present here. The number of Collembola in the different soil treatments, however, increased towards the end of this study, since it rained before the last sampling date. Collembolans recorded at this transect were *Proisotoma* sp. 3 (Isotomidae), *Brachystomella* sp. 1 (Brachystomellidae), *Sphaeridia* sp. 1 (Sminthurididae) and *Seira* sp. 1 (Entomobryidae) (Addendum A; Fig. 5.11).

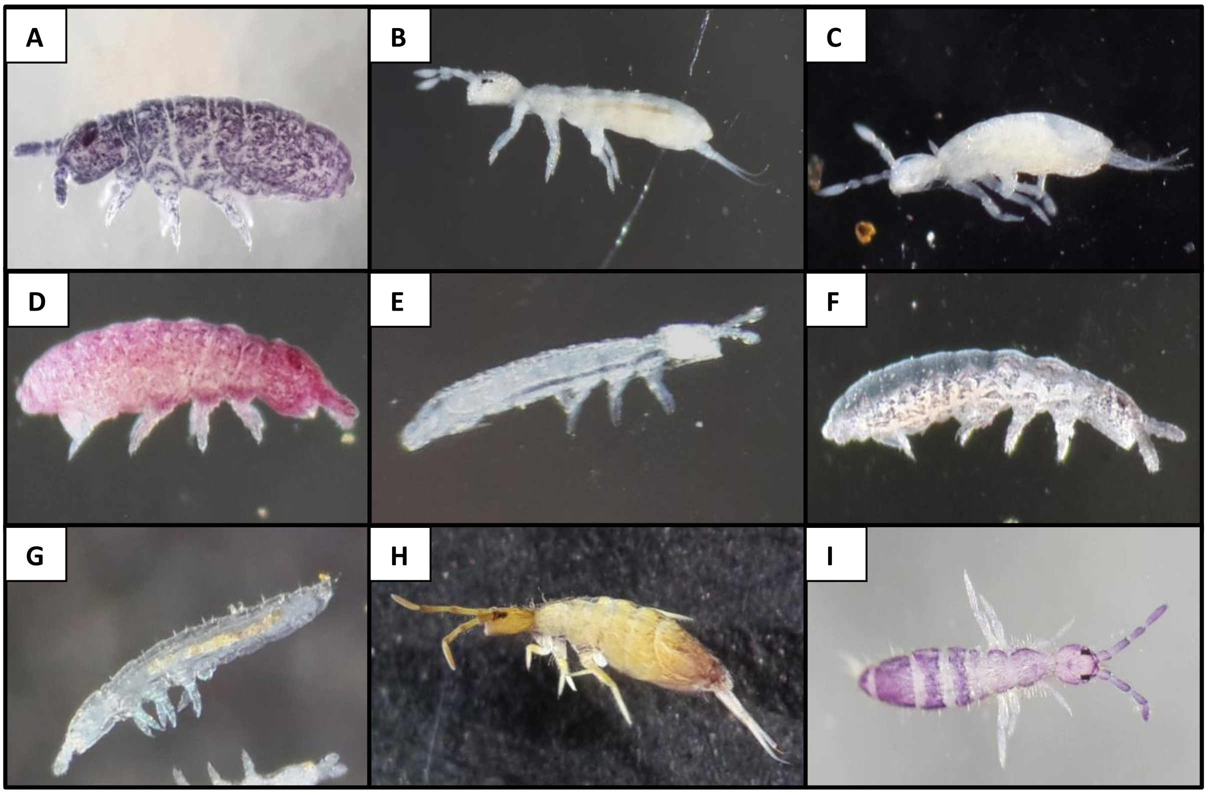
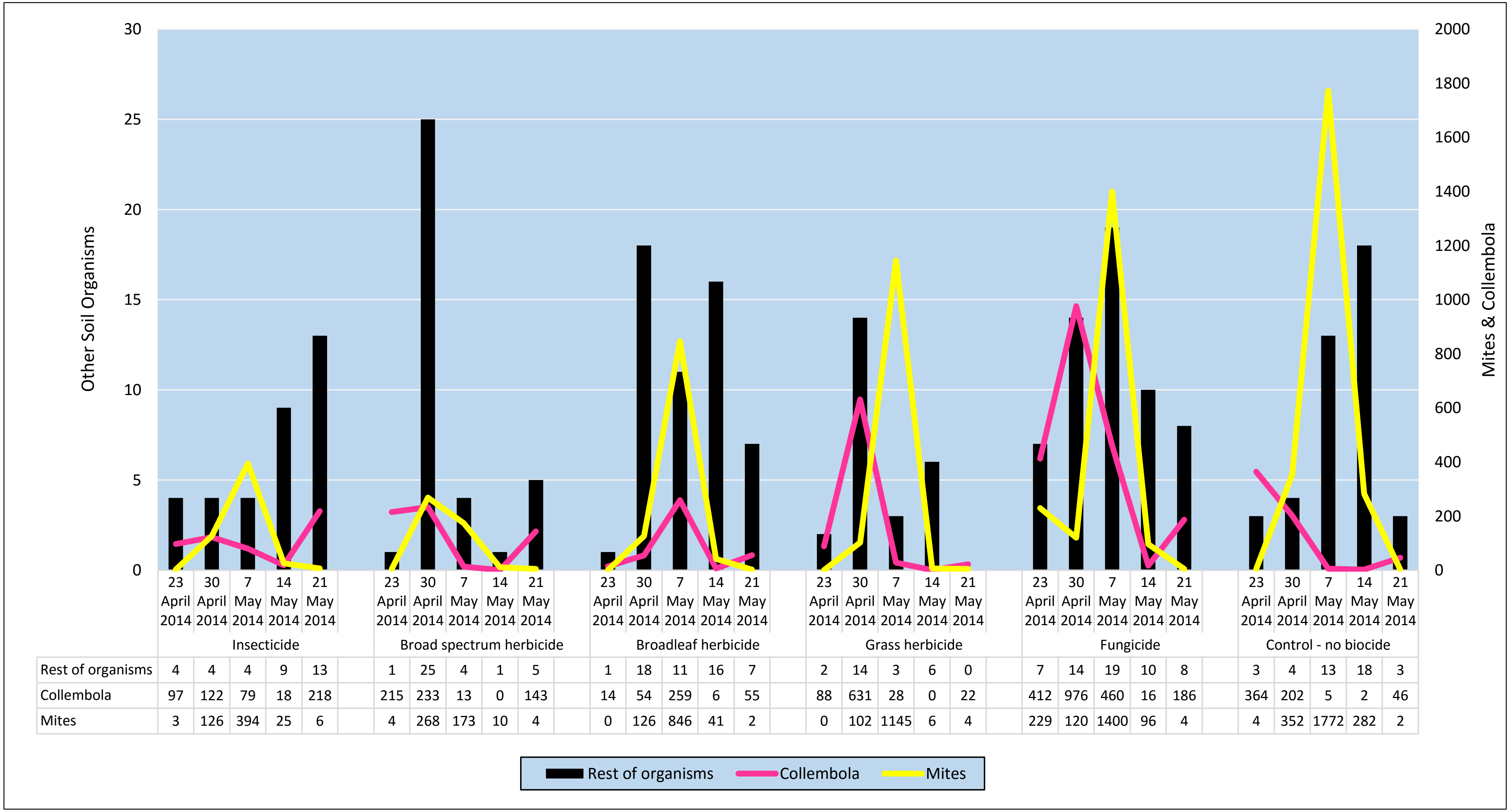


Fig. 5.11: Collembola representatives from the farm Paradys. A: *Brachystomella* sp. (Brachystomellidae); B: *Proisotoma* sp. (Isotomidae); C: *Pseudosinella* sp. (Entomobryidae); D: *Xynella* sp. (Hypogastruridae); E: *Isotomodes* sp. (Isotomidae); F: *Brachystomella* sp. (Brachystomellidae); G: *Tullbergia* sp. (Tullbergiidae); H: *Seira* sp. (Entomobryidae); I: *Capbrya* sp. (Entomobryidae).



	Insecticide					Broad spectrum herbicide					Broadleaf herbicide					Grass herbicide					Fungicide					Control - no biocide				
	23 April 2014	30 April 2014	7 May 2014	14 May 2014	21 May 2014	23 April 2014	30 April 2014	7 May 2014	14 May 2014	21 May 2014	23 April 2014	30 April 2014	7 May 2014	14 May 2014	21 May 2014	23 April 2014	30 April 2014	7 May 2014	14 May 2014	21 May 2014	23 April 2014	30 April 2014	7 May 2014	14 May 2014	21 May 2014	23 April 2014	30 April 2014	7 May 2014	14 May 2014	21 May 2014
Rest of organisms	4	4	4	9	13	1	25	4	1	5	1	18	11	16	7	2	14	3	6	0	7	14	19	10	8	3	4	13	18	3
Collembola	97	122	79	18	218	215	233	13	0	143	14	54	259	6	55	88	631	28	0	22	412	976	460	16	186	364	202	5	2	46
Mites	3	126	394	25	6	4	268	173	10	4	0	126	846	41	2	0	102	1145	6	4	229	120	1400	96	4	4	352	1772	282	2

Fig. 5.12: Fluctuations in soil faunal numbers in the presence of different biocide applications at the Paradys Experimental Farm of the University of the Free State in 2014. (See Addendum A for faunistic detail.)

iv) Odendaalsrus District (Eureka farm)

Collembola is a diverse group, contributing in many aspects of soil functioning. As most Collembola seem to feed on fungi, which are known to accumulate pollutants in their mycelia, they can form part of a bioaccumulation chain (Cortet *et al.* 1999). Some saprophagous collembolans have been ranked second when it comes to bioaccumulation of copper. It is therefore important when testing for bioaccumulation to keep in mind that variations in elements, concentration levels and the organisms affected will influence the outcome (Cortet *et al.* 1999).

At this locality collembolan numbers exceed those of mites at many of the sampling points (Fig. 5.13). This is due to the sensitivity of certain mite species to the presence of heavy metal pollution and the tolerance and avoidance mechanisms exhibited by some collembolan species (*e.g.* Posthuma & Van Straalen 1993).

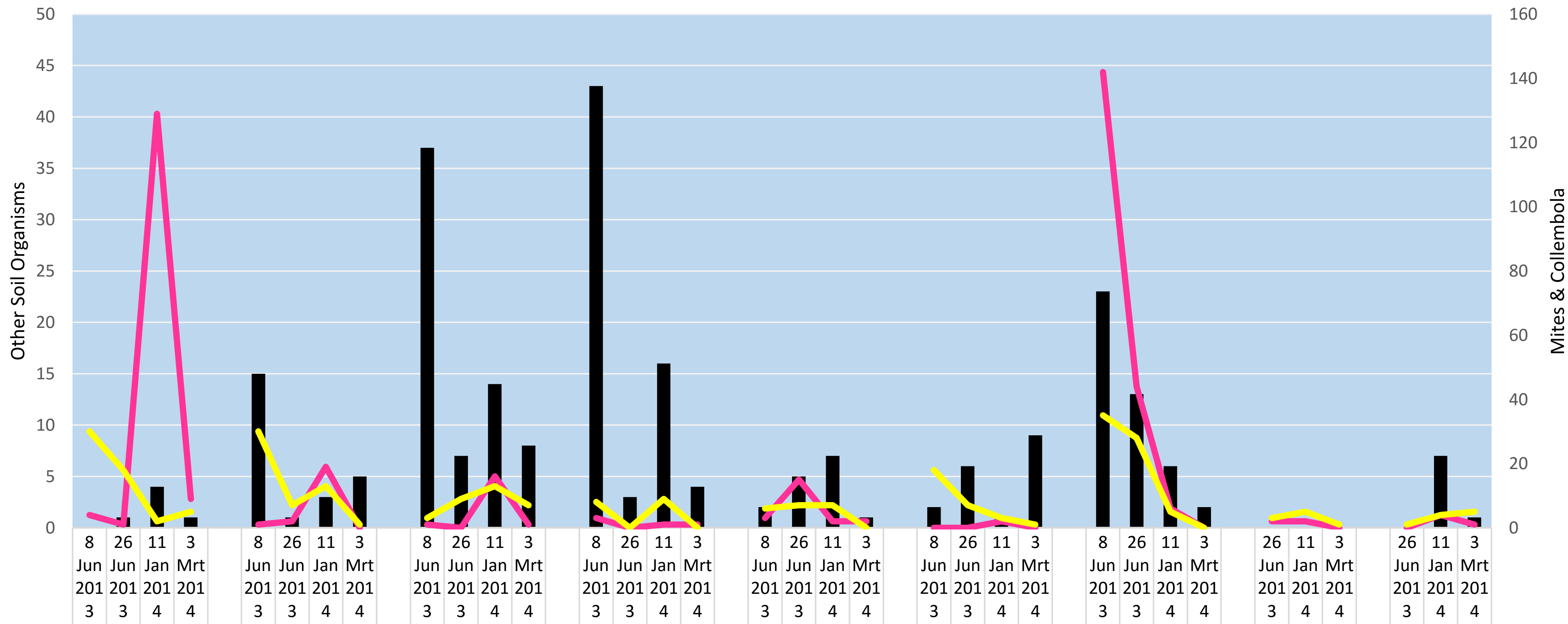
Sampling points 58 and 59 (Fig. 3.18) are located in a low lying part of the farm that was contaminated by the leaking tailings dam of a gold mine in 2000. At this site, both sampling points indicate an overall reduction in mites with a spike in collembolan numbers (Fig. 5.13). The spike in collembolan numbers noted on 11 January 2014 at sampling point 58, was due to very large numbers of Bourletiellidae sp. 2 (117 specimens). This sampling point also contained individuals of Bourletiellidae sp. 1, *Sphaeridia* sp. 1 (Sminthurididae), *Mucrosomia* sp. 1 (Isotomidae), *Seira* sp. 2 (Entomobryidae), *Capbrya* sp. 2 (Entomobryidae), *Hypogastrura* sp. 4 (Hypogastruridae) and *Cyphoderus* sp. 1 (Cyphoderidae). Sampling point 59 had a higher abundance of other soil organisms and simultaneously showed a decrease in collembolan numbers. This sampling point, as opposed to sampling point 58, contained certain different collembolan species. The total diversity recorded was Bourletiellidae sp. 2, *Sphaeridia* sp. 1 (Sminthurididae), *Mucrosomia* sp. 1 (Isotomidae), *Pseudosinella* sp. 1 (Entomobryidae) and *Lepidocyrtus* sp. 4 (Entomobryidae). It is interesting to note that many of these collembolans are phytophagous, with only a few mycophages (Addendum A).

Sampling points 54 and 52 overall showed relatively high numbers of soil organism individuals, but only a few mites and collembolan individuals (Fig. 5.13). These sampling points are at amongst reeds and weeds respectively. Sampling point 54 contained *Lepidocyrtus* sp. 4 (Entomobryidae), Entomobryidae sp. 2, *Mucrosomia* sp. 1 (Isotomidae) and *Hypogastrura* sp. 4 (Hypogastruridae) (Addendum A). All of these species are mycophagous. The collembolans present at sampling point 52 were either phytophagous or mycophagous and were represented by Bourletiellidae sp. 2, *Sphaeridia* sp. 1 (Sminthurididae), *Mucrosomia* sp. 1 (Isotomidae) and *Hypogastrura* sp. 4 (Hypogastruridae) (Addendum A; Fig. 5.14). Weeds generally have a higher nutritional value than reeds, which could be the reason for the phytophage incidence.

Sampling point 50 and 51 are situated in the natural veldt. Although sampling point 50 did not have a high abundance of fauna, a relatively low number of mites and collembolans was nevertheless still observed. Sampling point 51, however, indicated an overall clear decrease in number of other organisms, mites and Collembola. Collembola from sampling point 51 were comprised of *Sphaeridia* sp. 1 (Sminthurididae), *Mucrosomia* sp. 1 (Isotomidae), the latter being *Brachystomella* sp. 1 (Brachystomellidae) (Addendum A; Fig 5.13).

Sampling points 55 and 56 had minimal collembolan representatives and these were represented only by *Hypogastrura* sp. 4 (Hypogastruridae) and *Capbrya* sp. 4 (Entomobryidae) (Addendum A; Fig 5.13). These are highly polluted sites and these organisms must have some adaptation to be able to survive in such soils.

Overall little is known of the contamination effect of South African goldmine tailing dams on the environment and how far the trace elements they contain can be dispersed by the wind.



	Sampling point 58				Sampling point 59				Sampling point 54				Sampling point 52			
	8 Jun 2013	26 Jun 2013	11 Jan 2014	3 Mrt 2014	8 Jun 2013	26 Jun 2013	11 Jan 2014	3 Mrt 2014	8 Jun 2013	26 Jun 2013	11 Jan 2014	3 Mrt 2014	8 Jun 2013	26 Jun 2013	11 Jan 2014	3 Mrt 2014
Rest of Organisms	0	1	4	1	15	1	3	5	37	7	14	8	43	3	16	4
Collembola	4	1	129	9	1	2	19	0	1	0	16	1	3	0	1	1
Mites	30	18	2	5	30	7	13	1	3	9	13	7	8	0	9	0

	Sampling point 53				Sampling point 50				Sampling point 51				Sampling point 56			Sampling point 55		
	8 Jun 2013	26 Jun 2013	11 Jan 2014	3 Mrt 2014	8 Jun 2013	26 Jun 2013	11 Jan 2014	3 Mrt 2014	8 Jun 2013	26 Jun 2013	11 Jan 2014	3 Mrt 2014	26 Jun 2013	11 Jan 2014	3 Mrt 2014	26 Jun 2013	11 Jan 2014	3 Mrt 2014
Rest of Organisms	2	5	7	1	2	6	1	9	23	13	6	2	0	0	0	0	7	1
Collembola	3	15	2	2	0	0	2	0	142	44	6	0	2	2	0	0	4	1
Mites	6	7	7	0	18	7	3	1	35	28	5	0	3	5	1	1	4	5

Fig. 5.13: Fluctuations in the numbers of soil organisms sampled at various sites on the farm Eureka, which is located adjacent to a gold mine tailings dam. (See Addendum A for faunistic detail.)

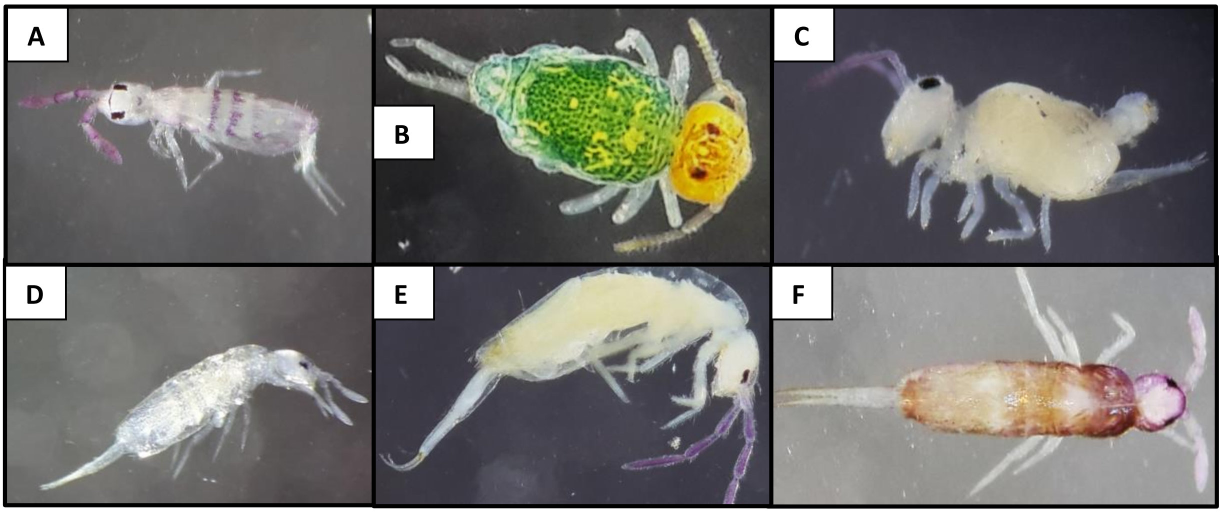


Fig. 5.14: Some Collembola representatives from the farm Eureka. A: *Capbrya* sp. (Entomobryidae); B-C: Bourletiellidae spp.; D: *Pseudosinella* sp. (Entomobryidae); E: *Seira* sp. (Entomobryidae); F: *Lepidocyrtus* sp. (Entomobryidae).

v) Bothaville District (Klein Brittanje farm)

At sampling points 64 – 66 (Fig. 3.23), the collembolan numbers were higher than that of the mites (Fig. 5.16 A). These samples were from an area that was converted from veldt to maize field during 2011. This could have been due to the large quantity of plant material that was worked into the soil, since the natural vegetation was sprayed with herbicides and the decomposition process may have been prolonged (see Chapter 3). As previously mentioned, Collembola are better adapted to function in such an environment. These sampling points were represented by high numbers of individuals from *Brachystomella* spp. (Brachystomellidae) and *Proisotoma* sp. 4 (Isotomidae) and lower numbers *Capbrya* sp. 2 (Entomobryidae), *Capbrya* sp. 3 (Entomobryidae), *Seira* sp. 2 (Entomobryidae) and *Cyphoderus* sp. 1 (Cyphoderidae) (Addendum A). The other fields surveyed were dominated by mites, with their numbers considerably higher than that of the Collembola at the sampling points.

Sampling point 63 is located in a fallow field. On 4 February 2013, the spike in collembolan numbers was due to high numbers of *Brachystomella* spp. (Brachystomellidae), as well as *Proisotoma* sp. 4 (Isotomidae), *Tullbergia* sp. 1 (Tullbergiidae), *Capbrya* sp. 2 (Entomobryidae), *Seira* sp. 2 (Entomobryidae) and

Friesea sp. 1 (Neanuridae) (Fig. 5.16 B). This was a very interesting sample, since it represented collembolans of three different trophic levels (Addendum A).

The sampling points that are in the natural veldt were dominated by mites (Fig. 5.17). These sites contained collembolan representatives from *Brachystomella* spp. (Brachystomellidae) and *Proisotoma* sp. 4 (Isotomidae), *Tullbergia* sp. 1 (Tullbergiidae), *Capbrya* sp. 2 (Entomobryidae), *Seira* sp. 2 (Entomobryidae) and *Cyphoderus* sp. 1 (Cyphoderidae) (Addendum A; Fig. 5.15). Fluctuations were small throughout sampling points 73 and 74, indicating more stable populations. Sampling point 67, however, was in a humus rich area and had high numbers of different oribatids (Fig. 5.17).

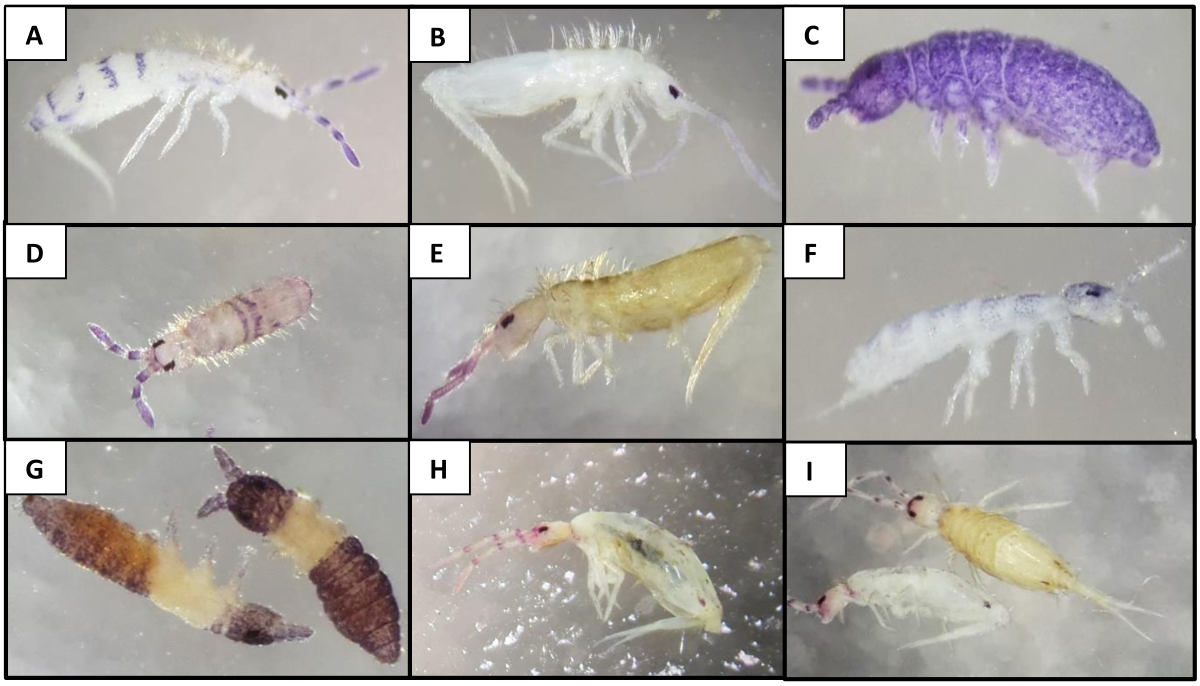


Fig. 5.15: Some Collembola representatives from the farm Klein Brittanje. A: *Capbrya* sp. (Entomobryidae); B: Entomobryidae sp.; C: *Brachystomella* sp. (Brachystomellidae); D: *Capbrya* sp. (Entomobryidae); E: *Seira* sp. (Entomobryidae); F: *Proisotoma* sp. (Isotomidae); G: *Friesea* sp. (Neanuridae); H-I: *Seira* sp. (Entomobryidae).

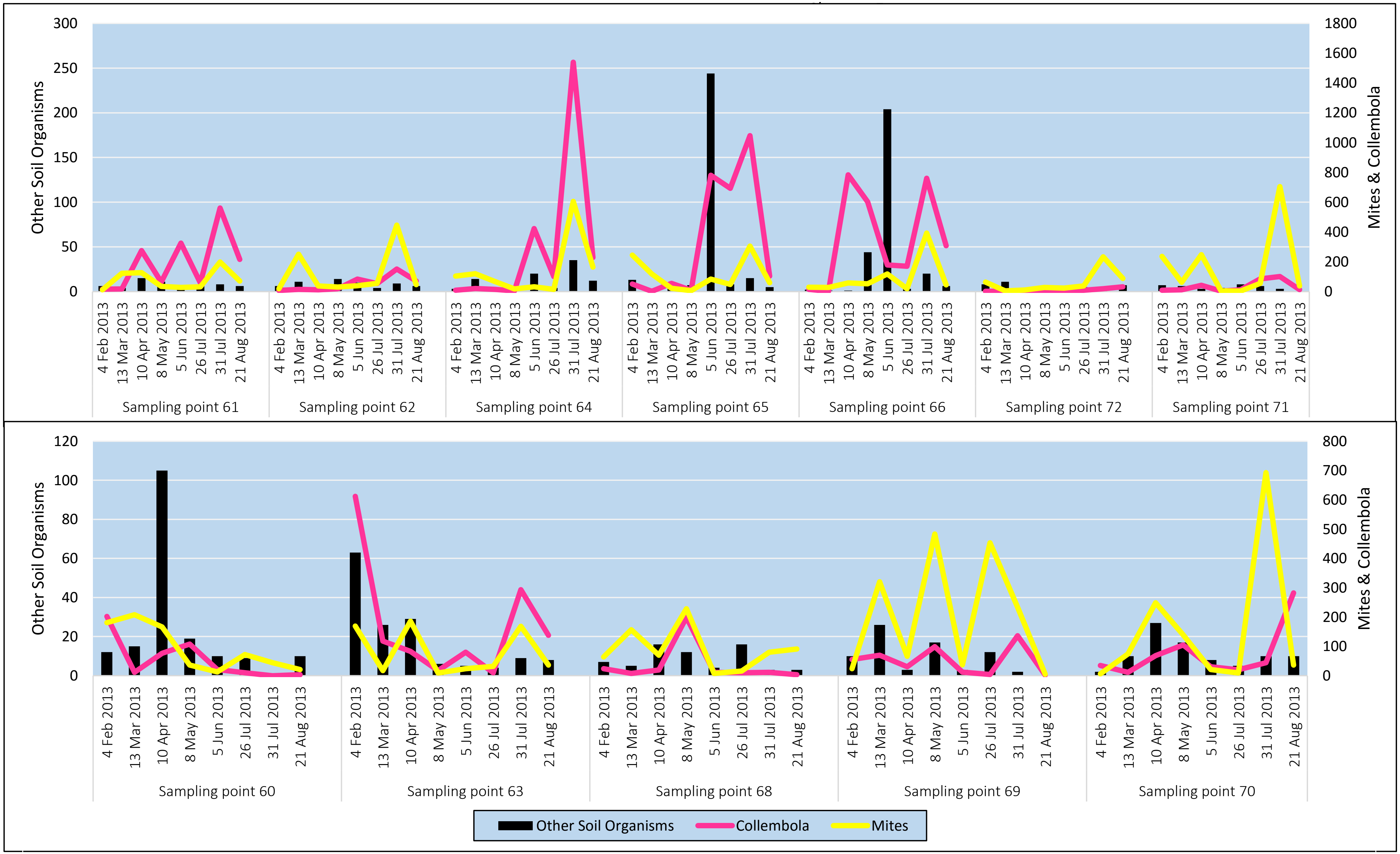
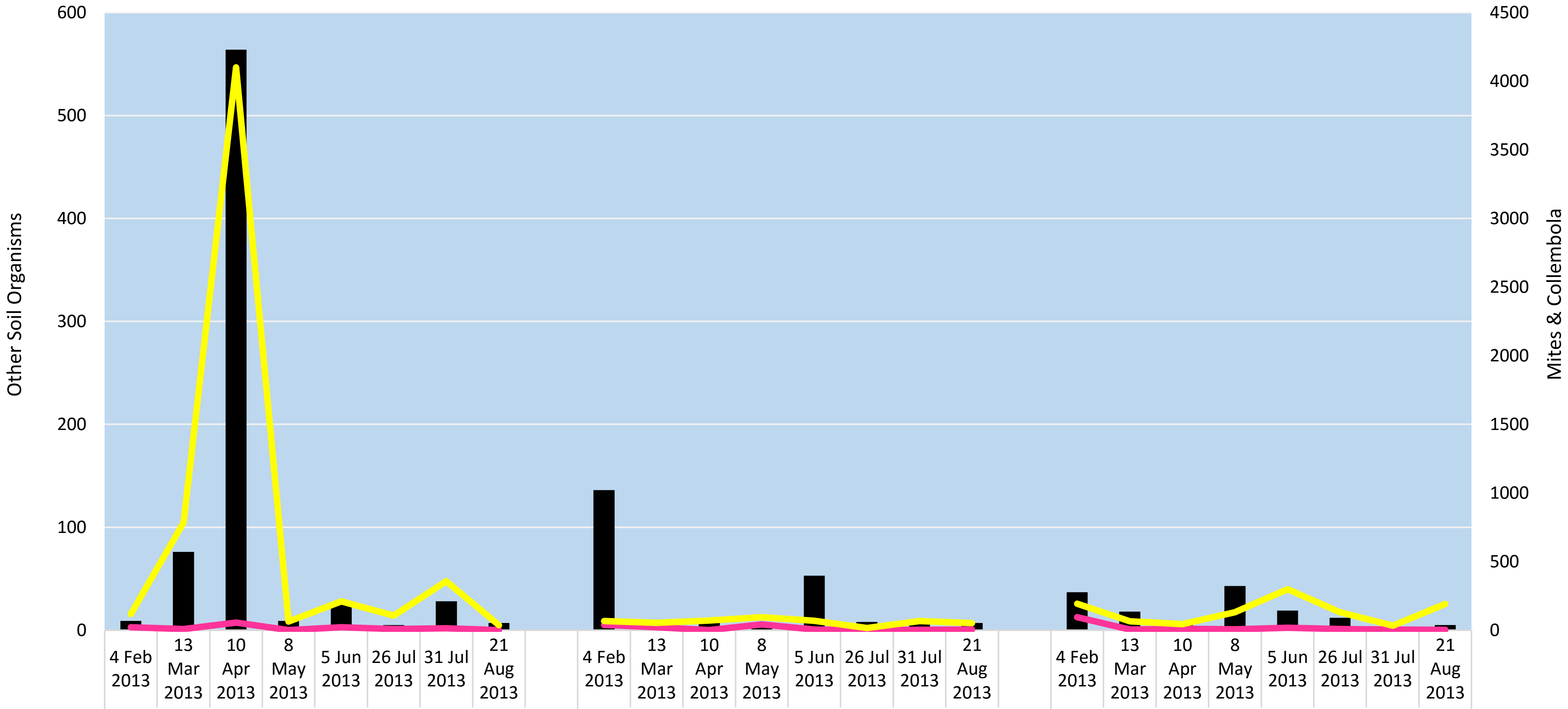


Fig. 5.16: Fluctuations in the numbers of soil organisms sampled at various sites on the farm Klein Brittanje during 2013. A: samples within fields cultivated with maize; B: fields that were left fallow after the maize was harvested in 2012. (See Addendum A for faunistic detail.) (Note: y-axis not the same scale)



	Sampling point 67: 4 Feb 2013	13 Mar 2013	10 Apr 2013	8 May 2013	5 Jun 2013	26 Jul 2013	31 Jul 2013	21 Aug 2013	Sampling point 73: 4 Feb 2013	13 Mar 2013	10 Apr 2013	8 May 2013	5 Jun 2013	26 Jul 2013	31 Jul 2013	21 Aug 2013	Sampling point 74: 4 Feb 2013	13 Mar 2013	10 Apr 2013	8 May 2013	5 Jun 2013	26 Jul 2013	31 Jul 2013	21 Aug 2013
Other Soil Organisms	9	76	564	9	27	5	28	7	136	7	12	7	53	8	8	7	37	18	6	43	19	12	2	5
Collembola	21	8	55	1	22	9	14	1	39	21	1	43	5	1	3	5	95	5	10	7	17	9	5	2
Mites	117	785	4101	62	209	106	357	37	66	54	70	94	69	15	66	51	193	64	44	131	298	130	33	191

Fig. 5.17: Fluctuations in the numbers of soil organisms sampled within the natural veldt on the farm Klein Brittanje during 2013. (See Addendum A for faunistic detail.)

d) Conclusion

Soils are inhabited by a wide variety of organisms in various size classes. These organisms interact with one another and their environment. Some of these organisms have proved to be beneficial to humans, since they can be used to evaluate soil health. One such organism is the mesofaunal collembolan species, *Folsomia candida*. Collembolans are also essential for the functioning of soils, since they are a wide-spread group that has representatives in all trophic levels. Some collembolans are opportunistic and will increase rapidly in the absence of predator and competition pressures. Other have the ability to feed on fungi that contain pollutants or to avoid such fungi. Bioaccumulation can influence the persistence of these hazardous chemicals in an environment. Certain collembolans can excrete these toxins successfully or store it in the epithelium which is lost during moulting.

In environments, including soils, disturbance disruptions can act as filters which select for certain species. This will obviously decrease diversity indices and therefore the complexity factor of a community. This, in turn, influences ecological functioning and the delivery of ecosystem services. More complex communities tend to be more stable and can buffer change better. In this study it was found that the characteristics of each study area allowed for certain species to occur in that specific area. When considering the distribution of collembolans, certain species were only recorded in certain areas, *viz.* *Mesaphorura* spp. (Tullbergiidae) were only sampled on the farm Vaaldam (on account of condensation effect after stubble-burning); *Xenylla* sp. 1 (Hypogastruridae) was only sampled on the farm Paradys (on account of herbicide tolerance); *Megalothorax* sp. 1 (Neelidae) was only sampled on the farm Thornberry (on account of its small size and the high clay components effect on pore spaces); Bourletiellidae sp. 1 and *Mucrosomia* sp. 1 (Isotomidae) were only sampled on the farm Eureka (on account of the ability to tolerate or avoid heavy metals) and *Friesea* sp. 1 (Neanuridae) (on account of high SOM) was only sampled on the farm Klein Brittanje. These examples demonstrate that every locality has selected for certain species to occur there and therefore it is important to keep in mind that all environments are different in their own way, albeit often subtle, and should therefore be analyzed and evaluated accordingly. Disturbances, such as agricultural practices, also influenced collembolan abundance and the severity of the fluctuations in the number of individuals observed.

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Chapter 6


"The nation that destroys its soil destroys itself"
~ Franklin Delano Roosevelt, Letter (1937) (1882 – 1945)

GENERAL CONCLUSION, RECOMMENDATIONS & FUTURE RESEARCH

a) General Conclusion

- Agriculture is placed under immense pressure to produce food for a growing human population and still maintain sustainability. This is only possible in healthy soil landscapes that contribute to crop yields due to their active ecological functioning.
- These services are provided by the activities of the biological component of soils, which include representatives from all five kingdoms. These beneficial organisms are unfortunately sensitive to certain disturbances and anthropogenic activities, and are therefore limited or even excluded on account of certain activities.
- Disturbances, anthropogenic or natural, therefore act as a filtering mechanism that select for certain organisms to be present in an ecosystem and, by excluding others, could reduce the much needed complexity of the soil community.
- Natural disturbances are usually beneficial, since it provides the possibility of environmental improvement. This is possible due to the sufficient recovery period that accompanies such an event. This is unfortunately not true for most anthropogenic disturbances.
- The severity, replication and sometimes permanency of anthropogenic disturbances therefore lead to an extreme filtering effect and could eliminate some organisms and their activities permanently.
- Some organisms are better adapted to occur in disturbed locations, therefore still providing ecosystem services, but only in a limited manner. Others are opportunistic and will reproduce and occupy these ‘open niches’ in vast numbers.

- The latter poses a problem in agriculture, since these are usually introduced species that outcompete the endemic species. Some of the major agricultural pests, *e.g.* the red spider mite (*Tetranychus urticae*), which reproduces rapidly in dry unfavorable conditions or after being unsuccessfully sprayed with insecticides that reduced competition and predator pressures, is a case in point.

- Herbicides also influence the diversity of soil mesofaunal groups, *e.g.* the very high number of *Hypogastrura* sp.1 (Hypogastruridae) observed after the use of a herbicide at the farm Koppieskraal. These organisms are probably introduced and opportunistic due to an ability to either evade or tolerate the herbicide, indicating a possible ecological trade-off and genetic mutation.

- At the experimental farm of the University of the Free State, all the biocides show an initial low number of individuals, followed by a later spike. This indicates that even though minimal functionality was lost, there were a reduction in the number of individuals. Due to the minimal loss of trophic levels, the rate of recovery would be faster as functional diversity was still maintained as explained by Jurburg & Salles (2015).

- Agricultural fields can be described as an area of 'chronic' disturbance, as there are usually multiple disturbances in each field per season, without a proper recovery period that would benefit the soil fauna.

- Agricultural practices such as tillage, biocide application and controlled stubble-burning reduced the complexity of the soil faunal community structures, leaving these communities more vulnerable and with lower resilience.

- Even though these practices had a decreasing effect in the soil mesofauna, it was noted that over time and in the absence or with minimal additional disturbances, the diversity would start to increase again. This is explained by hysteresis, whereby environments follow a successional pattern to restore itself to its previous condition.

- The recovery period is not only influenced by the type of disturbance or the severity here of, but also by the 'pre-disturbance' biotic and abiotic factors of the particular environment.

- Environmental factors also influence the faunal recovery period, since clay percentage, pH, humidity, soil organic matter, etc. can influence the persistence and severity of disturbances.

- Sites where stubble was incorporated into the soil showed a higher resilience to the effects of disturbances. The diversity of soil fauna stayed relatively constant after a field with stubble was ploughed. This was due to the high organic component that was already in the soil, since the stubble was incorporated into the soil after each season.

- Good management of agricultural practices increases the resilience of soil communities. This was indicated by the minimal influence of the drying and waterlogging effects a gradual slope within a maize field had on soil mesofauna.

- The effect of biocides, on observed soil mesofauna in agricultural fields that had already been disturbed and therefore experienced a lower resilience, proved to be more severe when compared to a single application applied in the natural veldt.

- Stubble-burning influence the vertical distribution of soil mesofauna and were responsible for the presence of the collembolan, *Mesaphorura* spp. (Tullbergiidae), in the top 15cm of the soil.

- Predators are benefitted by mechanical disturbances. They are opportunistic feeders and exploit the vulnerability of the organisms that have survived the particular disturbance. Since these prey organisms eventually find shelter and re-establish themselves, the predator diversity declines over time.

- A fallow-field system also seemed beneficial since high numbers of soil mesofauna were present in these fields and were probably still providing ecosystem services. This phenomenon reduces the chances of certain species dominating and reaching pest status, since the micro-niches are already filled and a high level of competition exists.

- It is, however, important that fallow fields receive proper management. At the farm Thornberry, one patch of a field was burnt and in the rest of the field the stubble was worked into the soil. Within two months' compaction increased to such an extent in the litter that the soil faunal diversity was greatly reduced. The stubble should rather have been retained above ground in order to assist in reducing compaction caused by strong winds.

- Specific events can influence soil organism diversity both directly or indirectly. Pollution is of major concern since the implications of such an event can be detrimental to an entire area. Tailing dams are used to store mine waste and are a large source of pollution in their environment. Due to seepage, leakage and wind distribution, heavy metals can be transported from these dams into the surrounding environment. These pollutants result in the loss of most soil biota and only a few, such as certain Collembola species, are adapted to such conditions. A few collembolan species were only sampled at this locality and are therefore presumed to show preference to these conditions, since they can probably evade or tolerate the pollutants as was indicated by avoidance studies done by Tranvik & Eijsackers (1989) and Fountain & Hopkin (2001). There is a high likelihood that these species are hardy and invasive. These species included *Hypogastrura* sp. 4 (Hypogastruridae), *Mucrosomia* sp. 1 (Isotomidae), *Lepidocyrtus* spp. 4, 5 & 6 (Entomobryidae), *Capbrya* sp. 4 (Entomobryidae) and Bourletiellidae sp. 1 and 2 (Addendum A). On the other hand, these conditions were detrimental to centipedes of the order Geophilamorpha which do not have a protective waxy layer to protect them from such harsh conditions.

- At the Paradys Experimental Farm two types of selection were observed. The collembolan *Xenylla* sp. 1 (Hypogastruridae), was only observed in the broad-spectrum and broadleaf herbicide applications, whereas the environmental selection favoured *Seira* sp. 1 (Entomobryidae) and *Proisotoma* sp. 3 (Isotomidae).

- Specific environmental conditions of areas also influence soil mesofaunal incidence, with Scolopendrellidae sp. 1 (Order: Symphyla) and the collembolans Bourletiellidae sp. 3, *Megalothorax* sp. 1 (Neelidae), *Lepidocyrtus* sp. 2 & 3 (Entomobryidae), *Proisotoma* sp. 4 (Isotomidae) and *Hypogastrura* sp. 1 & 2 (Hypogastruridae) only occurring on the farm Thornberry (with higher organic and clay components); *Mesaphorura yossi* (Tullbergiidae) only occurring on the farm Vaaldam (due to upwards movement on account of the condensation effect after subble-burning); *Tullbergia* sp. 2 (Tullbergiidae) and *Proisotoma* sp. 1 (Isotomidae) only represented at the farm Vaaldam which had a lower rainfall. Also the farm Klein Brittanje had the highest annual rainfall of these three areas and *Capbrya* sp. 3 (Entomobryidae), *Lepidocyrtus* sp. 1 (Entomobryidae), *Entomobrya* sp. 1 & 2 (Entomobryidae), Neanuridae sp.1 and *Friesea* sp. 1 (Neanuridae) were only sampled at this locality (with a fallow-field system which enhances the soil organic component). This farm also had the highest number of Araneae species (Addendum A).

- The importance of soil fauna is, however, not to have high abundance or high species richness, but to maintain a healthy soil community with representatives in all trophic levels. This will ensure a higher productivity level since most of the possible services could be provided and, together with a high species richness, the resilience of that community will be increased and the effect of disturbances reduced. This correlates with the findings of Nielsen *et al.* (2011) and Wall *et al.* (2012).

b) Recommendations

- Evaluate environmental factors that could influence the persistence of all disturbances before application.
- Reduce disruptive applications and make use of more friendly management strategies, such as the increase of soil organic material (SOM) to reduce the effect of disruptions that are necessary.
- Gather all the necessary information before deciding on a treatment program for pests, since a more specifically targeted approach would cause minimal damage to non-target organisms. Therefore do not use broad-spectrum treatments.

- Minimize chemical drift when biocides or fertilizers are applied, since even with minimal drift natural soil fauna populations could be harmed, which lowers the efficiency of refuge areas around fields. If these natural populations become unstable, pests could exploit the refuge areas.
- Enrich soils with organic additives, rather than inorganic chemicals, since soils with optimal functioning can provide services that improve yields and reduce the need for mechanical disturbances.
- Rather do not remove plant material from fields, since these materials provide stability to the soil, resources for soil fauna, lower the effects of other disturbances and reduce the chances of wind compaction and sandstorms. If these materials are to be burned, ensure that the ashes are washed or worked into the soil to enrich the soils with nutrients.

c) Future Research

During this study the following random research opportunities came to light:

i) <u>Polluted sites</u>

- Determine the pollutant, its spread and distribution mechanisms and determine all the other environmental factors that could influence its persistence and movement within the area.
    - Determine depth of pollutant in soil
    - Understand soil properties
    - Investigate the influences on groundwater
- Determine the biotic component and its ecology in the area, as well as the bioaccumulation factor within all biotic components (fauna and flora).

- Do laboratory tests on toxicity of the pollutant(s) and determine the mechanisms used by the biota to evade, tolerate or utilize the polluted resources.

ii) <u>Pesticide application sites</u>

- Determine the influence of different biocide applications on soil faunal groups and include fungi and bacteria in the analysis.
- Add soil analyses to determine the effect on soil fauna under different conditions, *i.e.* pH value, clay %, trace elements present, water applications, etc., and monitor changes in any of these factors over the course of the study.
- Investigate biocide application in combination with other agricultural related conditions, such as:
    - Mechanical disturbances – tillage
    - Chemical disturbances – additional biocides and fertilizers
    - Controlled stubble-burning
    - Number of replicates of the same biocide
    - Natural veldt
- This duration of the study should at least be a few years in order to properly track the extent of regular applications and recovery.

iii) <u>Different fertilizer application methods</u>

- Investigate the effect of fertilizer application methods on soil faunal groups and the succession process after the application.
- Make use of ammonia gas, liquid fertilizer, granular fertilizer and organic fertilizer and determine their effect on the soil fauna.

- Do field experiments and if possible, do replications in different soil types under the same climatic conditions.

iv) <u>Sandstorms: Distribution due to 'air borne' topsoil</u>

- Sandstorms remove large quantities of topsoil each year. Since a large proportion of the beneficial soil fauna occur in this part of the soil it is necessary to determine how such an event affects them.
- Field trials should be done, by collecting soil from these sandstorms. Extraction of soil organisms would then indicate how many of these organisms were transported per kilogram sand.
- By incorporating wind speed, humidity and certain laboratory trials, the distance 'traveled', survival and ecological displacement of the soil fauna could be determined.

v) <u>Survey of South African Collembola</u>

- Determine the distribution and incidence of collembolan species richness across South Africa.
- Add barcoding information on already described and new species.
- Examine gut contents of Collembola sampled from the environment to place all species within the correct trophic level.
- Determine generalist and specialized feeding strategies.

d) References

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Addendum A

Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms	Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Arachnida																								
Spiders	A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Order: Araneae																								
Pholcidae - *Smeringopus* sp. (Imm.)										E														
Linyphiidae - *Meioneta* sp. (Imm.)									A															
Linyphiidae - *Meioneta* sp. 1													A			G								
Linyphiidae - *Metaleptyphantes familiaris*									A									K						
Linyphiidae - *Limoneta sirimoni*				F																				
Linyphiidae - *Ostearius melanopygius*	A	B		F			A						A											
Linyphiidae - *Pelecopsis janus*													A		E	G								
Linyphiidae - *Mermessus fradeorum*	A																							
Linyphiidae sp. 1		B														G								
Hahniidae - *Hahnia tabulicola*																G								
Lycosidae - *Amblyothele* sp. (Imm.)																G								
Lycosidae - *Proevippa* sp. (Imm.)																		K						
Lycosidae - *Paradosa* sp. (Imm.)		B																						
Lycosidae - *Paradosa crassipalpis* (Imm.)																G								
Ammoxenidae - *Ammoxenus amphalodes*																				M				
Gnaphosidae - *Camillina cordifera*													A		E									
Gnaphosidae - *Camillina* sp. (Imm.)													A			G							I	
Gnaphosidae - *Drassodes* sp. (Imm.)															E	G								
Gnaphosidae - *Setaphis* sp. (Imm.)													A		E	G								
Gnaphosidae - *Zelotes fuligineus*																G								
Gnaphosidae - *Zelotes* sp. (Imm.)													A		E									
Gnaphosidae sp. (Imm.)						G							A											
Philodromidae - *Thanatus* sp. 1 (Imm.)															E			K					I	
Araneae - 1st Instar Immatures									A															
Mites & Tics																								
Superorder: Parasitiformes																								
Order: Ixodida																								
Argasidae - *Argas* sp. 1										E														
Order: Mesostigmata																								
Uropodidae sp. 1																G								
Uropodidae sp. 2												G												
Parasitidae - *Pergamasus* sp. 1	A												A	D		G							I	J
Rhodacaridae - *Gamasellopsis* sp. 1	A								A							G								
Rhodacaridae - *Protogamasellus* sp. 1	A	B	C	F		G	A	G	A	E			A		E	G				M	G	H	I	J
Rhodacaridae - *Protogamasellus* sp. 2	A			F	E	G			A	E		G	A		E	G	G	K	L	M	G	H	I	J
Rhodacaridae - *Protogamasellus* sp. 3	A			F	E	G			A	E			A		E	G	G	K	L	M	G	H	I	J
Rhodacaridae m.sp. 1									A				A											
Rhodacaridae - Nymph m.sp. 1	A					G			A	E			A		E	G	G		L	M	G	H	I	J

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Arachnida																									
Mites & Tics		A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Superorder: Parasitiformes																									
Order: Mesostigmata																									
Macrochelidae - *Macrocheles* sp. 1	⊕	A																	K			G			
Ascidae - *Gamasellevans* sp. 1	⊕	A			F	E	G			A	E	F	G	A		E	G					G	H	I	J
Ascidae - Nymph m.sp. 1	⊕	A					G			A	E	F		A		E	G	G		L	M	G	H	I	J
Phytoseiidae - *Typhlodromus* sp. 1	⊕	A					G			A	E			A		E	G					G	H	I	J
Dermanyssidae - *Laelaps* sp. 1	⊕																G								
Dermanyssidae - Laelaptinae m.sp. 1	⊕													A		E									
Laelapidae - cf. *Hypoaspis* sp. 1	⊕	A												A		E				L					
Mesostigmata m.sp. 1	⊕																G								
Mesostigmata m.sp. 2	⊕																G								
Mesostigmata Larvae m.sp. 1	⊕	A												A											
Mesostigmata Nymph m.sp. 1	⊕						G				E			A		E	G								
Superorder: Acariformes																									
Order: Trombidiformes																									
Suborder: Prostigmata																									
Bdellidae - *Spinibdella thori*	⊕						G						G	A		E	G					G			J
Bdellidae - *Spinibdella* sp. 1	⊕												G												
Bdellidae - *Spinibdella* sp. 2	⊕										E						G					G			
Cunaxidae - *Cunaxa* sp. 1	⊕	A			F		G			A	E			A		E	G		K	L					J
Cunaxidae - *Cunaxa* sp. 2	⊕	A					G						G			E	G				M	G	H		J
Cunaxidae - *Dactyloscheles* sp. 1	⊕										E												H		
Rhagidiidae Larvae m.sp. 1	⊕										E					E									
Eupodidae - *Eupodes* sp. 1	⧗	A			F	E	G			A	E		G	A		E	G	G	K	L	M	G	H	I	J
Eupodidae - *Eupodes* sp. 2	⧗						G										G					G			
Eupodidae - *Eupodes* sp. 3	⧗	A																							
Eupodidae - *Cocceupodes* sp. 1	⧗															E									
Tydeidae - *Brachytydeus* sp. 1	⧗	A			F	E	G			A	E	F	G	A		E	G	G		L	M	G	H	I	J
Tydeidae - *Brachytydeus* sp. 2	⧗									A			G				G								
Tydeidae - *Pronematus* sp. 1	⧗	A			F	E	G			A	E	F		A		E	G		K		M	G	H	I	J
Caeculidae - *Microcaeculus* m.sp. 1	⊕	A					G						G												J
Caeculidae - *Microcaeculus* m.sp. 2	⊕						G																		
Caeculidae - *Microcaeculus* m.sp. 3	⊕																								
Caeculidae Larvae m.sp. 1	⊕												G	A				G				G	H	I	J
Adamystidae - *Saxidromus* sp. 1	⊕						G										G								
Anystidae - *Anystis* sp.	⊕												G												
Anystidae - Erythracarinae m.sp. 1	⊕									A										L					
Anystidae - Erythracarinae m.sp. 2	⊕																								
Anystidae - Erythracarinae m.sp. 3	⊕									A															

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Arachnida																									
Mites & Tics		A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Superorder: Acariformes																									
Order: Trombidiformes																									
Suborder: Prostigmata																									
Anystoidea m.sp. 1	⊕															E	G								
Pseudocheylidae - *Anaplocheyles* sp. 1	⊕	A					G			A				A		E	G								
Paratydeidae - *Tanytydeus cristatus*	⊕	A					G																		
Erythraeidae - *Erythraeus* sp. 1	⊕	A					G																		
Erythraeidae - *Leptus* sp. 1	⊕													A			G	G							
Erythraeidae Larvae m.sp. 1	⊕															E									
Erythraeidae Nymph m.sp. 1	⊕												G				G								
Erythraeidae Nymph m.sp. 2	⊕						G							A		E	G								
Erythraeidae Nymph m.sp. 3	⊕																G								
Erythraeidae Nymph m.sp. 4	⊕						G									E	G								
Cohort - Parasitengonina m.sp. 1	⊕																G								
Trombidiidae sp. 1	⊕															E	G								
Microtrombidiidae - *Microthrombidium* sp. 1	⊕						G									E	G	G			M				
Microtrombidiidae Nymph m.sp. 1	⊕	A												A		E	G								
Raphignathidae - *Raphignathus* m.sp. 1	⊕																G								
Raphignathidae - *Raphignathus* m.sp. 2	⊕	A					G							A		E	G								
Raphignathidae m.sp. 1	⊕																G								
Tetranychidae - *Bryobia praetiosa*	▲						G										G								
Tetranychidae - *Tetranychus urticae*	▲	A					G							A		E	G			L					
Linotetranidae - *Linotetranus* sp. 1	▲	A					G						G	A		E	G	G		L	M	G	H	I	J
Linotetranidae - *Linotetranus* sp. 2	▲	A					G						G	A		E	G								
Cheyletidae - *Cheyletiella* sp. 1	⊕						G			A	E		G	A		E	G							I	
Cheyletidae - *Cheyletus* sp. 1	⊕															E			K			G			
Scutacaridae - *Imparipes* sp. 1	⧗	A								A	E			A		E	G					G	H	I	J
Scutacaridae - *Imparipes* sp. 2	⧗	A								A	E			A		E	G					G	H		J
Scutacaridae - *Scutacarus* sp. 1	⧗	A			F		G			A	E														
Tarsonemidae - *Hemitarsonemus* sp. 1	⧗	A			F	E	G			A	E	F	G	A		E	G	G	K	L	M	G	H	I	J
Prostigmata m.sp. 1	⧗	A												A		E	G								
Prostigmata m.sp. 2	⧗	A															G								
Order: Sarcoptiformes																									
Suborder: Endeostigmata																									
Nanorchestidae - *Nanorchestes* sp. 1	●	A			F		G			A	E			A		E	G								J
Nanorchestidae - *Nanorchestes* sp. 2	●	A					G			A	E			A		E	G	G	K			G	H	I	J
Nanorchestidae - *Nanorchestes* sp. 3	●	A					G			A															
Nanorchestidae - *Nanorchestes* sp. 4	●	A					G			A	E			A		E	G				M				
Nanorchestidae - *Speleorchestes meyeri*	●	A					G			A	E	F	G	A		E	G			L	M	G	H	I	J
Alicorhagiidae - *Stigmalychus veretrum*	●	A																							

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms	Vaaldam	Koppieskraal	Thornberry	Klein Brittanj	Eureka	Paradys
Class: Arachnida						
Mites & Tics	A B C F E G	A G	A E F G	A D E G	G K L M	G H I J
Superorder: Acariformes						
Order: Sarcoptiformes						
Suborder: Oribatida						
Brachychthoniidae - *Brachychthonius* sp. 1	A G					
Nanhermanniidae - *Nanhermannia* sp. 1			E			
Epilohmanniidae - *Epilohmannia* sp. 1	B		A	A G		
Euphthiracaridae - *Acrotritia* sp. 1			A			
Oppiidae - *Oppiella* sp. 1	A B C F G	A G	A E	A E G	G K L M	G J
Oppiidae m.sp. 1	A G		A	A E G		
Tectocepheidae - *Tectocepheus* sp. 1			A E	A E G		
Scutoverticidae - *Ethiovertex* sp. 1	G		A G	A		
Protoribatidae - *Protoribates* sp. 1	G		A E G	A E G		G H I J
Oribatulidae - *Oribatula* (*Zygoribatula*) sp.1			A E F G	A E G		
Oribatulidae - *Oribatula* (*Zygoribatula*) sp.2			A E G	A E G		
Oribatulidae - *Oribatula* (*Zygoribatula*) sp.3			A E G			
Oribatulidae - Nymph m.sp. 1			A E	A G		
Oribatulidae - Nymph m.sp. 2	A G		A E G	A E G		
Chamobatidae - *Hypozetes* sp. 1	A B G	A G	G	A D E G		
Galumnidae - *Galumna* sp. 1				A E		
Galumnidae Nymph m.sp. 1				A E		
Oribatida m.sp. 1						
Oribatida Nymph m.sp. 1	A G		A E G	A E G		H
Suborder: Astigmata						
Acaridae - *Caloglyphus* sp. 1	A		A E	A E		
Acaridae - cf. *Tyrophagus putrescentiae*	A F G		A E G	A E G	G K	G H I J
Acaridae - *Rhizoglyphus* sp. 1	A F	A	A E	A G		
Acaridae - *Rhizoglyphus* sp. 2	A			E		G
Acaridae m.sp. 1			E G	E G		
Acaridae m.sp. 2	A G		A E	G		
Acaridae m.sp. 3				A G		
Acaridae Nymph m.sp. 1	A		A	A E G		
Pseudoscorpions						
Order: Pseudoscorpiones						
Pseudoscorpiones m.sp. 1	G					
Pseudoscorpiones m.sp. 2	G					

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms	Vaaldam	Koppieskraal	Thornberry	Klein Brittanje	Eureka	Paradys
Class: Malacostraca						
Pillbugs and woodlice	A B C F E G	A G	A E F G	A D E G	G K L M	G H I J
Order: Isopoda						
Armadillidiidae m.sp. 1	G					
Oniscidea m.sp. 1	A	A		A		
Class: Chilopoda						
Centipedes	A B C F E G	A G	A E F G	A D E G	G K L M	G H I J
Order: Geophilamorpha						
Geophilamorpha m.sp. 1	A B	A	A E	A D E G		
Geophilamorpha m.sp. 2	A		A F	E G		J
Geophilamorpha m.sp. 3			A E			
Class: Symphyla?						
Symphylans						
Order: Symphyla						
Scolopendrellidae sp. 1			A G			
Class: Hexapoda						
Proturans						
Order: Protura						
Protura spp.	F			G		
Springtails						
Order: Collembola						
Suborder: Poduromorpha						
Brachystomellidae - *Brachystomella* sp. 1	A			A E G	G K	G H I J
Brachystomellidae - *Brachystomella* sp. 2			G	A E G		
Immatures - cf. Brachystomelldae	A			A E G	G L M	G H I J
Hypogastruridae - *Xenylla* sp. 1						H
Hypogastruridae - *Hypogastrura* sp. 1	A F G	A G				
Hypogastruridae - *Hypogastrura* sp. 2			A E G			
Hypogastruridae - *Hypogastrura* sp. 3			A E F G			
Hypogastruridae - *Hypogastrura* sp. 4					G K L	
Immatures - cf. Hypogastruridae	A G		E			
Neanuridae - *Friesea* sp. 1				A E		
cf. Neanuridae sp. 1				A		
Tullbergiidae - *Mesaphorura yosii*	A F					
Tullbergiidae - *Mesaphorura* sp. 1	A G	A				
Tullbergiidae - *Tullbergia* sp. 1				A E G		H I J
Tullbergiidae - *Tullbergia* sp. 2	A F					

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam	Koppieskraal	Thornberry	Klein Brittanje	Eureka	Paradys
Class: Hexapoda							
Springtails		A B C F E G	A G	A E F G	A D E G	G K L M	G H I J
Order: Collembola							
Suborder: Entomobryamorpha							
Isotomidae - *Proisotoma* sp. 1	⧗	A F G					
Isotomidae - *Proisotoma* sp. 2	⧗	A F	A				
Isotomidae - *Proisotoma* sp. 3	⧗						G H I J
Isotomidae - *Proisotoma* sp. 4	⧗			A E F G	A E G		
Isotomidae - cf. *Isotomodes* sp. 1	⧗						H I J
Isotomidae - *Mucrosomia* sp. 1	⧗					G L M	
Isotomidae - *Folsomides parvulus*	⧗	A G		A E F G			
Entomobryidae - *Seira* sp. 1	▲						G H J
Entomobryidae - *Seira* sp. 2	▲				A E G	M	
Entomobryidae - *Entomobrya* cf. *multifasciata*	▲	A G	A G	A E G			
Entomobryidae - *Entomobrya* sp. 1	▲				A E		
Entomobryidae - *Entomobrya* sp. 2	▲				A E		
Entomobryidae - cf. *Lepidocyrtus* sp. 1	⧗				A E		
Entomobryidae - *Lepidocyrtus* sp. 2	⧗			A			
Entomobryidae - *Lepidocyrtus* sp. 3	⧗			A E			
Entomobryidae - *Lepidocyrtus* sp. 4	⧗					K M	
Entomobryidae - *Lepidocyrtus* sp. 5	⧗					K	
Entomobryidae - *Lepidocyrtus* sp. 6	⧗					K	
Entomobryidae - *Pseudosinella* sp. 1	⧗				A E G	M	H J
Entomobryidae - *Capbrya* m.sp. 1	⧗	A					H I
Entomobryidae - *Capbrya* m.sp. 2	⧗			G	A E G		
Entomobryidae - *Capbrya* m.sp. 3	⧗				A		
Entomobryidae - *Capbrya* m.sp. 4	⧗					K M	
Entomobryidae sp. 1	⧗	A			A		
Entomobryidae sp. 2	⧗				A	K	
Cyphoderidae - *Cyphoderus* sp. 1	⧗				A E	G M	
Suborder: Neelipleona							
Neelidae - *Megalothorax* sp. 1	⧗			A			
Suborder: Symphypleona							
Sminthurididae - *Sphaeridia* sp. 1	▲	A G			A E G	G M	G H I J
Bourletiellidae sp. 1	▲					M	
Bourletiellidae sp. 2	▲					L M	
Bourletiellidae sp. 3	▲			G			
Diplurans							
Order: Diplura							
Japygidae sp. 1	⊕		A				

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Hexapoda																									
Silverfish		A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Order: Thysanura																									
Lepismatidae m.sp. 1	◎																G								
Grasshoppers & Crickets																									
Order: Orthoptera																									
Nymph - Gryllidae sp. 1	◎	A												A											
Earwigs																									
Order: Dermaptera																									
Labiduridae sp. 1	◎																							I	
Labiduridae sp. 2	◎																			L					
Termites																									
Order: Isoptera																									
Termitidae m.sp. 1	▬						G											G							
Termitidae m.sp. 2	▬										E		G												
Termitidae m.sp. 3	▬	A																							
Termitidae m.sp. 4	▬													A											
Hodotermitidae m.sp. 1	▬	A					G		G																
Hodotermitidae m.sp. 2	▬																G								
Bugs																									
Order: Hemiptera																									
Suborder: Heteroptera																									
Miridae - Deraeocorinae sp. 1	⊕	A							G																
Miridae - Phylinae sp. 1	▲	A					G		G																
Miridae sp. 1	▲						G			A															
Tingidae sp. 1	▲						G																		
Anthocoridae sp. 1	⊕	A									E			A											
Cydnidae sp. 1	▲						G																		
Lygaeidae - Geocorinae sp. 1	⊕																G								
Lygaeidae sp. 2	▲	A																							
Pyrrhocoridae m.sp. 1	▲						G																	I	
Nymph m.sp. 1 - cf. Lygaeidae	▲						G																H		J
Nymph m.sp. 2 - cf. Miridae	▲	A							G						D	E	G							I	
Nymph m.sp. 3 - Reduviidae	⊕																G								
Nymph m.sp. 4 - cf. Cydnidae	▲													A											
Nymph m.sp. 5 - cf. Lygaeidae	▲																					G	H	I	J
Nymph m.sp. 6	▲																	G						I	J
Nymph m.sp. 7 - cf. Lygaeidae	▲																	G							
Nymph m.sp. 8	▲						G																		
Nymph m.sp. 14	▲																G								

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Hexapoda																									
Bugs		A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Order: Hemiptera																									
Suborder: Heteroptera																									
Nymph m.sp. 15	▲																G								
Nymph m.sp. 17	▲																						H		
Nymph m.sp. 18 - Pentatomidae	▲																								J
Nymph m.sp. 19 - cf. Lygaeidae	▲																							I	
Nymph m.sp. 21 - cf. Lygaeidae	▲																								J
Nymph m.sp. 23 - cf. Lygaeidae	▲									A							G								
Nymph m.sp. 25	▲						G																		
Nymph m.sp. 36	▲																G								
Suborder: Auchenorrhyncha																									
Cercopidae sp. 1	▲						G																		
Cicadellidae m.sp. 1	▲	A					G		G					A				G							
Cicadellidae m.sp. 2	▲	A					G																		
Cicadellidae m.sp. 3	▲						G																		
Cicadellidae m.sp. 4	▲										E														
Nymph m.sp. 9	▲						G																		
Nymph m.sp. 10 - cf. Cicadellidae	▲																G					G			J
Nymph m.sp. 20 - cf. Cicadellidae	▲																					G	H		
Nymph m.sp. 24	▲																G								
Suborder: Sternorrhyncha																									
Psyllidae sp. 1	▲		B																						
Aphididae m.sp. 1	▲	A								A									K						
Aphididae m.sp. 2	▲																		K						
Diaspididae m.sp. 1 (♂)	▲										E														
Coccoidea Nymph m.sp. 1 - cf. Pseudococcidae	▲	A					G		G	A			G	A			G								
Coccoidea Nymph m.sp. 2 - cf. Pseudococcidae	▲								G												M				
Coccoidea Nymph m.sp. 3 - cf. Pseudococcidae	▲																			L					
Coccoidea Nymph m.sp. 4 - cf. Monophlebidae	▲																		K						
Coccoidea Nymph m.sp. 5 - cf. Pseudococcidae	▲																	G							
Coccoidea Nymph m.sp. 6 - cf. Pseudococcidae	▲						G																		
Coccoidea Nymph m.sp. 7 - Margarodidae	▲						G																		
Thrips																									
Order: Thysanoptera																									
Suborder: Tubulifera																									
Phlaeothripidae m.sp. 1 - cf. *Haplothrips* sp.	▲	A	B				G		G					A			G		K	L	M				
Phlaeothripidae m.sp. 2	⧗	A												A		E	G			L					
Phlaeothripidae m.sp. 3	⧗						G							A											
Phlaeothripidae m.sp. 4	⧗												G				G								

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam	Koppieskraal	Thornberry	Klein Brittanje	Eureka	Paradys
Class: Hexapoda							
Thrips		A B C F E G	A G	A E F G	A D E G	G K L M	G H I J
Order: Thysanoptera							
Suborder: Tubulifera							
Phlaeothripidae m.sp. 5	⧗			G	G		
Phlaeothripidae m.sp. 6 - cf. *Haplothrips* sp.	▲			A	A G		G H J
Immature m.sp. 2 - cf. Phlaeothripidae	⧗	G		G	G	M	G H
Immature m.sp. 3 - cf. Phlaeothripidae	⧗				G		H
Immature m.sp. 4 - cf. Phlaeothripidae	⧗				A G		
Immature m.sp. 5 - cf. Phlaeothripidae	⧗				E G		
Immature m.sp. 6 - cf. Phlaeothripidae	⧗				A		H
Suborder: Terebrantia							
Aeolothripidae sp. 1	⊕		G				
Thripidae m.sp. 1	▲	A B C G	A G	G	A E G	M	
Thripidae m.sp. 2	▲	A G		A G	A D E G	G K L M	G H J
Thripidae m.sp. 3	▲	A		G		M	J
Thripidae m.sp. 4	▲				A G		H
Thripidae m.sp. 5	▲			G			
Immature m.sp. 1 - cf. Thripidae	▲				G		
Booklice							
Order: Psocoptera							
Trogiidae sp. 1	⧗				E		
Liposcelididae sp. 1	▬	A G	A G	A E G	A D E G	G K L M	G H J
Immature m.sp. 1	⧗				A		
Immature m.sp. 2	⧗				G		
Beetles							
Order: Coleoptera							
Suborder: Adephaga							
Carabidae (Harpalinae) - *Harpalus* sp. 1	▲	A G		A E F	A E G		H
Carabidae (Pterostichinae) - *Pterostichus* sp. 1	⊕			G	G		
Carabidae - Pterostichinae sp. 1	⊕				A E G		J
Carabidae - Pterostichinae sp. 2	⊕			E F	E	K	
Carabidae - cf. Pterostichinae sp. 3	⊕	A		E G		L	
Suborder: Polyphaga							
Histeridae sp. 1	⊕	G			G		
Staphylinidae - *Philonthus* sp. 2	⊕					L	
Staphylinidae - *Philonthus* sp. 3	⊕				G		
Staphylinidae - Pselaphinae sp. 1	⊕			A	G		
Staphylinidae sp. 1	⊕	A B	A G	A E G	A E	K	
Staphylinidae sp. 2	⊕				A E	K L	

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Hexapoda																									
Beetles		A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Order: Coleoptera																									
Suborder: Polyphaga																									
Scarabaeidae (Aphodiinae) - *Aphodius* sp. 1	✦																						H		J
Scarabaeidae (Aphodiinae) - *Aphodius* sp. 3	✦	A		C	F								G	A		E	G	G		L			H	I	
Scarabaeidae (Aphodiinae) - *Rhyssemus* sp. 1	✦										E		G	A		E	G								
Scarabaeidae (Scarabaeinae) - *Onthophagus* sp. 1	✦																G	G							
Scarabaeidae - Melolonthinae sp. 1	▲	A																							
Elateridae - *Cardiotarsus acuminatus*	▲													A											
Elateridae sp. 1	▲													A											
Elateridae sp. 2	▲									A															
Lampyridae - *Lampyris* sp. 1		A																							
Dermestidae - *Anthrenus* sp. 1	◎																						H		
cf. Dermestidae sp. 2	◎	A																		L	M				
cf. Anobiidae m.sp. 1	▬	A										F		A			G			L			H		
cf. Anobiidae m.sp. 2	▬	A			F		G																		
Melyridae (Dasytinae) - *Astylus atromaculatus*	▲						G																		
Melyridae sp. 1	⊕	A					G																		
Nitidulidae - *Brachypeplus* sp. 1	⊕	A			F		G			A				A		E									
Nitidulidae sp. 1	▬	A												A			G								
Silvanidae sp. 1	⧗						G										G								
Cryptophagidae sp. 1	⧗	A																				G			
Cryptophagidae sp. 2	⧗		B							A	E	F		A	D	E	G								
Coccinellidae - *Scymnus* sp. 1	⊕																		K						
Coccinellidae - *Scymnus* sp. 2	⊕																		K						
Coccinellidae - *Scymnus* sp. 3	⊕													A											
cf. Mycetophagidae sp. 1	⧗	A											G												
Tenebrionidae - *Gonocephalum simplex*	▲													A		E									
Tenebrionidae - *Tribolium castaneum*	▬						G																		
Tenebrionidae - *Phanerotomea* sp. 1	▬																G								
Tenebrionidae (Tenebrioninae) - *Alphitobius* sp. 2	▬							A						A											
Tenebrionidae - *Zophosis* sp. 1	▬	A												A										I	
Tenebrionidae sp. 1	▬													A		E									
Tenebrionidae sp. 2	▬						G																		
Tenebrionidae sp. 3	▬		B				G																		
Anthicidae - *Anthicus* sp. 1	◎	A														E	G								
Anthicidae - *Anthicus* sp. 2	◎	A																							
Anthicidae - *Anthicus* sp. 3	◎	A								A	E									L					
Anthicidae - *Anthicus* sp. 4	◎										E						G								

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms	Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Hexapoda																								
Beetles	A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Order: Coleoptera																								
Suborder: Polyphaga																								
Anthicidae - *Formicomus* sp. 1	A					G	A									G								
Anthicidae - *Notoxus cucullatus*											F													
Anthicidae sp. 1										E			A											J
Anthicidae sp. 2										E														
Aderidae sp. 1															E									
Chrysomelidae - Alticinae sp. 1																		K						
Chrysomelidae - Bruchinae sp. 1	A									E														
Curculionidae sp. 1								G																
Scolytidae sp. 1													A				G			M				
Coleoptera Larvae																								
Campodeiform larvae m.sp. 1	A						A		A	E			A		E	G	G						I	J
Campodeiform larvae m.sp. 2 - cf. Carabidae	A			F						E	F		A		E	G								
Campodeiform larvae m.sp. 3 - cf. Dermestidae							A																	
Campodeiform larvae m.sp. 4	A								A				A		E	G					G	H		J
Campodeiform larvae m.sp. 5 - cf. Carabidae	A								A														I	
Campodeiform larvae m.sp. 6 - cf. Carabidae	A								A		F				E	G								J
Campodeiform larvae m.sp. 7 - cf. Carabidae												G											I	
Campodeiform larvae m.sp. 8 - cf. Carabidae																						H	I	J
Campodeiform larvae m.sp. 9 - cf. Carabidae	A									E		G												
Campodeiform larvae m.sp. 10 - cf. Carabidae										E														
Elateriform larvae m.sp. 1	A	B	C	F		G		G					A	D	E	G				M				
Elateriform larvae m.sp. 2	A	B	C	F					A				A	D									I	J
Elateriform larvae m.sp. 3 - cf. Elateridae	A			F					A				A	D	E	G						H	I	J
Elateriform larvae m.sp. 4																						H		
Elateriform larvae m.sp. 5 - cf. Tenebrionidae	A			F		G			A	E	F		A		E	G	G		L	M	G	H	I	J
Elateriform larvae m.sp. 6 - cf. Nitidulidae	A														E	G						H		
Elateriform larvae m.sp. 7 - cf. Cryptophagidae	A								A							G						H		
Elateriform larvae m.sp. 8 - cf. Tenebrionidae	A					G			A	E			A		E	G	G		L		G	H	I	J
Elateriform larvae m.sp. 9	A								A				A		E								I	J
Elateriform larvae m.sp. 10 - cf. Elateridae									A	E			A		E					M		H	I	J
Elateriform larvae m.sp. 11									A	E			A								G	H	I	
Elateriform larvae m.sp. 12 - cf. Tenebrionidae									A						E	G					G	H	I	
Elateriform larvae m.sp. 13	A											G			E	G	G							
Elateriform larvae m.sp. 14 - cf. Erotylidae	A																G							
Elateriform larvae m.sp. 15 - cf. Tenebrionidae	A												A			G	G	K	L					
Elateriform larvae m.sp. 16	A												A											
Elateriform larvae m.sp. 17	A																							

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam	Koppieskraal	Thornberry	Klein Brittanje	Eureka	Paradys
Class: Hexapoda							
Beetles		A B C F E G	A G	A E F G	A D E G	G K L M	G H I J
Order: Coleoptera							
Coleoptera Larvae							
Elateriform larvae m.sp. 18	▲				A		
Elateriform larvae m.sp. 19	▲			F	G		
Elateriform larvae m.sp. 20	▲			F	A E G		
Elateriform larvae m.sp. 21	▲				A E		
Elateriform larvae m.sp. 22 - cf. Elateridae	▲			A E	E G		
Elateriform larvae m.sp. 23 - cf. Elateridae	▲				A G		
Eruciform larvae m.sp. 4 - cf. Chrysomelidae	▲				A	K	
Eruciform larvae m.sp. 5	▲				A E G		
Scarabaeiform larvae m.sp. 1 - cf. Scarabaeidae	▲	A E			A E		H I
Scarabaeiform larvae m.sp. 2	▲	A G	G				
Scarabaeiform larvae m.sp. 3	▲	A					
Vermiform larvae m.sp. 3	▲	A G			G		
Vermiform larvae m.sp. 5	▲	G		G	G	L	G
Vermiform larvae m.sp. 13 - cf. Curculionidae	▲	A G			A	G	
Vermiform larvae m.sp. 24 - cf. Buprestidae	▲				G		
Lacewings		A B C F E G	A G	A E F G	A D E G	G K L M	G H I J
Order: Neuroptera							
Coniopterygidae sp. 1	⊕	A					
Wasps & Ants							
Order: Hymenoptera							
Ceraphronidae - cf. *Ceraphron* sp. 1	◊	A G		A	A E G		
Ceraphronidae - cf. *Aphanogmus* sp.	◊	A			G		
Ceraphronidae sp. 3	◊				G		
Braconidae sp. 1	◊	A					
Braconidae sp. 2	◊	A					
Braconidae sp. 3	◊	G					
Mymaridae sp. 1	◊						
Mymaridae sp. 2	◊	A					
Eulophidae - cf. Entedoninae sp. 1	◊	A					
Encyrtidae sp. 1	◊				A		
Encyrtidae sp. 2	◊	B					
Pteromalidae sp. 1	◊	A G					
Scelionidae - *Baeus* sp. 1	◊				A E G		
Scelionidae sp. 1	◊					K	
Platygastridae sp. 1	◊				G		J
Bethylidae - cf. Epyrinae sp. 1	◊	A G					
Bethylidae sp. 1	◊				G		

Addendum A

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Hexapoda																									
Wasps & Ants		A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Order: Hymenoptera																									
Bethylidae sp. 2	◇						G																		
Formicidae - Cerapachyninae m.sp. 1	⊕			C			G			A			G	A	D		G								
Formicidae - Cerapachyninae m.sp. 2	⊕	A									E			A		E			G			G	H		
Formicidae - Cerapachyninae m.sp. 3	⊕												G												
Formicidae - Dorylinae m.sp. 1	⊕	A					G		G				G		D		G				M		H		
Formicidae - Dorylinae m.sp. 2	⊕						G										G	G		L					
Formicidae - Ponerinae m.sp. 1	⊕	A												A		E	G		K						
Formicidae - Ponerinae m.sp. 2	⊕													A											
Formicidae - Ponerinae m.sp. 3	⊕	A					G		G						D										
Formicidae - Pseudomyrmecinae sp. 1	▲																	G							
Formicidae - Myrmicinae m.sp. 1	▲						G						G												
Formicidae - Myrmicinae m.sp. 2	▲						G							A	D		G								
Formicidae - Myrmicinae m.sp. 3	▲	A															G						H		J
Formicidae - Myrmicinae m.sp. 4	▲													A		E	G			L					J
Formicidae - Dolichoderinae m.sp. 1	▲												G												
Formicidae - Dolichoderinae m.sp. 2	▲												G						K						
Formicidae - Formicinae m.sp. 1	▲						G	A	G						D										
Formicidae - Formicinae m.sp. 2	▲	A							G							E	G								
Formicidae - Formicinae m.sp. 3	▲						G																		
Vermiform larvae m.sp. 7 - Formicidae							G							A			G								J
Moths																									
Order: Lepidoptera																									
Gelechiidae sp. 1	▲	A						A	G																
Tortricidae sp. 1	▲	A																							
Pyralidae m.sp. 1	▲	A																							
Crambidae m.sp. 1	▲	A					G				E														
Lepidoptera Larvae																									
Eruciform larvae m.sp. 1 - cf. Noctuidae	▲	A	B				G																		
Eruciform larvae m.sp. 2	▲	A															G	G		L					
Eruciform larvae m.sp. 3	▲	A																							
Flies																									
Order: Diptera																									
Suborder: Nematocera																									
Psychodidae sp. 1	▲	A											G												
Ceratopogonidae sp. 1	⬭	A									E														
Chironomidae sp. 1		A					G				E														
Simuliidae sp. 1	⬭												G												

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms		Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Hexapoda																									
Flies		A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Order: Diptera																									
Suborder: Nematocera																									
Cecidomyiidae sp. 1	▲	A	B		F		G	A		A			G	A			G			L	M				
Cecidomyiidae sp. 2 (♂)	▲	A					G							A			G								
Cecidomyiidae sp. 3 (Wingless)	▲															E									
Suborder: Brachycera																									
Dolichopodidae sp. 1	⊕	A																							
Phoridae sp. 1	◎	A																	K		M				
Phoridae sp. 4	◎									A			G												
Phoridae sp. 5	◎									A															
Phoridae sp. 6 (Wingless)	◎													A											
Anthomyiidae sp. 1	◎	A																							
Agromyzidae sp. 1	▲						G																		
Sepsidae sp. 1	▲															E									
Sepsidae sp. 2	▲										E														
Chloropidae sp. 1	▲	A																							
Chloropidae sp. 2	▲	A																							
Chloropidae sp. 3	▲																G								
Sphaeroceridae sp. 1	▲	A																							
Sphaeroceridae sp. 2	▲	A																							
Sphaeroceridae sp. 3	▲	A												A											
Sphaeroceridae sp. 4	▲																								
Sphaeroceridae sp. 5	▲																								
Drosophilidae sp. 1	⧗																			L					
Drosophilidae sp. 2	⧗																		K						
Diptera Larvae																									
Vermiform larvae m.sp. 1	▬	A	B					A	G					A	D					L	M				
Vermiform larvae m.sp. 2	▬	A					G							A		E	G							I	J
Vermiform larvae m.sp. 4	▬	A					G									E	G						H		J
Vermiform larvae m.sp. 6	▬													A		E	G						H	I	
Vermiform larvae m.sp. 8	▲																					G	H		
Vermiform larvae m.sp. 9	▬													A		E	G					G	H	I	
Vermiform larvae m.sp. 10	▬	A								A			G	A		E	G					G	H	I	
Vermiform larvae m.sp. 11	▬												G										H		
Vermiform larvae m.sp. 12	▲						G			A											M		H		
Vermiform larvae m.sp. 14 - cf. Bibionidae	▲	A														E									
Vermiform larvae m.sp. 15	▬	A														E	G		K						
Vermiform larvae m.sp. 16	▬	A														E									
Vermiform larvae m.sp. 17	▬	A								A				A		E	G		K						

Continued: Soil faunal groups recorded at the various sampling sites, with letters implicating their presence under specific conditions; A – maize field, B – potato field, C – cotton field, D – newly developed field, E – fallow field, F – 90min after stubble-burning, G – control in natural veldt, H – herbicide, I – insecticide, J – fungicide, K – canal wall at mine, L – mine spillage (2006), M – mine spillage (2000). Symbols represent trophic groups which are explained in Chapter 2 (See page 64).

Soil Organisms	Vaaldam						Koppieskraal		Thornberry				Klein Brittanje				Eureka				Paradys			
Class: Hexapoda																								
Flies	A	B	C	F	E	G	A	G	A	E	F	G	A	D	E	G	G	K	L	M	G	H	I	J
Order: Diptera																								
Diptera Larvae																								
Vermiform larvae m.sp. 18	A											G				G								
Vermiform larvae m.sp. 19	A																							
Vermiform larvae m.sp. 20	A																							
Vermiform larvae m.sp. 21	A																							
Vermiform larvae m.sp. 22						G							A		E									
Vermiform larvae m.sp. 23 - cf. Therevidae													A											
Class: Clitellata																								
Potworms																								
Order: Haplotaxida																								
Enchytraeidae spp.	A					G	A			E			A	D	E	G	G					H		

Addendum B

Soil mesofaunal species richness and abundance at 6 localities in the Free State, South Africa. Data were recorded between 2011 and 2014 under variable conditions (see Chapter 3, pp. 69-104)

Localities & Sampling Points	Vegetation Type	Agricultural Practices	Species Richness (Accumilated)	Abundance (min-max recorded)	Comments
Vaaldam (Fig. 3.2)					
Sampling points 1-3	Natural veldt	None	105	16 - 362	Fluctuations due to seasonal change
Sampling points 4-5	Bt. Maize	Agricultural practices noted in Fig. 3.4 and elevation	85	12 - 2161	Tillage observations
Sampling points 7&9	Bt. Maize		61	95 - 5831	
Sampling points 6&8	Non-Bt. Maize		87	135 - 1466	
Sampling points 11&13	Bt. Maize		74	50 - 6869	
Sampling points 10&12	Non-Bt. Maize		74	164 - 5109	
Sampling point 14	Non-Bt. Maize		43	27 - 1891	Higher elevation
Sampling point 15	Bt. Maize		46	72 - 916	
Sampling point 16	Non-Bt. Maize		44	280 - 1029	Lower elevation
Sampling point 17	Bt. Maize		39	157 - 1399	
Sampling points 18-23	Maize stubble	Stubble-burning	60	78 - 3461	
Sampling point 24-25	Potatoes (2012)	Minimal biocide application	13	3 - 70	Part of preliminary study; have lower values
Sampling points 26-29	Potatoes (2012)	Extensive biocide application	13	1 - 44	
Koppieskraal (Fig. 3.6)					
Sampling points 30-31	Maize stubble (2011-2012)	Ploughing	23	14 - 61	Part of preliminary study; have lower values
Sampling point 32	Natural veldt (2012)	None	14	15 - 59	
Sampling point 33	Natural veldt (2011-2012)	Herbicide application	21	3 - 548	
Thornberry (Fig. 3.9)					
Sampling point 34	Natural veldt	None	70	28 - 252	
Sampling points 35-36	Wheat stubble	Disc-ploughing	70	10 - 9078	Low abundance values due to compaction
Sampling points 37-38	Wheat stubble	Stubble-burning & disc-ploughing	53	12 - 479	
Sampling point 39	Maize	Various	57	7 - 2634	Clay = 26%
Sampling point 40	Maize		78	9 - 1466	Clay = 38%
Sampling point 41	Maize		61	69 -596	Clay = 23%
Paradys (Fig. 3.13)					
Sampling point 44	Natural veldt	Insecticide	57	52 - 477	
Sampling point 45	Natural veldt	General herbicide	49	11 - 526	
Sampling point 46	Natural veldt	Broadleaf herbicide	52	15 - 1116	
Sampling point 47	Natural veldt	Grass herbicide	42	12 - 1176	
Sampling point 48	Natural veldt	Fungicide	69	122 - 1879	
Sampling point 49	Natural veldt	None	52	51 - 1790	Fluctuations due to seasonal change

Addendum B

Continued: Soil mesofaunal species richness and abundance at 6 localities in the Free State, South Africa. Data were recorded between 2011 and 2014 under variable conditions (see Chapter 3, pp. 69-104)

Localities & Sampling Points	**Vegetation Type**	**Agricultural Practices**	**Species Richness** (Accumilated)	**Abundance** (min-max recorded)	**Comments**
Eureka (Fig. 3.18)					
Sampling points 50-51	Natural veldt	None	45	2 - 203	Fluctuations due to seasonal change
Sampling points 52-53	Natural veldt	Mine pollution	45	3 - 54	Spillage 2006
Sampling points 54-56	Natural veldt		47	1 - 43	Drainage canal
Sampling points 57-58	Natural veldt		43	6 - 135	Spillage 2000
Klein Brittanje (Fig. 3.23)					
Sampling point 60	Bt. Maize	Fallow 2013	76	33 - 395	Various agricultural practices
Sampling point 61	Bt. Maize	Cultivated 2012&2013	67	33 - 766	
Sampling point 62	Non-Bt. Maize	Cultivated 2012&2013	60	32 - 605	
Sampling point 63	Non-Bt. Maize	Fallow 2013	71	32 - 844	
Sampling point 64	Non-Bt. Maize	Cultivated 2012&2013	70	24 - 2179	
Sampling point 65	Bt. Maize	Cultivated 2012&2013	73	21 - 1365	
Sampling point 66	Bt. Maize	Cultivated 2012&2013	56	30 - 1172	
Sampling point 67	Natural veldt	None	130	45 - 4719	Fluctuations due to seasonal change
Sampling point 68	Bt. Maize	Fallow 2013	56	23 - 439	Various agricultural practices
Sampling point 69	Bt. Maize	Fallow 2013	68	7 - 599	
Sampling point 70	Non-Bt. Maize	Fallow 2013	66	34 - 747	
Sampling point 71	Bt. Maize	Fallow 2012	54	11 - 808	
Sampling point 72	Non-Bt. Maize	Fallow 2012	46	18 - 254	
Sampling point 73	Natural veldt	None	70	24 - 241	Fluctuations due to seasonal change
Sampling point 74	Natural veldt	None	92	39 - 334	