Belowground impacts of regenerative agriculture in a South African seed production system.

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ABSTRACT

Agricultural practices that are environmentally detrimental, is a serious global problem. Widespread deforestation as a means to expand agricultural areas and the overuse of agrochemicals causes rapid biodiversity loss, large scale soil erosion and agroecosystems to become stuck in negative feedback cycles. Agricultural management thus plays an important role in the stability and resilience of agroecosystems. Shifts towards more sustainable practices have been encouraged, with regenerative agriculture gaining a lot of traction over the years. It is crucial to understand the impact of various management strategies on various aspects of agroecosystems to gain a greater, more holistic understanding. A newly established long-term trial provided an ideal opportunity to explore and compare the impacts of regenerative agriculture with conventional agriculture within a cauliflower seed production system in the Western Cape of South Africa. Specifically, this study investigated the effects of different management strategies on soil fauna and the important soil processes they govern. The treatments included "conventional agriculture" (C) which was the use of agrochemicals and tillage, "mulching combined with compost" (MC), "cover cropping" (CC), and crop rotation. To determine the changes in soil fauna community assemblages and species richness, two components of the belowground fauna were investigated.

Firstly, arthropod diversity indices were compared between conventional agriculture and regenerative agriculture management strategies (Chapter 2). Four arthropod taxa were selected for study, providing information on the macrofauna and mesofauna of the area. These included ants, beetles, spiders, and springtails. Soil fauna were sampled every two months for a year, using pitfall traps. Differences in species richness and abundance of the four study taxa were assessed in relation to agricultural treatment, sites, crop lines, and sampling times. ANOSIM (Analysis of Similarities) was conducted to determine sample differences between the study factors of site, treatment, crop rotation and sampling time. The presence of particular species was assessed for associations between factors, to determine whether any species show influential characteristics associated with particular agricultural treatments, sites or seasons. It was found that total arthropod abundance and species richness was generally greatest during the pre-trial sampling event that took place in February 2021. Arthropod abundance and species richness responses were taxa specific and differed between field sites and sampling times. Arthropod communities within each site, treatment, crop line and season, were not more similar to the communities of different sites, treatments, crop lines and seasons. Several species of

interest were determined based on their presence within the study factors. Particularly, the spider *Pardosa crassipalpis*, displayed agrobiont behaviour within this agroecosystem and will be a key species of interest for further monitoring within the trial. The presence of this highly abundant species was particularly noted at the site Jondu, within mulching and compost treatments, and typically during the final (February 2022) sampling period. Furthermore, the abundance of species of springtails recorded within this agroecosystem is remarkable. Native Entomobryidae made up more than 50% of all abundances and more than half of all species recorded. These species fall within a group of collembolans that display remarkably high thermal tolerances and desiccation resistance, within such an arid agroecosystem.

Secondly, decomposition rate (k), remaining litter mass (g), carbon to nutrient ratios and soil mesofauna community compositions were compared between agricultural treatments, by means of a litter bag experiment (Chapter 3). Multivariate analyses based on decomposition rate with respect to temporal changes showed significant site, treatment and temporal effects on decomposition rate. On average, decomposition rates were highest for litter bags placed at the site Jondu. Conventional agricultural treatment showed significantly lower decomposition rates and mass loss than the other two treatments. Site and sampling times were the most important factors explaining the variation in carbon to nutrient ratios. Carbon to nutrient ratios showed overall decreases over time, with particular site-specific responses observed. Particularly interestingly, C/N ratios showed considerable site-specific responses, with litterbags at Jondu recording decreased C/N over time, compared to increased C/N at the other sites.

This study provided essential baseline information for the system. Treatment effects were not as evident as expected. However, due to the study being conducted within the first established year of the trial, this may be expected. Site effects were far more prominent than treatment or crop rotation effects. Differences in soil conditions between sites may be accounting for a great deal of the variation in arthropod community assemblages and litter decomposition. Continued assessment of the impact of agricultural management strategies and crop rotation as the trial progresses and becomes more established will prove useful in determining effects of regenerative agriculture on soil fauna and litter decomposition.

CHAPTER 1.
GENERAL INTRODUCTION

AGRICULTURE, SOIL HEALTH AND FOOD SECURITY

Food provision is an integral part of daily life. The inability to provide sufficient, nutritious food to the growing population is a global concern. It is estimated that by 2050, the human population will reach 9.73 billion people (FAO, 2017). There is major pressure on the agricultural sector to produce sufficient food to ensure sustained food security. Food production systems will need to double their global output to reach this desired outcome by 2050 (FAO, 2017). Historically, tremendous growth in food production has been achieved. During the Green Revolution, through agricultural intensification, global food production tripled in five decades (Tilman et al., 2001; Godfray & Garnett, 2014; Gomiero, 2016). Based on this historic growth, reaching required food production rates by 2050 seems achievable.

However, several constraining factors could limit sufficient agricultural growth (FAO, 2017). Previous agricultural intensification was achieved through land expansion and clearing, the use of synthetic inputs and extensive irrigation (Tilman et al., 2001; Arizpe, Giampietro & Ramos-Martin, 2011; West et al., 2014; Pywell et al., 2015; Pretty et al., 2018; Schreefel et al., 2020). Evidence suggests that the clearing of land for agricultural purposes is one of the largest causes of global biodiversity loss (Altieri & Nicholls, 2004; Iannotta et al., 2007; Scalercio et al., 2009; Hanson et al., 2016; Pretty et al., 2018; Schreefel et al., 2020). The use of inputs in the form of nitrogen fertilizers and pesticides results in excess greenhouse gas emissions, loading the biosphere with nutrients and pesticide pollution of waterways (Tilman et al., 2001; Arizpe, Giampietro & Ramos-Martin, 2011; de Vries & Bardgett, 2012; West et al., 2014; Schreefel et al., 2020). The extent of such input use tends to increase as arable land becomes more limited (Arizpe, Giampietro & Ramos-Martin, 2011). Inputs are also a costly means to increase yield. Freshwater resources have been consistently depleted for irrigation, soils have been degraded manifesting in large scale soil erosion, and greenhouse gases have led to climate forcing (Pywell et al., 2015; Pretty et al., 2018). Currently factors such as climate change, underinvestment in agriculture, economic gaps leading to gaps in technology and limited available arable land further contribute to the challenge of sufficiently increasing agricultural production (FAO, 2017). Thus, agricultural intensification has not aligned with soil mindfulness and sustainability (Gomiero, 2016).

As declared in the 68th UN General Assembly in 2015, soil sustainability is the key to ensuring adequate food provisions for the growing population (Montanarella, 2015). Land use and agricultural management must prioritise soil health to ensure that essential ecosystem services are delivered sustainably (Zhang et al., 2007; Gomiero, 2016). Soil health refers to the quality and condition of the soil resource (Kibblewhite, Ritz & Swift, 2008). Healthy agricultural soil, as defined by Kibblewhite, Ritz & Swift (2008), "is one that is capable of supporting the production of food and fibre, to a level and with a quality sufficient to meet human requirements, together with continued delivery of other ecosystem services that are essential for maintenance of the quality of life for humans and the conservation of biodiversity." Soil health and sustainable agriculture are often used in combination in the literature. Ecosystem services are defined by Daily (1997) as the "the conditions and processes through which natural ecosystems, and the species that make them up, sustain and fulfil human life."

Agroecosystems currently extend across approximately 40% of the world's land surface (FAO, 2017). Thus, a significant proportion of the world's ecosystems are managed by humans. These agroecosystems are essential providers of food, fibre and biofuel. However, agroecosystems rely on natural ecosystem processes (e.g., soil formation, nutrient cycling, and photosynthesis), as well as the regulation of these processes (e.g., climate regulation, pollination, and disease and pest control) to optimise and sustain the system's ability to ensure such provisions. Agroecosystems also produce several disservices that may reduce productivity (yield) or increase production costs (Tilman, 1999; Zhang et al., 2007; Lyytimäki & Sipilä, 2009; von Döhren & Haase, 2015). These include pest damages, undesirable species competition, nutrient runoff and erosion (Zhang et al., 2007; Lyytimäki & Sipilä, 2009; von Döhren & Haase, 2015). A degrading soil is an agricultural setback, reducing yields and requiring increased inputs from farmers. The global challenge is thus to increase food production by increasing the quantity and quality of crop yields but in a manner that prioritises soil health (Kibblewhite, Ritz & Swift, 2008; Jankielsohn, 2018). This would promote helpful ecosystem services, limit disservices and ensure sustainable food security (Zhang et al., 2007; Lyytimäki & Sipilä, 2009).

SOIL BIODIVERSITY

Soil health is directly impacted by soil biota, as they play an essential role in providing ecosystem services (Altieri, 1999). They are responsible for much of the nutrient cycling in the soil and through these processes they impact soil properties such as soil fertility and structure

(Altieri, 1999; Gonthier et al., 2014; Jankielsohn, 2018). Soil is a highly complex habitat typically showing the greatest diversity of terrestrial organisms (Decaëns, 2010; Pauli et al., 2016). Soil biodiversity is a major contributor as to whether beneficial services or disservices emerge in an ecosystem (Altieri, 1999; Zhang et al., 2007; Lyytimäki & Sipilä, 2009). However, many species of soil fauna are undescribed and understudied (Decaëns, 2010). This poses substantial challenges in understanding the ecology and biology of agroecosystems (Pauli et al., 2016). Since soils are integral to agricultural production and are the base of all ecosystems, understanding the dynamics within the soil system is crucial to ensuring sustainable management of soils (Pauli et al., 2016).

Research shows that biodiversity is crucial in the internal regulation of agroecosystems (Altieri, 1999). Insects are a hugely successful group of organisms in terms of species' richness and abundance (Jankielsohn, 2018). They are the most specious group of organisms on earth. With 39 orders of insects globally, they contribute to about 66% of all animal species (Samways, 1993; Stork et al., 2015; Jankielsohn, 2018) and more than 75% of current global biodiversity (Kim, 1993). It is estimated that only about 7% of insect species have been described (1 million species). This brings the potential number of global insect diversity to around eight million species (Jankielsohn, 2018). Insects display impressive variation among species (Samways, 1993; Jankielsohn, 2018), representing many different trophic niches and a range of ecological functions (Samways, 1993). This all contributes to their massive functional significance and their vital role in providing ecosystem services (Samways, 1993). Ecologically, insects are responsible for pollination, predation – herbivory and carnivory, nutrient cycling, and decomposition (Jankielsohn, 2018). A considerable proportion of global crops depend on insect pollination (Jankielsohn, 2018) to improve and stabilize crop yield. Pollinator diversity is known to increase seed set (Hoehn et al., 2008). Herbivorous arthropods play an essential role in accelerating the rate of nutrient cycling through the breakdown of plant biomass (Mattson & Addy, 1975; Belovsky & Slade, 2000; Hunter, 2001; Metcalfe et al., 2014), while predatory arthropods establish population control over herbivores, controlling the abundance of herbivorous insects that could become crop pests (Jankielsohn, 2018). Predatory arthropods are typically from the orders Araneae, Coleoptera, Hymenoptera, Odonata, Neuroptera, Dermaptera and Hemiptera. Insects also improve agricultural soil quality through their activity allowing for increased nutrient content and cycling within the soil. It has been shown that this nutrient cycling provided by soil fauna increases crop yield significantly more than fertilizer inputs (de Groot, Wilson & Boumans, 2002). Several insect taxa are also responsible for the

decomposition of organic waste. Coleoptera, particularly dung beetles, help increase soil nutrients (Macfadyen et al., 2015), and decrease greenhouse gas emissions (Nichols et al., 2008). Other taxa such as flies, ants and termites, assist the decomposition process by consuming or shreding dead plant matter (Evans et al., 2011). The tunnel system of ants and termites also improves soil water filtration and aeration (Evans et al., 2011). In the agricultural sector, crops cannot be produced without the ecosystem functions provided by insects.

Insects have been predominantly perceived as pests or potential pests, particularly in agroecosystems (Jankielsohn, 2018), when only less than one percent of total known insect species are considered as pests (Kim, 1993). Often this perception of insects undervalues their ecological importance (Kim, 1993; Jankielsohn, 2018). Nevertheless, herbivorous insects are capable of causing damage to crops, estimated at around 18% damage of global agricultural production (Losey & Vaughan, 2006). Humans manipulate agroecosystems in such a way that allows insect pests to flourish, uncontrolled by natural enemies (Jankielsohn, 2018). It has been shown that herbivorous arthropod abundances increase significantly in monoculture systems (Jankielsohn, 2018). The human disturbance of agroecosystems, by means of consistent destructive farming practices, creates an unstable environment for natural enemies (Kromp, 1989; Cotes et al., 2009; Pizzolotto et al., 2018). Without the suppression of pests by natural enemies, farmers resort to pest control through synthetic means which only promotes a positive feedback cycle. The increased use of synthetic measures and inputs shifts the soil quality, community structure and species richness to more unfavourable situations (Kromp, 1989; Robinson & Sutherland, 2002; Cotes et al., 2009; Thiele-Bruhn et al., 2012; Pizzolotto et al., 2018). Sustainable agriculture aims to create a management approach that allows for pest suppression by natural enemies. This can only be done by creating habitats that favour beneficial soil fauna and increase soil biodiversity. High soil biodiversity is an essential part of efficient ecosystem and soil functioning (Thiele-Bruhn et al., 2012). Conservation policy has started to shift to focus on conserving soil biodiversity because of their utmost importance in food provision (FAO et al., 2020).

Major threats to soil biodiversity are anthropogenic. In particular, agricultural intensification causes devastating biodiversity losses, especially since its effect is multiplied by the effects of other threats such as soil acidification, nutrient imbalances, soil salination, soil erosion, loss of soil organic matter (SOM) and soil organic carbon (SOC), and/or pollution (FAO et al., 2020). This in turn impacts soil biota abundance, community assemblage, distribution, species diversity and richness, functional diversity. Importantly, these anthropogenic disturbances do

not impact soil fauna equally, with some taxa showing more sensitivity to disturbance than others. A trend noticed is that agricultural intensification appears to affect larger body sized soil arthropods that are at higher trophic levels (Tsiafouli et al., 2015; FAO et al., 2020).

In this way, soil fauna and their diversity may be extremely useful indicators of the health and condition of agroecosystem soils.

BIOINDICATORS

The effects of agricultural land management can effectively be assessed through the use of bioindicators (Paoletti et al., 1992; Büchs, 2003; Duelli & Obrist, 2003; Jeanneret et al., 2014; Pizzolotto et al., 2018). Bioindicators are taxa or functional groups that indicate the status of the environment (Gerlach, Samways & Pryke, 2013). Bioindicators can be used to indicate local environmental change, evaluate environmental stress or even indicate the degree of biodiversity in an ecosystem (McGeogh, 1998; Gerlach, Samways & Pryke, 2013). Thus, bioindicators may play a useful role in evaluating the effects of agricultural management in agroecosystems (Pizzolotto et al., 2018). Several organisms can be useful bioindicators, however their aptness depends on the ecosystem and state of the environment (Gerlach, Samways & Pryke, 2013). Soil invertebrates are especially useful in agricultural systems, as they display sensitivity to soil disturbances (Holland & Luff, 2000; Magoba & Samways, 2012; Sadej et al., 2012; Sklodowski, 2014a, b). The diversity and ubiquity of soil invertebrates allows them to be effective bioindicators of local ecosystem biodiversity. This also aids in effectively understanding the biological complexity of soil systems (Pizzolotto et al., 2018). They show significant sensitivity to changes in soil conditions because of their small size. Their mobility also allows them to flee changing or undesirable conditions, making them effective environmental indicators (Gerlach, Samways & Pryke, 2013). Because invertebrates are extremely functionally diverse, various taxa can indicate specific environmental changes (Gerlach, Samways & Pryke, 2013). However, not all soil fauna have been used as bioindicators, this has been reserved to only a few well-known groups (Gerlach, Samways & Pryke, 2013). Spiders, certain groups of beetles, collembola, ants and wasps are shown to be useful as bioindicators in varying situations (Hutcheson, 1990; Magura, Horváth & Tóthmérész, 2010; O'Neill et al., 2010; Gerlach, Samways & Pryke, 2013; Pizzolotto et al., 2018). Often, the community structure of certain taxa are extremely useful indicators of environmental stress, change or biodiversity (Benhadi-Marín et al., 2020). It is important to

monitor the dominance of particular species in crops as they are potentially good indicators of arable agricultural habitats and can aid in monitoring the quality and sustainability of agroecosystems (Benhadi-Marín et al., 2020).

Collembola are vital bioindicators of many ecosystem dynamics. They are extremely diverse and can be highly abundant (Janion-Scheepers et al., 2015). They perform many significant regulatory roles in the soil such as decomposition (de Oliveira Filho et al., 2016) and are known to be sensitive to types of litter and litter depth (Magoba & Samways, 2012). Studies have also shown their sensitivity to pollutants (Geissen & Kampichler, 2004; Lins, Santos & Gonçalves, 2007; Son et al., 2007; Fiera, 2009; Nota et al., 2009; Tabaglio, Gavazzi & Menta, 2009; Xu, Ke, et al., 2009; Xu, Schleppi, et al., 2009), which enables them to indicate such environmental stress. They can be used to indicate habitat characteristics (Greenslade, 2007; Fernandes, Nessimian & de Mendonça, 2009; Gerlach, Samways & Pryke, 2013), land management (King & Hutchinson, 2007; Halaj, Halpern & Yi, 2009; Gerlach, Samways & Pryke, 2013), environmental change (Chagnon et al., 2001; Greenslade, 2007; Salamon et al., 2008; Barbercheck et al., 2009; O'Neill et al., 2010; Gerlach, Samways & Pryke, 2013), restoration efforts (Majer, Brennan & Moir, 2007; Gerlach, Samways & Pryke, 2013) and in the context of agroecosystems, they even prove useful as indicators of the use of genetically modified crops (Haughton et al., 2003). Often, Collembola community structure is a useful tool in monitoring the effects of land use change on soil health and quality (Gerlach, Samways & Pryke, 2013; de Oliveira Filho et al., 2016).

Spiders (Araneae) have often been used as bioindicators in ecosystems (Gerlach, Samways & Pryke, 2013), as they are important generalist predators in the soil ecosystem (Benhadi-Marín et al., 2020). They are typically abundant in agroecosystems (Marc, Canard & Ysnel, 1999) and considered as significant natural enemies against various crop pests (Wyss, Niggli & Nentwig, 1995; Hooks, Pandey & Johnson, 2003; Ghavami, 2008; Oelbermann & Scheu, 2009; Pekár et al., 2015). Only certain families of spiders are typically used as indicators, which is due to the limited taxonomic information and the difficulty in identifying species of spiders (Gerlach, Samways & Pryke, 2013). The more visible and easily identifiable spider families are used. Spiders are often used as indicators because of their taxonomic diversity (Cardoso et al., 2004a, b). They are also used to indicate habitat change (Perner & Malt, 2003; Kapoor, 2008; Magura, Horváth & Tóthmérész, 2010), habitat characteristics (Jeanneret et al., 2003; Buchholz, 2010), management success (Cattin et al., 2003; Cardoso et al., 2004a,b; Rodríguez et al., 2006; Scott, Oxford & Selden, 2006; Řezáč, Řezáčová & Pekár, 2007; Midega et al.,

2008; Horváth et al., 2009) , restoration success (Gollan et al., 2010), level of pollutants in an environment (Haughton et al., 2003; Jung et al., 2008; Seyyar et al., 2010) and in some cases they can indicate the impact of genetically modified crops (Lövei et al., 2002). In agroecosystems, it is important to determine spider community structure and the dominance of particular species (Benhadi-Marín et al., 2020).

Beetles (Coleoptera) are extremely taxonomically and ecologically diverse (Gerlach, Samways & Pryke, 2013), making them useful representatives of insects as a whole (Hutcheson, 1990). Because beetles as an order are so diverse, often specific families are used as bioindicators (Gerlach, Samways & Pryke, 2013). Beetles are mostly used to indicate habitat characteristics, habitat disturbance (Vásquez-Vélez et al., 2010), indicate potential pollution (García et al., 2010), monitor habitat management (Jacobs et al., 2010) and restoration (Babin-Fenske & Anand, 2010; Gerlach, Samways & Pryke, 2013). Many beetle families are beneficial in agroecosystems (Holland & Luff, 2000). Carabid beetles are especially useful indicators in agroecosystems (Jeanneret et al., 2014; Skłodowski, 2014a, b; Eyre, McMillan & Critchley, 2016) as they respond to artificial inputs in the soil (Holland & Luff, 2000; Büchs, 2003; Hanson et al., 2016). Carabid beetles are particularly sensitive to soil structure (Holland & Luff, 2000), soil management (Sadej et al., 2012; Sklodowski, 2014a, b) and pesticide use (Scalercio et al., 2009; Burgio et al., 2015). Any changes in these properties will affect their species distribution. They may also be useful indicators of ground-dwelling diversity. Many ground beetles are sensitive to environmental change caused by humans, making them potentially useful environmental and ecological indicators.

Hymenoptera are known to be impressive biological indicators (Gerlach, Samways & Pryke, 2013). Ants, in particular, are an extremely useful indicator group as they are diverse, abundant and ubiquitous, found in most habitats (Majer, 1983). Ants are particularly useful in indicating different aspects of a system. Ground-dwelling ants may potentially indicate overall ground level diversity, although this measure is variable in its reliability. Instead of focus on species richness indications, ants are more useful when evaluating their diversity to understand functional attributes and relative proportions (Majer, 1983, 1985; Gerlach, Samways & Pryke, 2013).

Thus, biological indicators can be useful measures of soil health in an agricultural landscape, where different management strategies are in place. They can give an indication to the presence or absence of beneficial species or invertebrate groups as well as indicate how the presence,

abundance or community structure of certain taxa may influence the biotic relationships within the entire agroecosystem.

REGENERATIVE AGRICULTURE

It is well established that land management practices have major effects on soil health with respect to the physical, chemical, and biological fertility of soils (Thiele-Bruhn et al., 2012) (Fig. 1.1). Agricultural practices and the management of soils also greatly impact communities of terrestrial invertebrates (Pizzolotto et al., 2018). Policies around agricultural production acknowledge the need for sustainability and environmental protection, as not to exceed the planet's carrying capacity (Ignaciuk et al., 2021). Increased efforts are being made to develop food production systems that attempt to reduce environmental damage, since agricultural intensification is often coupled with negative environmental impacts (Rockström et al., 2017). Agroecosystems require the use of low-input agronomic techniques that will promote positive contributions to the ecosystem, allowing for more stability (Pizzolotto et al., 2018; Pretty et al., 2018). This can be achieved through shifting agricultural production techniques such as use of agrochemicals and tillage, to more sustainable, soil-friendly practices such as cover cropping with nitrogen-fixing legumes, crop rotation, promotion of natural enemies in the system and reduced tillage (Pardini et al., 2002; Vukicevich et al., 2016; Pretty et al., 2018).

Agricultural intensification through conventional methods focusses on soil tillage as the primary method of preparing soil for crops (Petersen, 2002). By tilling the soils, weeds and pests are supressed and organic matter and fertilizer can be incorporated into the soil (Petersen, 2002). However, tillage impacts soil biophysical properties to varying degrees (Rodríguez et al., 2006; Roger-Estrade et al., 2010) and is extremely detrimental to soil communities (Petersen, 2002; Roger-Estrade et al., 2010). Tillage directly impacts many soil organisms. This soil disturbance can kill or severely damage soil fauna as well as expose them to potential predation (Roger-Estrade et al., 2010). Several studies show how tillage directly affects nematode, earthworm and Collembola communities (Petersen, 2002; Roger-Estrade et al., 2010; Ayuke et al., 2011). The effect of tillage on soil fauna is typically dependent on the extent of the tillage system used, the underlying soil properties of the system (Rodríguez et al., 2006; Roger-Estrade et al., 2010) and most particularly, the overall agroecosystem management (Snapp, Gentry & Harwood, 2010). It is also understood that tillage dramatically reduces fungal networks and biomass in agroecosystems, with systems showing a recovery in biomass when

tillage is abandoned (de Vries et al., 2007). Conventional agriculture also relies on agrochemical inputs to achieve high yields on smaller areas of land. The overuse of nitrogen inputs leads to overall increased N in the atmosphere, further contributing to global change through greenhouse gas emissions (de Vries & Bardgett, 2012). However biodiverse systems have greater nutrient use efficiency, resulting in reduced need for agrochemical inputs (Bardgett & McAlister, 1999) (Fig. 1.1). Fertilizers and agrochemicals also exert strong effects on soil biodiversity and functioning (Thiele-Bruhn et al., 2012). As agriculture intensifies, more land is converted to monoculture crop production and the use of artificial inputs increases, resulting in greater damage to local biodiversity (Chamberlain et al., 2000; Tilman et al., 2001; Attwood et al., 2008).

Achieving sustainable agriculture is attempted through various approaches and from various perspectives. Some approaches focus on sustainable intensification (Cole & McCoskey, 2013; Garnett et al., 2013), where crop yields are increased without additional environmental damage; whereas other approaches are more concerned with sustainable consumption (Garnett et al., 2013; Tilman & Clark, 2014; Schreefel et al., 2020), where global consumption rates are ultimately reduced to fit the capability of the biosphere. These sustainable narratives are achieved through farming approaches such as organic agriculture, climate-smart agriculture (Palombi & Sessa, 2013) and sustainable intensification (Palombi & Sessa, 2013; Schreefel et al., 2020). Studies have shown that agricultural systems with reduced soil tillage and reduced use of inputs, in the form of fertilizers and agrochemicals, promote self-regulating systems and higher biodiversity (Thiele-Bruhn et al., 2012). When comparing organic farming with conventional farming systems, that incorporate a similar tillage system, the organic farming approach appears to enhance soil nutrient cycling, encourage self-regulation in the soil system, and promote increased biodiversity (Mäder et al., 2002; Birkhofer et al., 2008; Jangid et al., 2008; Thiele-Bruhn et al., 2012). Currently, regenerative agriculture is gaining increased attention as a solution towards a more sustainable food system (Shelef, Weisberg & Provenza, 2017; Elevitch, Mazaroli & Ragone, 2018; LaCanne & Lundgren, 2018; Schreefel et al., 2020). Regenerative agriculture is often perceived in a variety of ways in the literature, without a transparent definition (Elevitch, Mazaroli & Ragone, 2018; Schreefel et al., 2020). From a review on regenerative agriculture, Schreefel et al. (2020) proposed a provisional definition of regenerative agriculture as “an approach to farming that uses soil conservation as the entry point to regenerate and contribute to multiple ecosystem services”. Elevitch, Mazaroli & Ragone (2018) highlight that regenerative agriculture needs to contribute to soil health and

promote self-renewal and resiliency in the system. Although regenerative agriculture is understood differently, there are several aspects that converge in the literature (Schreefel et al., 2020). Regenerative agriculture aims to increase and conserve biodiversity of agroecosystems, improve water retention and water cycling capacities and of the soil, enhance nutrient cycling and carbon sequestration, alleviate climate change, and improve soil quality (Elevitch, Mazaroli & Ragone, 2018; Schreefel et al., 2020; Beillouin et al., 2021; Giller et al., 2021). Regenerative agriculture strongly regards soil as the base with a clear focus on environmental health and improvement (Schreefel et al., 2020).

Studies have shown that invertebrate biodiversity can be conserved using a low input crop management strategy in agricultural landscapes that are highly intensified with little native vegetation remaining (Attwood et al., 2008). Low input management strategies and low-intensity land use can also potentially promote ecologically diverse invertebrate groups, such as predators and decomposers (Attwood et al., 2008). In addition, the response of soil fauna to different agricultural land use approaches may differ at varying depths in the soil strata (Attwood et al., 2008), as farming practices impact different soil strata in varying degrees. In no-tillage systems, more stable microclimates are created by ensuring crop residues remain on the soil surface. This provides a more suitable habitat for soil fauna than a tilled soil surface (Rodríguez et al., 2006). Cover cropping is a beneficial regenerative farming practice that aids in reducing soil erosion and providing nitrogen to crops if nitrogen-fixing legumes are used, as well as decreasing N and P losses (Kaspar & Singer, 2011). Cover cropping has the potential to increase soil organic carbon (SOC) by increasing the total residue carbon that is added to the soil (Kaspar & Singer, 2011). Cover crops also impact soil biota communities and abundance. Cover crops can impact soil microclimate by increasing surface cover and influencing soil temperature and water content (Mele & Carter, 1999; Reeleder et al., 2006; Kaspar & Singer, 2011). Cover crops also increase the inputs of nutrients and carbon into the soil, thus increasing the abundance and activity of soil fauna (Mele & Carter, 1999; Kaspar & Singer, 2011). Through adding increased surface cover, decomposition of organic matter is stimulated, and nutrients are added to the soil. The rate at which crop organic matter decomposes impacts the balance of soil carbon gains and losses (Kaspar & Singer, 2011). The use of surface mulches is another practice of regenerative and sustainable farming. Adding mulch, in the form of straw or compost, aids in suppressing weeds without the use of herbicides (Thomson & Hoffmann, 2007). Using mulch as ground cover can also impact soil quality, increase soil organic matter, increase soil water holding capacity and filtration, reduce surface evaporation thus impact

microclimate and stimulate invertebrate activity (Thomson & Hoffmann, 2007). Studies show that soil insect predator abundance increased with mulch application (Thomson & Hoffmann, 2007), particularly with spiders, beetles, and earthworms. However, invertebrate communities vary in their response to mulch application, especially regarding which type of ground cover is used (Thomson & Hoffmann, 2007).

In conclusion, regenerative agriculture can be an effective means of impacting soil health and encouraging beneficial synergies within the soil system.

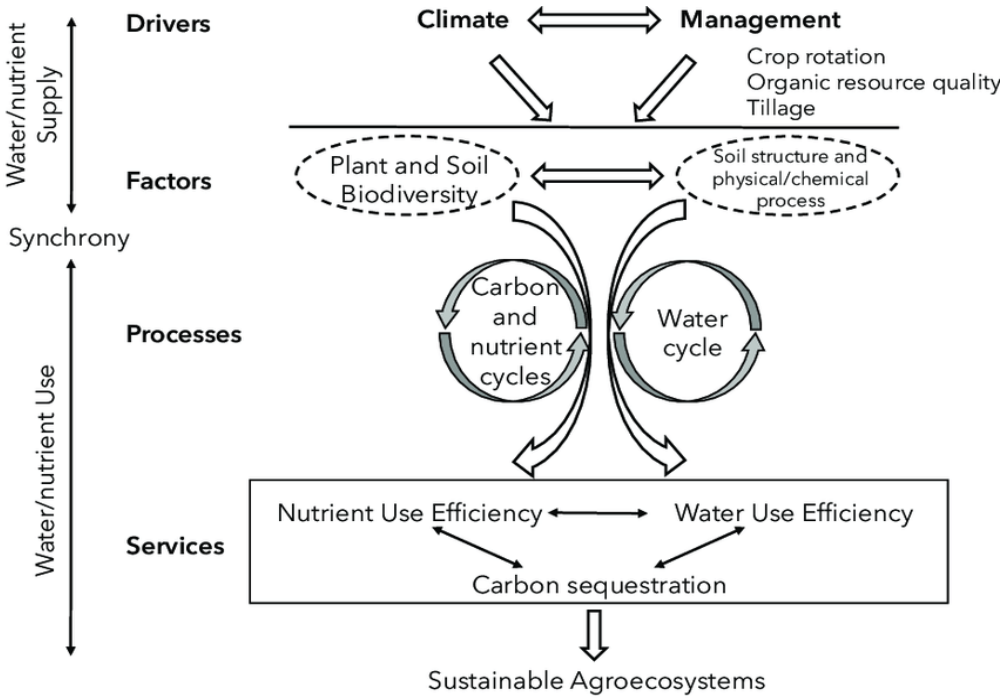

Fig. 1.1. Conceptual diagram on the relationships between management, plant and soil biodiversity, soil structure, and nutrient and water ecosystem use efficiencies. Source: Brussaard et al. (2007).

BROAD AIMS AND OBJECTIVES

The aims and objectives of the study are to examine the effect of regenerative agricultural practices (i.e., mulch, compost, cover cropping and crop rotation) and conventional agricultural practices (i.e., tillage and agrochemical inputs), in a cauliflower seed production system in the Western Cape, South Africa. More specifically, I aimed to:

i. Collect baseline information of the system's soil arthropod species and community composition, as little is known about the mesofauna and macrofauna of the area. Focus was placed on four main arthropod taxa: Ants (Hymenoptera: Formicidae), beetles (Coleoptera), spiders (Araneae), and springtails (Collembola).

ii. Determine the effect of these management practices on soil arthropod diversity, abundance, and community structure, with some focus on evaluating potentially influential species (Chapter 2).

iii. Determine the impact of agricultural management practices on decomposition rate of straw and the mesofauna present, with a particular focus on springtails (Collembola) (Chapter 3).

From these main aims, the following hypotheses are made:

1. Soil invertebrate diversity is expected to be higher over time where cover cropping and compost and mulch is applied, compared with conventional farming practices that use insecticides, synthetic fertilizers, and soil tillage, due to these practices creating more favourable microhabitats for soil arthropods.
2. Decomposition rate is hypothesized to be greater under cover cropping, and mulching and compost treatments, due to the increased soil moisture retention and favourable microhabitats created under these strategies.
3. Soil arthropod abundance and diversity is expected to be greatest under mulch and compost treatments, and lowest under conventional agriculture treatment.

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CHAPTER 2.
BELOWGROUND EFFECTS OF REGENERATIVE AGRICULTURE

ABSTRACT

Crop and food production are essential components of the ever-changing agricultural landscape across the world. Shifts to environmentally sustainable agriculture, with greater focus on soil health, is increasing. Since soil fauna is such an integral component of all ecosystems and responsible for maintaining many soil processes, this study assessed the soil biodiversity in a cauliflower seed production system. A six-year regenerative agriculture trial was implemented to understand the impacts of conventional agriculture, mulch and compost inputs, cover cropping, and crop rotation on soil arthropod community structure. Particular focus was given to ants, beetles, spiders, and springtails. The study was carried out on three privately owned farms (Ebenhaezer, Jondu and Sewester) near Lutzville, South Africa. Species richness and abundance of the taxa were determined from a total of 324 pitfall samples. Sampling was carried out every two months between February 2021 and February 2022. Community biodiversity was quantified using diversity indices, while an ANOSIM (Analysis of Similarity) was carried out to determine the relationships between soil fauna communities and agricultural treatment and crop rotation effects. For the macrofauna (ants, spiders, and beetles), 2696 individuals were found, distributed in 36 families. The families Formicidae, Tenebrionidae, Staphylinidae, and Lycosidae were the most abundant. Mesofauna (springtails) consisted of 7498 individuals from four families and nine species. Overall, higher species richness was recorded for macrofauna whilst higher abundance was found for mesofauna. Mean species richness for all taxa showed significant site and temporal differences, with degree of response being taxa specific. The ANOSIM further indicated significant correlations between arthropod communities and both site and sampling season ($p<0.001$). However, the relationships between communities and each factor are weak suggesting that samples within sites or sampling events are not more similar to each other. Finally, this study serves as baseline data for this agricultural long-term trial that has had no previous arthropod diversity data. Even though species composition was not strongly influenced by treatment or crop rotation, ongoing monitoring is critical in determining whether agricultural treatment and crop rotation effects become more pronounced once the trial is more established.

INTRODUCTION

Crop and food production are essential parts of the ever-changing agricultural landscape across the world (Bruins, 2009). Seed security is the cornerstone of food security, thus producing high quantities of quality seed is paramount (Bruins, 2009). However, due to increased environmental pollution and degradation, particularly of cultivated soils, as well as climate change, more emphasis is being placed on the need for agricultural production to be environmentally sustainable (Russo & Berlyn, 1991; Shahrajabian et al., 2021). The maintenance and protection of soils is critical, as it is one of the most important natural resources in agricultural systems (Martínez-Mera et al., 2019; Chamorro-Martínez et al., 2022).

A vital consideration to be made when shifting to sustainable agricultural strategies, is ensuring that seed quantity, seed quality, crop yield and producer income is maintained (Szparaga et al., 2019). Management strategies such as organic farming, push-pull technology, agroecology, climate-smart or conservation agriculture and sustainable intensification (or a combination of these) are all proposed mechanisms of increasing productivity in a way that limits further soil degradation (Khan et al., 2011; Godfray & Garnett, 2014; Lal, 2015; Descheemaeker, Reidsma & Giller, 2020; Jordon et al., 2022). In cropping systems, most of these strategies draw on similar practices to achieve their end goal: reducing soil tillage, promoting crop rotation to increase planned plant diversity, planting cover crops to protect soils between crops and increase soil organic matter (SOM), composting to rebuild SOM, mulching to conserve soil moisture, and limiting artificial inputs (Michels, Sivakumar & Allison, 1995; Scheunemann et al., 2015).

Soils are complex systems with a network of interacting factors (physical, chemical, and biological processes), all serving unique purposes to ensure that essential ecosystem services are delivered. However, through anthropogenic activities such as land-use change due to agricultural intensification and the use of agrochemicals in conventional agriculture, soil physicochemical and biological properties have been altered (Kiani et al., 2017; Chamorro-Martínez et al., 2022). Altered soil properties, in turn, modify soil microorganisms' density, diversity and distribution (Martínez-Mera et al., 2019). These soil microorganisms are critical components in the soil ecosystem, impacting ecosystem function and in turn affecting production sustainability (Zulu et al., 2022). Soil organisms play a variety of roles in impacting soil quality and characteristics, such as impacting soil formation, nutrient cycling, leaf litter

decomposition and biological soil fertility (Chamorro-Martínez et al., 2022; Zulu et al., 2022). Soil biodiversity (macrofauna, mesofauna and microfauna) is an important natural resource in agroecosystems that needs to be conserved. Diverse soil communities determine the functional diversity of agroecosystems, ultimately affecting the output of ecosystem services including the natural control of pest species (van Hamburg & Guest, 1997).

Agriculture is known to be negative to soil fauna (Zulu et al., 2022). Conventional tillage practices limit the availability of favourable habitats for soil organisms, through physical disruption, removal of SOM leading to exposure to extreme variation in temperature (Chamorro-Martínez et al., 2022; Zulu et al., 2022). In contrast, decreasing the intensity and frequency of tillage practices has been shown to contribute to an increase in herbivorous insects (Kladivko, 2001; Brévault et al., 2007; Henneron et al., 2015) as well as the diversity and abundance of generalist predators, such as spiders and carabid beetles (Schmidt & Rypstra, 2010; Rendon et al., 2015). The increase in herbivorous insects includes the increased prevalence of economically important crop pests (Kladivko, 2001; Brévault et al., 2007; Henneron et al., 2015). This is a potential risk factor for sustainable agriculture management. However, the conservation of generalist predators, that non-selectively feed on other arthropods, may help curb potential pest outbreaks (Schmidt & Rypstra, 2010; Rendon et al., 2015).

The impact of artificial fertiliser use on soil arthropods has been well documented. The rate of fertiliser application, the type of fertiliser used and the pH of the soil all compound to affect soil fauna communities (Ma, Brussaard & de Ridder, 1990; Wang et al., 2016). Organic fertilisers are generally beneficial to all soil organisms, in part due to the abundance of soil organisms found in the organic fertiliser itself (Curry, 1979). The regime of organic fertilising impacts abundance of soil arthropods differently (Kautz, López-Fando & Ellmer, 2006). The response to fertilization regimes tends to also be species specific, with springtails (Collembola) responding differently than mites (Cluzeau et al., 2012; Gruss, Twardowski & Hurej, 2018). Little is known about the impact of fertiliser management practices on soil arthropod communities in different cropping systems, especially in arid and semi-arid regions (Zulu et al., 2022).

Crop rotation could potentially help increase soil arthropod biodiversity, particularly in monoculture systems (Stinner & House, 1990; Jones et al., 2003; Meyer et al., 2019). Monoculture cropping systems could decrease the diversity of microhabitats, impacting the

assemblages of soil arthropods (Jones et al., 2003). Diverse crop rotations have been shown to have positive associations with several soil health indicators, such as organic carbon, bulk density, soil porosity and soil water content (Karlen et al., 2006; Mtyobile, Muzangwa & Mnkeni, 2020) The physiology, biochemical processes and metabolism of each cropping system impacts arthropod community assemblages differently (Holland & Luff, 2000; Sticht et al., 2008), with changes in species diversity likely between crops (Menta et al., 2020).Thus, changes in diversity, distribution and abundance of arthropod communities are likely to occur under crop rotation systems.

Monitoring the effects of agricultural practices by means of organisms that are sensitive to changes in land management is a very useful way of approaching such complex agroecosystem studies (Paoletti et al., 1992; Büchs, 2003; Duelli & Obrist, 2003; Jeanneret et al., 2003). However, studies focussed on the impact of cover crops, mulch, organic fertiliser and limited tillage on soil biodiversity and functional diversity in cropping systems in South Africa are limited (but see Eckert et al. (2020) for vineyards) and quantification methods are underdeveloped (Petchey & Gaston, 2002; Bàrberi et al., 2010; Winqvist et al., 2014). However, in natural systems several useful bioindicators are well established (McGeogh, 1998; Gerlach, Samways & Pryke, 2013). Invertebrates have been found to be useful environmental, ecological and biodiversity indicators, as their body size makes them sensitive to local environmental changes and their mobility allows them to move in response to changing conditions. Due to high invertebrate diversity and variability in functional characteristics, there is a wide variety of response specific invertebrate taxa (Gerlach, Samways & Pryke, 2013).

Ants (Hymenoptera: Formicidae), beetles (Coleoptera), spiders (Araneae) and springtails (Collembola) are all taxonomically diverse soil fauna. Ants, beetles, and spiders represent important macrofauna, responsible for most predation in agroecosystems (van den Berg & Dippenaar-Schoeman, 1991; Philpott & Armbrecht, 2006; Rouabah et al., 2014; Albertini, Pizzolotto & Petacchi, 2017), whilst springtails are an abundant and highly diverse group of prey (Hopkin, 1997; Rusek, 1998). Ants make up approximately 10% of the world's animal biomass and are ubiquitous globally, largely due to their remarkable social organization (Diamé et al., 2018). In agroecosystems, ants play a role in plant pollination, soil bioturbation, bioindication, and the regulation of crop pests (Diamé et al., 2018). Agricultural management and land-use intensity impact how suitable habitats are, thus impacting soil fauna community composition (Hanson et al., 2016). Ants are sensitive to habitat and land use change (Armbrecht, Rivera & Perfecto, 2005; Urrutia-Escobar & Armbrecht, 2013) and show strong

negative responses to agricultural intensification (Philpott & Armbrecht, 2006). The responses of ants to changes in agricultural management and intensification is known to be specific to functional groups (guilds). Ecological guilds are often a useful way to study the response of community assemblages to environmental changes, given that the taxonomic composition of the guild responds similarly to changes in the environment (Cardoso et al., 2011; Urrutia-Escobar & Armbrecht, 2013). Little literature is available on ants in South African cropping systems and their response to changes in agricultural practices, with most studies focusing on ants as pest species in vineyard systems (Addison & Samways, 2012).

Beetles, particularly ground beetles, have been suggested to be beneficial insects in agroecosystems (Holland & Luff, 2000) and important predators of destructive pests (Rouabah et al., 2014; Dinis et al., 2016; Albertini, Pizzolotto & Petacchi, 2017). Beetle communities within agroecosystems are impacted by agricultural management and land-use intensity (Schweiger et al., 2005; Eyre, Luff & Leifert, 2013; Palmu et al., 2014). Their response is species-specific and impacted by their functional diversity (Vandewalle et al., 2010; Hanson et al., 2016). Caballero-López et al. (2012) found that predatory, carnivorous beetles were more successful under high intensity agricultural management. This was distinct from the response of herbivorous beetles that preferred less managed habitats (Woodcock et al., 2010; Birkhofer, Wolters & Diekötter, 2014). Interestingly, the average body size of ground beetles negatively correlates with agricultural management intensity, with highly managed systems recording smaller beetle body size on average (Blake et al., 1994). Increased tillage could possibly have a negative impact on larger ground beetle species (Tsiafouli et al., 2015). Predation rates of soil predator communities can also be negatively correlated with body size (Rusch et al., 2015), potentially impacting pest control. Beetle species typically have different soil moisture requirements and preferences, and this impacts their emergence during overwintering periods (Holland et al., 2007; Hanson et al., 2016). Agricultural management that impacts soil moisture can in turn affect beetle community composition (de Vries et al., 2013). Thus, the main environmental factors impacting beetle species distribution are type of soil management (Sadej et al., 2012; Sklodowski, 2014a, b), use of pesticides (Scalercio et al., 2009; Burgio et al., 2015), and soil structure (Holland & Luff, 2000).

Spiders are an ubiquitous and abundant predator group (van den Berg & Dippenaar-Schoeman, 1991). They occupy a variety of guilds and display impressive functional diversity, making them essential role players in soil ecosystems (Dippenaar-Schoeman et al., 2013). Spiders are polyphagous predators and influence prey communities greatly through their predatory

adaptations (Nyffeler et al., 1990; Dippenaar-Schoeman et al., 2013). Owing to their ability of consuming large amounts of prey in short periods of time, spiders may experience great predation rates when prey are locally abundant. Spiders are also important predatory colonizers of crop systems. Early season spider assemblage has been shown to be useful in limiting pest abundance (van den Berg & Dippenaar-Schoeman, 1991; Dippenaar-Schoeman et al., 2013). Thus, they have the potential to regulate pest populations at endemic levels, helping prevent major pest species outbreaks. Changes in microhabitats may affect the spider community composition (Dondale & Binns, 1977; Rushton, Topping & Eyre, 1987; Frampton, van den Brink & Gould, 2000) showed how abiotic factors such as moisture content, impact spider community structure, with increased spider densities correlating with increased precipitation. The quality of the soil top layer is also an important factor indirectly impacting spider community structure. The quality of detritus available impacts the densities of prey such as springtails, mites, and dipterans, thus in turn regulating the abundance of spiders (Lensing, Todd & Wise, 2005). Spiders are also important agrobiont species in agroecosystems. Agrobiont species associated with various agroecosystems differ based on the structure of the system and the management strategies in place (Samu & Szinetár, 2002). Determining whether a species (and which species) is agrobiont in a system, is especially helpful as it helps understand how widespread the occurrence of the species in the particular crop and/or agricultural treatment is (Dippenaar-Schoeman et al., 2013).

Soil mesofauna (200 µm–2 mm) play a distinct and vital role in agroecosystems. Their activity is mostly concentrated within the top 20cm of the soil profile, and they impact decomposition rates and nutrient cycling in the soil, considerably (Bradford et al., 2002; Lensing, Todd & Wise, 2005; Menta et al., 2020). Springtails are a particularly abundant and influential group of soil mesofauna, that impact rates of leaf-litter decomposition, directly and indirectly (Lensing, Todd & Wise, 2005). They are also one of the main prey groups for generalist predators, particularly spiders (Chen & Wise, 1999; Lensing, Todd & Wise, 2005). The interaction between predator and prey is hugely influential within the soil system (Lensing, Todd & Wise, 2005). Springtails are an extremely useful group of soil fauna to study in response to changes in management and environmental factors as they are highly sensitive to physio-chemical changes and are affected by both above- and belowground environmental factors (Menta et al., 2020). Within aboveground factors, mesofauna presence is affected by plant cover, which sensibly impacts soil properties by shading and regulating soil temperature, allowing steady environmental conditions (Menta et al., 2020). It is known that there is a

positive correlation between springtail densities and soil moisture (Lensing, Todd & Wise, 2005). Springtails are also known to move laterally and vertically throughout the leaf litter, looking for favourable microclimates. Studies show that mesofauna, particularly springtails, discriminate between agricultural management systems (Filser et al., 2002; Maraun et al., 2003; Menta et al., 2020), however no consistent response has been determined. Brussaard et al. (1990) and Filser et al. (2002) found that springtail populations are more abundant under intensive management systems, opposed to sustainable systems, whereas Maraun et al. (2003) found that springtail populations are highly sensitive to disturbance. In other studies, sustainable agricultural practices appear to positively impact springtail community density and diversity (Winter, Voroney & Ainsworth, 1990; Dittmer & Schrader, 2000; Cortet et al., 2002; Petersen, 2002; Coulibaly et al., 2017). This is due to the availability of organic matter in the form of increased crop residues which has been shown to influence microclimate conditions and increase available resources for decomposers and springtails (Axelsen & Kristensen, 2000; Kladivko, 2001). Furthermore, Ngosong et al. (2009) found that springtail species richness was greater in systems that used mineral fertilizer as opposed to organic fertilizer. Thus, agricultural management notably, but variably, affects mesofauna community structure.

Using the focal taxa mentioned above, in the present study I aimed to determine the impact of conventional and sustainable agricultural practices in a cauliflower seed production system in South Africa on arthropod community distribution, diversity, and abundance.

METHODS

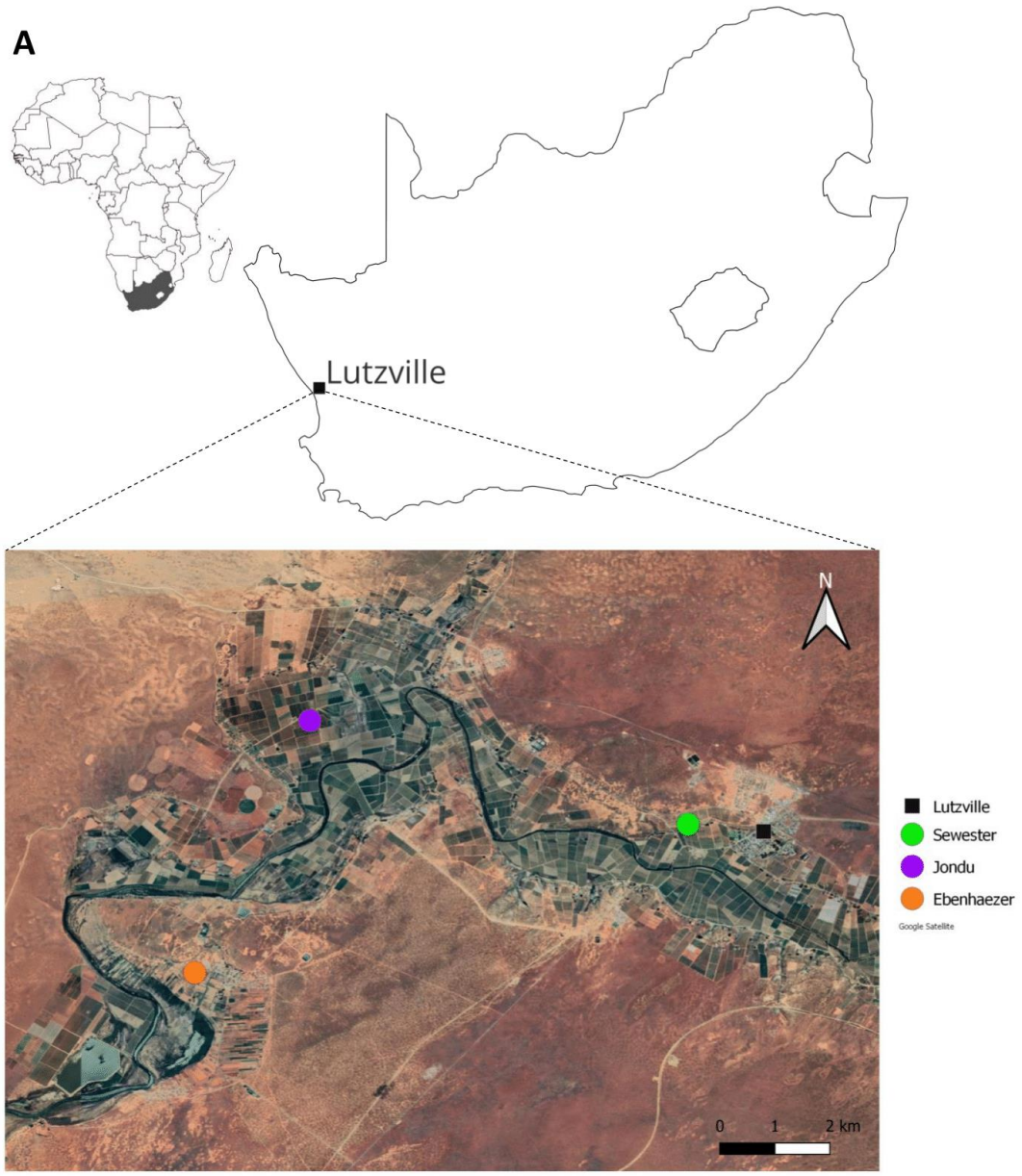
Study area and site selection

The Syngenta Foundation and The Nature Conservancy, embarked on a joint venture to establish a six-year cauliflower (*Brassica*) seed production field trial that focussed on incorporating regenerative agricultural practices. The study was conducted near Lutzville in the Western Cape Province of South Africa (Fig. 2.1A). The study consisted of three experimental field trial sites (~1 ha each), based on three separate farms: Sewester (-31.55628°, 18.33034°), Jondu (-31.53611°, 18.25777°) and Ebenhaezer (-31.58472°, 18.23611°) (Fig. 2.1A). The farms are situated in the semi-desert succulent Karoo Biome of the Western Cape. This region is one of two seed production areas of Syngenta Seeds in South Africa. Cauliflower, broccoli, and lettuce seed production are the main focus. The experiment was established in

the 2021 growing season to investigate the long-term effects of several regenerative agriculture practices on soil fertility, disease, crop and seed yield and soil arthropod communities.

Climatology, soil, and vegetation

As yearly precipitation is around 150-160mm, the whole agricultural area is dependent on water from the Clanwilliam Dam, derived from the catchment areas to the east. The water is distributed to the west by the Olifantsrivier. Irrigation was performed by drip irrigation. Based on soil samples obtained in November 2020 (pre-trial), initial field preparations involved no addition of lime or phosphorous prior to planting (S. Nieuwoudt, pers. comm). Differences in vegetation type were determined across the three study sites (Fig. 2.2). The Sewester and Ebenehaezer sites had underlying Namaqualand Strandveld vegetation (Mucina & Rutherford, 2006), whilst the Jondu site had Namaqualand Riviere vegetation. Thus, differences in soil and geology types across sites are evident. Sewester and Jondu sites were characterised by brackish soils, whilst Ebenhaezer was characterised by good sandy/ loamy soil, without brackishness. An addition of two tonnes of gypsum per hectare was recommended at all three experimental fields. However, deep cultivation of 1 ton gypsum per hectare was needed at Sewester and Jondu to alleviate crusting. No other immediate soil amelioration/fertilisation was required in all three fields as poor soils would help to evaluate the effects of the experimental treatments (see Appendix 1 for soil parameters tested). Due to logistical constraints, limited temperature and moisture data, across sites and treatments, were available during the study period.



B

312	322	332	311	321	331	Line 3
232	212	222	231	211	221	Line 2
122	132	112	121	131	111	Line 1

Fig. 2.1. Map of South Africa showing the study area, near Lutzville, Western Cape (A) and the map of Lutzville with experimental field trial sites labelled as follows: Sewester (green), Jondu (purple) and Ebenhaezer (orange). Plot design of each experimental field (B), with lines 1, 2 and 3 denoting irrigation lines. Plot number notation: the first number is the irrigation line, the second number is the experimental treatment, and the third number is the replicate of that treatment.

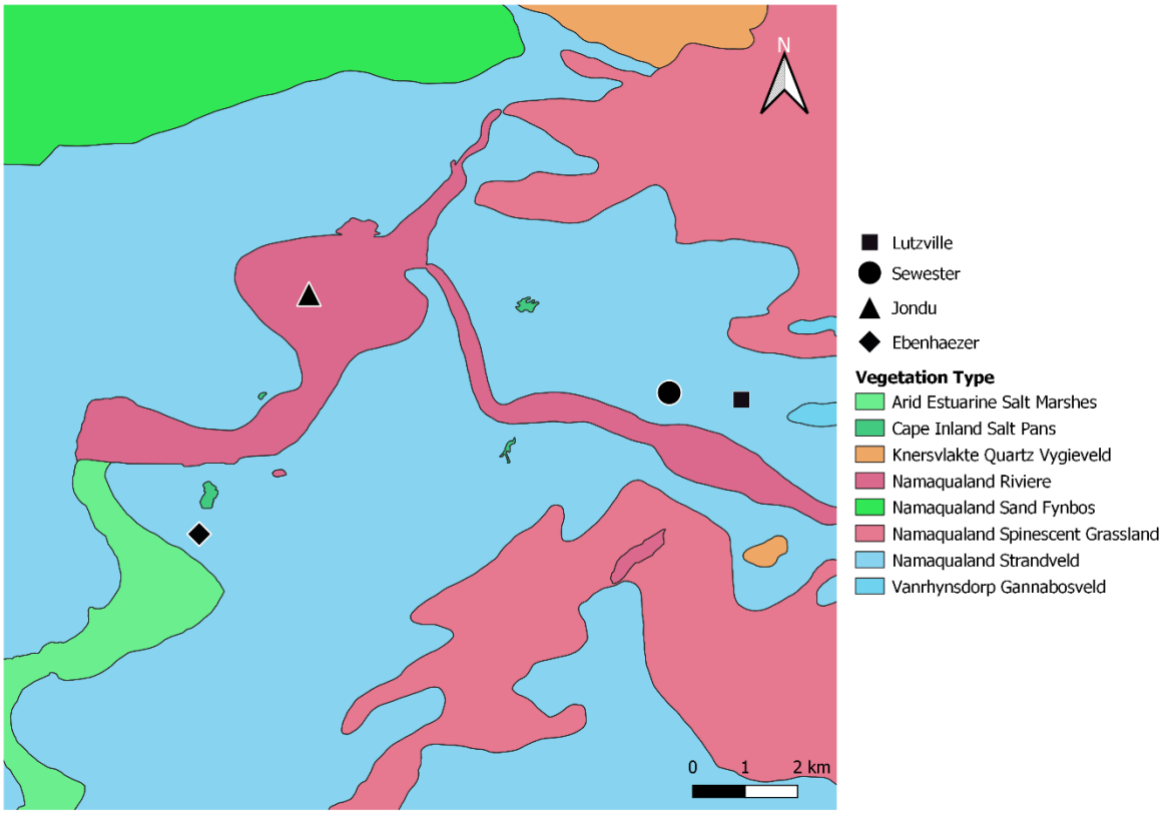


Fig. 2.2. Vegetation map of study sites (Mucina, Rutherford & Powrie, 2015; map by G. Walker).

Experimental plot design

As all experimental field trial sites were approximately the same size and dimension, it was possible to have the exact same layout of experimental plots in the three fields (Fig. 2.1B). The experiment was a split plot design with the experimental field being divided into three lines (Fig. 2.1B). Each plot within the experimental fields were provided with their own unique plot number notation (e.g., 111), with the first number denoting the irrigation line, the second number denoting the treatment applied and the third number denoting the treatment replicate. A 3-year crop rotation was imposed on the fields. Each irrigation line, denoted as line 1, 2 and 3 (Fig. 2.1B) is in a different year of the crop cycle (Table 2.1). Three agricultural treatments (conventional agriculture, cover crops, and mulch and compost combined) were randomized within each line, with two replicates. The treatments are represented by the second number in the plot number notation (Fig. 2.1B), with number one indicating conventional agriculture (C), number two indicating mulching and compost (MC) and number three indicating cover cropping (CC).

Treatment one (C) involved conventional field management and crop production. This included herbicide spraying before planting, removal of crop organic matter from field, extensive weeding during the season, fertigation with chemical fertilizer, use of chemical crop protection options. Before crops were planted, $15m^3$/ha of compost was ploughed superficially (10cm deep) into the soil. Treatment two (MC) involved adding double the amount of compost from treatment one ($30m^3$/ha) to the plots. Additionally, no more than $3m^3$/ha of kelp was added to the treatment plots. In addition, 3000kg/ha of straw mulch or equivalent was added into the plots. Ideally, ensuring that the soil was never bare. Fertigation with chemical fertilizer was avoided.

Treatment three (CC) involved covering the soil with growing cover crops. This included 25kg/ha of forage barley, 20kg/ha forage rye and 20kg/ha bitter lupins grown in the plots. Fertigation with chemical fertilizer was avoided.

Cauliflower production is nitrogen intensive, thus crop rotation for commercial cauliflower farming is essential. Crop rotation revolved around the growing of *Brassica* seed, cycled with the growing of several cash crops, such as tomato, watermelon, melon, and beans (Table 2.1). The trial made use of a three-year crop cycle. Preparation of the fields began a month before planting occurred. *Brassica* seed was to be planted in March and harvested in October for every year of the trial, however slight delays in 2021 resulted in *Brassica* planting occurring in April 2021 and harvesting in October 2021. This study began in February 2021, with a pre-trial sampling event, however trial preparations only began in March 2021. The study sites in February 2021 were characterized by dry and hot weather conditions, as well as bare experimental fields and sandy soils.

Table 2.1. Six-year crop rotation plan. Each irrigation line is in a different year of the crop rotation so that every year each field generates data of the whole crop rotation. Month refers to period of growing.

Year	Irrigation line	Crop cycle	Month
2021	1	Brassica seed	Apr 2021 – Oct 2021
2021	2	Tomato cash crop	Oct 2021 – Jan 2022
2021	3	Melon cash crop	Oct 2021 – Jan 2022
2022	1	Watermelon cash crop	Dec 2021 – Feb 2022
2022	1	Tomato cash crop	Oct 2022 – Jan 2023

2022	2	Melon cash crop	Oct 2022 – Jan 2023
2022	3	Brassica seed	Mar 2022 – Oct 2022
2023	1	Melon cash crop	Oct 2023 – Jan 2024
2023	2	Brassica seed	Mar 2023 – Oct 2023
2023	3	Beans cash crop	Dec 2022 – Feb 2023
2023	3	Tomato cash crop	Oct 2023 – Feb 2024
2024	1	Brassica seed	Mar 2024 – Oct 2024
2024	2	Beans cash crop	Dec 2023 – Feb 2024
2024	2	Tomato cash crop	Oct 2024 – Feb 2025
2024	3	Beans cash crop	Nov 2024 – Jan 2025
2025	1	Beans cash crop	Dec 2024 – Feb 2025
2025	2	Beans cash crop	Nov 2025 – Jan 2026
2025	3	Brassica seed	Mar 2025 – Oct 2025
2026	1	Tomato cash crop	Oct 2025 – Feb 2026
2026	2	Brassica seed	Mar 2026 – Oct 2026
2026	3	Watermelon cash crop	Dec 2025 – Feb 2026

Macrofauna and mesofauna sampling

Macrofauna and mesofauna were sampled using pitfall traps. Sampling took place every two months between February 2021 and February 2022 (February 2021, April 2021, July 2021, September 2021, November 2021, and February 2022), to obtain baseline arthropod data for one year (see Fig. A1a, b, c, d in Appendix 1 for site changes between 2021 and 2022). Sampling in this way meant that the different seasonal period of activity of each taxon could be determined. Pitfall sampling is the most standard and frequently used sampling method to survey ground-dwelling arthropods (Perner & Schueler, 2004; Schmidt et al., 2005; Shrestha & Parajulee, 2010; Hohbein & Conway, 2018). Pitfall traps (transparent plastic containers: Ø = 5.5cm, depth: 7.3cm) were put into the soil, flush to the soil surface, half-filled with 100% propylene glycol and left out for five days. At all sites, one trap was set up in the centre of each treatment block, in between rows of crops. Eighteen traps were utilized at each of the three sites, thus 54 traps in total, per sampling session. Rain covers were placed above the buried

pitfall traps during the winter months to prevent heavy rains from filling the containers with water. The rain covers were made from plastic lids (Ø = 12cm) and wire (1mm thick) threaded through the lids. These rain covers stood approximately 5cm above the pitfall traps (see Fig. A2a, Appendix 2).

Upon collection, samples were preserved in 96% ethanol for laboratory identification (see Fig. A2b, Appendix 2). All individuals of ants, beetles, spiders, and springtails were identified to morphospecies level, from hereon called species, based on each group's unique morphological characters and with the assistance of taxonomic experts of the specific taxon. Ants were mounted and identified under the microscope to genus level (Abusisiwe Ndaba, University of Cape Town, South Africa) using identification keys (Fisher & Bolton, 2016). Springtails were kept in ethanol and separated to morphospecies under the microscope. Representatives of the morphospecies were prepared in slides for further identification to genus level (Charlene Janion-Scheepers, University of Cape Town, South Africa) using identification keys (Janion-Scheepers, 2021). Spiders were identified to species, as far as possible, otherwise to genus level by Charles Haddad, University of the Free State, South Africa. Beetles were pinned and identified to genus level as far as possible by consulting the Coleoptera dry collections at the Iziko South African Museum in Cape Town, South Africa. All microscope work was done using Motic Stereo SMZ-168 microscope.

Statistical analyses

In this study, species richness refers to the number of species, and abundance to the number of individuals (Magurran, 2004). To understand sampling effort for each taxon at each site, species accumulation curves were obtained using the *vegan* (v2.6-2; Oksanen et al., 2022) package in R (v4.2.2; R Core Team, 2018). Total arthropod abundance and species richness across sampling seasons were visualized using the *ggplot2* (v3.3.6; Wickham, 2016) package. The effects of field sites, treatments, crop lines, and sampling times on arthropod species richness were assessed using Poisson regression models (GLM), since the response variable was counts. The complexity of full models was reduced through backward stepwise selection using the stepAIC function in the *MASS* package (v7.3-58.1; Venables and Ripley, 2002). Models were compared by Akaike's Information Criterion (AIC), with the final model having the lowest AIC (Murtaugh, 2009). To determine which arthropod species were associated with field sites, agricultural treatments, crop lines, or sampling seasons, the *indicspecies* (v1.7.12;

de Caceres & Legendre, 2009) package was used. The software PRIMER (Clarke & Gorley, 2006) was used to conduct ANOSIM (Analysis of Similarities) tests to test for significant differences in arthropod community composition between the study factors (Clarke, 1993). Furthermore, Bray-Curtis based NMDS (Non-Metric Multi-Dimensional Scaling) ordinations were used to visually compare the overall community structure of the four study taxa between samples.

RESULTS

Soil fauna abundance and species richness

Three hundred and twenty-four samples were collected during the first sampling year of the trial (February 2021 – February 2022). This resulted in 10194 soil arthropods from the four study taxa, namely ants, beetles, spiders, and springtails (Table 2.2). A total of 151 species, from 41 distinct families were identified. Beetles were the most species rich order, with the greatest family diversity. Spiders showed high diversity, considering spiders' species richness relative to abundance. Springtails displayed high abundances; however, this was contrasted with limited number of distinct species. For most taxa and sites, arthropod sampling was near completion (Fig. 2.3). Although evidently further sampling for certain taxa may be beneficial, as plateaus in some species' accumulation curves had not been reached.

Table 2.2. Abundance, family diversity, and species richness recorded across four invertebrate orders over a one-year sampling period.

Invertebrate group	Total abundance	Family diversity	Species richness
Ants	807	1	28
Beetles	1387	20	60
Spiders	502	16	54
Springtails	7498	4	9
Total	**10194**	**41**	**151**

Arthropod abundance and species richness showed temporal fluctuations during the study period (Fig. 2.4). These temporal responses in abundance and species richness were taxa specific, with springtails displaying far more consistent abundance patterns over the sampling year compared with ants, beetles, and spiders. Arthropod abundance and species richness was

notably high at the onset of the experimental trial (February 2021). However, April 2021 saw a considerable reduction in the abundance of all arthropod taxa, particularly noting the absence of spiders during this sampling session. Springtail and spider abundances increased during the study year from February 2021 to February 2022, with 132% greater springtail abundance and 444% greater spider abundance in February 2022. Springtail abundances were considerably greater than any other taxa throughout the study period. Springtail abundances from February 2021 were 260% and 2463% greater than beetle and spider abundances, respectively. However, spider species richness in February 2021 was 600% greater than springtail species richness. The greatest beetle and ant abundances were recorded at the onset of the trial, in February 2021. Abundances for these two taxa decreased during the study period, 360% and 160% reductions in the abundances of beetles and ants, respectively, by February 2022. Communities of beetles and ants had not yet resembled pre-trial abundances after one year.

Differences in species richness of arthropod species were found between field sites, agricultural treatments and sampling times (see Appendix 2 for Poisson regression (GLM) outputs). Ant species richness differed between sites, agricultural treatments and sampling seasons. Ebenhaezer had the fewest number of ant species recorded. This was noticed particularly during the February 2021 sampling period, where ant species richness was 131% and 216% greater at Jondu and Sewester, respectively, compared to Ebenhaezer (Fig 2.5). In total, 83 individuals of 12 species were sampled at Ebenhaezer, 342 individuals of 13 species at Jondu, and 382 of 22 species at Sewester. The mulching combined with compost treatment consistently showed the greatest mean species richness of samples across all three sites. The greatest number of ant species was sampled in February 2021 across all sites and treatments. Ant species richness steadily decreased between February 2021 and October 2021, reaching the fewest number of species recorded in October 2021. Following this, ant species richness steadily increased until February 2022. A total of 64 individuals were recorded between February 2021 and October 2021, which is vastly different to the 428 and 326 individuals sampled in February 2021 and February 2022, respectively. Ant communities showed no difference in mean sample species richness based on crop line, indicating little impact by crop rotation on the observed richness.

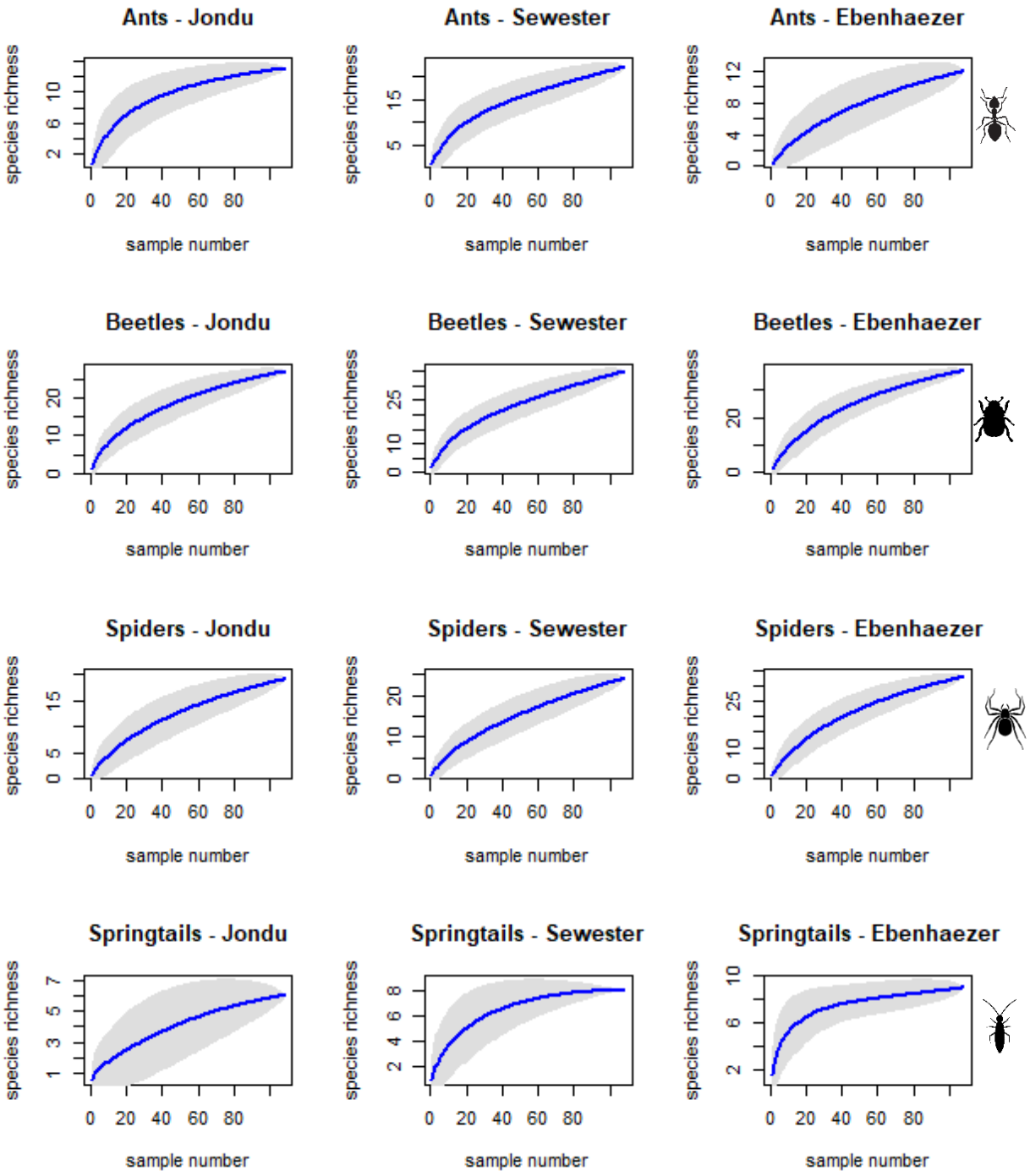


Fig. 2.3. Species accumulation curves depicting sampling effort for ants, beetles, spiders, and springtails across the three experimental sites (Sewester, Jondu and Ebenhaezer).

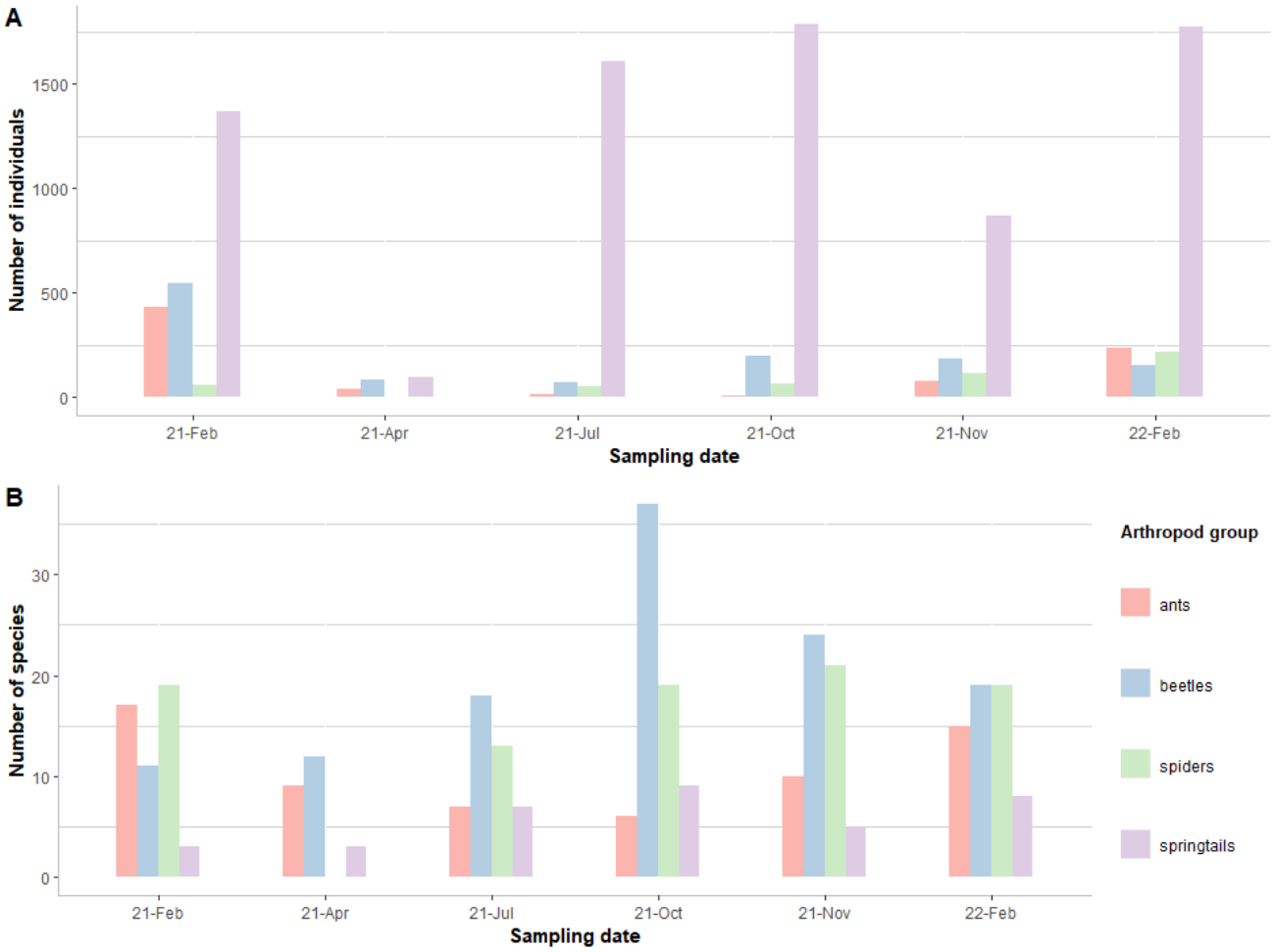


Fig. 2.4. Total abundance (A) and species richness (B) of the four arthropod groups studied (ants, beetles, spiders, and springtails), during each sampling event.

Beetle communities displayed somewhat similar responses in species richness. However, treatment effects on beetle species richness appeared insignificant, whilst crop rotation interacting with site and sampling time had an impact on observed differences (Fig 2.6). Mean beetle species richness was lower on average at Ebenhaezer and greatest at Sewester. Jondu showed the greatest total abundance of beetles, which was 33% more than at Sewester and 186% more than at Ebenhaezer. This was also evident across sampling times. Across the three sites, crop line 1 showed the fewest beetle species richness, whilst crop line 2 had the highest.

Species richness of spider communities showed some level of temporal and site effect (Fig 2.7). No treatment and crop line effects in species richness were detected. Species richness at the onset of the trial (February 2021) did not show any significant differences between spider communities at Ebenhaezer and communities at Sewester, however communities at Jondu were

considerably less species rich on average. Species richness of spider communities at Ebenhaezer showed more delayed recovery after April 2021's no presence. This observation was interestingly contrasted at Jondu that displayed great average species richness in July 2021 after no spider presence in April 2021. However, from October 2021 to February 2022, spider richness was greatest again at Ebenhaezer. Patterns of species richness in springtail communities appeared to mirror that of spider communities to some degree (Fig 2.8). Very similar site effects were observed, again with no detection of treatment and crop line effects in species richness. Springtail species richness at Ebenhaezer fluctuated temporally, with five times greater species richness on average observed in October 2021, and four times greater richness observed in February 2022, compared to February 2021. Conversely, springtail communities at Sewester showed declines in species richness over time. Communities at Sewester showed 10 times reduced species richness in February 2022 compared with 2021 and no springtails were observed at Jondu in February 2022. Total springtail abundance at Ebenhaezer was greatest, with a total of nearly 3354 individuals sampled. This was 40% greater than total abundance at Sewester and 91% greater than total abundance at Jondu. Taxa specific responses in abundance and species richness are evident, with treatment effects only noticed in ant communities, and impacts of temporal change showing distinct patterns between sites and arthropod taxa.

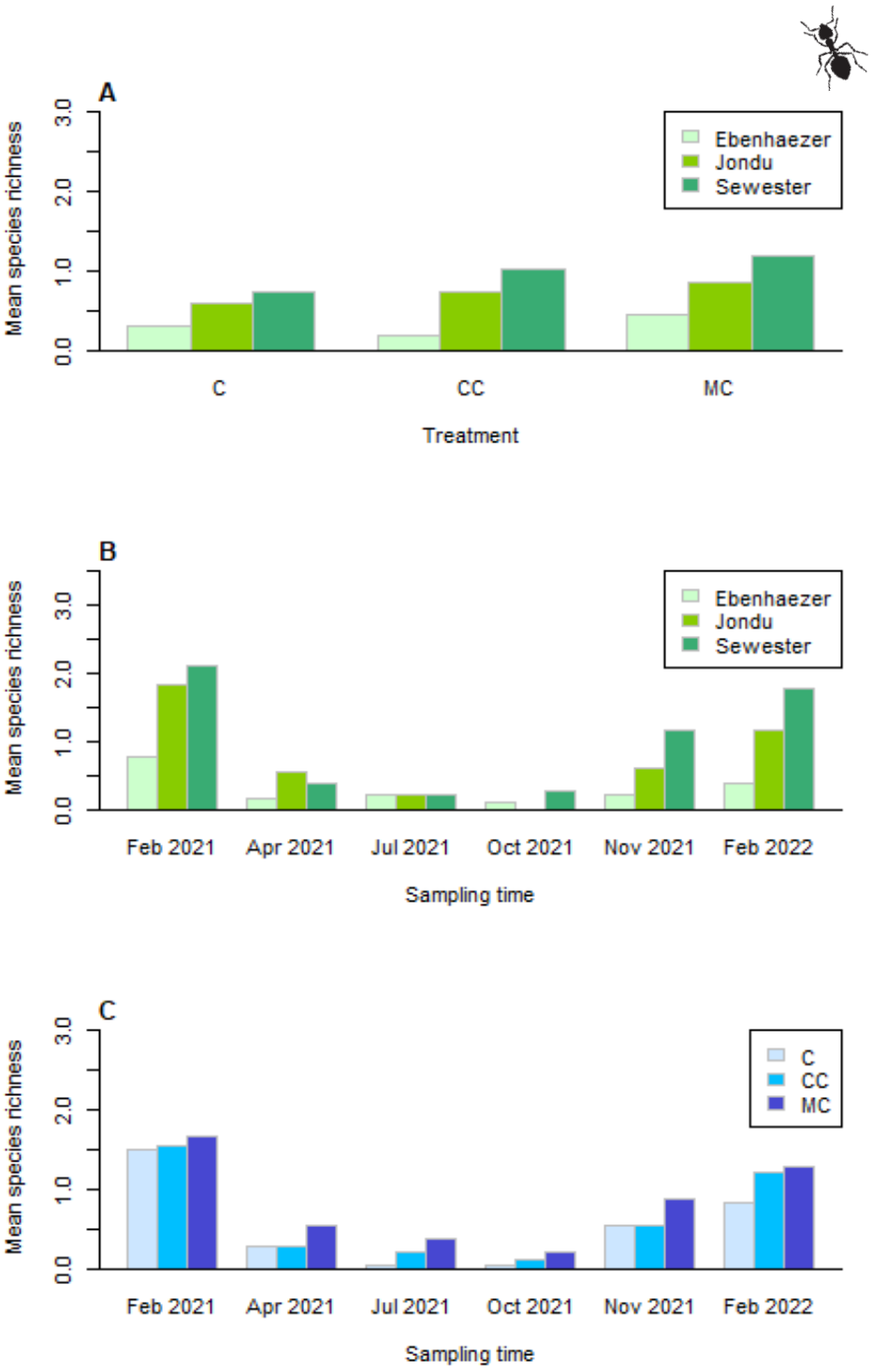


Fig. 2.5. Bar plot depicting ant species in relation to site and treatment (A), site and sampling time (B) and treatment and sampling time (C). Treatment refers to conventional strategies (C), cover cropping (CC), and mulch combined with compost (MC).

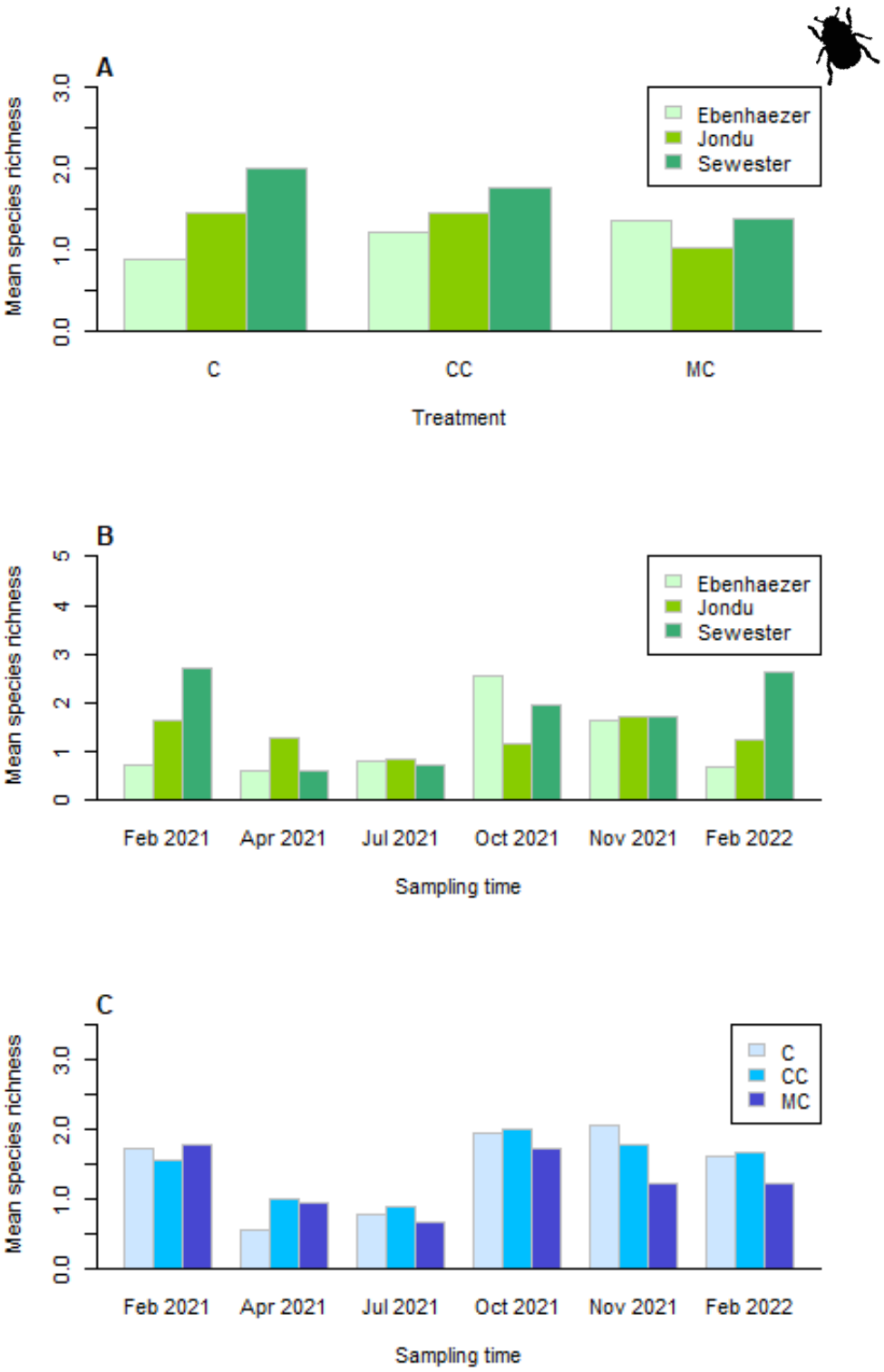


Fig. 2.6. Bar plot depicting beetle species richness in relation to site and treatment (A), site and sampling time (B) and treatment and sampling time (C). Treatment refers to conventional strategies (C), cover cropping (CC), and mulch combined with compost (MC).

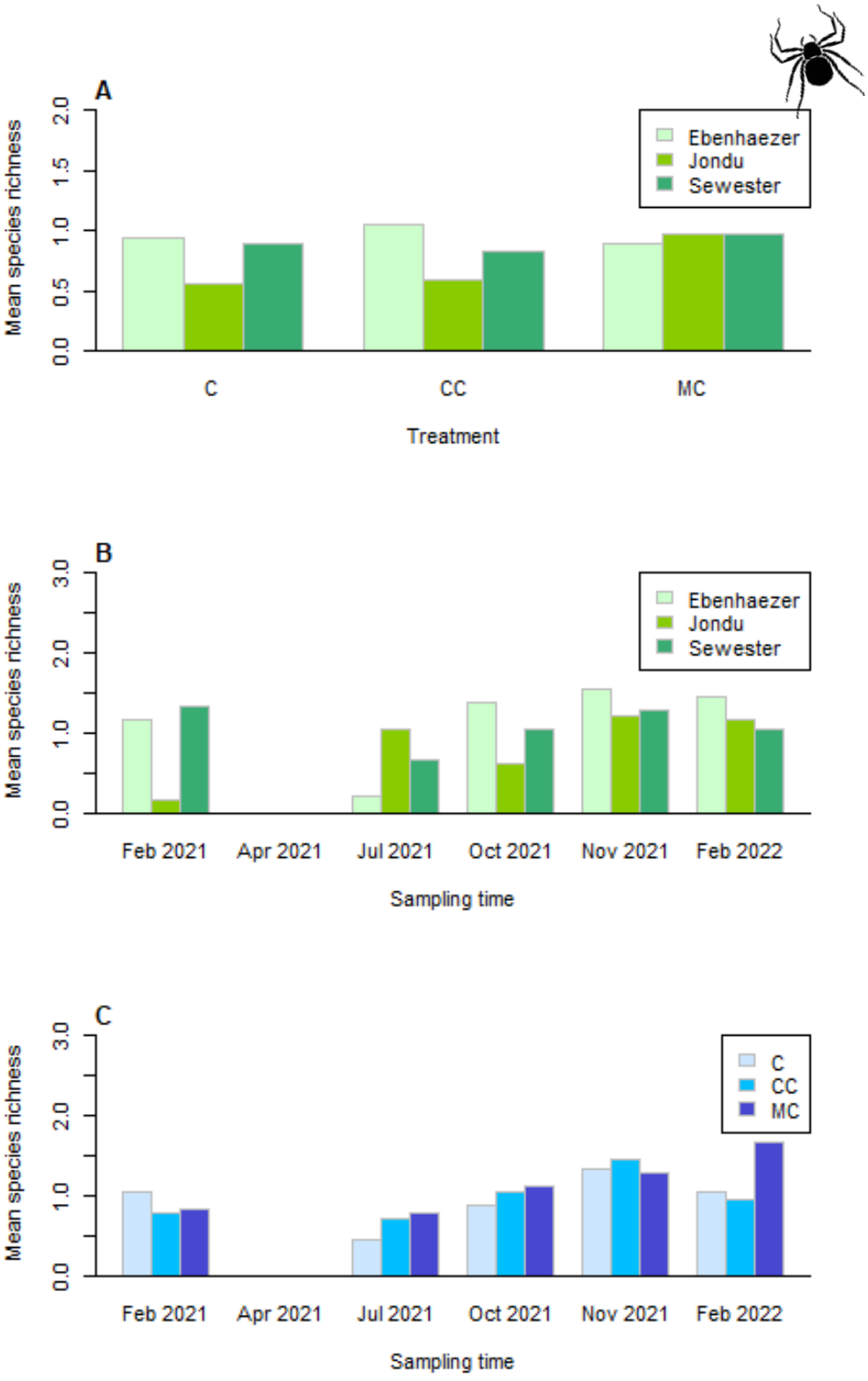


Fig. 2.7. Bar plot depicting changes in spider species richness in relation to site and treatment (A), site and sampling time (B) and treatment and sampling time (C). Treatment refers to conventional strategies (C), cover cropping (CC), and mulch combined with compost (MC).

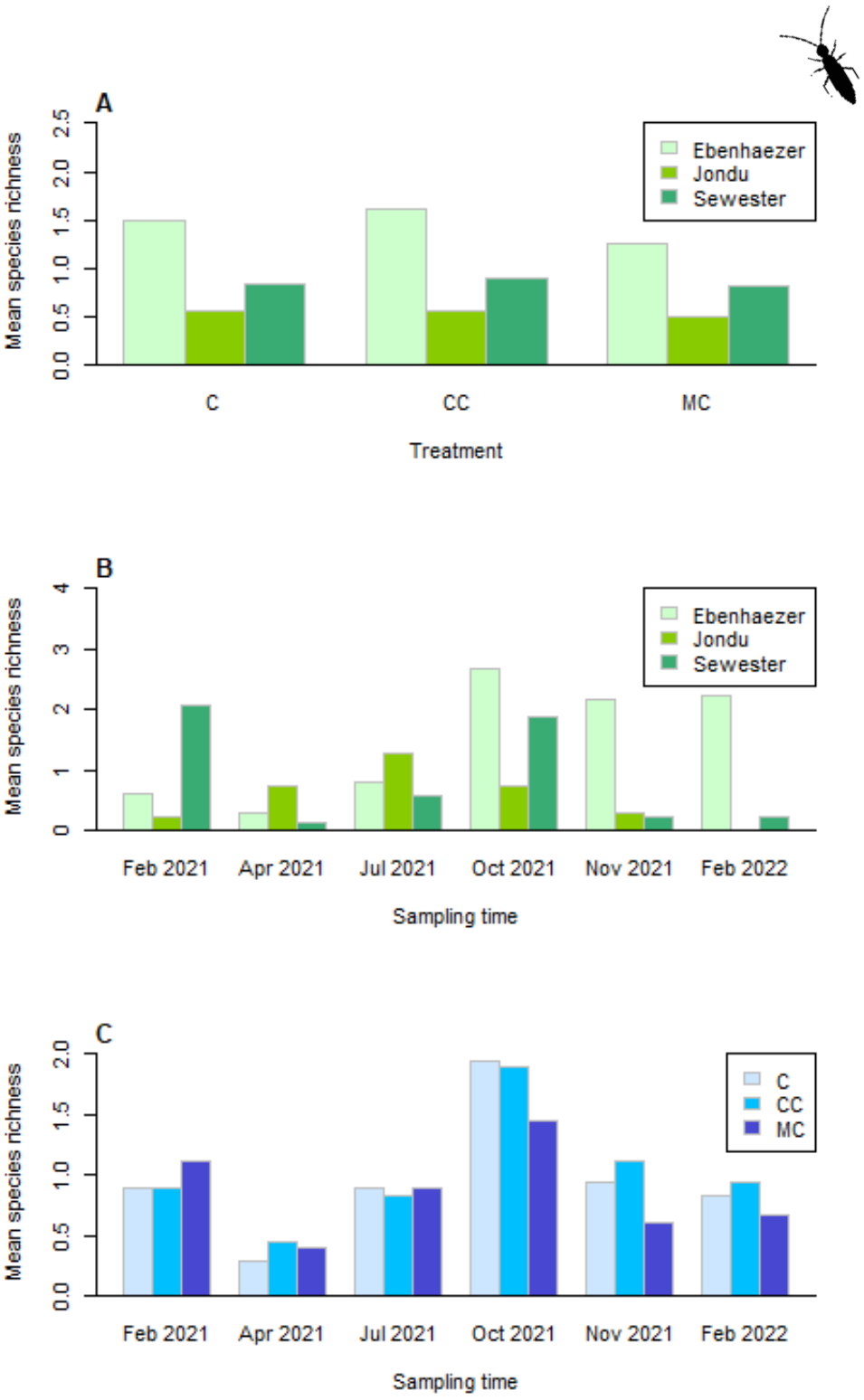


Fig. 2.8. Bar plot depicting changes in springtail species richness in relation to site and treatment (A), site and sampling time (B) and treatment and sampling time (C). Treatment refers to conventional strategies (C), cover cropping (CC), and mulch combined with compost (MC).

Arthropod community analyses

Analysis of similarities (ANOSIM) was used to detect possible dissimilarities in soil arthropod communities across three parameters: field site, treatment, sampling time. The ANOSIM results (Table 2.3) indicated significant responses to site and sampling time ($p<0.001$), across all four study taxa. No significant responses to treatment were observed, thus arthropod communities within a particular treatment were not more similar to each other than those between different treatments ($p>0.05$). For ant communities, field site showed no significant effect. The time of sampling indicated potential differences among samples from different sampling periods, however with some overlap ($R<0.03$). Beetle, spider, and springtail communities had significant site and sampling time effects. Site effects were weak in beetle and spider communities ($R<0.15$), and slightly greater in springtail communities ($R<0.3$). Springtail communities indicated greater among-site differences, however with some overlap. Sampling time had a greater effect on beetle and spider communities ($R=0.45$ & $R=0.3$, respectively), and a weak effect on springtail communities ($R<0.2$).

Non-metric multidimensional scaling (NMDS) ordinations of arthropod communities visually depicted the overall community structure of ants (Fig. 2.9), beetles (Fig. 2.10), spiders (Fig. 2.11), and springtails (Fig. 2.12). NMDS ordinations indicated no similarity of ant communities within sites, as no site clustering was evident. Some clustering based on time of sampling was noted, suggesting ant communities show seasonal differentiation. Seasonal differentiation was further observed in beetle and spider communities. In contrast, springtail communities show greater community differentiation based on field site rather than sampling time. However, all clustering based on sites and sampling times appear relatively weak, with limited pronounced clusters observed.

Table 2.3. ANOSIM results of NMDS ordination for ant, beetle, spider, and springtail communities across factors of site, treatment, sampling time and field line, with $p<0.001$ (***), $p<0.01$ (**), $p>0.05$ (ns).

	Ants		**Beetles**		**Spiders**		**Springtails**	
Factors	**R**	**p**	**R**	**p**	**R**	**p**	**R**	**p**
Site	0.053	ns	0.143	***	0.105	**	0.378	***
Treatment	0.001	ns	0.007	ns	-0.03	ns	-0.59	ns
Sampling time	0.267	***	0.454	***	0.302	***	0.161	**

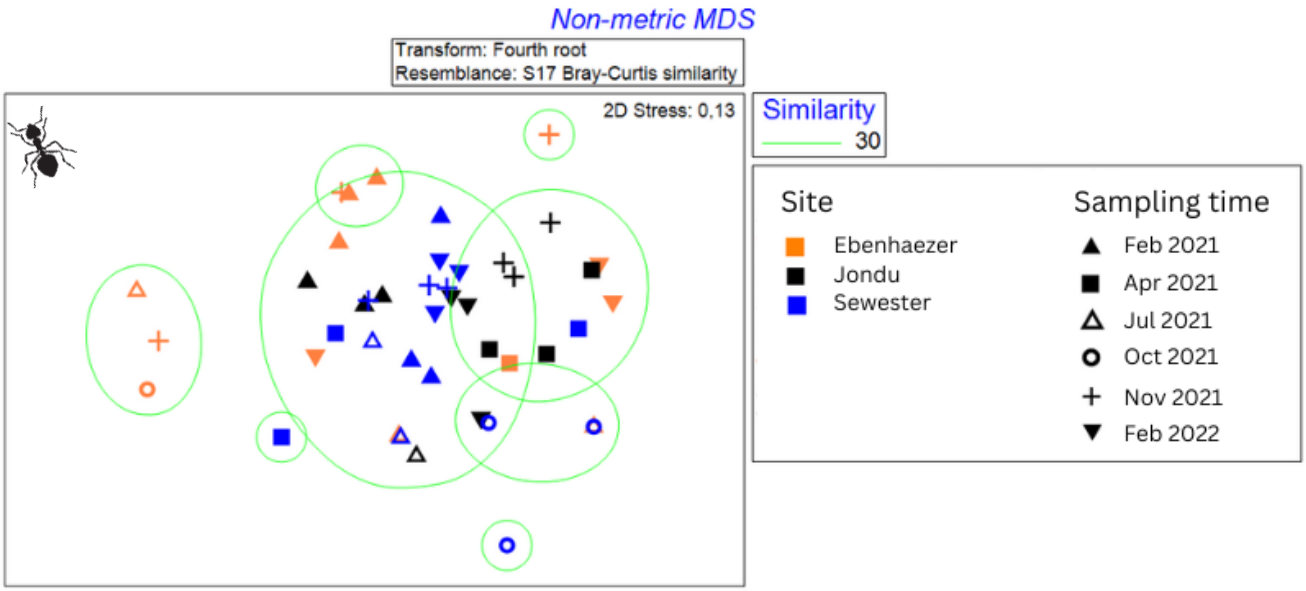


Fig. 2.9. Nonmetric multi-dimensional scaling plot of ant communities across field sites and sampling times (stress = 0.13).

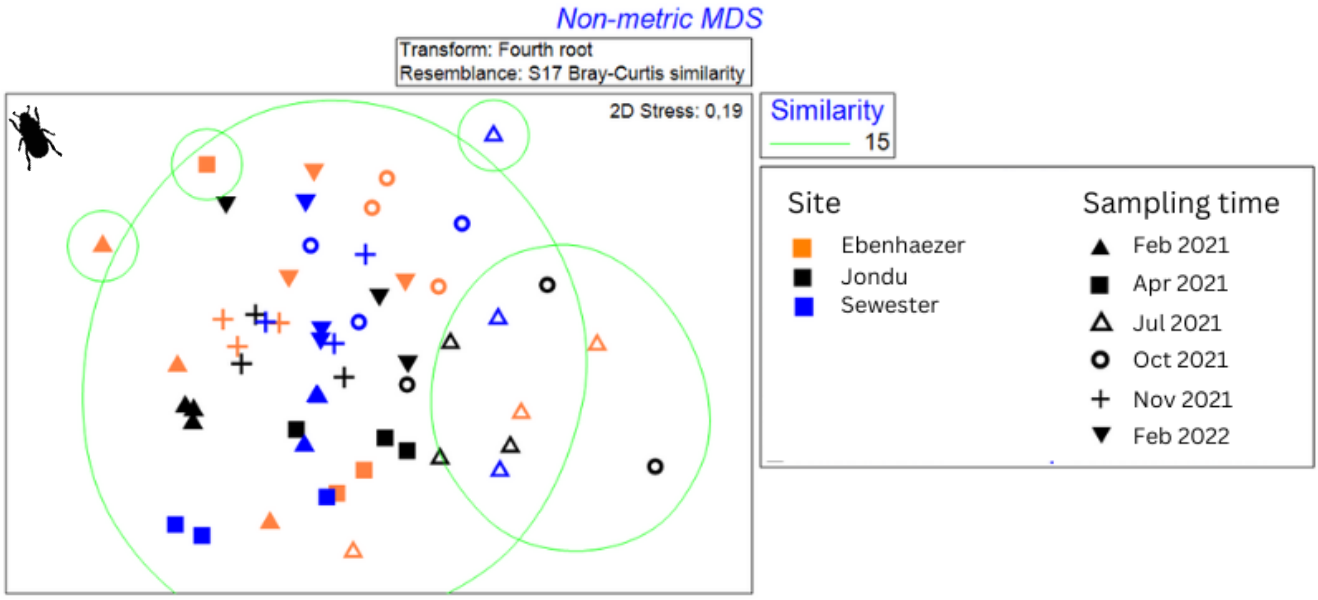


Fig. 2.10. Nonmetric multi-dimensional scaling of beetle communities across field sites and sampling times (stress = 0.19).

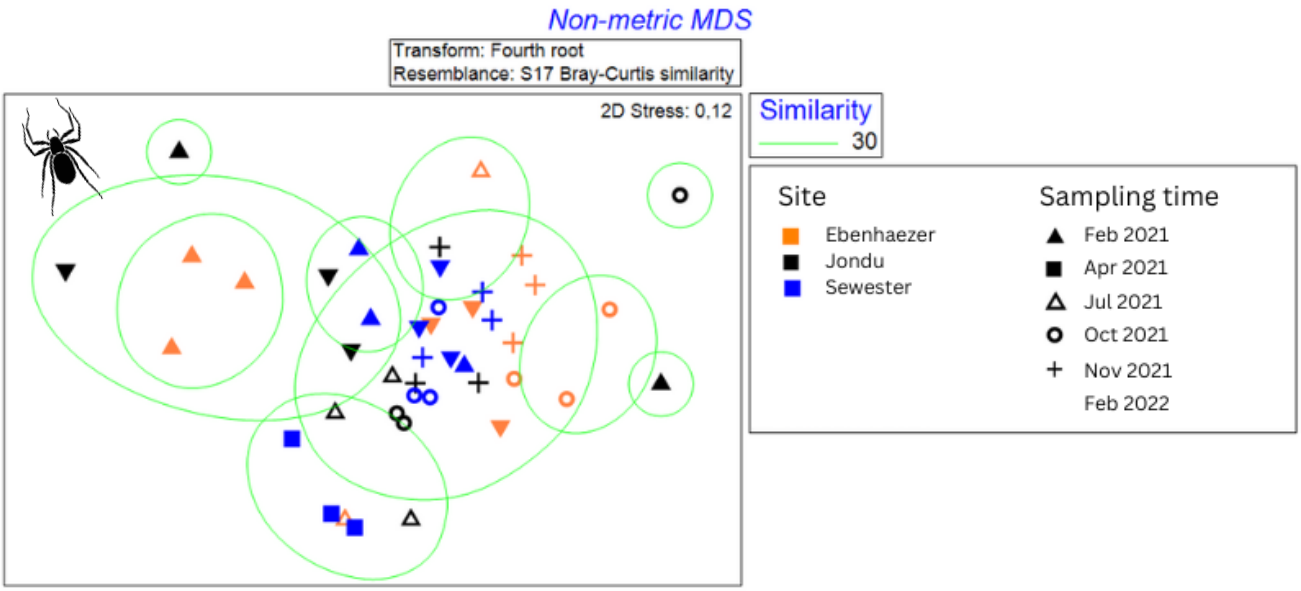


Fig. 2.11. Nonmetric multi-dimensional scaling of spider communities across field sites and sampling times (stress = 0.12).

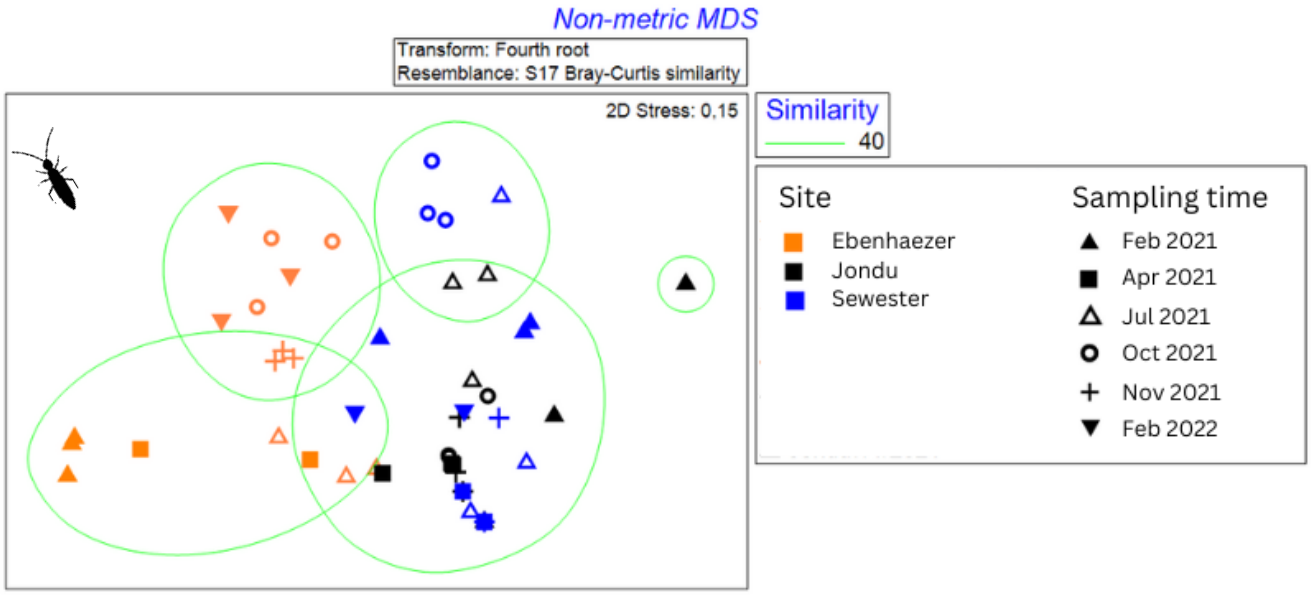


Fig. 2.12. Nonmetric multi-dimensional scaling of Collembola communities across field sites and sampling times (stress = 0.15).

Indicator species analyses

Indicator species for the ant, beetle spider and springtail communities were determined based on field site (Table 2.4), treatment (Table 2.5), field line position (Table 2.6) and sampling time (Table 2.7). Across the four soil fauna groups, 35 species showed strong associations with

experimental field site (Table 2.4). Three species of ants were strongly associated with site: *Tetramorium* sp. 1 was associated with Jondu field site; whilst *Cardiocondyla* sp. 1 and *Pheidole* sp. 1 were associated with Sewester field site. Fifteen of the 60 recorded beetle species were associated with site: Four species associated with Jondu, five with Sewester and six with Ebenhaezer. Eleven species of spiders showed strong associations with site: three at Jondu, two at Sewester, five at Ebenhaezer and one that was associated with both Sewester and Jondu. Several springtail species were associated with the Ebenhaezer field site, two *Entomobrya* spp. and two *Seira* spp. This association follows the high springtail abundance and species richness at the Ebenhaezer site, as discussed.

Ant, beetle, spider, and springtail communities showed limited association with agricultural treatment (Table 2.5). No species of ants or springtails were associated with any agricultural treatments, whilst only an association with the mulch/compost treatment from one beetle and one spider species, namely *Pardosa crassipalpis*, was observed. No associations were apparent for conventional agriculture (C) and cover crop treatments (CC).

Species associations with experimental field line (crop rotation) was just as limited (Table 2.6). A total of three species showed associations. Two spider species were associated with line three in the experimental fields and *Hypogastrura assimilis* was associated with field line one (i.e., cauliflower line). Beetles and ants showed no association with distinct crops.

Ant, beetle, spider and collembola communities appeared highly associated with sampling time, with 24 species across the four taxa associated with particular sampling times. Within the ant communities, *Tetramorium* sp. 2 showed associations with both April 2021 and February 2022. Beetle species show associations with every sampling event, apart from October 2021. Spiders and springtails were associated with the February sampling events (2021 & 2022) as well as July 2021. Interestingly, *Seira* sp. showed significant associations with the February sampling periods, whilst *Enotomobrya* sp. 1 was highly associated with the July sampling period.

Table 2.4. Indicator species of ants, beetles, spiders, and springtails based on experimental field site, with p<0.001 (***), p<0.01 (**), p<0.05 (*), p>0.05 (ns).

Site	Ants			Beetles			Spiders			Springtails		
	Species	**Stat**	**p**	**Species**	**Stat**	**p**	**Species**	**Stat**	**p**	**Species**	**Stat**	**p**
Jondu	*Tetramorium* sp. 1	0.224	*	*Aphodius* sp. 2	0.258	***	*Pardosa* sp. 3	0.21	**	-	-	ns
	-	-	ns	*Zophosis* sp. 2	0.240	***	*Mermessus fradeorum*	0.188	*	-	-	ns
	-	-	ns	*Zophosis* sp. 1	0.228	***	*Pardosa crassipalpis*	0.180	*	-	-	ns
	-	-	ns	*Formicomus* sp. 1	0.224	**	-	-	ns	-	-	ns
Sewester	*Cardiocondyla* sp. 1	0.325	***	*Anthicus hispidus*	0.357	***	*Ostearius melanopygius*	0.25	**	*Seira* sp. 1	0.328	***
	Pheidole sp. 1	0.199	*	*Tribalis* sp. 1	0.24	***	*Thanatus vulgaris*	0.218	**	*Parisotoma* sp. 1	0.145	**
	-	-	ns	*Anthicus biplagiatus*	0.24	***	-	-	ns	-	-	ns
	-	-	ns	*Sitophilus* sp. 1	0.19	**	-	-	ns	-	-	ns
	-	-	ns	*Bembidion* sp. 1	0.15	*	-	-	ns	-	-	ns
Ebenhaezer	-	-	ns	*Stips* sp. 1	0.323	***	*Psammoduon canosum*	0.326	***	*Seira* sp. 2	0.336	***
	-	-	ns	*Stenolophus* sp. 1	0.241	***	*Drassodes lophognathus*	0.299	***	*Entomobrya* sp. 2	0.251	***
	-	-	ns	*Phalacridae* sp. 3	0.178	*	*Cydrela* sp. 1	0.290	***	*Seira* sp. 3	0.185	*
	-	-	ns	*Trox* sp. 1	0.168	*	*Pelecopsis janus*	0.241	**	*Entmobrya* sp. 1	0.261	***
	-	-	ns	*Trox* sp. 2	0.168	*	*Nomisia* sp. 1	0.225	*	-	-	ns

	-	-	ns	*Corticaria* sp. 3	0.162	*	-	-	ns	-	-	ns
Sewester + Jondu	-	-	ns	-	-	ns	*Meteleptyphantes* sp. 1	0.173	*	-	-	ns

Table 2.5. Indicator species of beetles and spiders for agricultural treatment, with p<0.001 (***), p<0.01 (**), p<0.05 (*). Conventional agriculture (C), mulch/compost (MC) and cover crop (CC).

Agricultural treatment	**Beetles**			**Spiders**		
	Species	**Stat**	**p**	**Species**	**Stat**	**p**
C	-	-	ns	-	-	ns
MC	*Rhyterrhinus* sp. 1	0.158	*	*Pardosa crassipalpis*	0.215	*
CC	-	-	ns	-	-	ns

Table 2.6. Indicator species of ants, beetles, spiders, and springtails for field line position, with p<0.001 (***), p<0.01 (**), p<0.05 (*).

Field line	**Spiders**			**Springtails**		
	Species	**Stat**	**p**	**Species**	**Stat**	**p**
Line 1	-	-	ns	*Hypogastrura assimilis*	0.195	*
Line 2	-	-	ns	-	-	ns
Line 3	*Asemesthes* sp. 1	0.185	*	-	-	ns
	Trephopoda sp. 1	0.185	*	-	-	ns

Table 2.7. Indicator species of ants, beetles, spiders, and springtails for time of sampling, with p<0.001 (***), p<0.01 (**), p<0.05 (*).

Sampling time	Ants			Beetles			Spiders			Springtails		
	Species	**Stat**	**p**	**Species**	**Stat**	**p**	**Species**	**Stat**	**p**	**Species**	**Stat**	**p**
Feb 2021	-	-	-	*Sitophilus* sp. 1	0.377	*	*Cydrela* sp. 1	0.427	*	*Seira* sp. 1	0.455	*
	-	-	-	*Zophosis* sp. 2	0.367	**	-	-	ns	-	-	ns
Apr 2021	-	-	-	*Entiminae* sp. 1	0.424	**	-	-	ns	-	-	ns
Jul 2021	-	-	-	*Aphodius* sp. 2	0.399	**	*Meteleptyphantes* sp. 1	0.411	*	*Entmobrya* sp. 1	0.43	*
	-	-	-	*Brosyrus* sp 1	0.424	**	*Micaria* sp. 1	0.509	*	-	-	ns
	-	-	-	*Phalacridae* sp. 3	0.416	**	-	-	ns	-	-	ns
Oct 2021	-	-	-	-	-	-	-	-	ns	-	-	ns
Nov 2021	-	-	-	*Neocleonus* sp. 1	0.329	*	-	-	ns	-	-	ns
Feb 2022	-	-	-	*Lophyra* sp. 1	0.316	*	*Pardosa crassipalpis*	0.57	**	*Entomobrya* sp. 2	0.440	*

	-	-	-	*Tribalis* sp. 1	0.296	*	-	-	ns	*Seira* sp. 2	0.426	*
Feb 2021 + Apr 2021	-	-	-	*Anthicus biplagiatus*	0.337	*	-	-	ns	-	-	ns
	-	-	-	*Zophosis* sp. 1	0.287	*	-	-	ns	-	-	ns
Feb 2021 + Nov 2021	-	-	-	*Anthicus hispidus*	0.344	*	-	-	ns	-	-	ns
Apr 2021 + Feb 2022	*Tetramorium* sp. 2	0.417	*	-	-	ns	-	-	ns	-	-	ns
Jul 2021 + Oct 2021	-	-	-	*Stilbus* sp. 1	0.353	**	-	-	ns	-	-	ns
	-	-	-	*Corticaria* sp. 3	0.319	*	-	-	ns	-	-	ns
Oct 2021 + Nov 2021 + Feb 2022	-	-	-	*Gonocephalum arenarium*	0.428	*	-	-	ns	-	-	ns

DISCUSSION

Investigating different aspects of soil fauna (macrofauna and mesofauna), in agroecosystems, is important to better understand the role of arthropod communities in improving soil health. This study of agricultural soils adopting regenerative farming practices found 2696 individuals of macrofauna (ants, beetles, and spiders), and 7498 individuals of mesofauna (springtails). Springtails were the most representative in terms of abundance, however not in terms of species richness. The Coleoptera and Araneae were the most species rich orders, with 60 and 54 species, respectively.

Tenebrionidae and Staphylinidae were two of the most abundant beetle families present. Tenebrionidae are considered biological indicators of soil quality (Velasquez & Lavelle, 2019) while Staphylinidae are generalist predators, common in agroecosystems, feeding on ants, caterpillars, and insect eggs (Chamorro-Martínez et al., 2022). They also limit the growth of certain populations of crops pests (Chamorro-Martínez et al., 2022). Lycosidae was one of the most abundant spider families, of which *Pardosa crassipalpis* was highly abundant and comprised 50% of all spider abundances. Lycosids are highly mobile and adept hunters (Dippenaar-Schoeman et al., 2013). *Pardosa crassipalpis* is commonly found in a variety of agroecosystems, ranging from cotton to strawberry systems (Dippenaar-Schoeman et al., 2013). This species is also often highly abundant in agroecosystems and can be considered as an agrobiont in many systems (Dippenaar-Schoeman et al., 2013). Ants were also highly abundant. Ants are important biological indicators of disturbance (Majer, 1983; Gerlach, Samways & Pryke, 2013). They are also known to improve soil aeration, drainage, and decomposition through their role as soil engineers (Chamorro-Martínez et al., 2022). Springtails play an important role as prey and predators of microorganisms involved in litter decomposition. Entomobryidae (*Seira* and *Entomobrya* spp.) was the most abundant family of springtails, making up over 50% of all springtail abundances. Although still under investigation, at least one species of entomobryids is native to South Africa. The great abundance of Collembola found under these arid conditions is quite remarkable. The highly abundant Entomobryidae species are likely desiccation tolerant and adapted to these arid environments. Liu et al. (2020, 2021) found that the Collembola genus *Seira* have remarkably high critical thermal maxima (CT_{max}) and desiccation tolerance, which may partly explain their high species richness in hot, dry habitats such as the Cape Floristic Region.

It is known that vegetation structure and cover is known to be one factor influencing pitfall catches (Eyre, McMillan & Critchley, 2016). However, soil macrofauna and mesofauna densities and species composition showed limited association with treatment effects. Conventional agriculture, mulching and cover cropping had no significant impact on the distribution and composition of arthropod communities. Ant communities were an exception, with greater mean species richness of samples observed under mulching combined with compost treatments than under conventional agricultural strategies. Crop rotation, as described by the crop line, was also not a significant predictor of arthropod densities or species richness. Neither the presence of crops, or the type of crop planted (cauliflower, tomato, and watermelon) showed any significant impact on arthropod community assemblage.

Arthropod activity was more coupled with the experimental field site (Ebenhaezer, Jondu and Sewester) and with the season in which sampling events took place. To better understand community composition before the trial began, a pre-trial sampling event occurred in February 2021. This sampling event showed high total abundance of ants, beetles and springtails that corresponded with relative low species richness. During February 2021, the agricultural landscape was characterized by dry and hot weather conditions, and bare experimental fields characterized by sandy soil. Thus, dominance by a few species is not unexpected. The considerable reduction in arthropod abundances and species richness in April 2021 coincided with the planting of *Brassica* crops. No spiders were collected during the April 2021 sampling event, suggesting a high sensitivity of spiders to soil disturbance. However, spider populations appeared to recover and recolonize the agroecosystems subsequent to the disturbance. Substantially higher beetle species richness was recorded in crop line two during the November 2021 sampling period, which coincided with the planting of tomato crops. Springtail communities remained more consistent over the sampling period, in terms of abundance and species richness. The maintenance of high densities of important prey is thought to help sustain the diversity of macrofauna in the system (Root, 1973). In this study, although there were only nine species, there were 57 species of spiders, suggesting that high springtail abundance could be sustaining the high spider diversity. Furthermore, high densities of springtails suggest that processes such as decomposition and nutrient cycling are maintained (see Chapter 3).

The experimental field sites also appeared to considerably impact arthropod species activity, particularly for ants, beetles, and springtails. The Ebenhaezer site was characterized by considerably fewer ant and beetle species, however considerably greater number of springtails,

whereas abundance of springtails at Jondu was lowest. Due to the underlying vegetation type differing across field sites, the pronounced site effect was expected.

From the ANOSIM, cluster analysis and NMDS it was clear that samples did not cluster based on treatment and crop line, and clustering based on field sites and sampling times were weak. The slight site-by-site community clustering was potentially as a result of the differences in the underlying vegetation types and soil parameters across sites. Again, patterning based on environmental and treatment data may only become evident in arthropod communities in the future, with a one-year effect not being enough to showcase any distinctive associations. However, springtail community composition (along with the other invertebrate communities studied) seem more governed by experimental field site than any other factor. This is expected as site effects may still be pronounced within the first year of study before the system stabilizes and effects from treatment and crop rotation can potentially be observed.

Indicator species analyses provide insight into possible important associations between trial parameters and arthropod communities. *Pardosa crassipalpis* shows potential as an agrobiont in this agroecosystem, displaying high abundances and being highly associated with very specific environments, such as the Jondu site, the mulching and compost treatment and the February 2022 sampling period. Further surveying of abundance data would be beneficial in determining the role of this species as an indicator for this agroecosystem.

This study provides essential baseline data on the arthropod communities in an irrigated dryland agricultural system in South Africa, focused on regenerative agriculture. Understanding the arthropod diversity across a variety of factors will enable the monitoring and impact of agricultural management and the presence of beneficial arthropods. This will ensure that the natural enemy complex is maintained, thus promoting sustainable soil health which ultimately plays back into sustainable crop and seed production systems. As is the nature of surveying in the first year of a trial, limited patterns governing community assemblages are observed. However, continued monitoring will prove essential in furthering the knowledge of how these regenerative agriculture practices impact the soil fauna. Future work will also investigate the soil arthropod diversity in the natural vegetation in the region to enable comparisons between the experimental trial and the surrounding vegetation.

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CHAPTER 3.
IMPACTS OF REGENERATIVE AGRICULTURE ON STRAW MULCH DECOMPOSITION

ABSTRACT

Litter decomposition and nutrient cycling is a key process in ensuring soil fertility and maintain quality and yield in agroecosystems. Litter decomposition depends on climate, litter quality, physical and chemical site, and soil conditions as well as the size and composition of the decomposer community. Decomposer communities are very sensitive to land use, therefore changes in agricultural management strategies are known to impact soil fauna. This study assessed the effect of regenerative agricultural strategies, in a cauliflower seed production system, on litter decomposition by soil fauna. A litter bag experiment was conducted to understand litter decomposition dynamics over time and the potential influences by soil mesofauna. The study took place in Lutzville, Western Cape, on three privately owned farms (Ebenhaezer, Jondu and Sewester) for a period of five months from July 2021 to November 2021. Litter bags, with mesh sizes of 1.6mm, were filled with straw mulch obtained from the field, set out in sets of four within each experimental plot and collected periodically. Three treatments were defined: conventional agricultural, involving agrochemical inputs and tillage (C), mulching combined with compost (MC), and cover cropping (CC). The proportion of straw mulch remaining, decomposition rate, carbon to nutrient ratios and soil fauna species richness and abundance were measured. The proportion of litter remaining, and the corresponding decomposition rate was most significantly impacted by site and temporal changes. Proportion of remaining litter decreased over time, with highest decomposition rates being reflected for litter bags placed at Jondu. Treatment effects were also evident, with the proportion of litter remaining the greatest, and correspondingly the lowest decomposition rates under the conventional strategy treatment. However, litter placed under the cover cropping treatments showed greatest decomposition rate. Very little to no difference in carbon to nutrient ratios were observed between agricultural treatments and crop lines. Site and sampling time showed the greatest impacts on differences observed in carbon to nutrient ratios. Decreases in C/N ratio in litter bags were observed only at Jondu. The abundance and species richness of springtails were not significant in explaining the differences observed in decomposition rate and carbon to nutrient ratios. However, investigation into the community assemblage of springtails sampled from litter bags showed relatively strong community structuring based on

site, and no other study factor. Springtail communities within sites appeared more similar than those from different sites. These results highlight the influence of different agricultural management strategies on soil fauna and litter decomposition, and the influences of soil fauna communities on decomposition rate and nutrient uptake. Site differences remain large during the first year of the trial, thus assessing the effects of land management on litter decomposition by soil fauna is hugely important once the trial is more established.

INTRODUCTION

Crop and soil management are critical for sustainable food production. Soil fertility is a vital part of healthy and productive agroecosystems. Influx of soil organic matter (SOM), through organic fertilization or addition (and eventual decomposition) of crop residues, is a key component of ensuring soil fertility. Organic fertilization and addition of crop residues is well known to aid in maintaining SOM, improve soil water regulation and control erosion (Wilhelm et al., 2004; Lal, 2008; Diacono & Montemurro, 2010).

At large geographical scales, plant litter decomposition is regulated by climate and litter quality (Aulakh et al., 1991; Seneviratne, 2000; Sun et al., 2004; Cornwell et al., 2008; Bray, Kitajima & Mack, 2012; Wickings et al., 2012; Hobbie, 2015), whilst at the local scale it is controlled by the physical and chemical site and soil conditions (Zhang et al., 2016) and the size and composition of the decomposer community (Hättenschwiler, Tiunov & Scheu, 2005; García-Palacios et al., 2013; Martínez-García et al., 2021). In the decomposer community, litter decomposition is driven mostly by soil microbe activity (Hättenschwiler, Tiunov & Scheu, 2005). However, soil fauna, particularly soil mesofauna, help accelerate litter decomposition through litter fragmentation as well as modification of microbial community structure and activity (Brussaard et al., 1997; Smith & Bradford, 2003; Hättenschwiler, Tiunov & Scheu, 2005; Cole et al., 2006; Lavelle et al., 2006). García-Palacios et al. (2013) found that the absence of soil fauna negatively impacted litter decomposition rates in agroecosystems, causing a 30% decrease. The community structure of soil arthropods also influences the decomposition capabilities within the system, through population control of mesofauna by predators (Lensing, Todd & Wise, 2005). Springtails (Collembola) are important and abundant detritivores, influencing the rates of litter decomposition through their transportation of fungal spores and fungal grazing (Lensing, Todd & Wise, 2005).

Land use and management are particularly critical components controlling decomposition potential, as it can impact the soil nutrient stock, pH and biota communities (Steenwerth et al., 2003; Liiri et al., 2012; Fichtner et al., 2014). It is known that soil fauna are particularly sensitive to land use change (Cassani et al., 2021; Sabatté et al., 2021). Thus, agricultural management could be hugely impactful on the soil environment and the decomposer community, thus potentially affecting litter decomposition and mineralization. According to Giller et al. (1997) and Cassani et al. (2021), different agricultural practices have varying impacts on litter decomposition by micro, meso and macrofauna. Soil disturbance through tillage can impact ecosystem functioning and soil fauna biodiversity (Hendrix et al., 1992; Manetti et al., 2010; Cardinale et al., 2012). Changes in litter input, litter quality, soil structure and microclimatic conditions impact trophic interactions within soil food webs (Moore, McCann & de Ruiter, 2005; Sabatté et al., 2021). Thus, land management practices may impact soil faunal communities by affecting their food resources.

Moore et al. (2004) found that bacteria and their consumers were favoured under conventional tillage practices. In contrast, a greater balance between fungal and bacterial communities was observed under conservational tillage practices, due to conservational practices being less detrimental to soil microorganism communities. Moore et al. (2004) also found that a more balanced detrital soil food web proves to be more stable. Not only do soil tillage and land management practices impact soil microorganisms, but they also affect the mesofauna, and ultimately macrofauna communities (Manetti et al., 2010; Cassani et al., 2020). Management practices can also impact microclimatic conditions. This results in changes in arthropod communities as mesofauna, particularly springtails are sensitive to changes in moisture. Changes in the moisture content of the litter and soil can affect interactions between soil fauna and soil microbes by affecting their activity, population densities, and vertical stratification within the litter (Lensing, Todd & Wise, 2005). Ferguson and Joly (2002) found that experimental water supplementation increased springtail abundance. Moist habitats can indirectly benefit Collembola by enhancing the growth of fungi, their main food source. Hassall et al. (1986) found evidence of vertical stratification in response to changes in moisture. During low rainfall periods, Collembola moved into the humus layer, but showed migratory tendencies into the litter layer upon increased rainfall, in response to microorganismal blooms, with 30% remaining in the humus layer.

The use of organic mulching practices has been shown to be beneficial in agroecosystems. Mulching helps to suppress weeds, as well as to support soil quality, boost soil fertility,

improve soil water retention and support soil biota (Nakamoto & Tsukamoto, 2006; Thomson & Hoffmann, 2007; Ramos et al., 2010; Zheng et al., 2018; Webber et al., 2022), thereby potentially sustaining greater detritivore and decomposer community and increasing leaf litter decomposition (Jacometti, Wratten & Walter, 2010). Cover crops themselves may also benefit soil functional diversity through their nutrient-rich root exudates (Jiao et al., 2013), contribution to increased soil carbon (Steenwerth & Belina, 2008), and interactions with mycorrhizal fungi (Baumgartner, Smith & Bettiga, 2005; Turrini et al., 2017). Organic management in general has been shown to increase soil organic carbon (Gattinger et al., 2012) and increase soil microbial abundance and activity (Lori et al., 2017; Martínez-García et al., 2018).

A necessary component of determining agroecosystem resilience under different management practices is understanding the contribution of soil biodiversity to litter decomposition. Thus, in this chapter I aimed to assess the possible effects of agricultural soil management (conventional versus regenerative) on the litter decomposition process, soil arthropod community structure, litter N release over time, and carbon and nitrogen dynamics in soil.

METHODS

Information pertaining to site selection and field design can be found in chapter 2 (page 37).

Litter decomposition

The effects of agricultural management on soil fauna community composition and litter decomposition were examined for a period of five months in 2021, from July to November, by means of a litter bag experiment. Litter bags in this study were small cylindrical plastic containers with a height of 4 cm and a diameter of 7.5 cm, as used in Bengtsson et al. (2011). The bottom consisted of a steel net with mesh size 0.5 mm. Each trap had a removable lid with a bigger mesh size of 1.6 mm to allow mesofauna to enter the trap. Each litter bag was individually numbered. Straw mulch was used as the plant litter for the study, as this was readily available on site and used in the mulch combined with compost treatment (MC). The material was collected on site on 22 April 2021. The mulch was cut into 1 x 1 cm pieces and air dried for three days. The air-dried litter was then placed into the litter bags and weighed for initial mass, up to approx. 6.58 g (weighed to the nearest 0.1 mg) (range: 6.5358 g – 6.5896 g)

using an A&D semi-micro analytical balance (Phoenix GH series). The litter was not compressed and was allowed to maintain its normal volume and density. Five randomly chosen samples were used to estimate initial nutrient content. The litter samples showed initial nutrient concentration as follows, C: N = 22.50; and C: P = 88.4. The filled traps were stored dry at room temperature before being deployed in the field on 1 July 2021.

To examine the differences in decomposition rate and nutrient dynamics of straw mulch over time among the three farming treatments: Conventional agriculture (C), mulch/compost (MC) and cover cropping (CC), one set of four litter bag traps was placed in the middle of each treatment block at each of the three sites (Ebenhaezer, Jondu and Sewester). Each set contained three conventional litter bag traps and one exclusion trap that was covered in stocking material to prevent soil fauna from entering the trap. Thus, four traps were set out in 54 experimental blocks resulting in a total of 216 litter traps deployed across all sites. The traps were placed in the soil with the top of the trap at ground level. The traps in each set were placed within 3-4cm of each other.

The mass loss of litter over time was examined by setting up three distinct collection periods: 1 September, 16 October, and 29 November, i.e., after 62, 107, 151 days in the field. At each collection period, one randomly selected trap from each set was collected, while the remaining traps were left for later collection. The exclusion traps were left for the last collection date. An accidental farming management error resulted in the loss of some litter bags (five destroyed at Jondu, two at Sewester and two at Ebenehaezer). The data set thus comprises 207 of the original 216 traps. When traps were collected, they were taken out of the soil, sprayed lightly with water to keep soil fauna alive during transport, wrapped in aluminium foil, immediately placed in individual plastic bags, and stored in a cool, upright position in thermally insulated containers, until they were returned to the laboratory the following evening. Soil fauna were immediately extracted from the samples, upon arrival in the laboratory. The samples were placed face down onto Tullgren funnels for 7- 14 days until the litter was dry, to allow for the collection of soil fauna. After this extraction process, the litter samples in each trap were transferred to aluminium foil and oven dried at 60°C for six days to ensure that samples were dry before mass loss and chemical composition were determined.

Mass loss was determined as loss of organic matter from the traps. Dry weight was determined by weighing samples after oven drying. Many traps had accumulated mineral soil particles during the study period. These were removed from the dry litter using a 1 mm sieve. Dry litter

samples were then burned in a muffle furnace at 550°C for three hours. These samples were weighed to determine the ash free dry weight of samples, which was used to determine total mass loss of organic matter.

Carbon and Nitrogen isotopic analysis was conducted at the UP Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria, South Africa. All samples were weighed (1.1mg to 1.2mg) into tin capsules that had been pre-cleaned in toluene using a Mettler Toledo Mk5 microbalance. Samples were combusted at 1020°C using an elemental analyzer (Flash EA 1112 Series) coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany). All results are referenced to Vienna Pee-Dee Belemnite for carbon isotope values, and to air for nitrogen isotope values. Results are expressed in delta notation using a per mille scale using the standard equation (Coplen, 2011):

$\delta X(‰) = [(R_{sample}-R_{standard})/R_{standard}-1]$

where X= 15N or 13C and R represents 15N/14N or 13C/12C respectively. Phosphorus (P) and K, Na, Mg were measured using XRF (X-ray fluorescence) analysis of ground dried material.

Extracted arthropod samples were preserved in 96% ethanol for laboratory identification. All individuals were sorted into orders, with springtails being further sorted into morphospecies, from hereon called species, based on each group's unique morphological characters and with the assistance of taxonomic expert, Charlene Janion-Scheepers, University of Cape Town, South Africa and available identification keys (Janion-Scheepers, 2021). Furthermore, the abundance and species richness of springtails was determined.

Statistical analyses

Mass loss or organic matter was measured by the decomposition constant *k*, according to Olson (1963):

$$W_t = W_0 e^{-kt}$$

W_t = remaining mass of organic matter at time *t* (g)

W_0 = initial mass of organic matter (g)

k = decomposition constant (day $^{-1}$)

t = time (day)

This exponential model was linearized using the natural logarithm of the remaining litter (W_t/W_0) to obtain the decomposition rate (k):

$$k = \frac{-ln\left(\frac{W_t}{W_0}\right)}{t}$$

Carbon to nutrient ratios were calculated using the data from the chemical analyses. All ratios were square-root-transformed before statistical analysis, while k values were untransformed. In terms of diversity analyses within this study, species richness refers to the number of species and abundance to the number of individuals (Magurran, 2004).

The data was analysed in R (v4.2.2; R Core Team, 2018) with General Linear Mixed Models (GLMMs) using the *lme4* package (v1.1-31; Bates et al., 2015). Fixed factors were treatment, sampling time, site, crop line, springtail species richness and abundance and all their interactions, while sample plot was considered as random effect. Degrees of freedom were estimated with the Satterthwaite method using the *lmerTest* (v3.1-3; Kuznetsova, Brockhoff & Christensen, 2017) package. Model outputs for Carbon to nutrient ratios are available in Appendix 3. Relationships between response and dependent variables were visualized using the *ggplot2* (v3.3.6; Wickham, 2016) package. The effects of field sites, treatments, crop lines, and sampling times on springtail species richness were assessed using Poisson regression models (GLM), since the response variable was count data. The complexity of full models was reduced through backward stepwise selection using the stepAIC function in the *MASS* package (v7.3-58.1; Venables and Ripley, 2002). Models were compared by Akaike's Information Critera (AIC), with the final model having the lowest AIC (Murtaugh, 2009). To determine which springtail species were associated with field sites, agricultural treatments, crop lines, or sampling seasons, the *indicspecies* (v1.7.12; de Caceres & Legendre, 2009) package was used. The software PRIMER (Clarke & Gorley, 2006) was used to conduct ANOSIM (Analysis of Similarities) tests to test for significant differences in springtail community composition between the study factors (Clarke, 1993). Furthermore, Bray-Curtis based NMDS (Non-Metric Multi-Dimensional Scaling) ordinations were used to visually compare the overall community

structure of the four study taxa between samples (stress = 0.16). NMDS of springtail communities based on agricultural treatments can be found in Appendix 3.

RESULTS

Decomposition rate

Sampling time and field site were the most important factors explaining variation in the decomposition rate of straw mulch (Table 3.1). Mass loss of organic matter from litter bags was most rapid at Jondu, with less than 60% of original organic matter remaining after 107 days. The pattern of decomposition rates based on treatment strategy or crop line position, differed between sites, as indicated by the significant Site x Treatment and Site x Crop Line interactions (Table 3.1). Each site showed slightly different patterns in decomposition rates of litter placed under different treatments or in different crop lines. The abundance and species richness had no significant effect on changes in decomposition rate ($p>0.05$).

Table 3.1. Statistical (GLMM) analysis of decomposition rate in relation to the fixed factors treatment, crop line, sampling day, site, springtail species richness and springtail abundance as well as site and crop line interaction effects, with $p<0.001$ (***), $p<0.01$ (**), $p<0.05$ (*), $p>0.05$ (ns).

Factor	***df***	**Den*df***	***F***	**p**
Site	2	34.2	15.18	***
Treatment	2	33.2	5.90	**
Crop line	2	34.6	9.01	***
Sampling time	1	80.6	96.84	***
Springtail species richness	1	93.5	0.02	ns
Springtail abundance	1	99.2	2.94	ns
Site × Crop line	4	33.3	9.91	***
Site × Treatment	4	32.8	2.47	ns
Treatment × Crop line	4	32.9	3.13	*

Decomposition rates of litter placed under conventional agriculture (C), were consistently lower than those under the other two treatments, with cover cropping most often showing the

greatest rate of decomposition across the three sites (Table 3.2; Fig 3.1). Most litter had not fully decomposed after 151 days. Half-life of litter ranged from 195 days under cover cropping management strategy to 279 days under conventional management strategy (Table 3.2). Half-life of litter was 1.43 times longer under conventional management and 1.14 times longer under mulch and compost compared with the cover cropping strategy. However, because these values were calculated for only the first 151 days of decomposition, these values might be imprecise. Decomposition rate of litter differed between crop lines, however these patterns varied across sites. At Ebenhaezer, decomposition rates were greatest in crop lines 2 and 3 (Fig. 3.1.B), whereas at Jondu decomposition rate was greatest in crop line 1.

Table 3.2. Decomposition rate (k values) and half-life of litter for the three agricultural treatments, conventional management (C), mulching and compost (MC), and cover cropping (CC).

Treatment	***k* value (day $^{-1}$)**	**SE**	***n***	**Half-life (days)**	**SE**	***n***
C	0.00248	0.00027	46	279	581.8	46
MC	0.00311	0.00026	47	223	78.6	47
CC	0.00355	0.00030	51	195	100.1	51

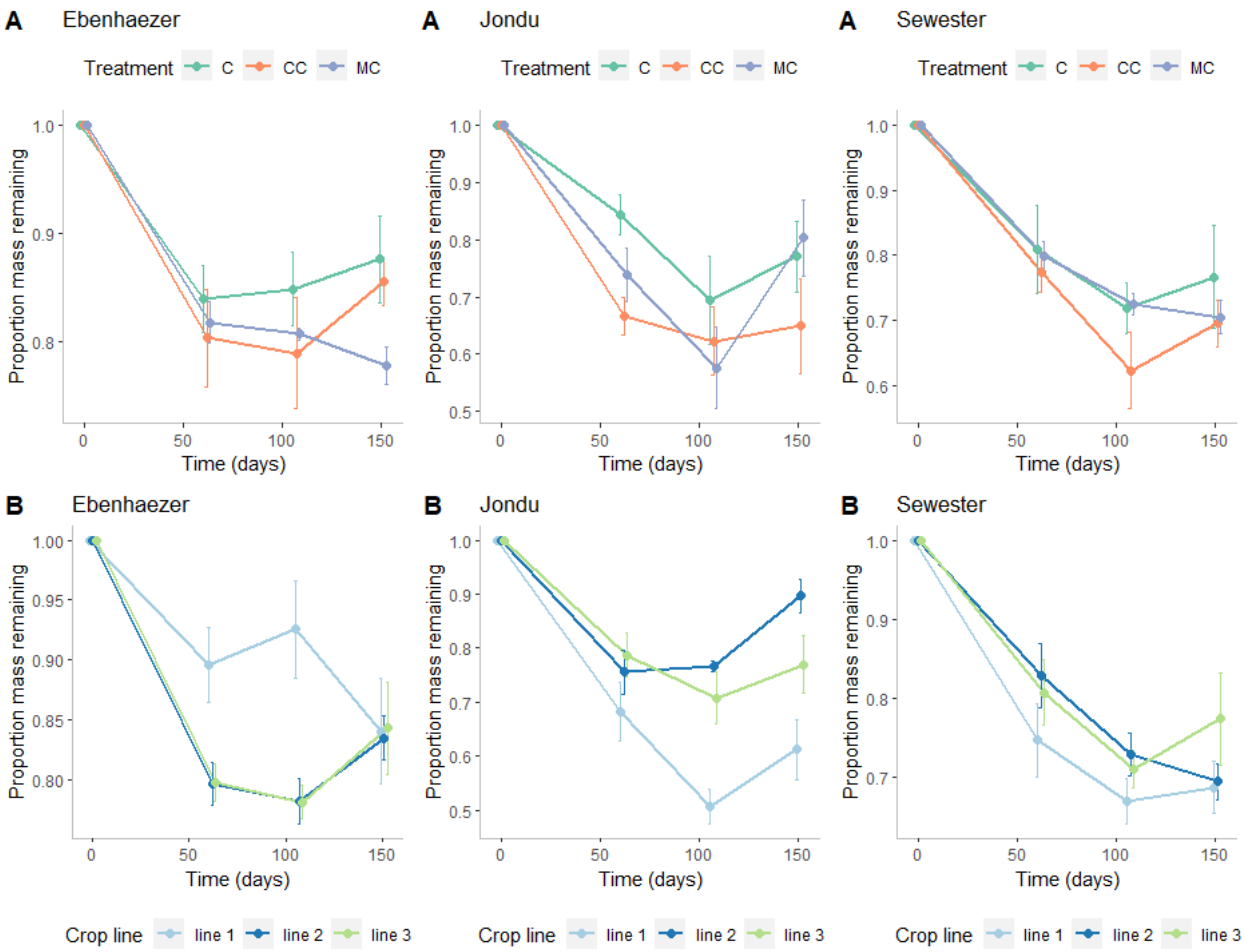

Fig. 3.1. Proportion of litter remaining in relation to time across three agricultural treatments (A) and crop rotation lines (B). Treatments refer to conventional agriculture strategy (C), mulching and compost (MC), and cover cropping (CC). Mass loss differed significantly between site, sampling days and crop line (Table 3.1). Bars represent standard error.

Stoichiometry during decomposition

Straw litter stoichiometry showed significant temporal changes (Fig 3.2, Fig. 3.3; Appendix 3). C/N showed significant site differences (Fig. 3.2), with C/N increasing over time at Ebenhaezer and Sewester and decreasing over time at Jondu. C/N also showed significant treatment differences. C/N was greater under conventional management (C) after 62 days but increased over time in mulch and compost treatments. C/N was lowest under cover cropping management. C/P (Fig. 3.3) showed significant temporal differences across sites. C/P at all sites increased over time until sampling day 107 and then decreased until sampling day 151. C/P was greatest at Ebenhaezer and particularly lowest at Jondu by day 151. There were significant temporal differences in C/K (Fig. 3.3). C/K consistently increased over time at Sewester. Jondu and Ebenahaezer showed increased C/K of litter between day 0 and 107 and

then sudden decreases until day 151. C/Mg of straw litter decreased significantly over time (Fig. 3.3). C/Mg also showed significant site differences, with Ebenhaezer having the greatest C/Mg of plant litter. Lastly, C/Ca showed no site or treatment differences (Fig. 3.3). Temporal differences were the most significant, with C/Ca decreasing between days 0 - 62, increasing between days 62 - 107, and then decreasing again. C/Ca was also greater in crop lines 2 and 3 compared to crop line 1. Springtail species richness and abundance had no significant effect on any carbon to nutrient ratios (see Appendix 3).

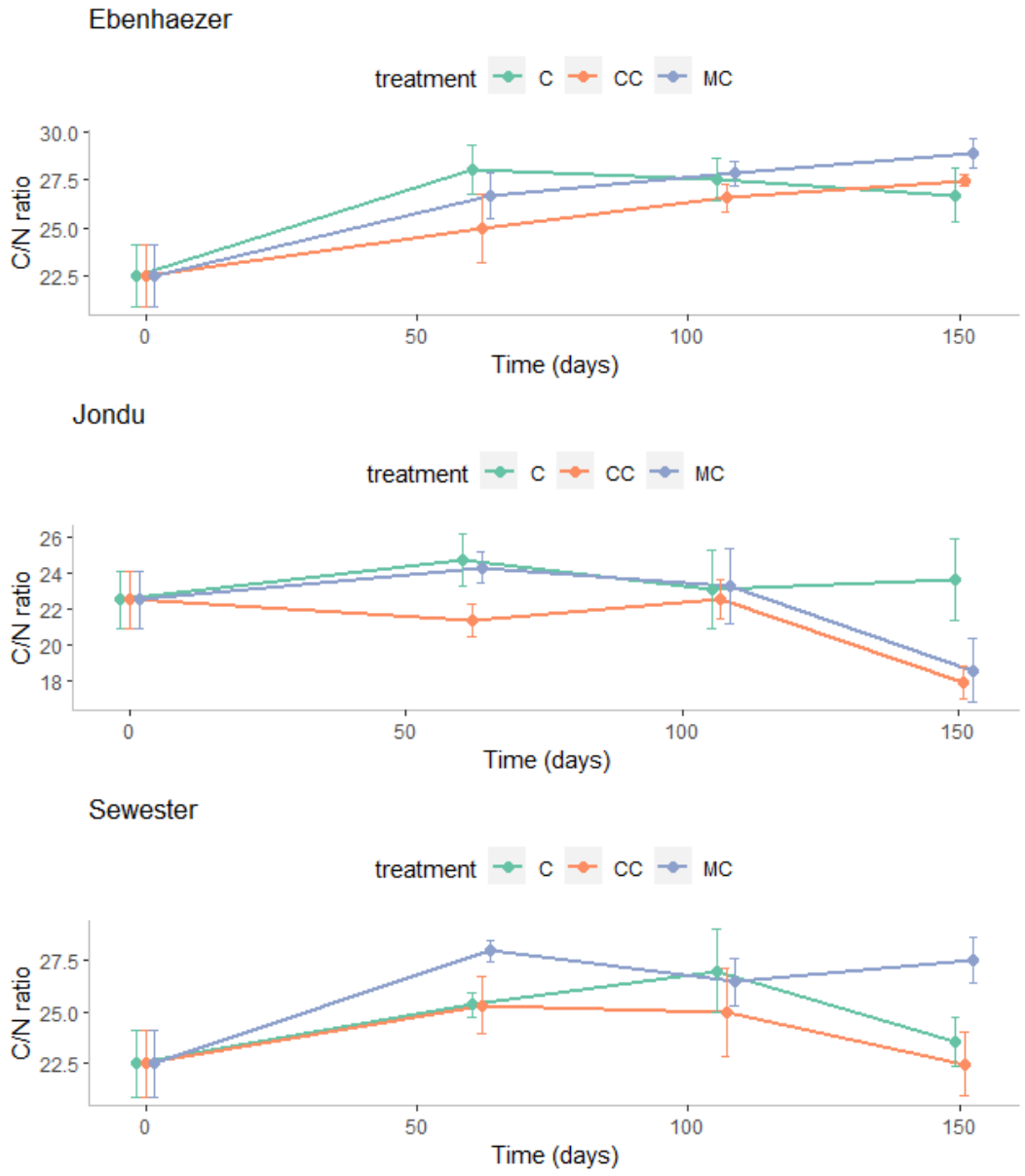

Fig. 3.2. Carbon to nitrogen ratios in relation to sampling time, across three field sites (Ebenhaezer, Jondu, and Sewester) and treatments (C, CC, MC). Nutrient ratios differed

significantly between sampling times, with some site and treatment effects as well (see Appendix 3).

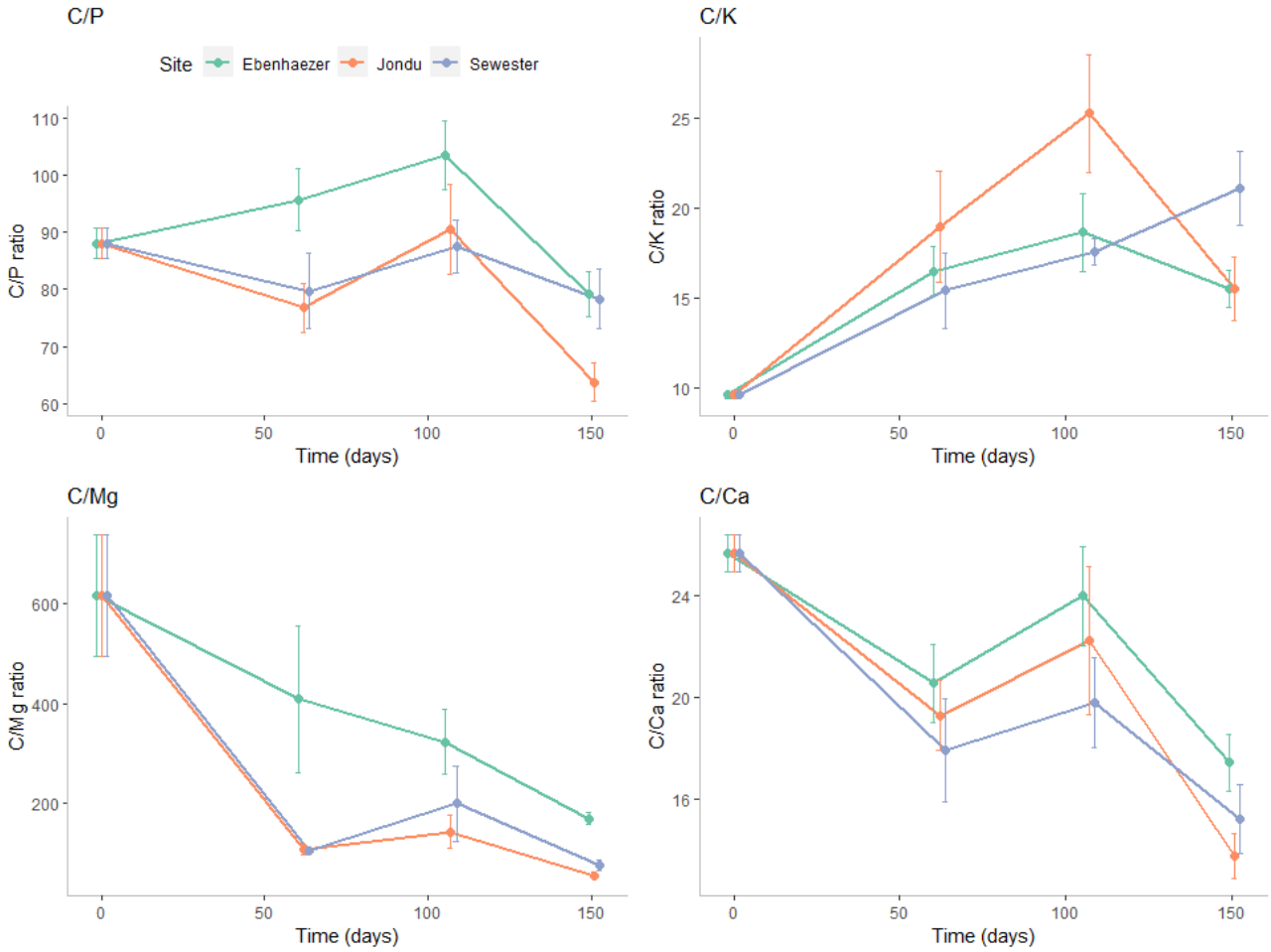

Fig. 3.3. Carbon to nutrient ratios in relation to sampling time, across three field sites (Ebenhaezer, Jondu, and Sewester). Nutrient ratios differed significantly between sampling times, with some showing site, treatment and/or line effects as well (see Appendix 3).

Springtail community analyses

The abundance and species richness of springtails sampled during the litter bag experiment were further investigated. A total of 10466 individuals of 15 springtail species were observed. Differences in total springtail abundance were observed between sites, treatments, and sampling times (Fig. 3.4). Interestingly, springtail abundance at Sewester, in the mulch and compost treatment, was significantly greater than abundances in any other treatment and at any other site. Some site and treatment differences were detected in the mean springtail species richness of samples. Mean springtail species richness at the Jondu site was typically 36% less

than that at Ebenhaezer, whilst species richness at Sewester was 235% greater than Ebenahezer, on average. Species richness for the cover cropping treatment was 21% less than the conventional agricultural treatment. Springtail species richness was, on average, greatest in the conventional treatment for all sampling times. Litter bags that were collected from the field after 107 days had the fewest number of springtail individuals and species (Fig. 3.2 B, C; Fig. 3.3 B, C).

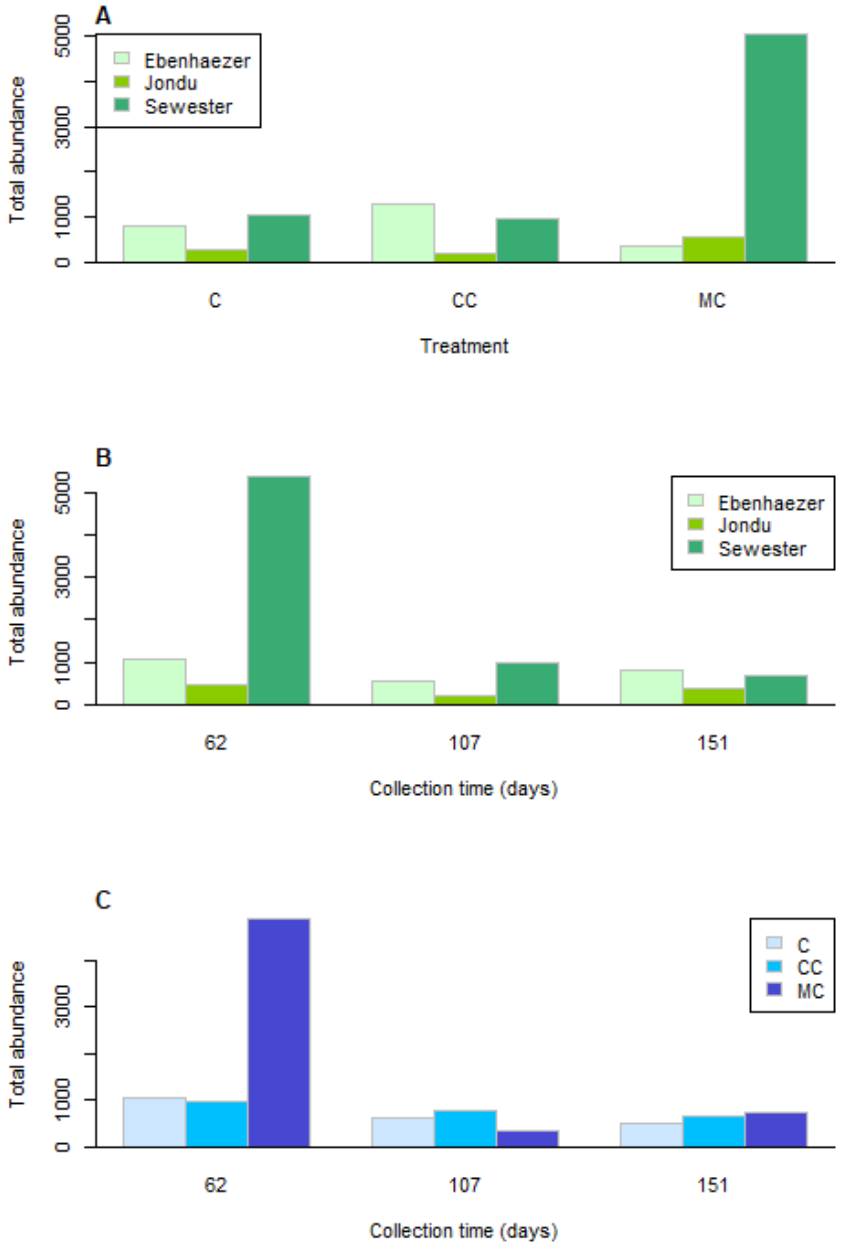

Fig. 3.4. Bar plot depicting changes in total springtail abundance in relation to site and treatment (A), site and sampling time (B) and treatment and sampling time (C). Treatment refers to conventional strategies (C), cover cropping (CC), and mulch combined with compost (MC).

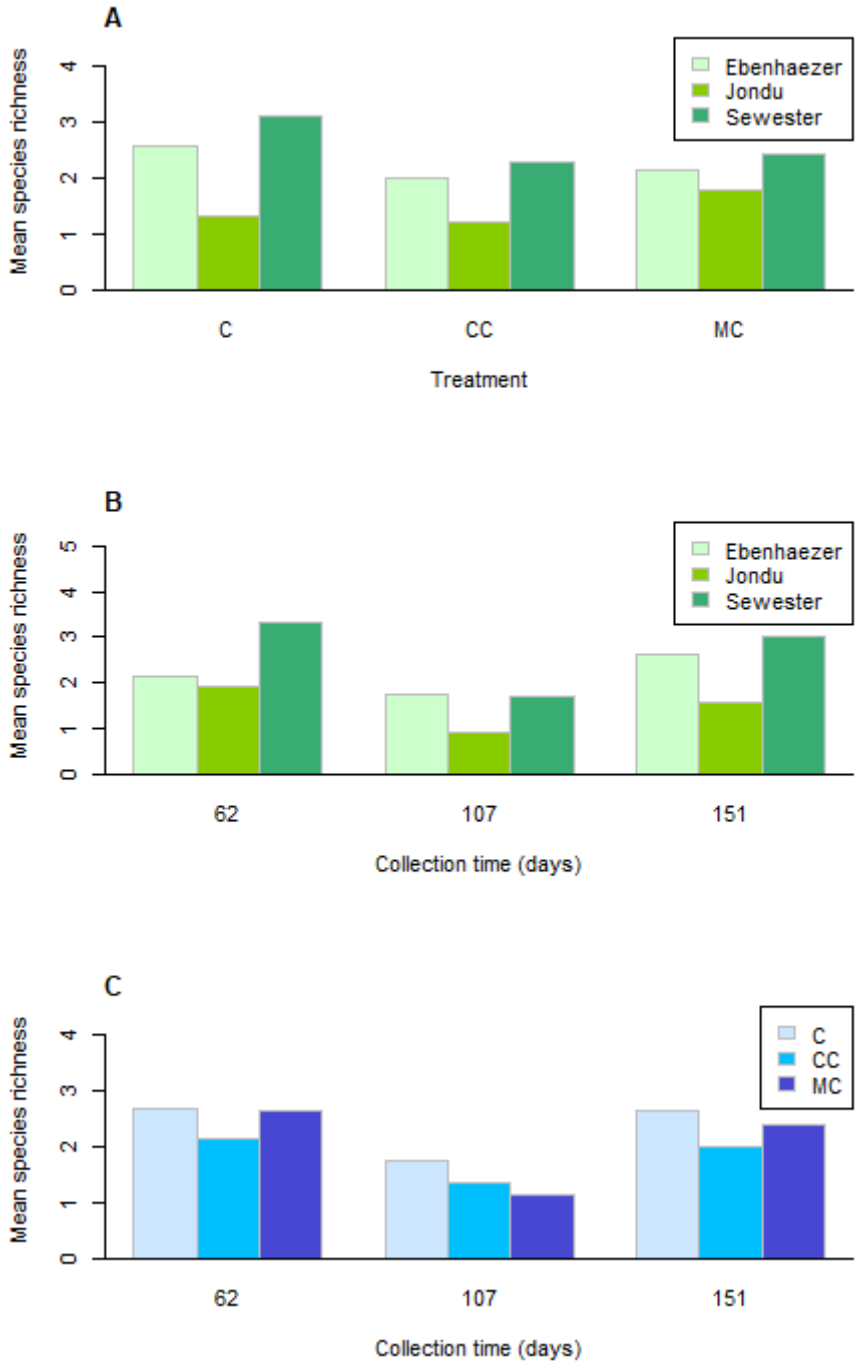

Fig. 3.5. Bar plot depicting changes in mean springtail species richness in relation to site and treatment (A), site and sampling time (B) and treatment and sampling time (C). Treatment refers to conventional strategies (C), cover cropping (CC), and mulch combined with compost (MC).

Analysis of similarities (ANOSIM) was used to detect possible dissimilarities in litter bag springtail communities across three parameters: field site, agricultural treatment, sampling time. The ANOSIM results (Table 3.3) indicated significant site effects ($p<0.001$), and insignificant treatment and sampling time effects ($p>0.05$). Site appeared to have had a relatively strong effect on springtail community similarity ($R=0.52$). Generally, samples from the same site were more closely distributed, within the NMDS, showing greater similarity of springtail communities within sites. However, overlap is evident.

Table 3.3. ANOSIM results of NMDS ordination for springtail communities from the litter bags across factors of site, treatment, and sampling time, with $p<0.001$ (***), $p>0.05$ (ns).

Factors	**R**	**p**
Site	0.52	***
Treatment	-0.045	ns
Sampling time	-0.027	ns

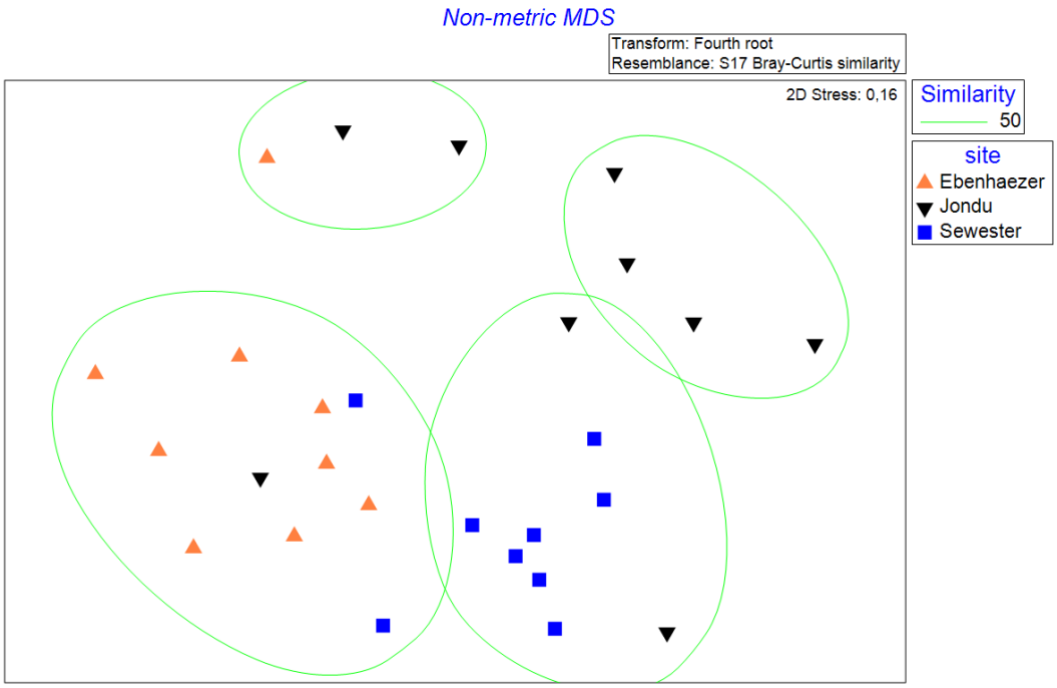

Fig. 3.6. Non-metric Multi-Dimensional Scaling (NMDS) plot of springtail communities clustered based on field sites (stress = 0.16).

Springtail indicator species analyses

The most abundant species of springtails were *Brachystomella* sp. 1, *Brachystomella* sp. 2, *Entomobrya* sp., and *Hypogastrura assimilis*. These species made up 9%, 10%, 17%, and 46% of all springtail abundances, respectively. Clear site, treatment, crop line and sampling time effects were observed across these four abundant species, however these effects appear species-specific (Fig. 3.7; Fig. 3.8). *Brachystomella* spp. were more highly abundant at the Ebenhaezer site than any other site (Table 3.4, Fig. 3.7). *Brachystomella* sp. 2 also showed high associations with crop line 2, however this did not coincide with any planting event. Entomobrya sp. did not show any strong associations with any of the study factors, and their abundances were relatively consistent across sites, treatments, crop lines and sampling times. *Hypogastrura assimilis* was the most factor effected species (Fig. 3.7). *Hypogastrura assimilis* showed high associations with the first litter bag sampling collection (i.e., after 62 days), the mulching combined with compost treatment, the Sewester site, and crop line 1 (i.e., cauliflower line) (Fig. 3.7; Fig. 3.8). The high abundance of *Hypogastrura assimilis* for these aforementioned factors, coincides with the total abundance spikes at the Sewester site, in the mulching combined with compost treatment, and in crop line 1 (Fig. 3.4). Further investigation into species-factor associations showed that only one species of springtail, *Proisotoma* sp., was associated with the conventional agriculture treatment. *Seira* sp. along with *Brachystomella* spp., as previously mentioned, were associated with Ebenahezer, whilst *Proisotoma* sp., *Parisotoma* sp., and *Hypogastrura assimilis* were associated with Sewester. No species associations were observed for Jondu.

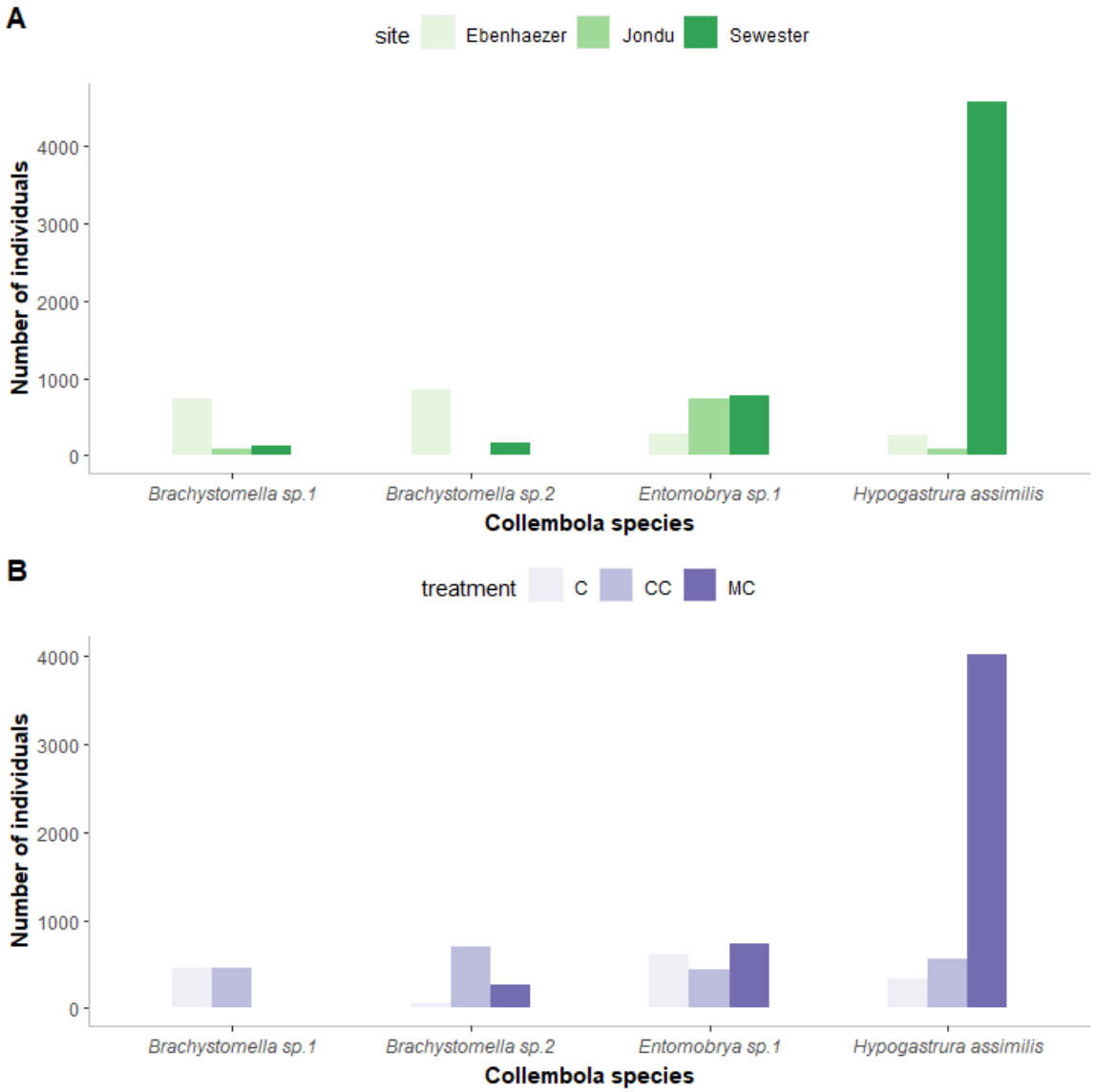

Fig. 3.7. Distribution of most abundant springtail species across experimental sites (A) and treatments(B).

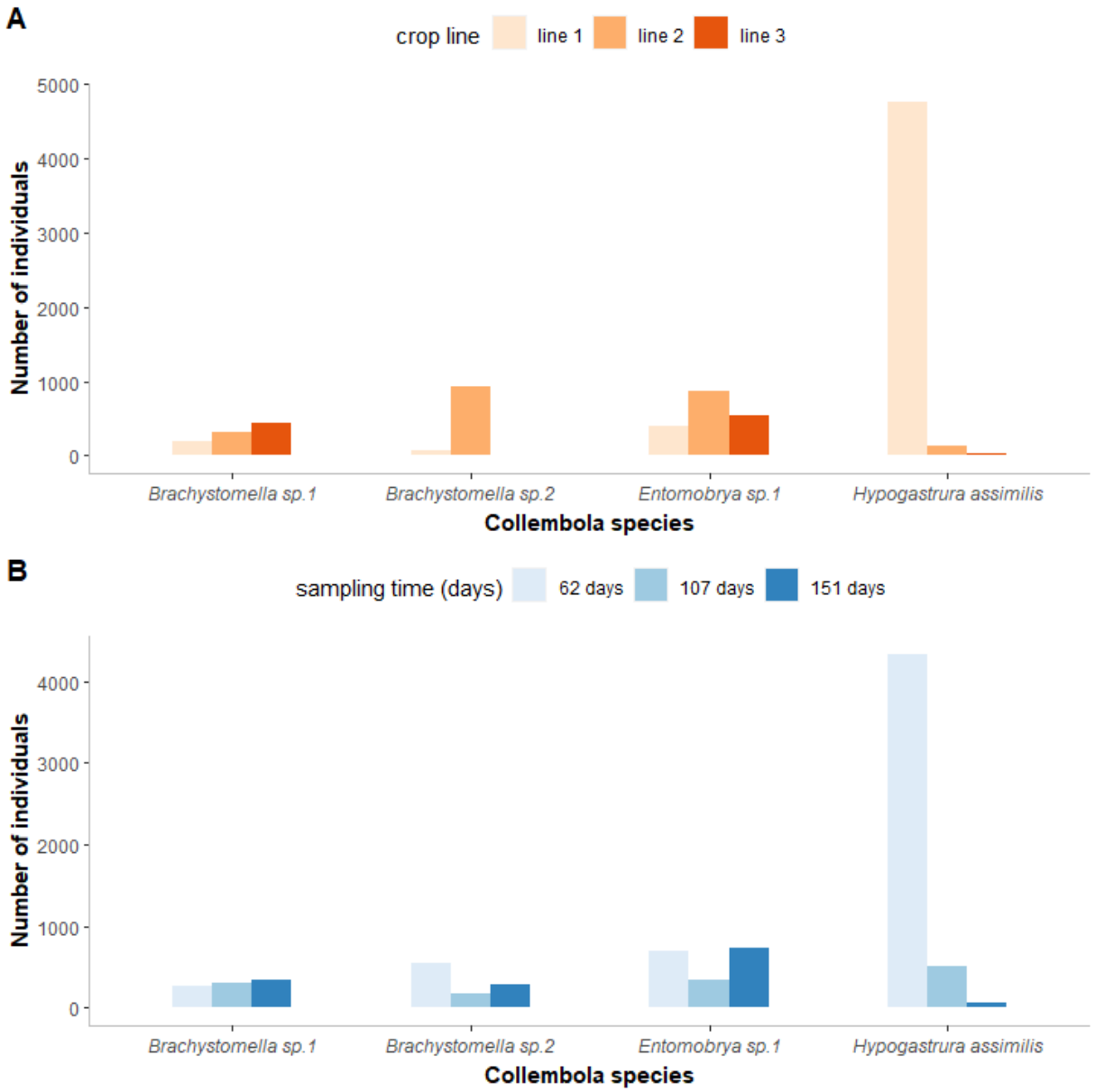

Fig. 3.8. Distribution of most abundant springtail species across crop lines (A) and sampling times(B).

Table 3.4. Springtail indicator species across sampling time, field sites, treatments, and crop lines with p<0.001 (***), p<0.01 (**), p<0.05 (*).

Factor	Species	Stat	p
Sampling time			
62 days	*Hypogastrura assimilis*	0.152	*
151 days	*Sphaeridia* sp.	0.148	*
Site			
Ebenhaezer	*Seira* sp.	0.362	***
Ebenhaezer	*Brachystomella* sp. 1	0.269	**
Ebenhaezer	*Brachystomella* sp. 2	0.203	*
Sewester	*Proisotoma* sp.	0.283	***
Sewester	*Parisotoma* sp.	0.177	***
Sewester	*Hypogastrura assimilis*	0.165	***
Treatment			
C	*Proisotoma* sp.	0.244	***
Crop line			
Line 1	*Hypogastrura assimilis*	0.173	***
Line 2	*Bracystomella* sp. 2	0.241	***

DISCUSSION

In this study the effect of agricultural management systems on litter decomposition by soil fauna was investigated in a seed production long-term trial. Litter decomposition under regenerative agricultural practices was almost always higher than under conventional management. Conventional management showed lowest litter decomposition rates and greatest half-life. This was in contrast to cover cropping and mulching strategies where decomposition rates were greatest and litter half-lives were lower. This follows the pattern of several studies suggesting that soil moisture retention is a key component driving decomposition rate as well as facilitating greater and more diverse soil fauna communities (Lensing, Todd & Wise, 2005; Ferguson & Joly, 2002). Conventional strategies likely had low soil moisture as the soil surface was exposed, with no organic matter to help create favourable microhabitats. However, treatment effects were also coupled with site effects on litter decomposition. Sites appeared to have a reasonable large effect on straw mulch decomposition.

The effects of agricultural treatments on litter decomposition also appeared to differ between sites, thus the underlying impact of site on litter decomposition played an important role. The significant differences between sites are to be expected within the first year of trial establishment, as pre-trial soil conditions between sites were not equal. Further investigation into soil parameters may help explain the exact driver of these site differences. However, continued assessment into litter decomposition processes between agricultural treatments will prove useful, especially once the trial is more established, and soil conditions have evened out somewhat.

During decomposition, carbon to nutrient ratios tended to decease over time. However, some site-specific responses were observed. C/N ratios decreased only for litter at Jondu, while litter at Ebenhaezer and Sewester increased in C/N over time. The decrease in carbon to nutrient ratios over time, is primarily a result of carbon-use efficiency and the degree of nutrient uptake by decomposers (Manzoni et al., 2008). Decreases in C/N ratio have also been shown to be a function of mesofauna contributions. Mesofauna have been shown to increase N in litterbags through the deposition of faecal material or by enabling fungal transfer of N (Jacobs et al., 2011; Lummer, Scheu & Butenschoen, 2012). However, no significant effect of springtail species richness and abundance was observed for temporal changes in all carbon to nutrient ratios. The loss of nitrogen and phosphorous from litter bags into the surrounding soil is primarily a result of N and P mineralization. N and P mineralization was more inclined to occur at Ebenhaezer and Sewester. Concentrations of Mg, Ca, and P typically increased over time. This is likely as a result of a net transport of nutrients from the surrounding soil into the litter bags (Bengtsson et al., 2011; Lummer, Scheu & Butenschoen, 2012). In contrast, concentrations of K decreased throughout the study period at all sites. This is often thought to be as a result of the high dissolvability of K (Bengtsson et al., 2011).

Springtail community structure appeared relatively strongly associated with field sites, with springtail communities showing some distinction between sites. However, no differences in springtail community composition were observed between treatments and litter bag sampling times. The site Sewester and the mulching treatment had the greatest abundance and species richness of springtails. This observation was largely driven by *H. assimilis* which showed significant factor-specific responses to Sewester and the mulching and compost treatment. Indeed, a closely related species, *H. manubrialis,* was found to dominate nutrient rich *Galenia africana* litter bags in a similar study (Leinaas et al., 2015). In addition, a negative correlation

between high abundances of this invasive species and indigenous species suggested some form of competitive exclusion of indigenous species (Leinaas et al., 2015). In the present study, high association of *H. assimilis* to very specific environments was found within the agroecosystem (Table 3.4). Thus, further investigation into the use of the species as an indicator as well as the interaction with indigenous species will be useful.

The results suggest variable responses in litter decomposition between sampling sites and agricultural treatments. There is considerable variation in the carbon use efficiency and nutrient uptake between sites and seasons. The differences in decomposition rates and C/N ratios between sites are likely a result of differences in the underlying vegetation, soil, and geology types across the three sites, since springtail community composition showed little effect. However, evaluating the community structure of several mesofauna taxa may provide more insight into the role of mesofauna in litter decomposition. Further assessment of the impacts of environmental conditions on soil fauna communities and decomposition rate will prove useful in obtaining a more complete view of the mechanisms driving these responses.

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CHAPTER 4.
CONCLUSION

This study set out to determine the impacts of conventional and regenerative agricultural practices on soil health, within a cauliflower seed production system. Soil health in this study focussed on soil fauna and soil processes such as litter decomposition and nutrient cycling. To determine whether regenerative agricultural strategies impacted soil health, I chose to evaluate the impact of agricultural management strategies on soil arthropod community composition, abundance, and species richness. I also evaluated the effect of agricultural management strategies on litter decomposition rate, mass loss of litter, carbon to nutrient ratios of litter and soil mesofauna communities. Although there are several studies documenting the impacts of management strategies in agroecosystems, particularly between "till" and "no till" treatments, very few studies on impacts of regenerative agriculture on soil fauna in seed production systems have been done, especially in South Africa.

In this study I investigated the impacts of regenerative agricultural practices on soil arthropod communities, with measures such as species richness and abundance. Multivariate analyses were used to assess the differences noticed in these indices across treatments, sampling sites, crop rotation lines and sampling days. Furthermore, I evaluated the decomposition rate of straw litter, as well as the corresponding carbon to nutrient ratios, under the various agricultural treatments, sampling sites, sampling times and crop rotation lines by means of General Linear Mixed Models (GLMM).

The main aims of this study were:

i. To determine whether agricultural treatments impact arthropod species richness and abundance of ant, beetle, spider, and springtail communities, and evaluate the extent of the impact.
ii. To determine whether soil arthropod communities within a specific site, under a specific treatment, or within a specific season were more similarly structured.
iii. To determine whether any arthropod taxa could be used as agroecosystem bioindicators, based on specific factors.

iv. To determine whether the mass loss and decomposition rate of straw litter showed differences between agricultural treatments, sampling sites, and/or sampling days.

Main findings

Arthropod communities showed a great degree of diversity and abundance across the four study taxa. Within these four taxa, 10194 individuals were sampled, representing 41 families and 151 species. The degree to which arthropod communities differed in their abundance and species richness was assessed across field site, treatments, crop rotation lines and sampling seasons. Responses in abundance and species richness were evidently taxa specific. Ant communities displayed differences in abundance and species richness across field sites, agricultural treatments, and sampling times, with mean ant species richness being greatest at Sewester and under mulching treatments and lowest at Ebenhaezer and in conventional treatments. Ant abundance decreased over the study period, with the last sampling period reflecting a return to pre-trial abundances. Beetles showed a similar temporal pattern to ants, also decreasing over time but returning to pre-trial abundances in February 2022. Beetle species richness was also significantly greater at Sewester, although agricultural treatments had no effect on beetle abundance and species richness. Notably, substantially higher beetle species richness was recorded in crop line two during the November 2021 sampling period, which coincided with the planting of tomato crops. Spiders and springtails similarly showed no differences in species richness between treatments or crop lines, although site and sampling season differences were observed. Springtail total abundance was the greatest of the four taxa, whilst spider species richness was the greatest. ANOSIM (Analysis of Similarities) results provided no evidence for any community clustering with sites or sampling seasons. Furthermore, the indicator species analyses provided valuable information pertaining to potentially influential arthropod species. *Pardosa crassipalpis* (Araneae) was recorded in high relative abundances within the agroecosystem and displayed specific factor associations. *Pardosa crassipalpis* was highly associated with the Jondu field site, with the mulching combined with compost treatment, as well as with the February 2022 sampling period. The presence of native and desiccation tolerant springtail species was another remarkable record. *Seira* sp. showed significant associations with the Sewester and Ebenhaezer sites, as well as the February sampling periods. Since February is one of the hottest and driest months of the year in this region, high associations with desiccation tolerant species are expected. Furthermore, *Entomobrya* sp. were highly associated with the July sampling period. Springtail species richness and abundance during

July were also high, suggesting increased springtail activity due to increased rainfall and soil moisture.

The mass loss and decomposition rate of straw mulch show temporal differences as expected, with longer time in field resulting in greater mass loss. Decomposition rate over time was rapid within the first 50 days of sampling and then became more gradual. Site location and agricultural treatments also impacted the degree of mass loss and the decomposition rate of straw mulch, with site-specific responses observed. Straw mulch placed under a conventional agricultural management resulted in far less mass loss over time and lower decomposition rates compared with the regenerative agriculture treatments. This suggests that cover cropping and mulch as well as compost inputs aided in facilitating greater and faster decomposition of litter. This could be caused by possible differences in physical and chemical soil conditions in regenerative treatment plots as opposed to conventional treatment plots. Springtail species richness and abundance had no significant effects on straw mulch decomposition rate or the changes in carbon to nutrient ratios over time. Further evaluation of the community compositions of several mesofauna taxa may provide more insight into potential interactions between mesofauna and litter decomposition. The temporal dynamics of proportion of carbon to nutrients within the litter help provide useful information into what processes may be driving such changes. The decreasing C/N ratio of litter observed in Jondu alone could be a result of increased carbon use efficiency, nitrogen uptake, and the influx of nitrogen by soil fauna. Conversely, increased C/N and C/P over time for litter at Sewester and Ebenehaezer is likely a result of N and P mineralization and leaching or plant uptake. Differences in carbon and nutrient dynamics with litter could suggest important site-specific soil arthropod responses. Treatment and crop rotation effects on decomposition rate and carbon to nutrient ratios were limited.

Springtail communities, sampled from litter bags, showed strong site-specific associations, potentially indicating differences in the physical and chemical conditions between the field sites owing to differences in the underlying vegetation types for each site. The abundances of *Hypogastrura assimilis* were especially sensitive to all study factors. Thus, this species may be indicative of specific environmental conditions, and worth considering for further study within this trial.

Since this study has taken place within the first year since the trial was established, treatment and crop rotation effects are expected to be less prominent than what might be seen in a few

years. It is suggested that owing to large site differences, due to differences in soil physical and chemical conditions, responses in decomposition and soil faunal communities are more impacted by site location. However, with continued temporal monitoring, treatment and crop rotation effects may take hold in a more established field trial.

Limitations and recommendations

Site selection, design of experimental field trial and the management thereof, proved to create certain caveats. Due to the design of the trial, each treatment, crop, and site combination had only two replicates. Since each factor had a likely effect on arthropod diversity measures and decomposition rate, having only two replicates for this three-way combination proves too little to substantially deduce and infer any significant relationship. Limited temperature and moisture data was available across sites for the study due to insufficient temperature and moisture probes. It would prove useful for future studies within this trial, to obtain temporal temperature and moisture data across sites and treatments. The management of the trial created a few setbacks in the litter bag experiment. As each farm is privately owned and treatments and cropping were carried out by each individual farm, some litter traps were destroyed on site due to accidental management errors. However, such logistical errors are expected when conducting research within such a collaborative environment, and accidental errors were kept to a minimum as far as possible.

Including a natural ecosystem alongside the study investigating the effects of agroecosystem management on soil fauna communities, would have provided greater insight into differences in soil fauna communities between a baseline (i.e., the natural site) and the experimental treatments. This would have also provided insight into possible rare arthropod observations, and species occurrences or absences that tend away from expected in the natural system. Future studies using this trial will include sampling the natural systems around the farms.

Arthropod abundance and richness were sampled by means of pitfall traps. Pitfall traps are the most common approach for sampling ground-dwelling arthropods, especially in environments with sandy soil such as present in this study system. However, several studies have shown the limitations of such trapping methods in determining a full and all-round understanding of the arthropod community of the area. Pitfall trapping is a preferred method due to the ease of handling and the high-density trapping capability (Perner & Schueler, 2004; Schmidt et al., 2005; Shrestha & Parajulee, 2010). However, pitfall trapping efficiency is known to be

sensitive to species' population density, often only accounting for highly abundant species, with rare species unobserved. Pitfall trapping is also sensitive to species locomotive capabilities, with more mobile species, more likely to be sampled (Perner & Schueler, 2004; Niemelä & Kotze, 2009; Schirmel et al., 2010; Zhao et el., 2013). Thus, pitfall trapping may provide unreliable estimations of species abundance and richness. However, if the number of pitfall traps per experimental plot were increased, this may have allowed for a more reliable estimation of arthropod community dynamics.

Within the decomposition experiment, litter bags were kept in the field for 151 days. This was four times less than the shortest half-life of the litter. Leaving the litter bags in the field for an extended period of time would have proved useful in assessing the full extent of temporal impacts and the decomposition dynamics of straw mulch. Also studying litter decomposition of straw mulch in varying seasons would provide a further insightful layer to the study. Furthermore, measuring the community structure of all taxa collected in the litter bags, not only springtails, should be done in the future.

As this study was conducted within the first year of the experimental trial, such results and caveats are expected. This study provides extremely useful baseline information in an understudied system in South Africa. Further assessment of these dynamics over time as the trial progresses will provide valuable information regarding the positive impacts of regenerative agriculture on soil health, particularly soil fauna diversity and density, within seed production systems.

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SUPPLEMENTARY INFORMATION

APPENDIX 1

Fig. A1a. Site changes at Ebenhaezer experimental field from pre-trial in February 2021 to one year later in February 2022.

Fig. A1b. Site changes at Jondu experimental field from pre-trial in February 2021 to one year later in February 2022.

Fig. A1c. Site changes at Sewester experimental field from pre-trial in February 2021 to one year later in February 2022.

Fig. A1d. Tomato cash crop at Ebenhaezer and Jondu in February 2022.

Table A1a. Soil parameters (mg nutrient/kg soil) across field sites, evaluated between April 2021 and February 2022.

Sampling time	Site	Active C	Available N	P	K	Mg	Ca	pH
April 2021	Ebenhaezer	74.84	31.52	103.17	253.39	2.02	3.13	7.38
April 2021	Jondu	38.88	76.69	199.83	547.89	6.49	12.15	7.52
April 2021	Sewester	54.28	27.14	152.06	273.11	8.86	19.04	8.04
Sep 2021	Ebenhaezer	23.27	36.09	101.22	304.89	1.90	3.38	7.07
Sep 2021	Jondu	45.33	42.86	185.89	588.17	6.33	12.99	6.86
Sep 2021	Sewester	20.37	17.63	146.89	304.28	9.22	19.35	7.17
Oct 2021	Ebenhaezer	-	-	107.5	276.56	1.77	2.76	7.28
Oct 2021	Jondu	-	-	199.72	694.22	6.47	11.35	7.43
Oct 2021	Sewester	-	-	154.67	332.72	9.16	17.70	7.82
Feb 2022	Ebenhaezer	15.73	26.45	95.33	304.33	2.17	2.77	7.38
Feb 2022	Jondu	40.48	69.47	165.28	532.61	6.53	11.91	7.52
Feb 2022	Sewester	15.88	67.75	129	286.2	8.30	19.57	8.03

Table A1b. Fertilizer records for November 2021 – February 2022 (15 weeks), implemented through drip irrigation. All sites received the same programme. **Dosage** and (date implemented) are provided.

Treatment	**Trichoderma**	**Amino K**	**Seabrix**	**Nourish liquid Fert (4:1:6)**	**$CaNO_3$**	**KNO_3**	**K_2SO_4**
Conventional (C)	**60 m*l*** (13 & 20 Dec)	**1.5 *l*** (13 & 20 Dec)	**510 m*l*** (13 & 20 Dec)	-	**2kg** (Weekly, Nov-13 Dec) **25kg** (Every second week, 13 Dec – 7 Feb)	**25 kg** (13 Dec, 10 Jan; 31 Jan)	**25 kg** (7 Feb)
Mulch & compost (MC)	**60 m*l*** (13 & 20 Dec)	**1.5 *l*** (13 & 20 Dec)	**510 m*l*** (13 & 20 Dec)	**2.7 *l*** (1, 15, 29 Nov) **8.5 *l*** (Every second week, 13 Dec – 7 Feb)	-	-	-
Cover cropping (CC)	**60 m*l*** (13 & 20 Dec)	**1.5 *l*** (13 & 20 Dec)	**510 m*l*** (13 & 20 Dec)	**2.7 *l*** (1, 15, 29 Nov) **8.5 *l*** (Every second week, Dec – Feb)	-	-	-

APPENDIX 2

Fig. A2a. Pitfall traps placed in a cauliflower seed production agroecosystem consisting of plastic containers buried in the ground, and a rain cover to prevent the trap being flooded during rainfall.

Fig. A2b. Laboratory extraction of pitfall trap contents and transfer from propylene glycol to 96% ethanol, ready for species identification.

Table A2a. Responses of ant species richness in relation to field site, treatment, and sampling time modelled with Poisson regression GLM, with $p<0.001$ (***), $p<0.01$ (**), $p>0.05$ (ns).

Factor	Estimate	Robust SE	p	LL	UL
Intercept	-0.53	0.20	***	-0.93	-0.13
Jondu	0.84	0.19	***	0.47	1.21
Sewester	1.15	0.19	***	0.78	1.51
CC	0.19	0.17	ns	-0.15	0.52
MC	0.42	0.17	*	0.08	0.76
April 2021	-1.45	0.22	***	-1.87	-1.02

July 2021	-1.96	0.27	***	-2.49	-1.43
Oct 2021	-2.50	0.41	***	-3.30	-1.69
Nov 2021	-0.86	0.20	***	-1.25	-0.47
Feb 2022	-0.35	0.17	*	0.69	-0.01

Table A2b. Responses of beetle species richness in relation to field site, treatment, and sampling time modelled with Poisson regression GLM, with p<0.001 (***), p<0.01 (**), p<0.05 (*), p>0.05 (ns).

Factor	**Estimate**	**Robust SE**	**p**	**LL**	**UL**
Intercept	-0.29	0.33	ns	-0.94	0.36
Jondu	0.80	0.36	*	0.10	1.51
Sewester	1.33	0.34	***	0.65	2.00
Treatment CC	0.03	0.12	ns	-0.21	0.26
Treatment MC	-0.14	0.12	ns	-0.38	0.11
Apr 2021	-0.17	0.43	ns	-1.00	0.67
Jul 2021	0.07	0.44	ns	-0.78	0.93
Oct 2021	1.26	0.38	**	0.52	2.00
Nov 2021	0.80	0.37	*	0.07	1.53
Feb 2022	-0.08	0.47	ns	-1.00	0.84
Jondu x Apr 2021	-0.06	0.47	ns	-0.98	0.86
Sewester x Apr 2021	-1.33	0.51	**	-2.32	-0.34
Jondu x Jul 2021	-0.73	0.51	ns	-1.74	0.27
Sewester x Jul 2021	-1.40	0.52	**	-2.43	-0.37
Jondu x Oct 2021	-1.59	0.48	**	-2.52	-0.65
Sewester x Oct 2021	-1.60	0.45	***	-2.49	-0.71
Jondu x Nov 2021	-0.74	0.45	ns	-1.61	0.14
Sewester x Nov 2021	-1.26	0.43	**	-2.11	-0.41
Jondu x Feb 2022	-0.20	0.53	ns	-1.24	0.85
Sewester x Feb 2022	0.04	0.51	ns	-0.96	1.04

Table A2c. Responses of spider species richness in relation to field site, treatment, and sampling time modelled with Poisson regression GLM, with $p<0.001$ (***), $p<0.01$ (**), $p>0.05$ (ns).

Factor	Estimate	Robust SE	p	LL	UL
Intercept	0.08	0.25	ns	-0.41	0.58
Jondu	-1.95	0.57	***	-3.07	-0.82
Sewester	0.13	0.26	ns	-0.39	0.65
Treatment CC	0.03	0.15	ns	-0.26	0.33
Treatment MC	0.17	0.14	ns	-0.11	0.45
Apr 2021	-18.45	0.33	***	-19.09	-17.81
Jul 2021	-1.66	0.61	**	-2.86	-0.46
Oct 2021	0.17	0.32	ns	-0.45	0.80
Nov 2021	0.29	0.33	ns	-0.36	0.93
Feb 2022	0.21	0.29	ns	-0.35	0.78
Jondu x Apr 2021	1.95	0.66	**	0.64	3.25
Sewester x Apr 2021	-0.13	0.43	ns	-0.97	0.70
Jondu x Jul 2021	3.50	0.82	***	1.89	5.12
Sewester x Jul 2021	0.97	0.67	ns	-0.35	2.28
Jondu x Oct 2021	1.12	0.67	ns	-0.18	2.43
Sewester x Oct 2021	-0.41	0.41	ns	-1.22	0.40
Jondu x Nov 2021	1.70	0.65	**	0.44	2.97
Sewester x Nov 2021	-0.33	0.41	ns	-1.13	0.47
Jondu x Feb 2022	1.73	0.66	**	0.44	3.03
Sewester x Feb 2022	-0.45	0.41	ns	-1.26	0.36

Table A2d. Responses of springtail species richness in relation to field site, treatment, and sampling time modelled with Poisson regression GLM, with $p<0.001$ (***), $p<0.05$ (*), $p>0.05$ (ns).

Factor	Estimate	Robust SE	p	LL	UL
Intercept	-0.49	0.19	**	-0.86	-0.08
Jondu	-1.01	0.48	*	-1.95	-0.07
Sewester	1.21	0.19	***	0.84	1.59
Apr 2021	-0.79	0.58	ns	-1.93	0.35
Jul 2021	0.24	0.25	ns	-0.24	0.73
Oct 2021	1.47	0.20	***	1.08	1.89
Nov 2021	1.27	0.23	***	0.82	1.71
Feb 2022	1.29	0.21	***	0.87	1.71
Jondu x Apr 2021	1.97	0.75	***	0.49	3.44
Sewester x Apr 2021	-2.13	0.89	***	-3.87	-0.39
Jondu x Jul 2021	1.51	0.52	***	0.49	2.53
Sewester x Jul 2021	-1.55	0.45	***	-2.44	-0.66
Jondu x Oct 2021	-0.29	0.52	ns	1.31	0.72
Sewester x Oct 2021	-1.56	0.25	***	-2.05	-1.07
Jondu x Nov 2021	-1.04	0.62	ns	-2.27	0.18
Sewester x Nov 2021	-3.49	0.50	***	-4.46	-2.52
Jondu x Feb 2022	-17.09	0.55	***	-18.16	-16.02
Sewester x Feb 2022	-3.52	0.61	***	-4.70	-2.33

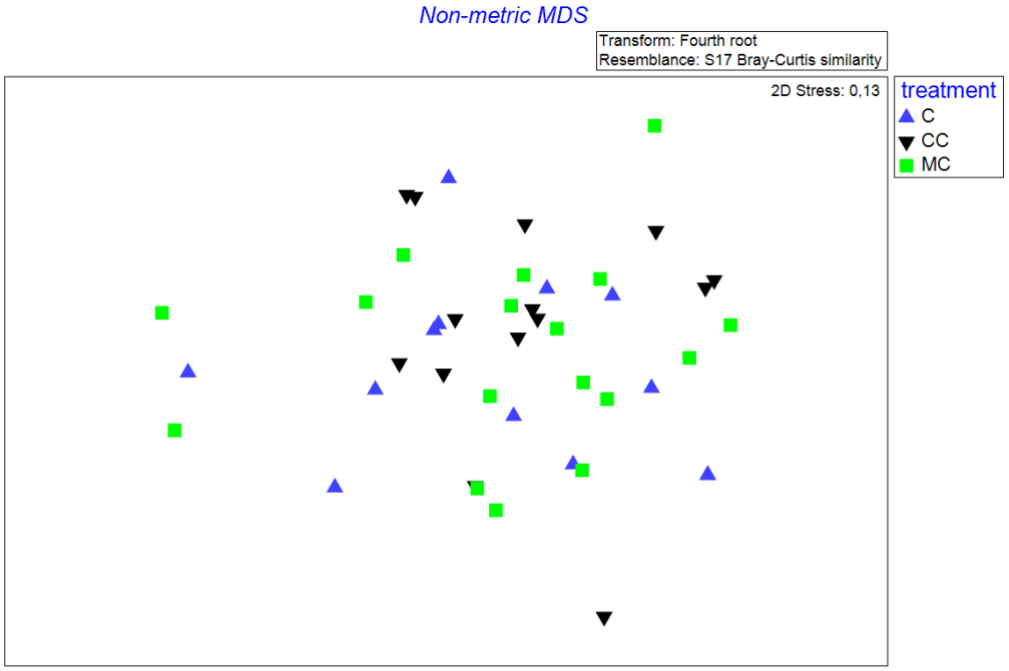

Fig. A2c. Nonmetric multi-dimensional scaling of ant communities across agricultural treatments (stress = 0.13).

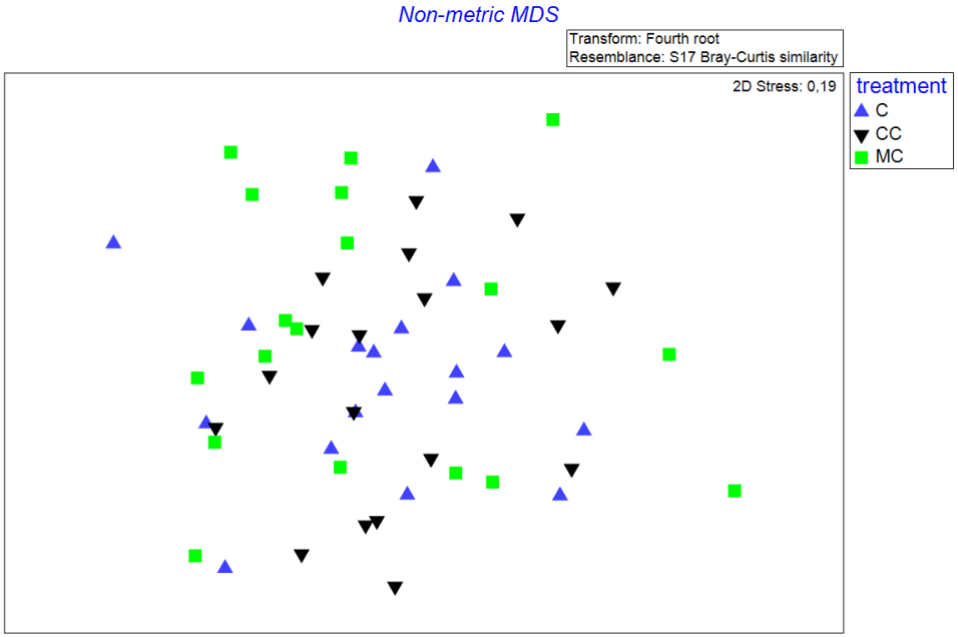

Fig. A2d. Nonmetric multi-dimensional scaling of beetle communities across agricultural treatments (stress = 0.19).

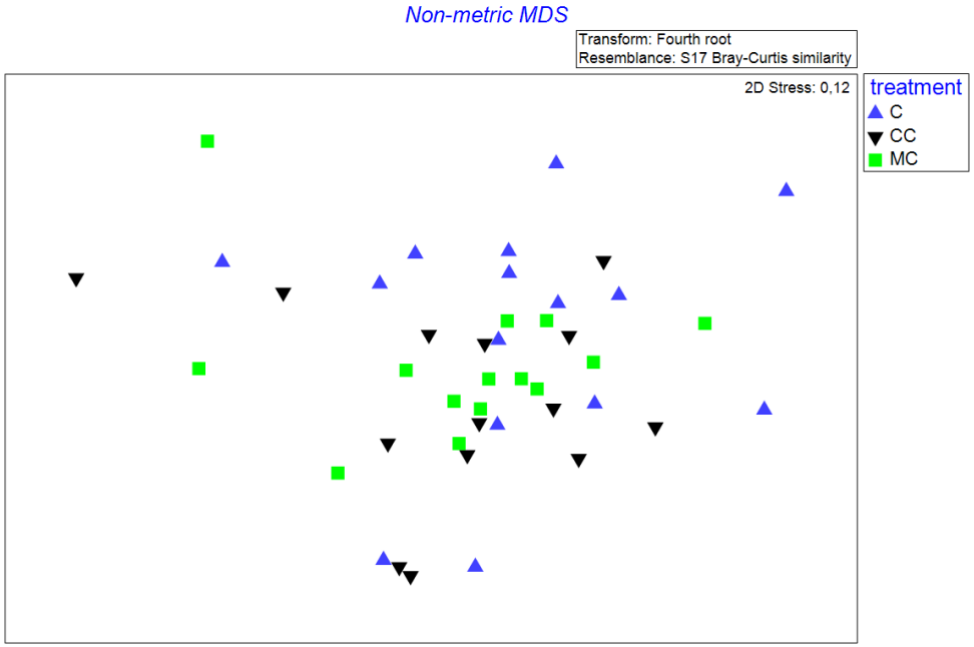

Fig. A2e. Nonmetric multi-dimensional scaling of spider communities across agricultural treatments (stress = 0.12).

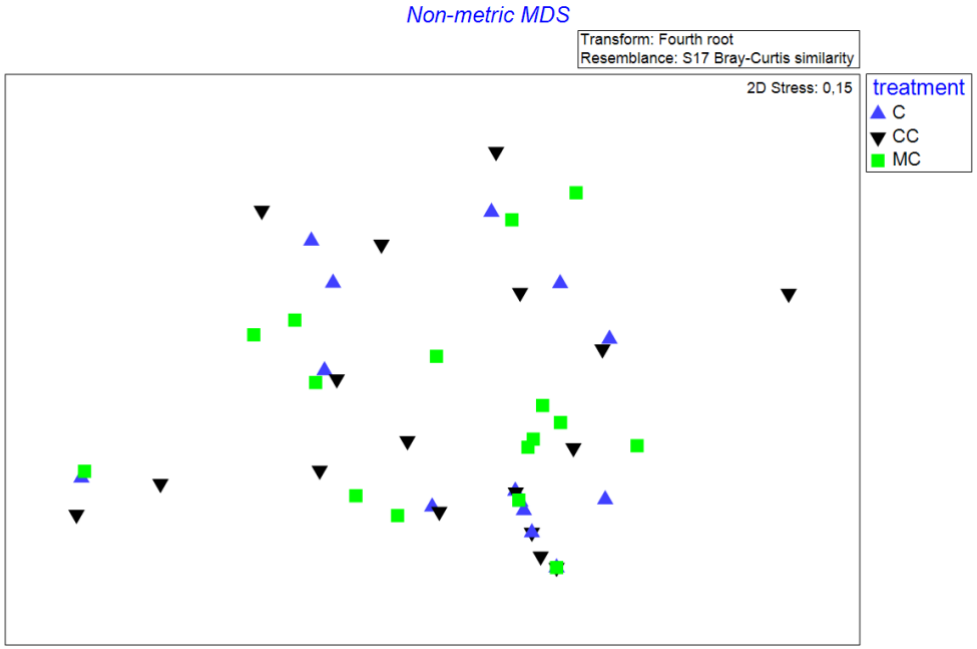

Fig. A2f. Nonmetric multi-dimensional scaling of springtail communities across agricultural treatments (stress = 0.15).

APPENDIX 3

Fig. A3a. Litter bags installed in a cauliflower seed production agroecosystem consisting of a set of four traps.

Fig. A3b. Collection of litter bags from study site. Traps were sprayed with water and wrapped in aluminium foil before being transported to the laboratory for mesofauna extraction.

Fig. A3c. Extraction of mesofauna from litter bags using Tullgren funnels.

Below are the GLMM outputs of carbon to nutrient ratios in response to agricultural treatment, crop line, field site, and sampling time.

Table A3a. C/N ratio, with p<0.001 (***), p<0.05 (*), p>0.05 (ns).

Factor	***df***	**Den*df***	***F***	**p**
Site	2	38.8	19.00	***

Sampling time	2	73.6	2.58	ns
Treatment	2	36.9	4.31	*
Crop line	2	37.3	3.05	ns
Springtail species richness	1	103.4	0.45	ns
Springtail abundance	1	106.7	0.25	ns
Site × Sampling time	4	72.3	2.33	ns

C/N in litter was greater under conventional management after 62 days, and lowest under cover cropping, but mulching/compost showed greatest C/N after 151 days.

Table A3b. C/P ratio, with p<0.01 (**), p>0.05 (ns).

Factor	***df***	**Den*df***	***F***	**p**
Site	2	41.9	7.97	**
Sampling time	2	77.3	7.21	**
Treatment	2	40.1	1.89	ns
Crop line	2	40.3	1.01	ns
Springtail species richness	1	104.7	0.96	ns
Springtail abundance	1	106.9	0.18	ns
Site × Sampling time	4	76.1	2.30	ns

C/P in litter decreased over time from pre-trial. Ebenhaezer showed the greatest C/P of the three sites.

Table A3c. C/K ratio, with p<0.05 (*), p>0.05 (ns).

Factor	***df***	**Den*df***	***F***	**p**
Site	2	36.5	0.38	ns
Sampling time	2	72.4	1.71	ns
Treatment	2	34.7	1.58	ns
Crop line	2	35.2	4.19	*
Springtail species richness	1	105.2	0.02	ns
Springtail abundance	1	106.3	2.85	ns

Sampling time × Crop line	4	71.4	0.29	ns

C/K in litter increases over time, with some interactions between sampling time, treatment, and site.

Table A3d. C/Mg ratio, , with p<0.001 (***), p>0.05 (ns).

Factor	***df***	**Den*df***	***F***	**p**
Site	2	43.7	16.97	***
Sampling time	2	80.2	8.47	***
Treatment	2	41.6	0.51	ns
Crop line	2	42.1	1.86	ns
Springtail species richness	1	105.9	0.58	ns
Springtail abundance	1	110.1	0.00	ns

C/Mg in litter decreased over time and was significiantly less than pre-trial. C/Mg was greatest at Ebenhaezer and indifferent between Jondu and Sewester.

Table A3e. C/Ca ratio, with p<0.01 (**), p<0.05 (*), p>0.05 (ns).

Factor	***df***	**Den*df***	***F***	**p**
Site	2	39.0	2.32	ns
Sampling time	2	78.3	7.40	**
Treatment	2	37.6	1.45	ns

Crop line	2	38.1	3.69	*
Springtail species richness	1	107.0	0.45	ns
Springtail abundance	1	107.0	0.03	ns
Crop line × sampling time	4	77.1	0.27	ns

C/Ca decreases over time from pre-trial but is greater in lines 2 and 3 compared to line 1.

Table A3f. Responses of springtail species richness in relation to field site, sampling time, and treatment modelled with Poisson regression GLM, with p<0.001 (***), p<0.01 (**), p<0.05 (*), p>0.05 (ns).

Factor	Estimate	Robust SE	p	UL	LL
Intercept	0.94	0.19	***	0.57	1.30
Jondu	-0.44	0.14	**	-0.71	-0.17
Sewester	0.16	0.12	ns	-0.08	0.40
Sampling time	-0.00	0.00	ns	-0.00	0.00
Treatment CC	-0.24	0.11	*	-0.48	-0.01
Treatment MC	-0.10	0.13	ns	-0.36	0.15

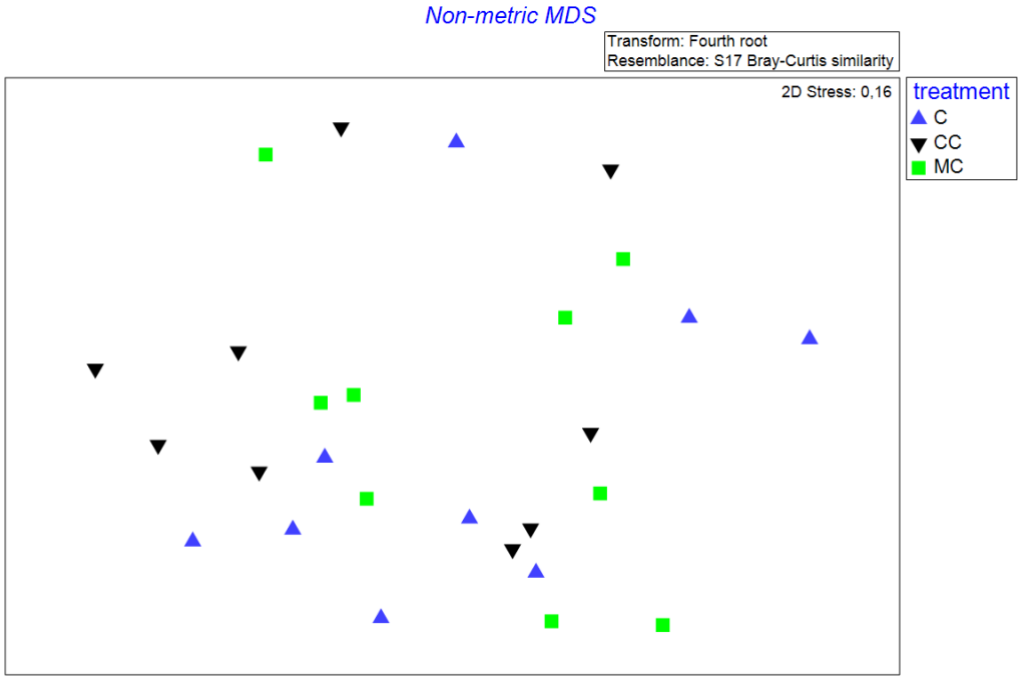

Fig. A2f. Nonmetric multi-dimensional scaling of springtail communities, sampled from litter bags, across agricultural treatments (stress = 0.16).