Belowground success: Collembola as indicators of restoration progress following active and passive restoration

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Abstract

Due to the unprecedented changes and fragmentation of ecosystems caused by human land uses and exploitation, ecological restoration is an increasingly essential topic in the field of conservation. In South Africa, a predominantly semi-arid country, invasive tree species negatively impact the limited water resources by reducing runoff and disrupting water-related ecosystem services. Private organizations, as well as the Western Cape government, have been clearing riparian areas of alien invasive trees along rivers in the Western Cape since 1995. Areas investigated in this study included three riparian sites along the Berg River in the Western Cape, habitats that are also vulnerable to environmental changes. The study focussed on monitoring restoration efforts by the Berg and Breede River Rehabilitation Programme, which involved clearing of alien invasive trees along the Berg River, followed by either active restoration (follow-up clearing and monitoring) or passive restoration (no treatment after alien plant removal). Despite soils being so vital in the field of restoration, most studies to date have investigated the aboveground impacts of restoration, with few studies on invertebrate taxa, especially belowground soil fauna. This study assessed Collembola (springtail) communities between active and passive restoration sites in comparison to invaded, non-restored sites as reference sites. Sampling was conducted during winter (2020) and winter and spring (2021) to consider the seasonal effects. From a total of 250 samples, 77,880 individual specimens and 34 morphospecies were collected and identified. Results showed that Collembola assemblages differed significantly among the different restoration treatments, especially between actively restored and invaded sites. Results from the study further suggested that active restoration may be the most effective method for bringing these communities closer to their natural state, since active restoration was found to be the most significantly different from invaded sites in terms of Collembola community composition (Kruskal-Wallis, $p<0.05$; ANOSIM, $p<0.05$ between active and invaded sites). The highest number of native Collembola was recorded at Bosplaas, with approximately 1,250 individuals per m^2 (p-value < 0.005) during spring 2020 and spring 2021 ($p<0.05$), also indicating clear seasonal differences for native Collembola species. This suggested more efforts are needed to include seasonal effects within a restoration context, for future studies on Collembola. Additionally, more native Collembola were found in 2021 than in 2020. These findings may also suggest a lag phase, with belowground fauna taking longer to recover post-restoration compared to aboveground plants. These results were also found to be similar to the responses of microorganisms in other restoration studies, this link between Collembola and microfauna also deserves further and more in-depth consideration within the field of restoration. Similarly, implications of restoration efforts on long-term soil chemical characteristics deserves careful consideration for implementing restoration practices. Additionally, variations in microhabitat conditions and litter cycling between native and invasive plants contributed to the differences in Collembola communities, further highlighting the importance of addressing invasive aboveground species and intricate ways how they affect the belowground environment.

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Chapter 1

Introduction

The value of soil and belowground ecosystems: A global and local context

Soils are amongst the most diverse ecosystems on Earth (Orgiazzi et al., 2016). Although often overlooked, soil is a vital part of any ecosystem and key to sustainable agriculture and food security (Rüdisser et al., 2015). Soils are formed and maintained by geological processes as well as by biotic organisms living in the soil, although we still have limited knowledge on the dynamics of these interactions (Wurst et al., 2012; Janion-Scheepers et al., 2016). However, soils are unable to function as part of a healthy and productive ecosystem without soil organisms and biodiversity (Wardle et al., 2004; Bardgett and van der Putten, 2014). Soils have a porous structure which increases surface area as well as extremely variable amounts of organic material, food, water and chemicals (Orgiazzi et al., 2016). This provides a wide range of habitats to a multitude of organisms.

Diverse biological communities inhabit the soil, comprising a wide range of life-forms and functions. These range from macro- to micro-levels depending on the physical and chemical characteristics of the given soil, as well as climate and vegetation (Orgiazzi et al., 2016). A single gram of soil can contain millions of individuals including several thousand species of bacteria, protozoa, tardigrades, rotifers, nematodes, mites, worms, insects, burrowing animals, plant roots, fungi, lichens and springtails (Orgiazzi et al., 2016; Kendzior et al., 2022). Species richness, composition and abundances of these organisms vary among ecosystems depending on many factors which often include temperature range, soil moisture content, acidity, availability of nutrients as well as the nature of organic substrates (Orgiazzi et al., 2016). In addition to these organisms, soil microbial structures, metabolites, mobile genetic elements such as viruses and phages, as well as relic DNA found in the soil habitat, all together make up the soil microbiome (Berg et al., 2020; Kendzior et al., 2022). In recent decades, pressures on soils, as well as soil dwelling organisms residing in these habitats have been on the rise. With a continuously rising global population, there has also been an increased demand for intensified agriculture as well as the accompanied use of fertilizers, pesticides and monocultures. These unsustainable agricultural practices, in addition to global climate change, increased soil erosion as well as the loss of aboveground diversity within ecosystems all have negative impacts on soil dwelling organisms (Orgiazzi et al., 2016).

Soils are remarkably complex and diverse, representing 59% of all life on Earth (Anthony et al., 2023). In recent years, more attention has been given to the importance of the soil microbiome and its biodiversity in ecosystem functioning (Orgiazzi et al., 2016; Kendzior et al., 2022). The soil microbiome plays a fundamental role in the relationship between healthy soils, climate, and humans (Kendzior et al., 2022). Within the soil microbiome, millions of soil dwelling organisms help promote vital ecosystem services such as the decomposition of dead organic matter as well as nutrient cycling which in turn influences our climate (Orgiazzi et al., 2016). Studies have shown that soil biodiversity is a critical component in the maintenance of multifunctionality of ecosystems, such as the ability of ecosystems to provide multiple ecosystem services within terrestrial ecosystems (Manning et al., 2018; Guerra et al., 2020).

Human impacts such as global climate change, soil erosion, alien species invasions, losses in local biodiversity and increasing rates of nitrogen deposition due to agricultural activities lead to both above and belowground biodiversity loss and also have negative effects on soil-dwelling organisms (Bardgett and Wardle, 2010). Habitat disturbances such as the establishment of invasive plants can have dramatic effects on vegetation community structure within an ecosystem (Richardson et al., 2007; Liu et al., 2012). These disturbances also have profound effects on other ecosystem characteristics and functioning, even to the extent that they can alter soil chemical characteristics, such as soil pH and nitrogen content (Richardson et al., 1992, 1996; Richardson and van Wilgen, 2004; Liu et al., 2012) and may therefore also have significant impacts on invertebrate assemblages inhabiting the soil and its surface (Liu et al., 2012). For example, a recent study by Chiappero et al. (2024) synthesized effects of land-use on soil fauna abundance and richness globally, by compiling data from 260 publications. The study found that abundances of most soil taxa as well as richness of Acari and Collembola were greatly reduced by land use changes, and these effects were also dependant on changes in soil properties as well as being partially dependant on changes in aboveground vegetation (Chiappero et al., 2024). Plant community structure and functioning has indeed also been found to cause significant effects on the distribution of soil-dwelling invertebrate assemblages (Wardle et al., 2004; Bardgett and Wardle, 2010).

Soils and belowground ecosystems still remain the least studied terrestrial habitats with little known or understood about their history and function in the natural environment (Decaëns et al., 2006, 2010). Although studies on soil biodiversity are increasing globally (Orgiazzi et al., 2016), limited knowledge exists for several soil taxa due to a lack of macroecological, biogeographical and taxonomic data for soil biodiversity and soil related functions (Guerra et al., 2020). At the same time, these ecosystems are also becoming the most degraded (Wardle et al., 2004). Several studies suggest that losses of biodiversity due to degradation in most given ecosystems reduces ecosystem functioning and impairs the stability of soil ecosystems over time (Cardinale et al., 2012; Bardgett and van der Putten, 2014). Most studies to date have focussed more on aboveground biodiversity and it's functioning rather than investigating belowground systems, even though these systems are crucial to the functioning of the environment (Eisenhauer et al., 2011).

Soil biodiversity has also been omitted in many global biodiversity assessments and policies (Cameron et al., 2018). This is most probably due to the lack of comprehensive information on soil biodiversity and its importance, especially on a global scale, as well as the difficulty in performing comprehensive assessments of soil biodiversity (Cameron et al., 2018). However, there is a growing awareness among scientists and policymakers on the importance of soil biodiversity for the supply of ecosystem services (FAO, 2020), although there is still limited knowledge on the taxonomy, systematics and biogeography of understudied soil taxa (Decaëns et al., 2010; Guerra et al., 2020). There has been an increase in large scale assessments (Cameron et al., 2018), such as the African Soil Microbiology Project (Ramirez et al., 2015). The development of additional databases on soil biodiversity is starting to take hold in order to address these knowledge gaps (Erdelen, 2020), also in part through the Global Soil Biodiversity Initiative (Cameron et al., 2018). However, increased efforts are still needed as well as additional global datasets on soil taxa such as mesofauna. This will allow more detailed analyses of local and global soil biodiversity (Cameron et al., 2018).

Importance of above- and belowground diversity

Scientists are becoming increasingly aware of the importance of both above- and belowground components of ecosystems as well as the ecological linkages and feedback mechanisms between the two, ultimately controlling ecosystem processes (Wardle et al., 2004; Bardgett and van der Putten, 2014; Wagg et al., 2014). Research regarding feedback mechanisms of soil biodiversity into the aboveground biosphere and its importance is rapidly growing (Wall et al., 2015). Global efforts to preserve and restore soils will not only contribute to saving entire ecosystems as well as helping reduce greenhouse gas emissions by sequestering more carbon (Parry et al., 2007; Singh et al., 2010; Wagg et al., 2014), but will also contribute to a decrease in soil-borne diseases and provide medicine for humans, plants and animals (Wall et al., 2015).

The soil microbiome has been shown to play a significant role in the climate system, because it is a primary driver of terrestrial greenhouse gas fluxes and soil carbon dynamics (Singh et al., 2010; Kendzior et al., 2022). Soils play a major role in storing and cycling carbon, while they also release greenhouse gases such as carbon dioxide (CO_2) and methane. Soils store more than 80% of the global carbon stock (Parry et al., 2007). Rising temperatures could accelerate rates of soil microbial respiration and in turn increase the release of CO_2 into the atmosphere, thus promoting positive feedback on climate change (Jenkinson et al., 1991; Bardgett and Wardle, 2010; Singh et al., 2010; Eisenhauer et al., 2012). Studies have also shown that temperature dependence of decomposition vary with organic matter quality and therefore the quality of plant material entering the soil (Luo et al., 2001; Bardgett and Wardle, 2010).

At a fine scale level (centimetres to meters), spatial patterns of soil biota can often be explained by variations in the physical and chemical properties of the soil, such as soil water, and carbon-nutrient availability, along with the identity of dominant plant species (Bardgett and van der Putten, 2014). Different plant species vary in the quantity and quality of resources and organic matter they return to the soil (Wardle, 2002; Bardgett and van der Putten, 2014), and this also determines how efficiently and quickly microbes and fauna can decompose their litter. The pattern of nutrient release from different plant species therefore also differs greatly. This in turn has a major influence on nutrient cycling and plant nutrition, formation of soil organic matter, quality of the soil organic matter, ecosystem carbon storage as well as which communities of soil organisms are found there (Wardle et al., 2004; Bardgett and Wardle, 2010). Individual plant species such as invasive plants may therefore have important effects on soil biota and the processes that they regulate (Wardle et al., 2004).

Despite historical recognition of the contribution of soil organisms to soil fertility, the key role that belowground organisms and their biotic interactions play in determining an array of very crucial ecosystem processes have only recently gained more attention (Bardgett and Wardle, 2010). In addition, a very small percentage of soil biota and soil microorganism species have been identified (Orgiazzi et al., 2016). Decisions surrounding the management of natural resources are often made without considering the local soil biota. This requires a detailed understanding of ecosystem functioning in the specific region and often requires knowledge of which species are involved, where they occur, how they interact, as well as their implications on ecosystem services (MEA, 2005; Cardinale et al., 2012). Poor decisions can do ecosystems more harm than good, reducing ecosystem functionality, reducing ecosystem services and subsequently leading to damaged, less resilient ecosystems (MEA, 2005; Cardinale et al., 2012).

Knowledge of soil biodiversity is a vital aspect when restoring degraded habitats in order to increase biodiversity, which is crucial for healthy ecosystem functioning (Palmer et al., 2008). However, there is little understanding of the functional consequences of belowground biodiversity loss (Bardgett and van der Putten, 2014). This research is especially important, as belowground soil communities are extremely complex and diverse (Bardgett and van der

Putten, 2014). At a local scale, belowground biodiversity is often found to be much richer than what is found in vegetation or aboveground fauna (Decaëns et al., 2010). Our knowledge of soil life is therefore growing, but still very limited compared to what is known about aboveground biodiversity (Bardgett and van der Putten, 2014).

Background and importance of riparian ecosystems

Riparian habitats or zones can be described as the fringes and banks along rivers and streams (Naiman and Decamps, 1997; Naiman et al., 1998; Tererai et al., 2013), and are often described as being restricted to these areas (Capon, 2020). However, in ecological terms, riparian ecosystems also encompass a wider array of habitats, including riverbanks, floodplains and wetlands, as well as dry rivers and lake beds, and phases of intermittent wetlands (Brock et al., 2006; Capon, 2020). Riparian ecosystems are characterized primarily as ecotones, or transition zones between terrestrial and aquatic habitats (excluding marine habitats). These aquatic habitats may also consist of either lotic (flowing) or lentic (standing) waters (Naiman et al., 1998; Capon, 2020).

Riparian habitats often have an abundance of unique flora and fauna, differing significantly from their nearby aquatic and terrestrial neighbours (Naiman and Decamps, 1997). Riparian ecosystems are also characterized by a high degree of habitat heterogeneity over local, as well as catchment scales (Stromberg et al., 2007; Capon, 2020). Due to spatial and temporal variation in hydrological regimes such as rainfall and flooding events (Capon, 2020), as well as distinct abiotic characteristics, riparian zones often support highly productive and species rich ecosystems (Capon and Pettit, 2018; Capon, 2020). Riparian ecosystems represent a high biodiversity of aquatic, amphibious and terrestrial species. The distribution of biota also reflects the variation in climate, geomorphology and geology (e.g., types of soil) (Capon, 2020). Riparian vegetation thus shows a spatially heterogenous structure with unique hydrophytic vegetation that varies in both structure and function from terrestrial vegetation close by (Capon, 2020; Van Zitters, 2021).

Vegetation and native floral diversity in these habitats support important ecosystem services, such as providing shade, acting as a buffer zone that filters sediments, controlling nutrients in the river, purifying the water and stabilizing stream banks (Barling and Moore, 1994; Richardson et al., 2007). This also helps with flood prevention, water retention, as well as providing unique habitats to local fauna (Richardson et al., 2007; Tererai et al., 2013; Van Zitters, 2021). Riparian habitats also provide a corridor for movement of biota, and serve as a channel or conduit for the movement of materials between differing adjacent clearly defined ecosystems (Naiman and Decamps, 1997; Macdougall and Turkington, 2005; Esler et al., 2008). These systems are dynamic, driven by natural disturbances such as frequent flooding of riverbanks (Naiman and Decamps, 1997; Esler et al., 2008). Natural disturbances maintain the ecological integrity of riparian habitats through acting as an essential restructuring agent

(Naiman, 2010; Van Zitters, 2021). However, these habitats are especially vulnerable to invasion by alien plants due to their dynamic hydrology and ease of invasive propagule transport (Holmes et al., 2005; Richardson et al., 2007; Esler et al., 2008; Van Zitters, 2021).

Intact riparian vegetation plays important roles in maintaining ecosystem health and services. Riparian habitats are also described as “critical transition zones” (Ewel et al., 2001), as they process significant fluxes of materials and organisms from ecosystems nearby, to which they are closely connected (Ewel et al., 2001; Holmes et al., 2005). These areas are often small components of large watersheds, and difficult to manage as managers may not have control over the entire river system (Ewel et al., 2001). However, the value of these ecosystem services provided to society, is disproportionately high relative to the area they occupy (Hunter et al., 2017; Capon, 2020; Van Zitters, 2021).

Habitat disturbances and effects of invasive plants on riparian ecosystems

Riparian habitats are also often the focus of intensive human activity and present as a challenge for managers and restoration ecologists. Many types of anthropogenic disturbances can adversely influence these ecosystems (Richardson et al., 2007; Esler et al., 2008), such as altering the flow of the river by building dams for agriculture, as well as soil being altered by nearby agricultural activities. These disturbances make it easier for invasive plants to establish along riverbeds (Huston, 2004; Lake and Leishman, 2004; Esler et al., 2008), and similarly for invasive invertebrates to establish (Samways et al., 1996). Invasive plants grow well in these frequently disturbed environments, taking advantage of opportunities provided by both natural flooding events as well as additional anthropogenic disturbances (Richardson et al., 2007). As most rivers flow through human settlements, there are also multiple opportunities for the establishment and spread of invasive plants along rivers which act as linear corridors that meander through the landscape (Huston, 2004; Richardson et al., 2007). Riparian habitats receive inputs of matter from large areas along the river, which also then accumulates, concentrates and exacerbates any human-induced impacts and disturbances (Richardson et al., 2007; Esler et al., 2008).

Invasive alien plants threaten riparian systems by affecting water-related ecosystem delivery (Richardson and van Wilgen, 2004; Richardson et al., 2007; Rebelo et al., 2015). In semi-arid countries like South Africa (van Zyl, 2003), invasive tree species have a negative impact on the country’s scarce water resources by reducing run-off (Holmes et al., 2005). Woody alien invasive plants in the Berg and Breede catchments have been found to be both aggressive and successful (Rebelo et al., 2022). Plants that invade riparian habitats in South Africa are mostly tall trees with a higher water consumption than the original native vegetation (Dye and Poulter, 1995; Dye et al., 2001; Esler et al., 2008). In winter and all-year rainfall areas, species of Australian *Acacia* (e.g., *Acacia mearnsii, A. longifolia* and *A. saligna*) and *Eucalyptus* (especially *Eucalyptus camaldulensis*) transform and alter riparian ecosystems (Forsyth et al., 2004; Holmes et al., 2005). Other less threatening invasives in the area also include non-transformers such as *Pinus pinaster* (Cluster Pine) and *P. radiata* (Monterey Pine) (van Wilgen et al., 2020). One characteristic of invasive *Pine* trees is that they do not resprout after felling, and don't require additional treatments such as herbicides for effective management, they are therefore easier and less costly to control (Fill et al., 2016).

Transformer alien species such as *A. mearnsii* (black wattle) alter soil chemistry by elevating soil nitrogen levels and can also facilitate the colonization of uncharacteristic native plant species or secondary alien species (Gwate et al., 2021). In areas where soil nitrogen levels have increased, grasses can have an enhanced competitive advantage and may become dominant after alien clearance (Holmes et al., 2005). Invasions by trees such as *E. camaldulensis* (river red gum) have been especially recognised as becoming an increasingly large problem in South Africa, especially in riparian zones (Tererai et al., 2013). Invasions of these systems can ultimately result in a change of native species composition, structure and function by driving ecosystems into alternate states or novel ecosystems (Huston, 2004; Richardson et al., 2007; Esler et al., 2008). *Eucalyptus* species are heavy water-users and alter local conditions, creating situations of water-repellence and allelopathy in soils (Ruwanza et al., 2013; Holmes et al., 2020). Eucalypts also tend to form dense canopies, producing dense layers of litter that suppress germination and growth of other plant species (Ruwanza et al., 2013; Holmes et al., 2020). As a result, native vegetation is suppressed, outgrown and replaced by alien woody plants along riverbeds (Rebelo et al., 2021). This also leads to reduction in river flows owing to the larger biomass of alien compared to native plants (Holmes et al., 2005). Increased biomass of invasive plants also leads to increased intensity as well as frequency of fires, which in turn negatively impacts native seed germination and a lowers native plant biomass. This also in turn creates even less competition for invaders (Gaertner et al., 2012; Rebelo et al., 2021).

Riparian zones are especially vulnerable to invasives such as *A. mearnsii.* These invasive species are aggressive invaders of river channels and wetland habitats including riparian zones and river catchment areas, which gives them access to water throughout the year (Rebelo et al., 2015). Once native plants in these areas have been displaced by invaders, underlying soil or peat beds, if present, are exposed, dry out and start to erode rapidly (Rebelo et al., 2015). Soil erosion is also elevated in areas densely invaded by alien trees, as ground cover in the form of native plants that also provide surface stability is excluded by the dense alien canopy. Invasive alien plants impact catchment hydrology and sediment yield, therefore affecting river geomorphology indirectly via surface runoff as well as directly where they invade river banks and channels (Holmes et al., 2005), therefore decreasing the catchment's ability to absorb extreme rainfall events, and increasing the degradation of its water filtering service (Rebelo et al., 2015).

The establishment of invasive plants into riparian systems can also have significant impacts on invertebrate assemblages that inhabit the belowground environment as well as the soil surface (Liu et al., 2012). Many studies to date have also confirmed that invasive plants have considerable effects on the quantity and quality of resource inputs to decomposer organisms as well as their role in ecosystem processes, which ultimately affects soil nutrient availability (Bardgett and Wardle, 2010). Invasive plants have been found to alter litter production cycles into the environment as well as nutrient inputs into the soil (De Almeida et al., 2022). Several invasive plant species have also been described to release allelopathic compounds into their environment (Weidenhamer and Callaway, 2010; De Almeida et al, 2022), enabling them to uniquely affect soil biochemistry and nutrient cycling through leaf litter and root exudes, as well as altering linkages with belowground biota and their associated functions such as mineralization (Weidenhamer and Callaway, 2010; De Almeida et al., 2022). These plant-soil feedbacks can further promote seedling establishment of invasive species (Aldorfová et al., 2020; De Almeida et al., 2022), and also inhibit germination of some native plants (Ruwanza et al., 2015). In general, higher organic matter content in soil has also been associated with higher abundances of Collembola (Harta et al., 2021). Collembola are also generally sensitive to desiccation and therefore respond significantly to changes in temperature and humidity (Kaersgaard et al., 2004; De Almeida et al., 2022). Non-indigenous vegetation may cause increased litter layers as well as different temperature and humidity conditions, also in-turn promoting the success of invasive invertebrate species such as invasive Collembola which thrive in these conditions (Simberloff, 2006; Liu et al., 2012).

Restoration efforts

To date, degradation across ecosystems has occurred to such an extent that it has led to the onset of intervention strategies and consequently, the United Nations has declared 2021-2030 as the "Decade of Ecosystem Restoration". Restoration can be defined as any environmental repair activity to achieve system recovery relative to an appropriate reference model such as an unharmed native ecosystem, or traditional cultural ecosystem (Gann et al., 2019). Ecosystem restoration is a broad term included in various approaches to the management of ecosystems (Woodworth, 2017; Gann et al., 2019). Interventions include reduced impacts, remediation (management activity aiming to remove sources of degradation, for example the removal or detoxification of toxic substances or excess nutrients from soil and water) rehabilitation (improving and reinstating a level of ecosystem functioning, leading to renewed provision of ecosystem services, even if from a novel, non-native ecosystem) and ecological restoration, that aims to improve biodiversity, ecological integrity and ecosystem services (Gann et al., 2019). There is, therefore, a spectrum of interventions that support ecosystem integrity and recovery, and their use depends on the degree of degradation experienced by the target ecosystem (Gann et al., 2019; Van Zitters, 2021). The Society for Ecological Restoration's restorative continuum helps to visualise this spectrum (Gann et al., 2019). The concept of the restorative continuum is broad and incorporates all forms of ecosystem repair described above (Gann et al., 2019). It is therefore a useful model for setting appropriate goals as well as associated actions across a broad spectrum of degradation (Holmes et al., 2020). Although restoration and rehabilitation interventions likely reflect a more continuous spectrum of restoration practices, the dichotomy between these two helps to visualize approaches (Chazdon et al., 2021; Van Zitters, 2021).

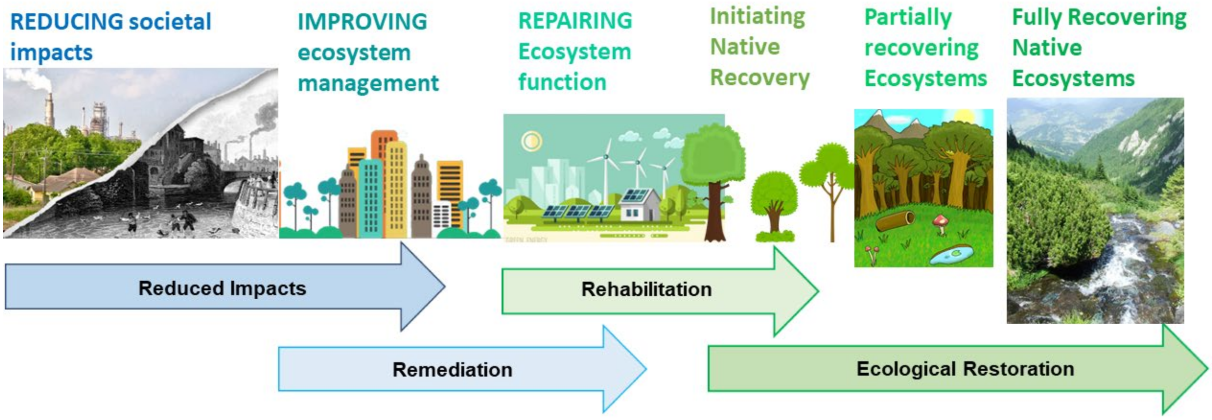

Figure 1: The restorative continuum, redrawn and adapted from Gann et al. (2019). It shows various interventions that can be made for ecosystem recovery. Images used under Creative Commons Licence.

As you move from left to right on the continuum (Fig. 1), ecological health and biodiversity outcomes, as well as quality and quantity of ecosystem services, increase. It is also noted that ecological restoration can also occur in urban, agricultural, and industrial landscapes (Gann et al., 2019). This continuum defines a range of interventions from the most damaged ecosystems, where the goal is to simply reduce negative impacts, through to the least damaged ecosystems where the goal is full recovery (Gann et al., 2019; Van Zitters, 2021). The overall goal of ecosystem restoration as stated by the UN declaration, is to greatly improve the restoration of degraded landscapes, to mitigate and adapt to the effects of climate change as well to improve and sustain the flow of ecosystem goods and services that are essential for human well-being (Gann et al., 2019; Holmes et al., 2020). Ecological restoration is also more narrowly defined as a process which returns an ecosystem to a close approximation of a reference condition, the extent of which is to be determined by the project goals (Pastorok et al., 1997; Van Zitters, 2021).

Rehabilitation is a closely related term which is more often applied to relatively degraded sites or where no historical data are available to establish a reference condition, or where it is no longer possible to recover native species composition. Rehabilitation can be defined as the process of recovering to a pre-determined degree of ecosystem structure and functionality on degraded sites, leading to increased ecosystem service provision to society

(Woodworth, 2017; Van Zitters, 2021). Clearing of invasive alien species through passive restoration is a first step in the right direction for restoring degraded habitats. It has been unclear whether it will be effective as a single management practice for the complete re-establishment of native riparian communities (Gaertner et al., 2012).

Interventions such as invasive alien tree removal and follow-up control of possible invasive seedling recruitment are one of several different types of ecological infrastructure interventions, within the 'ecological infrastructure intervention – ecosystem services' (EII-ES) framework, outlined in Rebelo et al., (2021). This framework was developed and used to build a conceptual model of ecosystem properties, as well as processes and ecosystem services and how they interact in relation to ecological infrastructure investments, in other words, how do the functioning of ecosystems and their provision of ecosystem services improve, based on investment and efforts in ecosystem restoration (Rebelo et al., 2021). Although progress has been made in this field, there is still a need for better baseline data collection, as well as monitoring during and after ecological restoration practices, in order to establish empirical evidence for the benefits of different restoration practices (Rebelo et al., 2021).

Active and passive restoration practices

Active and passive restoration practices are commonly associated with restoration work (Gann et al., 2019), and are also applied to rehabilitation (Van Zitters, 2021). Passive restoration can be defined as a management approach that relies on spontaneous succession after the degrading disturbance, for example, after invasive plants have been removed (Gann et al., 2019; Holmes et al., 2020). During passive restoration, there is no further human-assisted treatment following alien plant removal, thus restoration progress depends on spontaneous succession (i.e., natural recovery). Active restoration is where native plant communities are actively re-established following the removal of alien invasive vegetation. This normally involves human interventions such as re-planting of native species and additional practices to facilitate the recovery of native plants, also including irrigation, follow-up monitoring and clearing practices (Holl and Aide, 2011; Gann et al., 2019; Holmes et al., 2020; Van Zitters, 2021).

Over the past few decades, a link between alien woody invaders and the significant reductions in water supply in the South Africa's Core Cape Subregion (CCS) has been recognised (Richardson and van Wilgen, 2004; van Wilgen et al., 2016; Holmes et al., 2020; Rebelo et al., 2021). With South Africa being a largely water scarce country, the government funded Working for Water programme (WfW) was initiated in 1995, also following decisions to phase out plantation forestry along the Berg River during the 1990's (Fill et al., 2016). The WfW invested in invasive alien plant control and saving water as a vital ecosystem service, as well as creating employment (van Wilgen et al., 1998; van Wilgen and Wannenburgh, 2016). The WfW programme then expanded countrywide (Holmes et al., 2020). This programme is one of the world's largest initiatives to clear watersheds (including the Berg river catchment area) of invasive trees, with the goal to restore the structure, diversity and function of riparian ecosystems, as well as joint aims to enhance ecological integrity, social development and to create jobs within local communities (van Wilgen et al., 1998). In these areas including the Berg river, control of invasive alien plants was implemented by using appropriate mechanical, chemical and biological methods (Holmes et al., 2005). This was under the assumption that the targeted ecosystems, mostly riparian, would self-recover through spontaneous succession to a more natural state once alien plants have been removed (van Wilgen and Wannenburgh, 2016; Holmes et al., 2020).

The Working for water programme (WfW) has also been removing *Acacia* trees such as *A. mearnsii, A. longifolia* and *A. saligna* and *Pinus* trees such as *P. pinaster* along the upper Berg river catchment. Clearing methods used by the WfW programme focussed solely on alien control, rather than ecosystem restoration (Holmes et al., 2008) and at the time, no research had been published on the consequences of mechanical and chemical alien control methods on native vegetation recovery (Holmes et al., 2005). Approaches to effectively promote native vegetation recovery may therefore deviate from their recommended approach (Holmes et al., 2008). In 2008 the South African National Biodiversity Institute (SANBI) was contracted by the WfW in order to address this gap by developing species specific control programmes, initially focussing on alien plant species that were not yet invaders (Wilson et al., 2013; van Wilgen et al., 2020). Later efforts also focussed on invasive plants that were already widespread in the country, however sufficient resources and adequate monitoring in order to achieve eradication were not always in place (Wilson et al., 2017).

The Berg river project for clearing invasive trees was initiated in 2001 (Fill et al., 2016). By that time about 25% (~1900 ha) of approximately 7500 ha of the area, was covered by *Pinus* species plantations, and about 11% (~650 ha) of the area was covered by dense alien *Acacia* species (Fill et al., 2016). Significant amounts of money had been spent on follow-up and clearing (ZAR 28 million for *Pinus*, and over ZAR 21 million for *Acacia* trees, Fill et al., 2016). Following assessment of the restored area and restoration activities, it has been found that *Pinus* trees have been reduced to 419 ha of medium to dense coverage (5,58%). However, *Acacia* trees have actually increased by 174 ha. This was likely due to germination after fires, of which several has occurred in the catchment during this period (Fill et al., 2016). Young *Pinus* species was also still found across most of the Berg river catchment.

Studies have found that active restoration with careful follow-up clearing and continuous monitoring and evaluation of progress, may be more effective in promoting better ecosystem recovery, especially when previous invasions are dense or long-term (Gann et al., 2019; Holmes et al., 2020). Appropriate clearing methods also depend on the species being eradicated. For example, *Pinus* species have different characteristics than *Acacia* species, due to their inability to resprout following felling. However, seeds are released from cones following felling, also wind dispersed by winged seeds, and this requires burning after 1-2 years after felling to keep resultant seedlings from maturing (Holmes et al., 2000). *Acacia* species produce high abundances of seeds that build up in soils, they are more difficult and costly to control, as burning will only stimulate seedling germination and dramatically increase the number of seedlings that germinate following fires (Fill et al., 2016). For *Acacia*, initial felling is followed by the application of herbicides in order to prevent mass resprouting (Fill et al., 2016).

Without the re-growth of native vegetation, ecosystems are often prone to re-invasion by the same alien or secondary alien species (Holmes et al., 2005; Nsikani et al., 2018). A good explanation as to why alien plants regrow so effectively after thorough clearing efforts is the issue of soil legacy effects and native soil seed banks, with the latter found to decrease in native species abundance following invasions (Vosse et al., 2008). Legacy effects include measurable changes in soil biological, chemical or physical conditions (Corbin and D'Antonio, 2012; Nsikani et al., 2018). Ecophysiological traits of invasive nitrogen fixing woody species include high growth rates and seeds with long dormancy periods which contribute to soil legacy effects after clearing. A growing number of studies (Corbin and D'Antonio, 2012; Rodriguez-Echeverria et al., 2013; Nsikani et al., 2018) have described how soil legacy effects present barriers to restoration following the removal of invasive-nitrogen fixing woody species, while others have presented potential management actions to address these barriers to restoration (Fill et al., 2016; Nsikani et al., 2018).

There is still little understanding of how to correctly monitor and rehabilitate habitats after the successful removal of invasive plants, which is due to the high biodiversity and complexity within these habitats (Richardson et al., 2007). Various factors may play roles in the successful re-establishment of native riparian ecosystems. Previous studies on these invasions have investigated the above-ground structural effects and re-establishment of native plant communities along riparian sites (Holmes et al., 2005; Esler et al., 2008; Tererai, 2012; Tererai et al., 2013; Van Zitters, 2021). Most of the studies took place over a relatively short time frames following restoration, relative to natural ecosystem trajectories, and were focused on the earlier stages of succession following restoration (Esler et al., 2008). In more recent years, clearing of invasive plants along the Berg River was also implemented by other government departments as well as private organizations (i.e. LandCare, The Nature Conservancy) who also made good progress through local and regional clearing efforts (Van Zitters, 2021). Along the Berg River, a private organization called GreenIntaba led by Johann van Biljon as a part of the Berg River Improvement programme also carried out both active as well as passive clearing methods on various riparian sites which were investigated in this study (Horn, 2020).

In conjunction with the Western Cape Government, they have been removing *Eucalyptus* trees (*E. camaldulensis*) (Ruwanza et al., 2013; Holmes et al., 2020) and other alien invasive plants in the riparian zone by means of felling and ringbarking, as well as mechanical methods, followed by actively and passively restoring these habitats in some of the sites along the Berg river to improve river flow, bank stability and water quality. Their methods excluded the possible negative effects of chemicals and herbicides.

Four to five years after these interventions, the study by Van Zitters (2021) investigated the impacts of rehabilitation on vegetation composition at 11 sites along the Berg and Breede Rivers, including the current three sites being investigated in this study. The study found that these small-scale active rehabilitation efforts significantly improved native vegetation community composition in the short term and provided some insights on the effects of abiotic conditions and investment on rehabilitation effects (Van Zitters, 2021). However, the study had some constraints such as being within a very short period following restoration and focussing only on above-ground vegetation.

In this study, I investigated the belowground effects of restoration by the Berg River Improvement program (Horn, 2020), following the removal of woody tree invasions, over a time span of two years, and how this also translates onto aboveground vegetation structure.

Collembola as bioindicators to measure restoration success

Bioindicators can be defined as taxa whose community structure reflects the state and functioning of their surrounding environment (Gerlach et al., 2013). They can be used to evaluate the effects of ecosystem stress or disturbances as well as to evaluate the effectiveness of ecosystem recovery under different management practices (Gerlach et al., 2013). Studies have been investigating the efficacy of different biological indicators to evaluate the success of rehabilitation projects (Ruiz-Jaen and Aide, 2005; Londe et al., 2017). These included species richness of plants, ants, birds, seedling recruitment, litterfall structure, canopy cover or basal area which all were studied as indicators to measure restoration success (Ruiz-Jaen and Aide, 2005; Suganuma and Durigan, 2015; Londe et al., 2017; Van Zitters, 2021). Despite invertebrates being excellent bioindicators (Gerlach et al., 2013), they are not often used in restoration studies. To date, the recovery of ecosystems in terms of insect abundances were mostly focused on aboveground herbivores and their direct interactions with host plants (Maoela et al., 2016, 2019).

Many studies that use invertebrates as bioindicators in restoration studies include plant-pollinator interactions and above-ground insect abundance (Forup and Memmott, 2005; Mitchell et al., 2009; Borchardt et al., 2021). For example, a study by Watts and Didham (2006) conducted in New Zealand, investigated the impact of wetland habitat loss and isolation on a specific insect-plant interaction. They experimentally placed potted *Sporadanthus ferrugineus* (Restionaceae) at increasing distances from an intact habitat to test the effects of isolation and habitat fragmentation on the colonization rates and herbivory of *Batracheda* species (Lepidoptera: Coleophoridae) (Didham, 1997; Watts and Didham, 2006). The results revealed that even moderate isolation, such as distances over 400 meters from an intact wetland habitat, caused the insect-plant interactions to nearly collapse (Watts and Didham, 2006). Other examples include the investigation of insect herbivores in forests as bioindicators (Watts and Didham, 2006; Moreira et al., 2007). For example Moreira et al. (2007) found that galling insects in restored areas of the highly fragmented Brazilian Atlantic Forest showed low abundances in intermediate sites of *Myracrodruon urundeuva* trees, suggesting that this plant had a negative impact on the native plant community, and hinting towards allelopathic activity of the plant (Moreira et al., 2007). The study concluded that the plant decreased native species establishment and consequently also reduced associated native herbivore richness, thus galling insects were effective tools as bioindicators to evaluate environmental quality (Moreira et al., 2007).

Many other studies that use biological indicators were also highlighted in the global study by Borges et al. (2021). They found that a growing number of studies are using ecological indicators of which the most researched taxa included Hymenoptera (31.8%), Coleoptera (27.9%), Lepidoptera (12.9%), Araneae (9%), Nematoda (8.4%), Annelida (6.4%), Hemiptera (6.4%), Orthoptera (5.8%), undifferentiated invertebrates (10.3%), and also lastly, Collembola (5.1%). However, there was a lack of information on community or population-level measures of restoration success, including community structure, biomass, and species dominance. The study also identified further consequences of these knowledge gaps and highlighted the need for knowledge in all aspects of terrestrial invertebrates as restoration indicators. Few studies have focussed on smaller soil-dwelling invertebrates, specifically Collembola (springtails) as bioindicators in restoration projects. This is partly because of their small size (2-4mm), high abundance, and the taxonomic difficulty in species identification. Snyder and Hendrix (2008) highlighted the importance of soil biodiversity in returning functionality to restored ecosystems which has been previously under-appreciated, further highlighting the roles of larger detritivores such as earthworms, millipedes and isopods in assessing restoration progress. The study also investigated their functioning as bioindicators to help accomplish restoration goals. However, the above-mentioned study did not include smaller inconspicuous detritivores such as Collembola, which can be highly valuable as bioindicators. One example where Collembola were considered is a more recent study by Arenhardt et al. (2021), who evaluated the capacity of litter Insecta and Collembola in pasture restoration in the Atlantic Forest Biome as bioindicators. Collembola followed a successional pattern of restoration within sampled areas and was indeed an effective method of restoration monitoring (Arenhardt et al., 2021).

Collembola are among the most abundant soil-dwelling microarthropods (Eisenhauer et al., 2011). They can have densities of up to 60 000 individuals per m^2 in grasslands alone (Eisenhauer et al., 2011). Collembola are excellent indicators of soil biodiversity, as they have an abundance of species that take up a wide range of ecological niches (Cassagne et al., 2003). They are also very sensitive to changes in their habitat and respond to a wide range of environmental and ecological influences (de Filho et al., 2016). Collembola communities have been found to be influenced by disturbances such as changes in soil humus content, soil chemistry and pH (Cassagne et al., 2003), soil tillage (de Filho et al., 2016), fluctuations in temperature and moisture regimes (Huhta and Hänninen, 2001; Jucevica and Melecis, 2006), disturbances such as overgrazing, fire regimes (Janion-Scheepers et al., 2016) and intensive farming (Leinaas et al., 2015). Collembola can further be used as bioindicators to assess the soil quality and the effectiveness of rehabilitation (de Filho et al., 2016).

Collembola play different vital roles in ecosystem functioning, such as facilitating litter decomposition as well as the formation of soil microstructure (Rusek, 1998). They are responsible for about 30% of total soil invertebrate respiration processes (Orgiazzi et al., 2016). These small arthropods feed dominantly on fungi, and mobilize nutrients locked up in microbial biomass, thereby also affecting plant nutrition (Rusek, 1998; Eisenhauer et al., 2011). Different species of Collembola vary in terms of habitat preferences and can be divided into different life forms (Orgiazzi et al., 2016). These include epidaphic (surface-dwelling), euedaphic (soil-dwelling), and atmobiotic (plant-dwelling) Collembola (Orgiazzi et al., 2016; Pollierer and Scheu, 2017). Collembola feed on fungal spores and hyphae as well as living plant tissue, fine roots, bacteria and decaying plant material, although some species are also predators and feed on nematodes as well as other Collembola (Eisenhauer et al., 2011; Orgiazzi et al., 2016). By these feeding mechanisms, they have a significant influence on soil microbial ecology, soil fertility and nutrient cycling (de Filho et al., 2016). Although detailed ecological studies for this group are still lacking, knowledge about them is increasing in the Cape Floristic Region of South Africa Biome (Liu et al., 2012; Janion-Scheepers et al., 2015), and they have significant diversity and endemism in South Africa (Janion-Scheepers et al., 2021; Janion-Scheepers et al., 2015). In South Africa, 124 species out of 61 genera and 17 families of Collembola have been recorded, of which about 25% are introduced. Most of these, about 77 species, have been documented in the Western Cape (Liu et al., 2012; Janion-Scheepers et al., 2015).

In previous studies related to the Berg river riparian zone in the Western Cape, monitoring of riparian habitats after alien tree removal and subsequent restoration efforts have focused on the above-ground structural effects and re-establishment of native plant communities (Tererai, 2012; Ruwanza et al., 2013; Tererai et al., 2013; Van Zitters, 2021). In this study, I investigate the belowground effects of invasive alien plant species removal and active restoration, by comparing Collembola assemblages between different rehabilitated and disturbed sites.

In particular the main aims of this study were:

1) To compare the differences in Collembola diversity in study sites following active restoration (alien clearing and revegetation), and passive restoration (alien clearing and spontaneous succession).
2) To compare Collembola diversity results with aboveground plant data from previous studies.

The following hypotheses were tested:

1) There is a difference between Collembola diversity in active and passive restoration areas as well as between all restored compared to invaded areas.
2) There are more alien Collembola present in invaded, non-restored areas and more native Collembola present in restored compared to invaded areas.
3) Aboveground plant invasive status may directly or indirectly affect Collembola diversity.

This study will help to further understand the extent of the belowground impacts of plant invasions and the implications this may hold for ecosystem restoration.

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Chapter 2

Abstract

Due to the unprecedented transformation and fragmentation of ecosystems, resulting from anthropogenic land uses and exploitation, ecological restoration is becoming much-needed in conservation. South Africa is a largely semi-arid country, with invasive tree species having a negative impact on the country's scarce water resources by reducing run-off and affecting water-related ecosystem delivery. Since 1995, private organizations and the Western Cape government have been clearing riparian areas of invasive alien trees along riparian zones in the Western Cape. The areas of study included three riparian sites along the Berg River in the Western Cape, habitats that are sensitive to environmental changes. The Berg and Breede River Rehabilitation Programme has been clearing alien invasive trees along the Berg River, followed by either active restoration (follow-up clearing and monitoring) or passive restoration (no treatment after alien plant removal). Most studies to date have focused on the aboveground impacts of restoration, with limited research on invertebrate taxa, particularly belowground soil fauna. However, soils play a crucial role in successful restoration efforts. This study assessed the Collembola (springtail) communities between active and passive restoration sites in comparison to invaded non-restored sites as reference sites. Sampling was conducted during winter (2020) and winter and spring (2021) to consider the seasonal effects. From a total of 250 samples, 77,880 individual specimens and 34 morphospecies were identified. Active restoration was significantly different from invaded sites (Kruskal-Wallis, $p<0.05$; ANOSIM, $p<0.05$ between active and invaded sites) in terms of Collembola community composition, and may therefore be the most effective method in restoring these communities closer to a natural state. However, there was no significant difference between passive restoration and invaded sites (Dunn tests: $p >0.05$). The highest density (about 1,250 individuals per m^2) of native Collembola were found at Bosplaas farm (Kruskal-Wallis chi-squared = 14.626, df = 2, p-value < 0.005), during spring 2020 and spring 2021 ($p<0.05$), also indicating clear seasonal differences for native Collembola species. In addition, higher densities of native Collembola were found in 2021 than in 2020 at Bosplaas farm. These results may also indicate a lag phase, and that the belowground fauna may take much longer to recover after restoration than aboveground plants. Different microhabitat conditions and litter cycling between native and invasive plants were also found to be an additional driver of Collembola community composition.

Introduction

Habitat restoration in riparian zones

Riparian zones are dynamic, disturbance-driven ecosystems that support a variety of life history strategies. These unique systems are home to a diversity of uniquely specialized disturbance-adapted species (Naiman and Decamps, 1997; Holmes et al., 2005). Riparian zones are also considered critical transition zones, as they affect a large area of adjacent ecosystems by processing substantial fluxes of materials from their closely connected neighbouring ecosystems (Ewel et al., 2001; Holmes et al., 2005). These habitats are also extremely vulnerable to invasion by alien plants (Tererai et al., 2013) and are thus important in the field of conservation (Tererai, 2012). Many types of human disturbances can adversely influence these riparian zones (Richardson et al., 2007), such as altering the flow of the river by building dams for agriculture, soil being altered by nearby agricultural activities, as well as planting of invasive alien plants near rivers. Invasive alien plants, once established along rivers and streams, can have adverse impacts in vulnerable riparian habitats further downstream (Simberloff, 2006; Liu et al., 2012). In addition to decreasing the catchment's ability to absorb extreme rainfall events, they can also increase the degradation of its water filtering services (Rebelo et al., 2015). Invasive plants can lead to further soil erosion and alteration of soil properties such as pH as well as plant-litter and soil nutrient inputs (Weidenhamer and Callaway, 2010; De Almeida et al., 2022). Invasive plants also displace indigenous vegetation, causing soil legacy effects (Nsikani et al., 2018) and ultimately affecting soil- and litter-dwelling invertebrates (Rusterholz et al., 2014; Leinaas et al., 2015; De Almeida et al., 2022).

Rehabilitation is one of many restorative activities along a restorative continuum that also includes ecological restoration and its associated activities (Gann et al., 2019). Both ecological restoration and rehabilitation aim to contribute to improving ecosystem integrity and social-ecological resilience (Gann et al., 2019). While rehabilitation is a process of recovering to a pre-determined degree of ecosystem structure and functionality on degraded sites, leading to increased ecosystem service provision to society (Woodworth, 2017; Van Zitters, 2021), ecological restoration aims at achieving the highest level of ecosystem recovery possible (Gann et al., 2019). Restoration can be more narrowly defined as a process which returns an ecosystem to a close approximation of a reference condition, the extent of which is to be determined by certain goals set out in restoration projects (Pastorok et al., 1997; Van Zitters, 2021). In this study, we focussed on the terms active and passive restoration as forming part of a rehabilitation project.

Active restoration is where native plant communities are planted and re-established, in this case, following removal of alien invasive vegetation, while passive restoration is where alien plant removal is not followed by any additional treatment (Atkinson and Bonser, 2020; Holmes et al., 2020). Passive restoration, also referred to as "natural regeneration," relies on natural spontaneous succession and can often be used as the most cost-effective approach in restoration studies, but only when the potential for natural recovery is high (Gann et al., 2019). This can be effective in ecosystems where invasion has not been too severe and invasion history is relatively recent, these ecosystems are then able to self–restore through spontaneous succession (Holmes et al., 2020). This method usually works well in headwater and transitional riparian ecosystems, if the surrounding catchment contains relatively intact vegetation and has native propagules that may persist within its seedbanks (Vosse et al., 2008), or that can be water dispersed from upstream areas (Galatowitsch and Richardson, 2005; Holmes et al., 2020). Seeds in riparian soils are dispersed by many natural processes, such as dispersion by floodwater, burying seeds underneath sediments, as well as animal burrowing and seed burial. Many seeds can remain dormant until suitable conditions for germination develop (Holmes et al., 2005). These seedbanks are an important component of ecosystem recovery by passive restoration.

However, in some severely degraded areas, topsoil may have been removed and sediment material washed away as river dynamics have been so drastically changed by alien plants that the ability of the catchment to absorb extreme rainfall events has also been largely reduced. Frequent natural flooding of these areas during heavy seasonal rains are then followed by more habitat loss and erosion (Rebelo et al., 2015). In some areas where dense stands of invasive trees have grown over a longer period, certain biotic or abiotic thresholds may have been breached, leaving such areas unable to recover solely from passive restoration practices (Richardson et al., 2007). Here, invasive species could have left behind legacy effects (Nsikani et al., 2018). In such cases, native plant species may have been eliminated from aboveground communities and soil seed banks, invasive plants have established creating novel seed banks and/or biophysical conditions such as soil microbial communities, soil properties, organic matter, litter and soil dwelling organisms remain altered following removal of invasive plants (Nsikani et al., 2018; Holmes et al., 2020). Once one of these biotic thresholds have been crossed, active restoration interventions are needed (Gaertner et al., 2012; Gann et al., 2019; Holmes et al., 2020). In South Africa, this is usually the case where dense stands of *Eucalyptus camuldensis* have established (Ruwanza et al., 2013; Holmes et al., 2020). In this instance, the damage of removing invasive plants is usually high, thus all biotic and abiotic damage needs to be corrected to suit the original/natural state of the ecosystem by re-introducing most of its original biota and re-vegetating the area with its native plants (Gann et al., 2019).

Impacts of disturbances on soil fauna such as Collembola have been found to be related to invasive plants and litter composition. It is also known that changes in soil pH can cause significant changes in Collembola community structure, and that some species have narrower pH tolerance than others (van Straalen and Verhoef, 1997; van Straalen, 1998; Cassagne et al., 2003; Harta et al., 2021). Invasive Collembola species in South Africa are typically associated with nutrient-rich organic soils (Fjelberg, 1998; Leinaas et al., 2015), and occur much less frequently in nutrient-poor sites across the Western -Cape (Janion, 2012; Liu et al., 2012; Janion-Scheepers et al., 2015; Leinaas et al., 2015). Indigenous Collembola, which are able to utilize low-quality litter in natural areas, have been found to be completely displaced by invasive Collembola in nutrient-rich, invaded areas. Certain invasive Collembola species, such as *Hypogasturra manubrialis*, can also be competitive and completely displace indigenous Collembola from their original areas (Leinaas et al., 2015). From a restoration point of view, indigenous Collembola may be provided a spatial refuge in areas with low-quality litter, which their invasive counterparts are unable to utilize. These may include possible native riparian areas near the invaded site, therefore enabling them to slowly re-colonise sites following restoration. However, it is evident that high-nutrient plant-litter, even after alien plant removal, will favour certain species of invasive Collembola over native Collembola (Leinaas et al., 2015).

Both active and passive restoration approaches use natural recovery processes and require ongoing adaptive management until recovery is attained (Gann et al., 2019). It is also important to note that the effects of abiotic variables on riparian vegetation recovery have not been fully explored for all climatic regions of South Africa (Holmes et al., 2005; Van Zitters, 2021).

Importance of native riparian vegetation in riparian zones

Riparian ecosystems are well adapted to frequent environmental disturbances, which form a natural part of these environments (Stella and Bendix, 2018). These are usually characterized by hydrogeomorphic processes such as flooding and droughts, as well as herbivory and fire (Montgomery, 1999; Bendix and Hupp, 2000; Stella and Bendix, 2018). Frequent disturbances result in a pulse of mortality, reduced biomass and altered physical conditions (Pickett et al., 1987; Stella and Bendix, 2018). Native riparian ecosystems may frequently experience temporary setbacks due to natural disturbances such as flooding. However, these disturbances may benefit the long-term ecosystem functioning of these ecosystems (Naiman et al., 2005; Van Zitters, 2021). For example, natural flooding events cause a fluvial disturbance which sustains a diversity of riparian assemblages and transitioning plant communities (Naiman et al., 2005; Van Zitters, 2021). Patterns of drought and flooding events affect seasonal availability of water, which also in turn affects the survival and spatial and temporal distribution of riparian vegetation over landscapes. Over the long term, this also results in disturbance-driven and resilient native species recruitment sites along riparian zones (Naiman et al., 2005; Van Zitters, 2021).

Intact native riparian vegetation provides unique habitats, stabilizes streambanks and filters sediments and nutrients from surrounding catchments (Ewel et al., 2001). These highly productive systems are also important in maintaining natural river flow, nutrient and water filtering, flood buffering, climate regulation and other water-related ecosystem services. The type of plants naturally adapted to riparian habitats in winter rainfall regions of the Western Cape, typically consume less water than alien invasives that establish in these systems (Dye and Poulter, 1995; Dye et al., 2001; Esler et al., 2008). Natural vegetation also grows in areas that would otherwise be left barren by alien plants which can release allelopathic compounds, inhibiting local seedling growth as well as altering local soil biochemical structure (Ruwanza et al., 2015; De Almeida at al., 2022). The dense canopies also block sunlight (Ruwanza et al., 2013; Holmes et al., 2020), leaving native sun-loving plants to be unable to grow underneath. This causes the soil to only be suitable for invasive tree species and their litter, leaving the understory of these alien riparian forests to become mostly bare soil, which then subsequently gets eroded away with no roots from ground cover keeping the soil intact (Holmes et al., 2005). In addition, native riparian vegetation is very distinct compared to their surrounding fire-prone fynbos counterparts, although it occurs under similar macroclimatic conditions (Boucher, 1978; Holmes et al., 2005). This vegetation can be characterized by a relatively high cover of broad-leafed woody plants, similar to a forest or thicket biome

(Holmes et al., 2005), but also has a high cover of fynbos elements, such as Restionaceae and Ericaceae. It has been described as ‘closed scrub fynbos’, ’hygrophilous mountain fynbos’, as well as ‘broad sclerophyllous closed scrub’ (Holmes et al., 2005; Mucina and Rutherford, 2006).

Effects of alien invasive plant invasions on riparian zones.

Riparian habitats have undergone extensive degradation over the years as a result of human impacts (Richardson et al., 2007; Esler et al., 2008), such as altering the flow of the river by building dams for agriculture and soil being altered by nearby agricultural activities. Riparian ecosystems are prone to invasions by invasive species, which are often facilitated by disturbance-related effects and thrive in frequently disturbed environments (Lake and Leishman, 2004). The establishment of invasive plants can often result in a complete change of the native species composition, structure and function by driving ecosystems into alternate states (Huston, 2004; Richardson et al., 2007; Esler et al., 2008). Invasive plants such as *Eucalyptus camaldulensis* have been especially problematic in South Africa, especially in riparian zones (Tererai et al., 2013). Other invasive plants that occur in these areas include Australian *Acacia* species, such as *A. mearnsii, A. longifolia* and *A. saligna* (Forsyth et al., 2004), as well as long established pine species including *Pinus pinaster* (Cluster Pine) and *P. radiata* (Monterey Pine), which are remnants from plantations prior to 1990 (van Wilgen et al., 2020). Alien plant transformations in riparian habitats have been shown to create significant declines in ecosystem integrity and water-related ecosystem services (Richardson and van Wilgen, 2004). Invasive plants also consume more water, leading to reductions in overall runoff and river water supply throughout the year.

Invaded riparian zones and their immediate sub-catchments have therefore been targeted for alien clearance by the National Working for Water Programme (WfW) since 1995 (van Wilgen et al., 1998; van Wilgen and Richardson, 2012; van Wilgen and Wannenburgh, 2016). Clearing methods used by the WfW programme focussed mostly on clearing alien invasive plants in riparian areas, using rehabilitation methods and an area based approach rather than a species- based approach of ecosystem restoration (Holmes et al., 2008; Van Zitters, 2021; van Wilgen et al., 2020). This was under the assumption that these ecosystems could self-repair once alien invasive trees have been removed (Esler et al., 2008). Through this initiative, an initial estimated 7% of closed stand invasions in riparian zones have been cleared, leading to significant increases in water yields in these areas (Esler et al., 2008). However, individual species has not yet been targeted.

Studies have since found that in areas where dense, closed alien stands have established over longer periods of time, thresholds may have been passed, leaving such ecosystems unable to recover following the removal of the aliens (Holmes et al., 2008). These systems required additional methods of restoration to recover to a more natural state. Without appropriate interventions to promote native vegetation recovery, ecosystems are often subject to re-invasion by the same alien or secondary alien species (Holmes et al., 2005). In 2008 the South African National Biodiversity Institute (SANBI) was contracted by the WfW in order to close this gap by developing species specific control programmes. Following the Berg-river project which was initiated in 2001 (Fill et al., 2016), it has been found that *Pinus* trees have been reduced from 1900 ha (25% coverage) to 419 ha of medium to dense coverage (5,58%). In contrast, *Acacia* tress have actually increased by 174 ha. This has been attributed to germination from soil seed banks after fires which has occurred in the catchment during this period (Fill et al., 2016). Young *Pinus* species was also still found across most of the Berg river catchment. Although efforts were not completely ineffective, after a decade of restoration efforts invasive alien plants have not yet fully eradicated in the region (Fill et al., 2016).

Measuring restoration success

For efficient, cost-effective and adaptive rehabilitation of riparian zones across many spatial and temporal scales, it is also important to use evidence-based practices (Ntshotsho et al., 2011). Due to restoration practices being relatively more expensive compared to other methods, practitioners also need to justify the expense with solid evidence (Ntshotsho et al., 2011). As riparian ecosystems provide irreplaceable ecosystem services to both nature and humans, ecological restoration has the potential to reverse land degradation, increase ecosystem resilience as well as deliver crucial ecosystem services on a local and global level (Wortley et al., 2013). There is however still some uncertainty surrounding how efficient restoration programs are (Suding and Hobbs, 2011; Wortley et al., 2013).

Three conditions are needed for the advancement and establishment of evidence-based rehabilitation, which include the collection of baseline data, clearly defining the project goals, and lastly adequate and relevant monitoring (Ntshotsho et al., 2011). Monitoring and evaluation are key components of generating the needed evidence surrounding the efficiency of restoration efforts (Van Zitters, 2021), and has been shown to improve the success and efficiency of interventions (Webb and Erskine, 2003). Studies evaluating the recovery of riparian vegetation following intervention are becoming increasingly recognised (González et al., 2015). Evidence through evaluation is also seen as a critical steppingstone for the undertaking of future rehabilitation projects and other interventions (Cook et al., 2017). A rehabilitation project is seen as successful when certain predefined goals from the start of the project are being met (Palmer et al., 2007; Gann et al., 2019).

Different indicators can be used to evaluate the success of rehabilitation projects. Bioindicators can be defined as taxa whose community structure reflects the state and functioning of their surrounding environment (McGeoch and Chown, 1998; Gerlach et al., 2013). They can be used to evaluate the effects of ecosystem stress or disturbances as well as to evaluate the effectiveness of ecosystem recovery under different management practices (Gerlach et al., 2013). These include animal and plant species richness, seedling recruitment, canopy cover or basal area (Ruiz-Jaen and Aide, 2005; Suganuma and Durigan, 2015; Londe et al., 2017; Van Zitters, 2021). Insect diversity has been used to measure the recovery of ecosystems (Samways et al., 1996), but has mostly focused on aboveground herbivores and their direct interactions with host plants. For example, a study by Watts and Didham (2006), which was conducted in New-Zealand, examined the impact of wetland habitat loss and isolation on a specific insect-plant interaction. The colonisation rates and herbivory of *Batracheda* species (Lepidoptera: Coleophoridae) on potted *Sporadanthus ferrugineus* (Restionaceae) were experimentally placed at increasing distances from an intact habitat, to test for the effects of isolation and habitat fragmentation (Didham, 1997; Watts and Didham, 2006). Results showed that even a moderate degree of isolation, for example above 400m from an intact wetland habitat, led to insect-plant reactions to almost fully collapse (Watts and Didham, 2006). Moreira et al. (2007) highlighted the importance of insect herbivores (galling insects) in forests as bioindicators in programs of conservation and environmental impact. Low abundances of galling insects were found in intermediate sites of *Myracrodruon urundeuva* trees in restored areas of the highly fragmented Brazilian Atlantic Forest. The study found that this specific plant has a negative impact on the native plant community, also suggesting allelopathic activity of the plant (Moreira et al., 2007). The study concluded that the plant decreased native species establishment and consequently also reduced associated native herbivore richness. Galling insects were therefore effective tools as bioindicators to evaluate environmental quality (Moreira et al., 2007).

Many more studies on restoration indicators are also highlighted in Borges et al. (2021). This global study detected that a growing number of studies are using ecological indicators of which the most researched taxa included Hymenoptera (31.8%), Coleoptera (27.9%), Lepidoptera (12.9%), Araneae (9%), Nematoda (8.4%), Annelida (6.4%), Hemiptera (6.4%), Orthoptera (5.8%), undifferentiated invertebrates (10.3%), and also Collembola (5.1%). Many restoration scenarios, especially in temperate coniferous forests or taiga biomes affected by pollution or urbanization, have been inadequately studied (Borges et al., 2021). Certain techniques, such as brushwood transposition and research in large or old areas, are understudied. There is also a lack of information on community or population-level measures of restoration success, such as community structure, biomass, and species dominance. The study highlighted the consequences of these knowledge gaps and emphasizes the need for comprehensive knowledge of terrestrial invertebrates as restoration indicators (Borges et al., 2021).

Measuring restoration success: this study

The Berg River Improvement Plan (Horn, 2020) is part of the broader Berg and Breede River Rehabilitation Programme (Van Zitters, 2021) which has been removing alien *Eucalyptus* trees and other alien invasive plants since 1995 (van Wilgen and Wannenburgh, 2016) as well as actively restoring habitats in some of the sites along the Berg River River flow and quality. The Berg and Breede River Rehabilitation Programme was initiated by the Western Cape government in 2013 (Van Zitters, 2021). The project is regarded as the first of its kind in terms of the scale of its investment for the active restoration of areas in the Fynbos Biome (Van Zitters, 2021). The program has been extensively clearing invasive alien plants such as *Eucalyptus* and *Acacia* species at various sites along these rivers using mechanical methods. Further interventions were implemented focussing on small-scale revegetation of multiple native species at the various sites (Van Zitters, 2021). Their key objectives were to restore riverbank stability, flood attenuation and aid in the recovery of the riparian zone (Van Zitters, 2021). Invasions by *E. camaldulensis* into riparian habitats are major threats to conserving natural biodiversity in the Western Cape. The species has been found to transform native riparian habitats in both the Western Cape as well as other parts of the world, by changing the composition, structure and functioning of these ecosystems (Tererai et al., 2013).

Although invasion ecology tends to focus on the larger and more conspicuous invaders such as aboveground plants, invasions also occur within the inconspicuous microfauna inhabiting soils (Ehrenfeld and Scott, 2017). Further impacts of invasive plants on native soil – and litter dwelling invertebrates as well as microfauna has not received much attention, despite their important role within ecosystem functioning (Wardle et al., 2004; Bardgett and Wardle, 2010; Decaëns et al., 2010; Eisenhauer et al., 2011). Invasive plants are also known to release allelopathic compounds into their environment through leaf litter and root exudes (Weidenhamer and Callaway, 2010; De Almeida et al, 2022), therefore altering soil properties and microbial communities, as well as impacting nutrient cycling and ecosystem processes (Callaway et al., 2004). *Acacia* and *Eucalyptus* trees cause changes in soil chemistry as well as alterations in soil and biogeochemical processes such as amount, timing as well as chemistry involved in local plant litter production and nutrient cycling. This can significantly alter native soil microbial communities and their functioning (Slabbert et al., 2014; Jacobs et al., 2020). They negatively affect natural rhizosphere microbes and beneficial symbionts by accumulating pathogens and suppressing natural microbial communities through allelopathic interactions with invasive symbionts (Callaway et al., 2008; Coats et al., 2014). This further alters linkages with belowground biota such as Collembola and their associated functions such as mineralization (Weidenhamer and Callaway, 2010; De Almeida et al., 2022). Collembola has also been found to disperse microbial and plant propagules within their digestive system while foraging, and have a very close relationship with soil microbial communities (Potapov et al., 2020). Invasive plants can also lead to thicker litter layers and altered temperature and humidity conditions, which, in turn, favour the proliferation of invasive invertebrate species, such as invasive Collembola, which thrive under these conditions (Simberloff, 2006; Liu et al., 2012).

This study investigated the effectiveness of restoration on belowground soil biodiversity after the removal of alien invasive plants by using Collembola as bioindicators, specifically between active and passive restoration sites along a riparian zone in the Western Cape, South Africa. Collembola (springtails) are found to be excellent indicators of soil biodiversity, as they are highly abundant and diverse, and include species that take up a wide range of ecological niches (Cassagne et al., 2003). They are sensitive to changes in their habitat and respond to a wide range of environmental and ecological influences (de Filho et al., 2016). The study compared rehabilitated, invaded, and, where available, native sites to determine how Collembola communities respond to these various habitat disturbances.

Overall, this study investigated the belowground impacts of various restoration treatments that were conducted in the Berg River catchment area. This was done by comparing Collembola communities between sites actively restored (by the removal of invasive plants and replanting of native vegetation) to sites that were passively restored (through the removal of alien invasive trees without the replanting of vegetation). These sites were also compared to uncleared sites with invasive alien vegetation.

Aims of the study

1) To compare native and invasive Collembola species richness and abundance between three rehabilitated sites within a riparian zone in the Fynbos biome of the Western Cape. Specifically, Collembola communities were compared between active restoration sites, passive restoration sites, one native site and sites with invasive alien vegetation where no clearing attempts have been made.
2) To determine if Collembola species richness and abundance varied with plant status (i.e., native vs invasive plants) and abiotic environmental factors such as temperature, humidity and pH.
3) To determine if there were possible seasonal effects on Collembola species richness and abundance (although seasonal data was limited to one site).

Hypotheses

1) Species richness and abundance of invasive Collembola are higher in invaded sites than in restoration sites.
2) Species richness and abundance of Collembola differ significantly between different restoration treatments (i.e., active and passive restoration) as well as invaded sites.
3) Collembola species richness and abundance will be higher in sites with higher humidity.
4) Collembola species richness and abundance may be affected by plant invasive status, which could also be implied if there is a significant difference in assemblage composition, abundances or species richness of Collembola found among samples beneath native plants compared to samples beneath invasive plants.

Materials and Methods

Study sites

The Berg River flows in the north-eastern part of Cape Town in the Western Cape of South Africa (Tererai, 2012). It is described as a perennial river with high water levels. The banks are also frequently flooded in winter during May and until September (Tererai, 2012). Sites selected for this study are in various stages of rehabilitation following the removal of alien invasive *Eucalyptus* trees (*E. camaldulensis*) (Table 1), which have previously been present for more than 50 years (Tererai, 2012). The Berg River Improvement program (Horn, 2020), in conjunction with the Western Cape Government, has been removing these trees and other alien invasive plants as well as actively restoring habitats in some of the sites along the Berg River to improve river flow and quality.

The area has a mainly Mediterranean-type climate, the annual rainfall is about 550 mm and temperatures range from 11°C to 22°C during winter months (Tererai, 2012). Soil and rock types in the catchment area include dominating sandstone and quartzites belonging to the Cape supergroup, which typically have nutrient-poor lithologies (Midgley et al., 2003). The three main study sites that were sampled during this study, Bosplaas, Grootverlangen and Kleinplasie (hereafter called farms) are located along the Berg River (Fig. 1, Table 1), and they represented areas where long-established invasions have occurred and where invasive species have been removed (Tererai, 2012). Each farm contained two smaller rehabilitation sites: one site that has been actively restored (i.e., native plant communities were planted and re-established following removal of alien invasive vegetation) and one site with passive restoration (i.e., no treatment after removal of alien invasive vegetation). In addition, one native site (remnant native riparian forest) was identified at Grootverlangen along the Berg River, where the area was actively conserved and maintained by a local farmer. This area was only sampled in 2020 and was flooded and inaccessible during 2021 sampling.

In 2021, severely degraded invaded riparian sites were selected as reference sites along the riverbeds, close to the sites (within a range of about 1-2 kilometres) where active and passive restoration was conducted. These degraded sites were used as a baseline to compare the restoration progress of restored sites. Invaded sites are located on the farms Lamolor next to Bosplaas, Bloekomsrus across the river from Grootverlangen, as well as Hephzibah farm next to Kleinplasie. A timeline of sampling (Fig. 2) and maps showing sampled sites (Figs. 3 to 7) are presented.

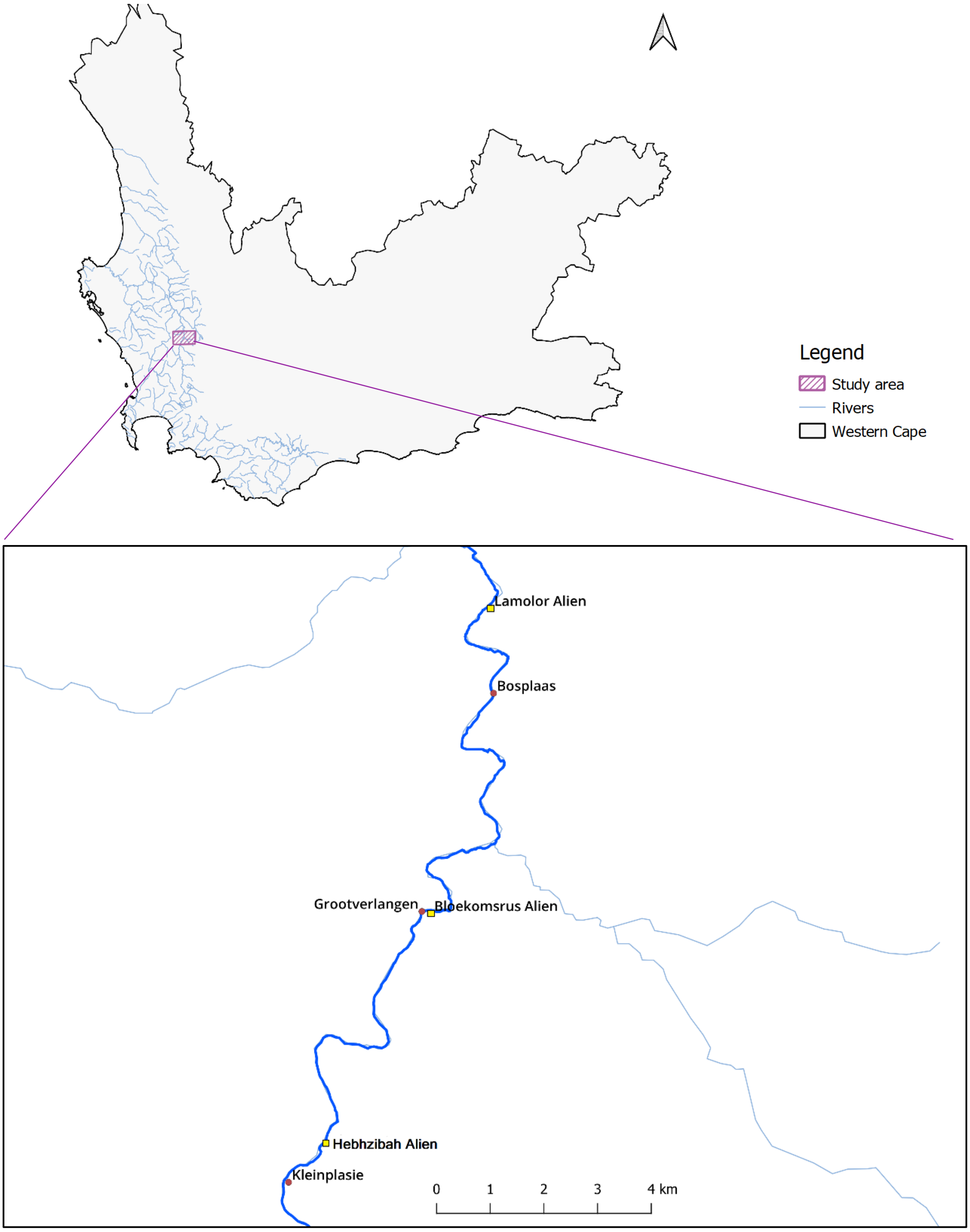
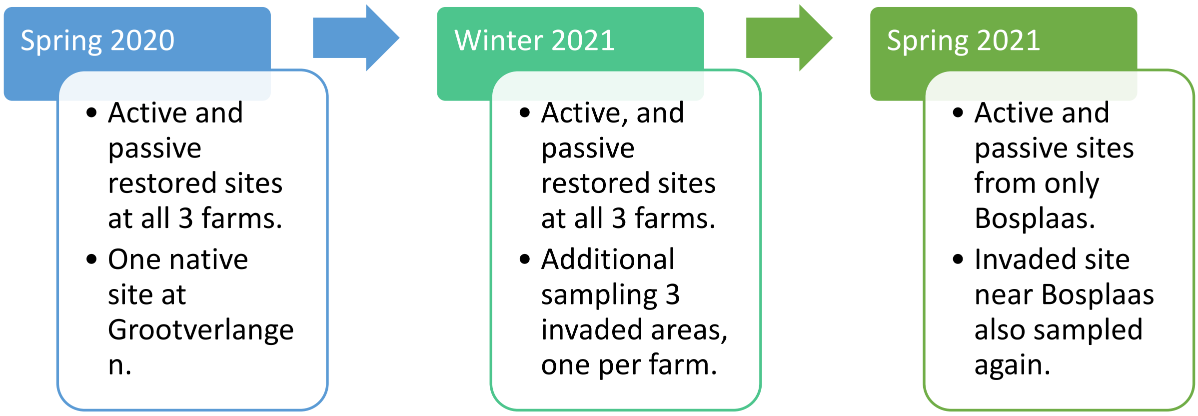


Fig. 1: Map showing location of farms with study sites.

Table 1: Description of the selected study sites in the Berg River Catchment area, Western Cape, South Africa (Van Zitters, 2021).

Site	Location	Size (m^2)	Clearing Method	Start date of rehabilitation	Sampling events in this study
Kleinplasie	33°27'10.30"S 18°57'0.06"E	98 773	Fell and removed; fell and stacked burn.	23 Jun 2016	Spring 2020 - active and passive restoration sites. Winter 2021 – active and passive restoration sites, alien invasive site
Grootverlangen	33°24'27.31"S 18°58'36.28"E	5 023	Fell and removed	29 Sep 2017	Spring 2020 - native forest, active and passive restoration sites Winter 2021 - active and passive restoration sites, alien invasive site
Bosplaas	33°22'15.91"S 18°59'27.99"E	8 080	Fell and removed; fell and stacked burn.	19 Jun 2016	Spring 2020 - active and passive restoration sites Winter 2021 - active and passive restoration sites, alien invasive site Spring 2021 - active and passive restoration sites, alien invasive site



Spring 2020

- Active and passive restored sites at all 3 farms.
- One native site at Grootverlangen.

Winter 2021

- Active, and passive restored sites at all 3 farms.
- Additional sampling 3 invaded areas, one per farm.

Spring 2021

- Active and passive sites from only Bosplaas.
- Invaded site near Bosplaas also sampled again.

Fig. 2: Timeline of sampling events and sites sampled during in this study.

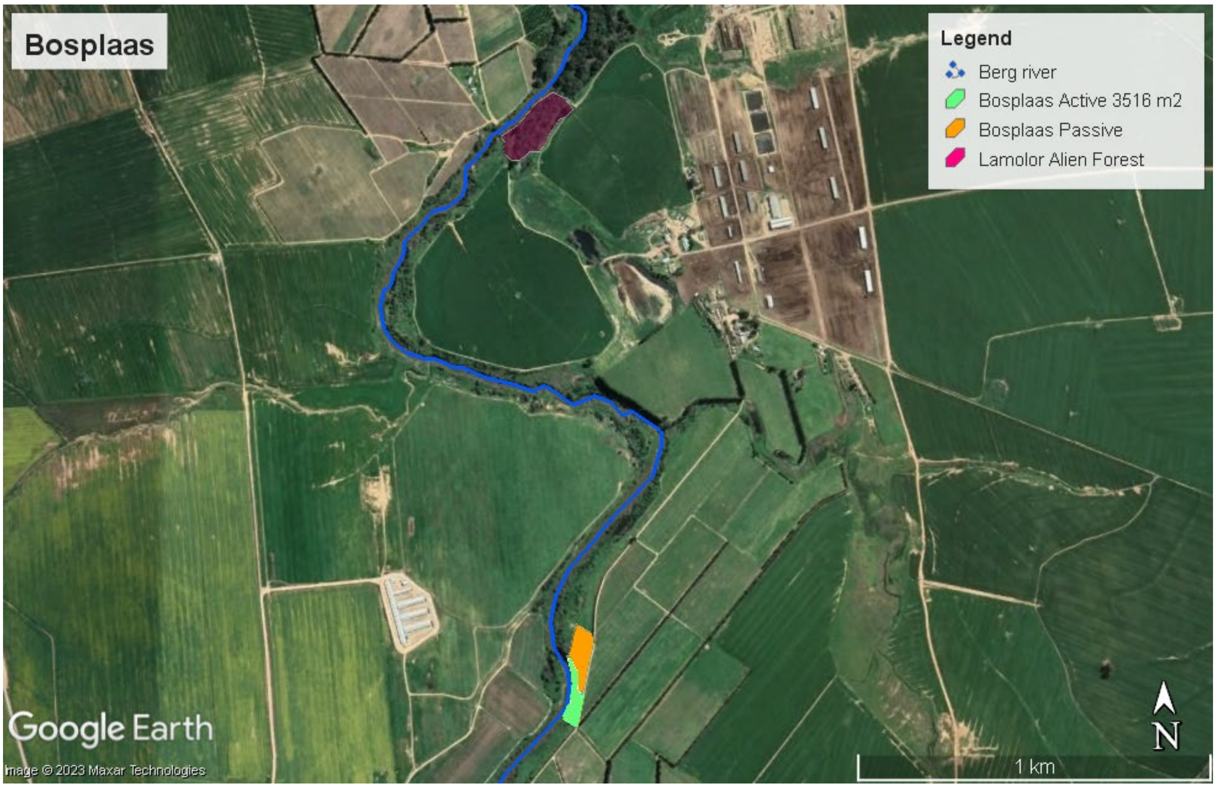


Fig. 3: Map showing restored (passive and active restoration) and invaded sites at the Bosplaas farm.

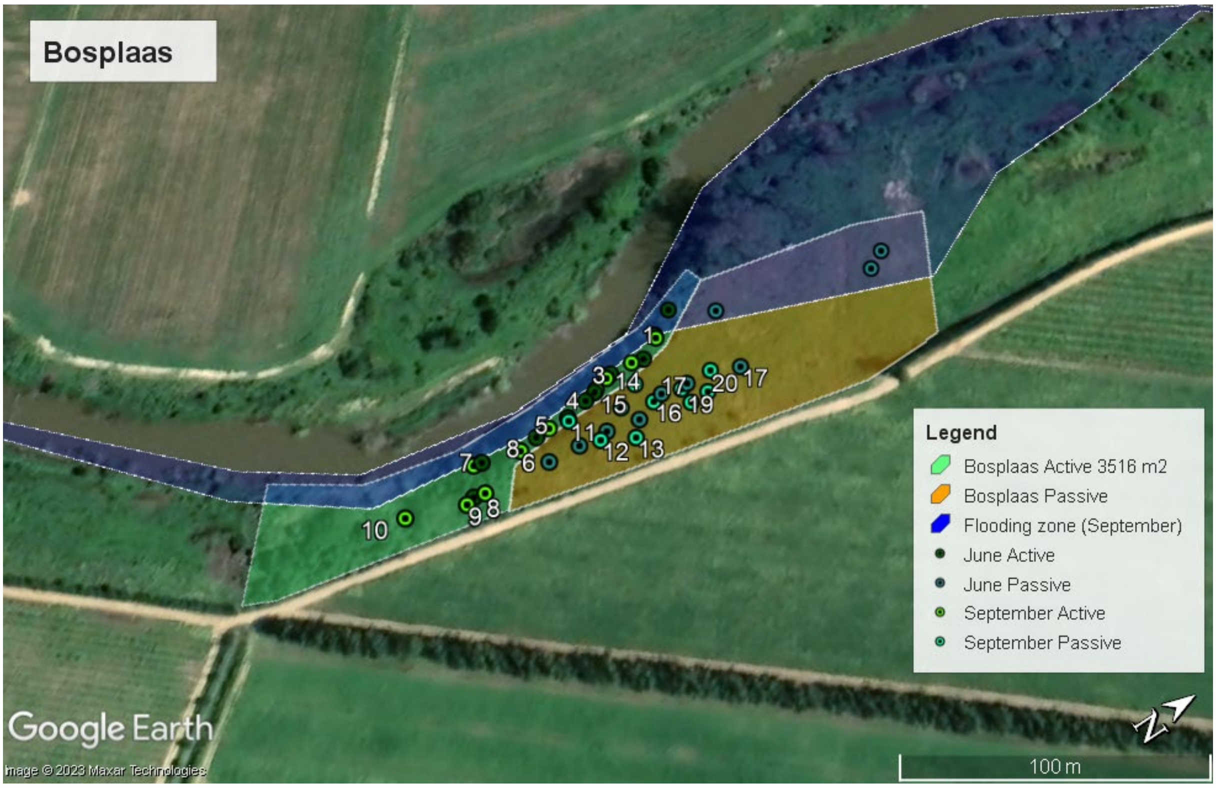


Fig. 4: Map of restored sites at the Bosplaas farm. Circles represent sampling points at different sampling times.

Fig. 5: Map of invaded sites at the Bosplaas farm. Circles represent sampling points at different sampling times.

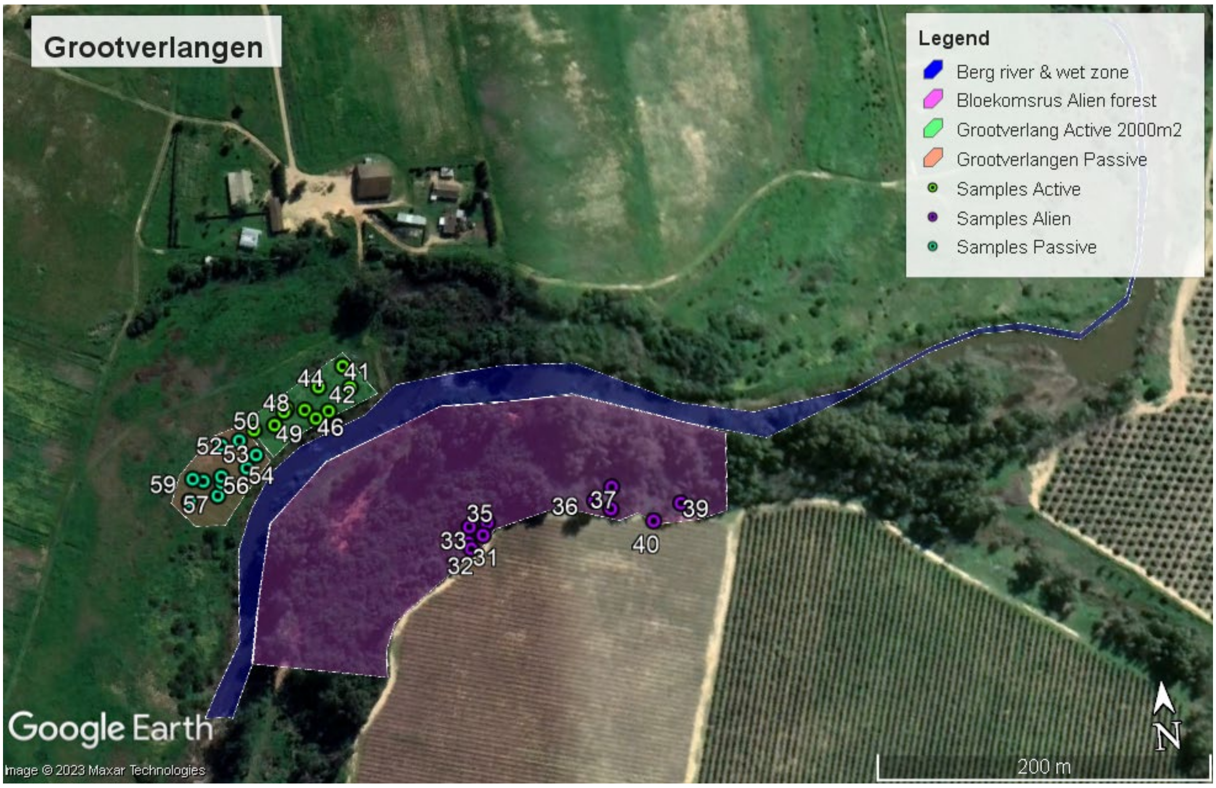


Fig. 6: Map of restored and invaded sampling sites at the Grootverlangen farm. Circles represent sampling points.

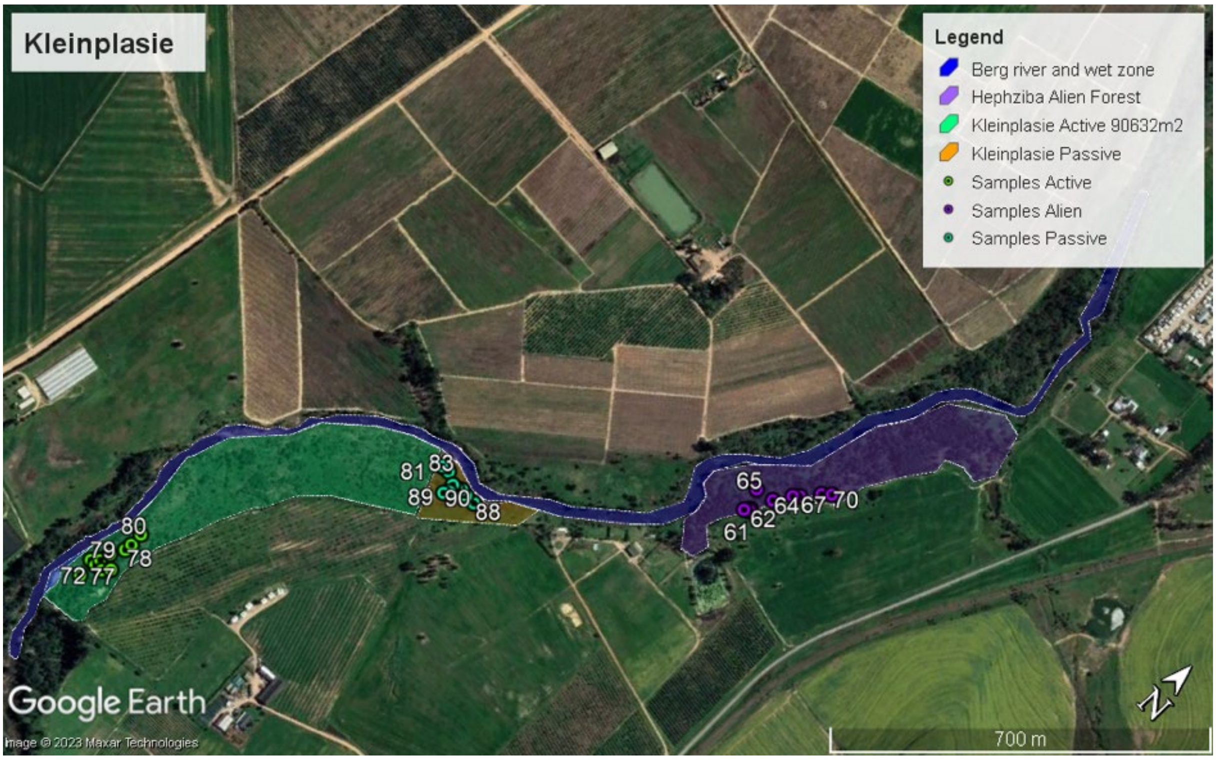


Fig. 7: Map of restored and invaded sites sampled at the Kleinplasie farm. Circles represent sampling points.

Sampling

Sampling was conducted in Spring (August 2020) and Winter (June 2021) at the farms Grootverlangen, Bosplaas and Kleinplasie, sampling also took place once more in September 2021 at Bosplaas sites (Fig. 3). The other sites could not be sampled again due to logistical constraints (i.e., time and costs). At each of the three farms, an alien invaded site was also sampled in 2021 (Table 1, Fig. 3). Invasive alien plants from the study sites and their invasion densities included 30% *Eucalyptus camaldulensis,* 10% *Acacia saligna* (Port Jackson), 50% *A. mearnsii* (Blackwattle), and about 2% of *Pinus* sp. including *P. radiata* (cluster pine) and *P. pinaster* (tall pine)*.* Flooding occurred during August 2021, causing some invaded sites to be left with no topsoil or plant litter, as they were situated well below flood lines (Fig. 8). In this study, the August and September sampling events will be referred to as Spring, while the June sampling event will be referred to as Winter (Fig. 1).

Fig. 8: Examples of invaded sites flooded during the study.

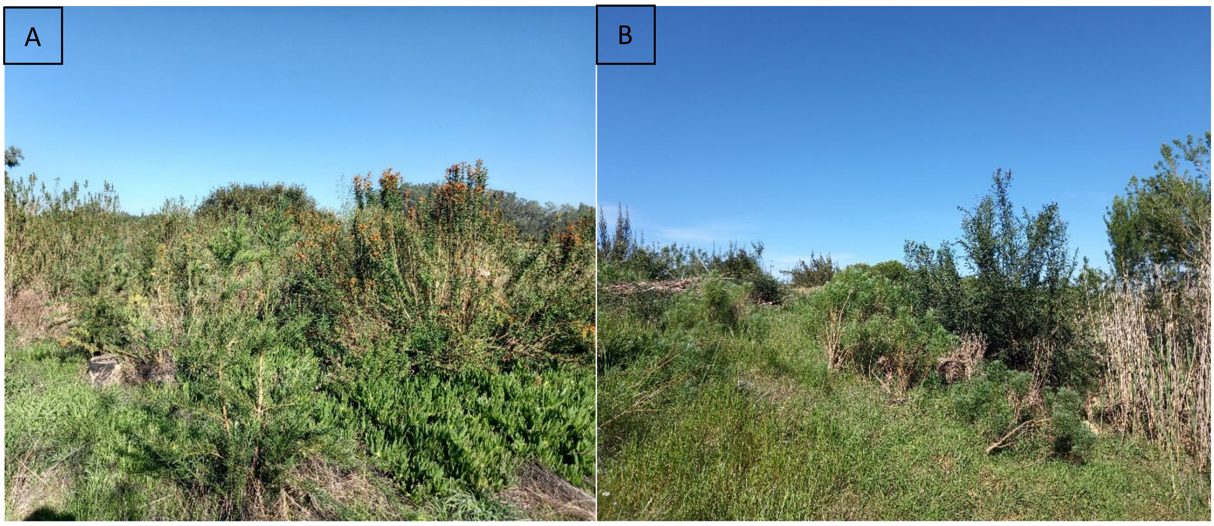


Fig. 9: Examples of an active restoration site, at Grootverlangen (A) and a passive restoration site (B), at Kleinplasie, during winter 2021. Both sites experienced some flooding before these photos were taken.

Soil samples were collected from each site using the following methods, to represent within-soil taxa which are more directly linked to the properties of the soil (Cassagne et al., 2003). At each location, 10 samples were collected from active restoration and 10 from the passive restoration sites. In 2021, this also included an additional 10 samples from a nearby invaded site. At each site, two transects were laid out at least 10 m apart. At each transect, five samples were taken 10 m apart by a 10 cm long yellow hand shovel, litter was also included with each sample and included by covering the top layer as scooped up by the shovel by hand before placing it into a container. At each sampling point, temperature and humidity were recorded using a handheld Vaisala temperature and humidity meter (Vaisala, Vantaa, Finland). At Grootverlangen, 10 samples were also taken from the native riparian forest (hereafter called the native site) in 2020. Each sample consisted of 1 L (year 2020) or 500 ml (year 2021) of the top 10 cm of soil and vegetation litter, sampling areas per sample also consisted of approximately 0.196 m^2 in 2020 and 0.090 m^2 in 2021. In 2021 samples were taken in smaller volumes due to high abundances observed in the 2020 sampling period and to reduce the time sorting and identifying specimens (due to time constraints). Samples were placed into a small square container with a lid. These were all placed in cooler boxes and transported back to the laboratory at Stellenbosch University. A total of 70 samples were taken in 2020 (including 10 samples from one native site), and an additional 180 samples (including additional invaded sites) in 2021. All samples were taken to the lab and extracted using Berlese-Tullgren funnels at the Department of Conservation Biology and Entomology, Stellenbosch University. Soil and litter were placed in a funnel on top of a layer of mesh (2mm) with a vial filled with 99.9% ethanol below (Fig. 10). The extraction method relied on drying of the soil from the top layer down (without light), this takes about seven days for the soil to completely dry. As Collembola are sensitive to desiccation (Kaersgaard et al., 2004), they will borrow deeper to find moisture and eventually fall through the mesh.

Across both years, each sample was taken close to (less than 0.5 m) a certain plant species and along with its litter, some invasive and some native. Plant species were recorded at each sampling point and categorized into invasive or native (referred to as “plant status”) by a botanist (S. Kritzinger-Klopper), as well as consulting plant data from Van Zitters (2021). This was also to include plant status as a parameter, due to some restored sites having remnant invasive plants, while some invaded sites had native vegetation.

Soil was collected at each site for chemical analysis following methods by Liu et al. (2012). Soil was collected randomly at two sampling points within each site and mixed into a single 450g sample (Liu et al., 2012). Soil samples used for chemical analysis were sampled with a 10 cm hand shovel, after litter has been cleared, as soil samples do not include plant litter. Each sample was dug to about 10 cm deep in an approximately 10 cm^2 sized hole, before being placed into a 450g ziplock bag. Soil samples were further analysed by a commercial service provider (Bemlab Pty. Ltd., Strand, South Africa). Soil chemical analysis was performed according to standard methods at BemLab, as was also highlighted in Kamutando et al. (2017). The pH was measured in saturated soil extracts (SSE). Total nitrogen (N) and carbon (C) were quantified through combustion at 1350°C. Total phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were extracted using $HCl\text{-}HNO^3$ after combustion at 550°C for 3 hours, and then measured using inductively coupled plasma optical emission spectrometry (ICP-

OES). Ammonium (NH^4) and nitrate (NO^3) were extracted with 2M potassium chloride (KCl) and diluted before analysis with a flow injection analyzer (FIA). Detailed soil characteristics and related metadata are provided in Supplementary Dataset Table A1. Analysis gave results for soil type, pH, available H+ ion concentration (cmolc/ kg) as well as available K (mg/ kg), % Carbon, and exchangeable Na, K, Ca and Mg cations in the soil (Table A1).

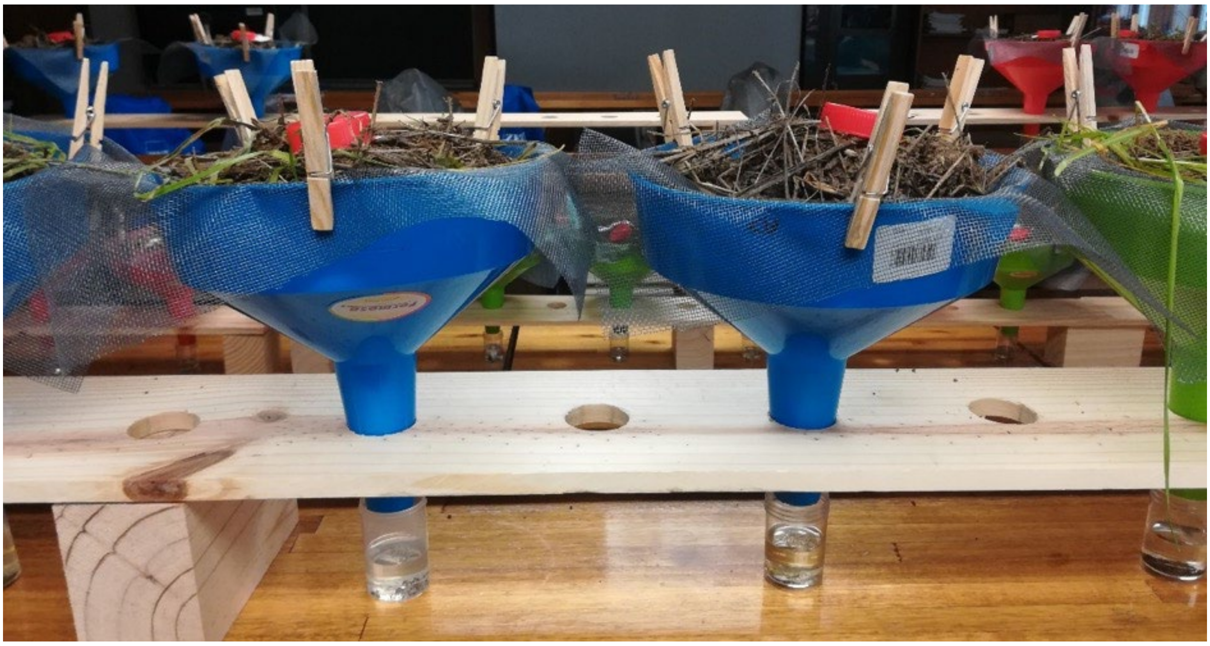


Fig. 10: Example of Berlese-Tullgren funnel setup used in this study.

Collembola Identification

Collembola were sorted into morphospecies (hereafter called species) using a Zeiss Stemi 305 stereo light microscope (Zeiss, Oberkochen, Germany). The morphospecies approach is widely used in describing the springtail fauna in South Africa, as many Collembola species are still undescribed (Janion-Scheepers et al., 2020). Four specimens of each morphospecies were mounted onto microscope slides using standard methods (Hopkin, 2007). They were cleared of colour pigments in lactic acid and also, if necessary, using potassium hydroxide. The specimens were then mounted onto microscope slides using Marc Andre II mounting medium (Cunningham, 1972). Specimens were then observed using a Zeiss compound microscope (Leica, Wetzlar, Germany). Morphospecies were sorted and identified using available identification keys (Hopkin, 1997; Potapov, 2001; Hopkin, 2007; Fjellberg, 2007; Janion-Scheepers, 2022). Genera or species were further identified by C. Janion-Scheepers and M. Potapov.

DNA barcoding was used to confirm species identification. DNA barcoding employs a sequence diversity in short, standardized gene regions to aid species identification and discovery in large assemblages (Ratnasingham and Hebert 2007). The cytochrome c oxidase (CO1) gene is used as a primary barcoding sequence for species delineation of members of the animal kingdom (Hebert et al. 2003; Savolainen et al. 2005; Ratnasingham and Hebert 2007) and has been successfully used in Collembola species delimitation and for the identification of invasive species (Porco et al., 2012). A total of 190 specimens (95 from each year) were individually photographed, placed in 96% ethanol in a 95-well microplate. Specimens were then sent to the Canadian Centre for DNA Barcoding (Ratnasingham and Hebert, 2007). Standard DNA barcoding protocols were followed and successful sequences were used to further determine species identity where possible. Sequences were compared to the springtail sequences available through the Collembola of South Africa (COLSA) project on the Barcode of Life Data System (BOLD) V4 (www.boldsystems.org). These individuals were assigned to uniquely identifiable species based on morphological characteristics or a barcoding gap of at least 2.5% (Meyer and Paulay, 2005; Janion-Scheepers et al., 2018). From these results, species that had sequences present in BOLD from other distant regions such as Europe, were considered invasive species (Janion-Scheepers et al., 2018). Species that did not have sequences available on BOLD, or represented only from individuals collected across South Africa, were considered native.

Collembola diversity and density

Collembola diversity was assessed using species richness as well as diversity indices. The Shannon (Shannon and Weaver, 1949) and Simpson Diversity Indices were calculated using the *vegan* package. The Shannon-Wiener index value ranges between 0 and 5, while the Simpson index ranges between 0 and 1 (Simpson, 1949; Ortiz-Burgos, 2016). For both these indices, the higher the value, the higher the diversity.

To evaluate patterns over the two-year sampling period as a whole, community parameters were calculated at the site level. According to the methods in Potapov et al. (2023), sample abundances of all sites from both years were recalculated to density per square meter using sampling area as sampled in the two respective years (0.196 m^2 for 2020 and 0.09 m^2 for 2021).

Statistical analysis

Analysis was conducted to determine the effect of active and passive restoration on Collembola species richness and abundance compared to non-restored sites. All analyses were undertaken in R V.4.0.2 (R Core Team, 2022). Sample-based accumulation curves (SACs) were generated by the randomisation of sample order with 100 permutations using the *vegan* package (v. 4.2.1; Oksanen et al. 2019) to evaluate the adequacy of sampling effort of Collembola species in the active and passive restoration and native sites per farm from 2020 sampling (Figs A1-A3), as well as active, passive and invaded sites per farm from 2021 sampling (Figs A5-A7). This was done to determine whether sampling was done to completion. Sampling is deemed adequate when the SAC has reached a plateau beyond a particular number of samples, as no further species in an assemblage will be added (Gotelli and Colwell, 2001), whereas steep curves reflect low levels of sampling completeness.

Data were examined for normal distribution using the Shapiro–Wilks normality test, which was performed using the *dplyr* package. The data for all farms were found to be not normally distributed. Data from 2020 turned out to have some unequal sample sizes, the data from different farms and sites were also heteroscedastic (didn't have the same variances), therefore violating assumptions needed to perform an ANOVA or nested ANOVA analysis (Cottingham et al., 2005; Miller, 1986).

Statistical methods were further undertaken also similar to methods of Harta et al. (2021) for highly skewed distributions. Significant differences between the groups could have been evaluated by Tukey's pairwise posthoc test (Copenhaver and Holland, 1988). However, as the required assumptions were violated (normality tests $p < 0.05$), the nonparametric Kruskal-Wallis test was used with Dunn's pairwise post hoc test instead (Dunn, 1964). Significant differences in abundances/species richness and diversity indices between farms and study sites/treatments (i.e., active, passive, invaded and native) were tested using the Dunn's post hoc test for two groups (i.e. Active and Passive restoration) and the Kruskal-Wallis analysis where more than two groups were assessed (i.e. Active, Passive and Native/Invaded sites). Kruskal-Wallis is useful as it is non-parametric and does not require the data to fit a normal distribution (Dunn, 1964). Following Kruskal-Wallis, significant differences between groups were assessed using the Dunn test and the *dplyr* and *FSA* package (Dunn, 1964).

The Dunn post hoc test (Dunn, 1964) and Kruskal-Wallis tests were undertaken to test if the distributions between types of restoration practices have the same median, and if there is any significant difference between treatments (native site, active restoration, passive restoration and invaded sites) as sampled per year and site.

Collembola assemblages between sites (i.e. different restoration treatments) were further compared using community analyses (Cassagne et al., 2003; Liu et al., 2012). Collembola abundance data were converted to densities (m^2) prior to analysis, in order to compare all the data as sampled across the two years and to enable comparison with other studies, for example Potapov et al. (2023). Collembola densities were then square-root transformed to smooth the distribution (Clarke and Warwick, 2001; Enríquez et al., 2018), and therefore minimize the possible effects of very abundant species and to weigh common and rare morphospecies equally (Liu et al., 2012). Non-Metric Multi-Dimensional Scaling (NMDS) using Bray-Curtis transformed data (Bray and Curtis, 1957; Hoyle and Harborne, 2005) and the *vegan* package in R were used to visualize: 1) Collembola assemblages between different restoration treatments; 2) Collembola assemblages in native and invasive plants within each study site per year; and 3) compare Collembola assemblages per farm between restoration treatments and seasons. Points were plotted based on dissimilarity, thus the further apart, the more dissimilar the assemblages are. NMDS plots were accompanied by a stress value, indicating how well the plot visualized the data in the displayed dimensions. The lower the stress value, the better the plot is at visualizing the data. Stress values close to or ≥ 0.2 should be interpreted with caution and higher values may even render the plot unusable and ineffectual (Clarke, 1993).

A one-way Analysis of Similarity (ANOSIM) was undertaken using the *vegan* package to determine if there is a significant difference between Collembola assemblages in active, passive and invaded sites, as well as for differences among native and invasive plants (Clarke and Warwick, 2001). The resulting R value ranges in value between 0 and 1. The closer the R value is to 0, the more similar the community, and the more it approaches 1, the more dissimilar the community. The R-value can further be interpreted as adapted from Goss-Gouza (2015) with the following explanation: 0< R<0.25- no/weak dissimilarity, 0.25<R<0.5- somewhat dissimilar, 0.5<R<0.75- reasonable dissimilarity, and 0.75<R<1- strong dissimilarity (Goss-Souza, 2015; Jacobs, 2023).

To investigate further where specific species were significantly associated with each type of restoration, a multi-level pattern analysis was run using the *indicspecies* package, using mean abundance data to calculate an indicator value (IndVal) (Dufrêne and Legendre, 1997; Mouillot et al., 2002, De Ćaceres and Legendre, 2009). The indicator index value together with permutation tests (set to 9999 permutations) allowed the identification of different groups of restoration types and farms that were more strongly associated with observed Collembola species (De Ćaceres and Legendre, 2009). The resulting value ranges between 0 and 1. The greater the value, the greater the association between a certain species and restoration treatment. Species with IndVal values ≥ 0.7 (or 70%) will be considered indicative of a particular variable (Dufrêne and Legendre, 1997; Mouillot et al. , 2002).

To determine the effect of environmental parameters including soil pH, temperature and humidity as well as plant status per restoration type on Collembola, a Generalized Linear Model (GLM) was used with a quasi-Poisson distributed errors and log link function using the *MASS* package (Ver Hoef and Boveng, 2007). Species richness and abundance were included as response variables, and the above-mentioned environmental characteristics as explanatory variables. A Tukey HSD post-hoc test was run using multcomp package to differentiate which groups are significantly different. GLM results were plotted using *ggplot2* to visualize the effects of variables such as temperature and humidity on Collembola richness and abundance. Regression lines and confidence interval were fitted according to model parameters.

Further analyses were undertaken to investigate correlations and effects of environmental parameters, using Spearman's correlation matrix and the *psych* and *corrplot* packages (Revelle, 2004; Simko, 2021). Correlation plots were generated from environmental data. According to Touhidul Islam (2021), the value of the correlation coefficient (r) lies between -1 to +1. When the value of r=0 there is no relation between the variables, if r=1 variables are perfectly positively correlated, and if r=-1 they are perfectly negatively correlated. When the r value is 0 to 0.30 there is a negligible correlation. If r is 0.30 to 0.50 there is a moderate correlation, and values of r=0.50 to 1 are highly correlated.

A Principal Component Analysis (PCA) was conducted on all farms and environmental variables to assess their relationship and their contribution to restoration group differentiation. This can help visualize the effects of different environmental parameters as well as seasonal effects and plant status and their contribution to variance in the data. PCA places all samples in a space defined by two or more axes (Kassambara, 2017). All environmental variables are included on the same axis in space, enabling the comparison between variables and finding to what extent these variables were responsible for differences between samples.

Results

Collembola species richness and abundance

A total of 77,880 individual Collembola specimens and 34 species from 12 families were identified from 250 samples (Table 2). Out of the 34 species identified, 14 were found to be native to South Africa, while 20 were alien species (Table 2). The Order Entomobryomorpha consisted of 16 species from two families, the order Neelipleona of one species. Poduromorpha of 10 species from five families, and the order Symphypleona of nine species from six families. Of the 190 specimens submitted, 88 were successfully barcoded (Fig. A1, A2, Table A2). These include 19 morphospecies from eight families. Example images of successfully barcoded Collembola are shown in Fig. A1, with all successfully barcoded families also shown in Fig. A2. Species accumulation curves for active, passive, native and invaded treatments either reached or approached an asymptote for spring 2020 (Fig. A3, A4) except for Kleinplasie (Fig. A5), as well as for all farms in winter 2021 (Fig A6, A7, A8) and Bosplaas in spring 2021 (Fig. A9)

From all Collembola sampled over two years as well as two seasons in the second year, spring 2020 had the highest density of native Collembola present during the study (Fig. 11,12,13 Table A4) with about 1,250-2,500 individuals per square meter on average, although densities of native species were much lower than that of invasives. Bosplaas farm (Fig. 11) had overall the most native Collembola present when compared to the other two farms (Table A5, Kruskal-Wallis chi-squared = 14.626, df = 2, p-value < 0.005), and it was also found that spring 2021 in the Bosplaas farm had higher densities of native Collembola compared to spring 2020 in the Bosplaas farm (Fig. 11, Table A6, Kruskal-Wallis chi-squared 8.2584, df = 2, p<0.05). Active sites at all farms in winter 2021 had higher densities of Collembola when compared to the invaded sites (Fig 11, 12, 13, Table A9, Kruskal-Wallis chi-squared = 16.634, df = 2, p-value = 0.0002444, p<0.05), with maximum densities ranging between about 12 000 and 12 500 individuals per m^2.

When comparing all alien and native Collembola among all treatments and all farms, there were no differences in densities between active and passive restoration (Table A7). However, in spring 2020, both treatments differed significantly from the native site (Fig. 11,Table A8, Kruskal-Wallis chi-squared = 14.976, df = 2, p<0.005) and in Winter 2021, mean Collembola densities differed significantly between active and invaded sites (Table A9, Kruskal-Wallis chi-squared = 16.634, df = 2, p<0.0005). There was no significant difference in abundance between farms or types of restoration neither in Spring 2020 (Table A10, Kruskal-Wallis chi-squared = 2.108, df = 2, p-value = 0.348, p>0.05), nor in Winter of 2021 (Table A11, Kruskal-Wallis chi-squared = 1.8627, df = 2, p-value = 0.394, p>0.05).

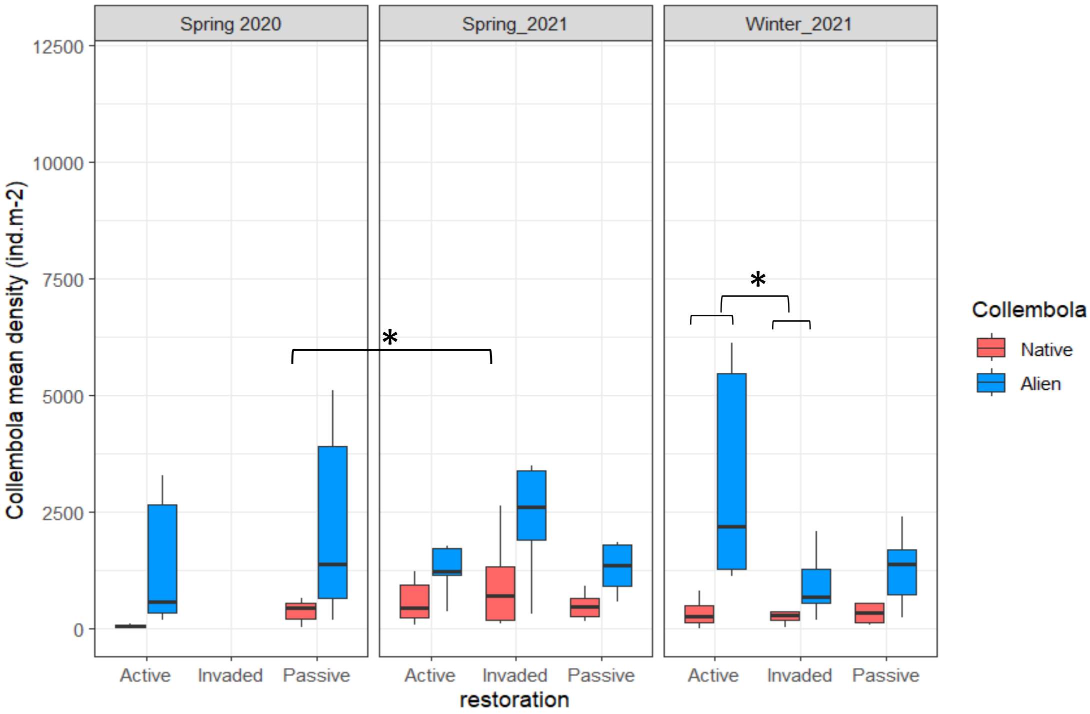


Fig. 11: Mean native and alien Collembola densities (ind.m^{-2}) in active and passive restoration sites at Bosplaas in Spring 2020, Winter 2021, and Spring 2021, also including invaded sites in winter 2021 and spring 2021. Error bars indicate standard error. Asterisks indicate significant differences between groups (P < 0.05) according to the results of a Dunn test.

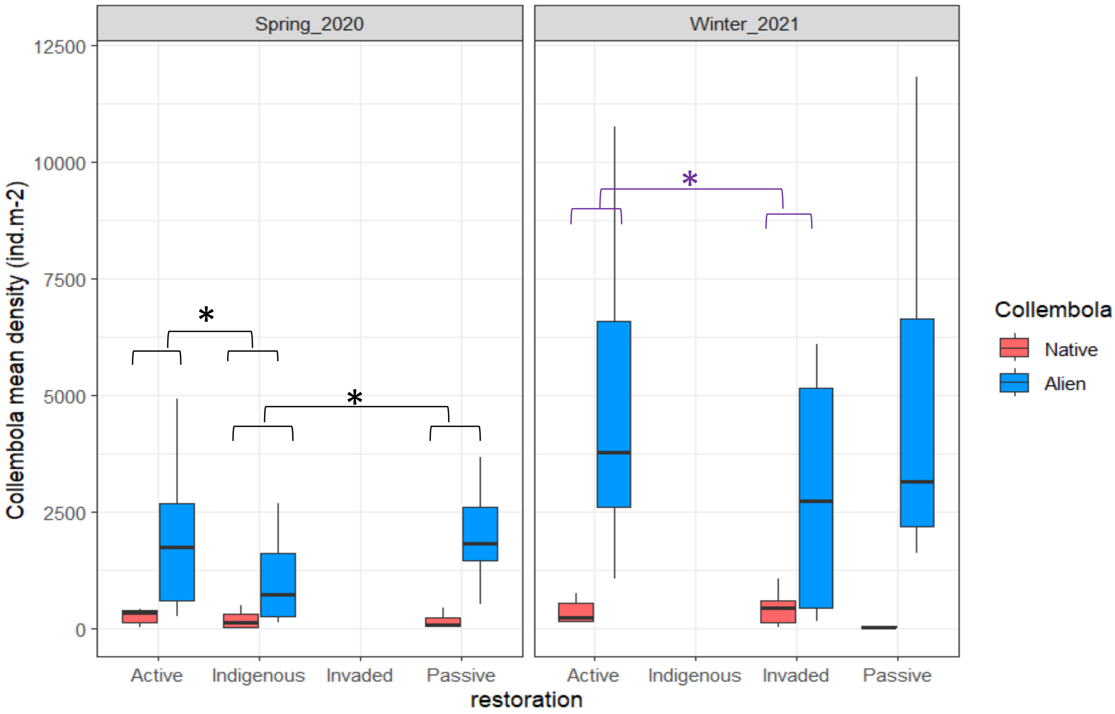


Fig. 12: Mean native and alien springtail densities (ind.m^{-2}) at Grootverlangen, with an additional native site (indicated as “Indigenous”) in Spring 2020 and an invaded site added in Winter 2021. Error bars indicate standard error. Asterisks indicate significant differences between groups (P < 0.05) according to the results of a Dunn test.

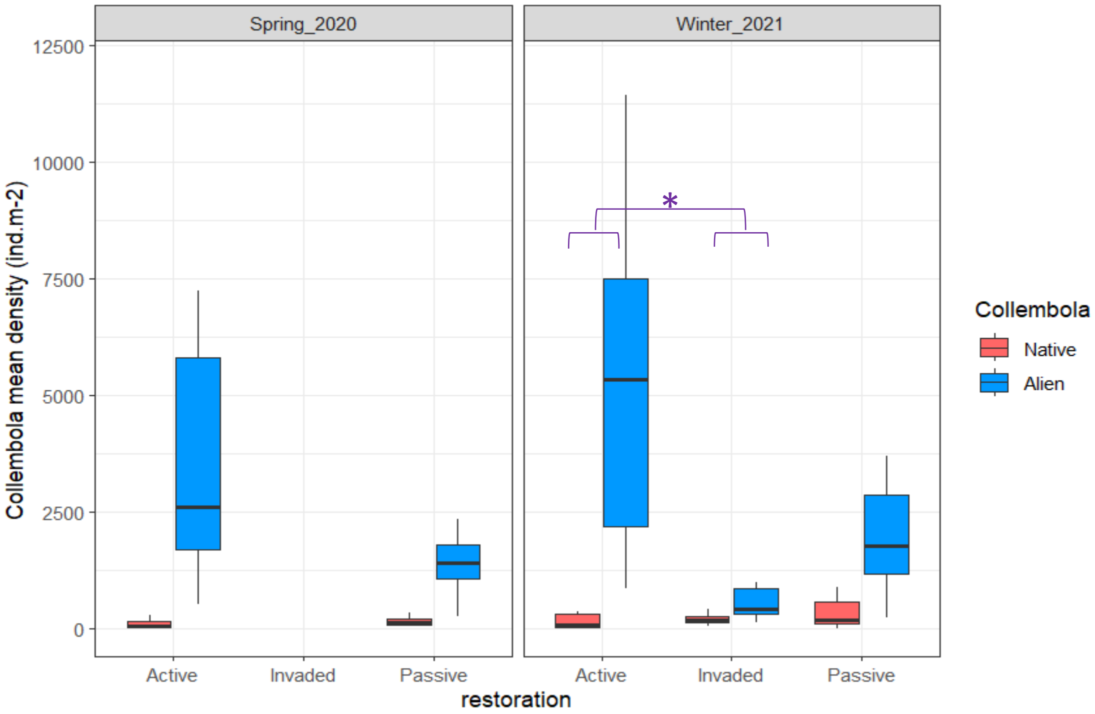


Fig. 13: Mean native and alien springtail densities (ind.m^{-2}) at Kleinplasie, with an additional invaded site added in Winter 2021. Error bars indicate standard error. Asterisks indicate significant differences between groups ($P < 0.05$) according to the results of a Dunn test.

Table 2: Collembola species collected in this study, as well as their alien/native status.

Order	Family	Species	Status
Entomobryomorpha	Entomobryidae	*Entomobrya* cf. *atrocincta*	Alien
		Entomobrya sp. 1	Native
		Entomobrya sp. 2	Alien
		Entomobrya sp. 3	Alien
		Entomobrya sp. 4	Alien
		Entomobrya sp. 5	Alien
		Lepidocyrtus sp. 1	Alien
		Lepidocyrtus sp. 2	Alien
		Sinella sp.	Native
	Isotomidae	*Ballistrura schoetti*	Alien
		Cryptopygus sp.	Native
		Folsomides sp.	Native
		Hemisotoma sp.	Alien
		Isotomorus sp.	Alien
		Parisotoma sp. 1	Native
		Parisotoma sp. 2	Native
Neelipleona	Neelidae	*Megalothorax* sp.	Alien
Poduromorpha	Brachystomellidae	*Brachystomella* sp.	Alien
		Setanodosa sp.	Native
	Hypogastruridae	*Austrogastrura* sp.	Native
		Ceratophysella denticulata	Alien
		Hypogastrura cf. *manubrialis*	Alien
	Neanuridae	*Neanura muscorum*	Alien
		Neanuridae sp.	Native
		Pseudachorutinae sp.	Native
	Tullbergiidae	*Tullbergia* sp.	Native
Symphypleona	Arrhopalitidae	*Arrhopilates* sp.	Native
	Bourletiellidae	*Bourletiellidae* sp.	Alien
	Dicyrtomidae	*Dicyrtomina* cf. *ornata*	Alien
	Katiannidae	Sminthuridae *sp.*	Native
	Sminthurididae	*Sphaeridia* sp.	Alien
	Sminthuridae	*Sminthurinus* cf. *elegans*	Alien
		Sminthurinus sp. 1	Native
		Sminthurinus sp. 2	Alien

In Spring 2020, Shannon and Simpson index of Collembola diversity were significantly higher in active and passive restoration sites than in the native site (Fig. 14).

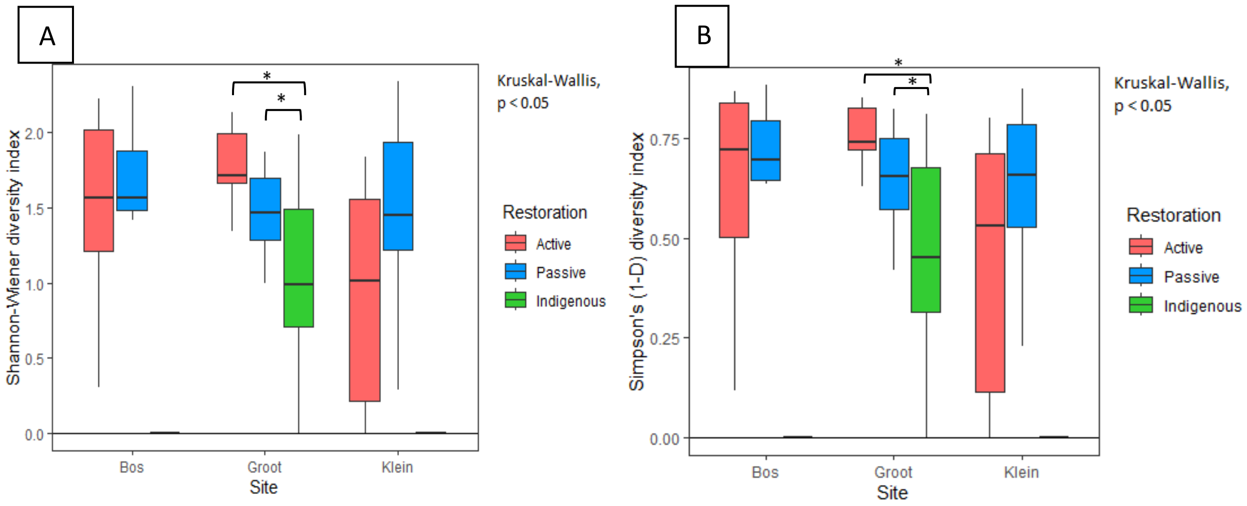


Fig. 14: Shannon's (A) and Simpson's (1-D) (B) diversity indexes for Collembola between different types of restoration (active, passive, native) and farms in spring 2020. One native site is included for the Grootverlangen farm only. Error bars indicate standard error. Asterisks indicate significant differences between groups (P < 0.05) according to the results of Dunn test.

In Winter 2021, Shannon index of Collembola diversity was significantly higher in active and passive restoration sites than in invaded sites at Bosplaas and Grootverlangen farms, and significantly lower in active restoration sites than in invaded sites at Kleinplasie farm (Fig. 15). Simpson index followed similar trends, except at Grootverlangen farm where it was significantly lower in passive than in active and invaded sites (Fig. 15).

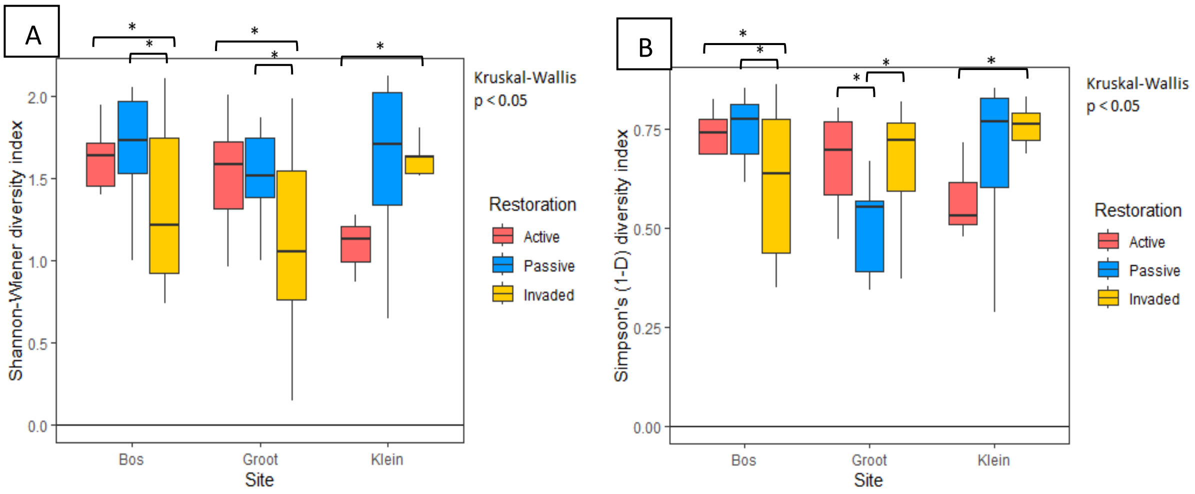


Fig. 15: Shannon's (A) and Simpson's (1-D) (B) diversity indexes for Collembola between different types of restoration (active, passive, invaded) and farms in Winter 2021. Error bars indicate standard error. Asterisks indicate significant differences between groups (P < 0.05) according to the results of Dunn test.

Collembola communities according to restoration types and plant status

All NMDS ordinations between Collembola communities according to plant status and restoration treatment had a stress value less than 0.2 in Spring 2020 and Winter 2021 (Fig. 16). In Spring 2020, communities associated to native and invasive plants had a strong dissimilarity at Grootverlangen (R=0.513), while communities between types of restoration were less dissimilar (R=0.2685) (Fig. 16A). At Kleinplasie, there was a higher between-group variation between types of restoration (R=0.4359) (Fig. 16C). At Bosplaas, communities regarding plant status were somewhat dissimilar (R>0.25), and communities between different restoration treatments were less dissimilar (Fig. 16E).

In Winter 2021, Collembola communities between native plants and invasive plants were found to be somewhat dissimilar (R>0.25) at Kleinplasie (Fig. 16B), but had no or a weak dissimilarity at the Bosplaas site (R<0.25) (Fig. 16F). Communities at the Grootverlangen site had a negative R value, which also implies there was a weak, non-significant dissimilarity between plant status and Collembola communities at that specific site. This could also be due to a lack in equal amounts of native and invasive plant data sampled among sites, especially at Grootverlangen (Fig. 16AB), where sampling was done near very few native plant species.

Type of restoration gave strongly significant results for all three sites, with R values between 0.4 and 0.5, indicating that there is a very high between group variations (Fig. 16). These results also gave very low p-values, even less than 0.005, showing highly significant dissimilarities in the compositions between these communities among different types of restoration.

Collembola communities according to years and seasons

NMDS plots for each site for both seasons had a stress value higher than 0.2. ANOSIM test results did show significant dissimilarities, with R values between 0.4 and 0.5, indicating a very high between-group variation (Fig 17,18,19). ANOSIM results also showed that communities had no dissimilarities or very weak dissimilarities between year and season for all three sites, which could have influenced these stress values. Grootverlangen Collembola communities over two years showed some significant dissimilarities between restoration sites, with some dissimilarity between restored sites and invaded sites (Fig 17). Kleinplasie had the most dissimilarity between treatments from all other sites (Fig. 18). Overall, there were significant results for all three sites, with R values between 0.4 and 0.5. These results also gave p-values less than 0.05, showing significant dissimilarities in the compositions between these communities among different types of restoration as well as invaded sites.

Indicator species

From the multi-level pattern analysis using combined replicate abundance data from each sampling year, no species were found to reach the 0.7 (70%) indicator value, to be considered indicative of any location, site or restoration type (Table 3) or any combination of variables. When repeated for Bosplaas seasonal data, one species, *Sminthurinus* cf. *elegans,* was found to be indicative of active and passive restoration treatments for both winter and spring at the Bosplaas site (Table 4).

Environmental parameters

The soils at invaded sites exhibited a generally slightly lower pH compared to restored sites, with pH values ranging from 4.3 to 4.7 for invaded sites. In contrast, the pH at restored sites ranged from 4.9 to 5.4 (Table A1). Soils in invaded sites were all loam, while in restored sites, they had a sandier texture and consisted of sand or loam, with significantly higher H^+concentrations and therefore higher acidity (Table A1). For Spring 2020, the PCA based on environmental factors showed that sites were significantly different between the three types of restoration, namely active, passive and native sites (Fig. 20; total inertia: axis 1 = 38.6%, axis 2 =24.5%). The first axis of the PCA was associated to temperature and plant status, and the second axis was associated to site and soil pH. Thus, active restoration was characterized by slightly less acidic soil. Collembola distributions in active sites were strongly affected by plant status. In contrast, passive sites were characterized by slightly lower pH, as well as higher temperatures, while plant status was also a significant factor in these sites. Collembola in the natural site was characterized by slightly higher humidity and lower temperature and the communities found here were localized within this one site.

Similarly, for Winter 2021, the PCA showed that sites under different restoration types were clearly separated (Fig. 21; total inertia: axis 1 = 43.9%, axis 2 =26.3%). The first axis was associated to temperature and humidity and the second axis to plant status and soil pH. In both cases, plant status appeared to be a significant factor explaining differences between sites. Here, active sites were also characterized by higher soil pH. Passive sites were characterized by slightly lower pH, with varying temperature and humidity. Communities in active and passive sites were characterized by higher soil pH and were strongly affected by plant status in comparison to invaded sites, which were characterized by lower soil pH and higher humidity. Both active and passive sites have a wide range of temperature and humidity conditions. Communities in invaded sites are exposed to a much narrower range of temperature and humidity.

Additionally, results of PCA using data of both years combined are presented in Fig. A10. Here, communities were separated across a gradient, with active sites and the one native site being distinguished by different plant status and higher soil pH, higher humidity and lower temperatures, as well as seasonal differences. Passive sites were also strongly influenced by temperature and humidity conditions. Invaded sites were less influenced by plant status and characterized by lower pH, higher humidity and lower temperatures. “Site,” which in this case referred to farms caused a negligible difference; effects of different farms as well as possible associated differences could therefore be ruled out in comparison to environmental effects. For example, although Grootverlangen differed somewhat in terms of alien plant removal practices and didn’t have the additional “stack and burn” treatment as the other two farms (Table 1), it didn’t cause any notable effects on Collembola species distributions.

For the Spearman’s correlation matrix, some correlations were observed (see Figs 22-23). In 2020 a strong positive correlation was observed between species richness and mean temperature, with a value of 0.68 (Fig. 22), also a strong negative correlation between humidity and mean temperature, with a value of -0.64 (Fig. 22). The 2021 data had a moderate negative correlation between species abundance and mean temperature (Fig. 23). There was also a moderate negative correlation between mean temperature and pH.

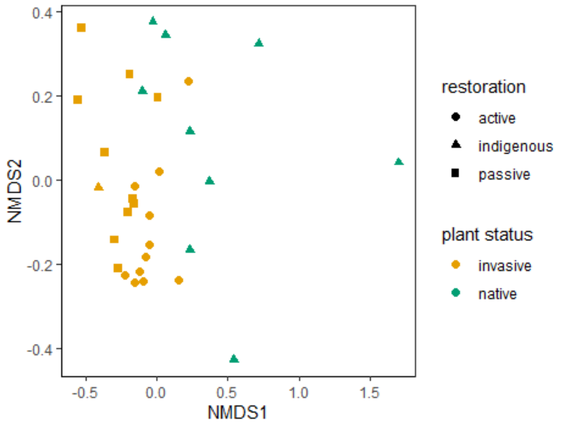
According to the GLM results, environmental effects, namely humidity and temperature, also had some significant effects on Collembola species richness and abundances for both 2020 and 2021 data. For species richness between active, passive and invaded site treatments as a factor, humidity had significant effects only within invaded sites (Fig. 24A, Table A12). For species abundances, there were significant negative values for both passive and invaded sites and mean humidity (Table 13B). There was however no effect for plant invasive status as a factor of humidity on Collembola species richness or abundances (Tables A14B, A15, Tukey p values >0.05). For collembola species richness with restoration as a factor of temperature, passive restoration was found to have some differences for temperature (Table A16B), while species richness and abundances in invaded sites were significantly affected by temperature (Table A17B, Figs 24C, D).

Spring 2020

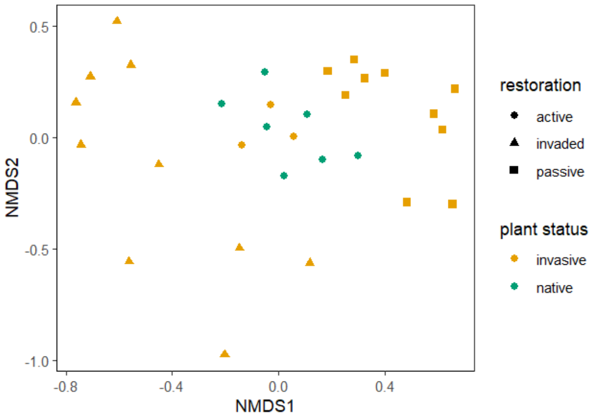
Winter 2021

Grootverlangen

A

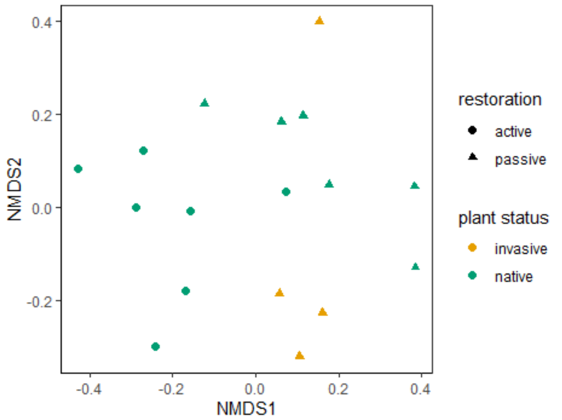


B

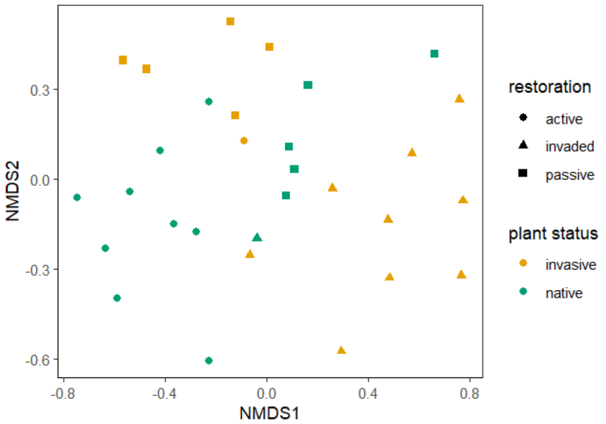


Kleinplasie

C

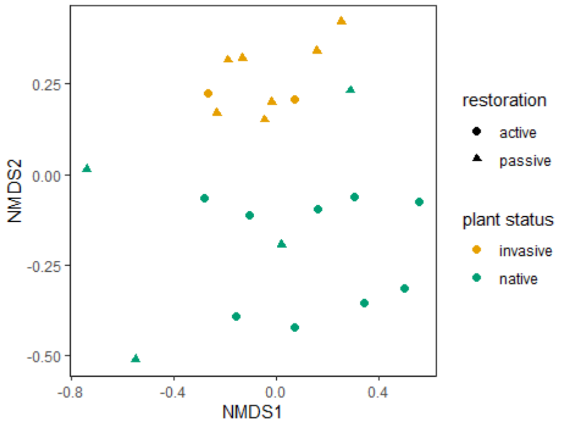


D



Bosplaas

E



F

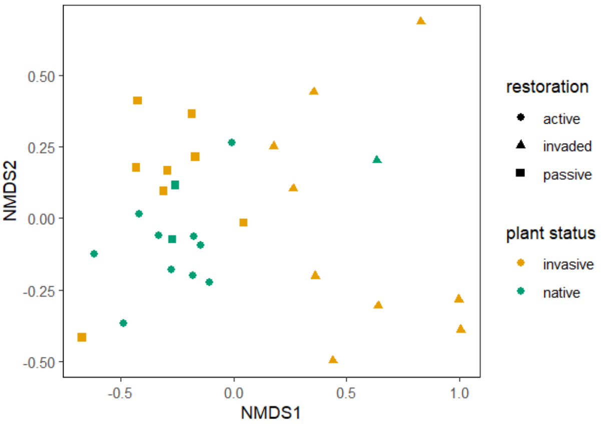


Fig. 16: NMDs on Collembola species densities according to restoration types and plant status in the sites sampled during Spring 2020. ANOSIM: Grootverlangen (A): Stress: 0.1552737, Plant status R: 0.513, p <0.005, Restoration R: 0.2685, p<0.05; Kleinplasie (C): Stress: 0.1651612, Plant Status R: 0.2445, p< 0.05, Restoration R: 0.4359, p<0.005; Bosplaas (E) Stress: 0.1726693, Plant status R: 0.3318, p< 0.005, Restoration R: 0.2047, p<0.05. NMDs results from Winter 2021, showing Anosim test results for both plant status and type of restoration, individually included as factors. Grootverlangen (B): Stress: 0.1488083, Plant status R: -0.2486, p> 0.05, Restoration R: 0.4587, p<0.005; Kleinplasie (D): Stress: 0.1771, Plant Status R: 0.1724, p< 0.05, Restoration R: 0.4416, p<0.005; Bosplaas (F): Stress: 0.1574, Plant status R: 0.0949, p< 0.05, Restoration R: 0.4587, p<0.005.

Grootverlangen

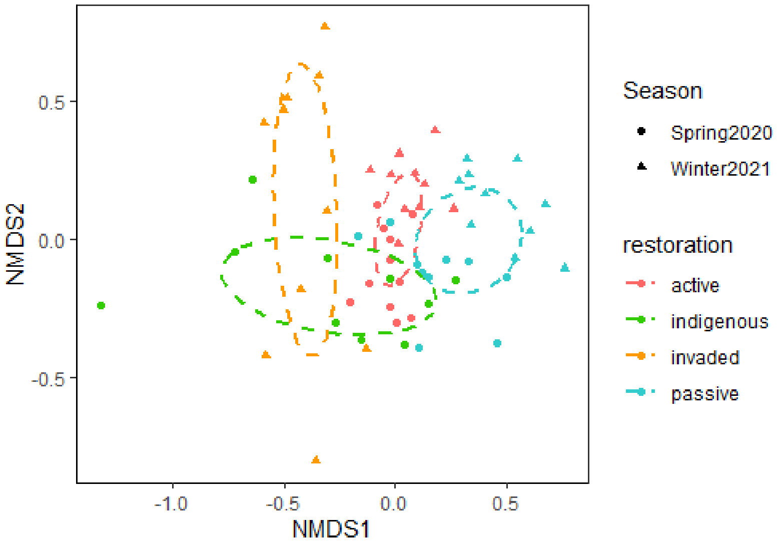


Fig. 17: NMDS of Collembola species densities at Grootverlangen showing active and passive restoration sites, native site and invaded sites over two years. Points represent relative densities, with different colours showing the restoration type and symbols the season of sampling. Ellipses show confidence for the different restoration groups. Stress: 0.2212, R: 0.4287, p<0.05.

Kleinplasie

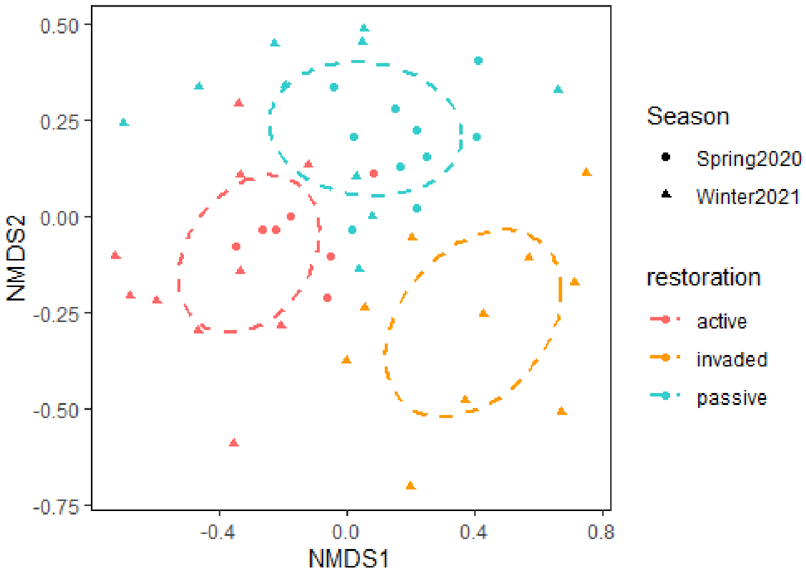


Fig. 18: NMDS of Collembola species densities at Kleinplasie showing active and passive restoration sites and invaded sites over two years. Points represent relative densities, with different colours showing the restoration type and symbols the season of sampling. Ellipses show confidence for the different restoration groups. Stress: 0.2018, R: 0.4411, p<0.05.

Bosplaas

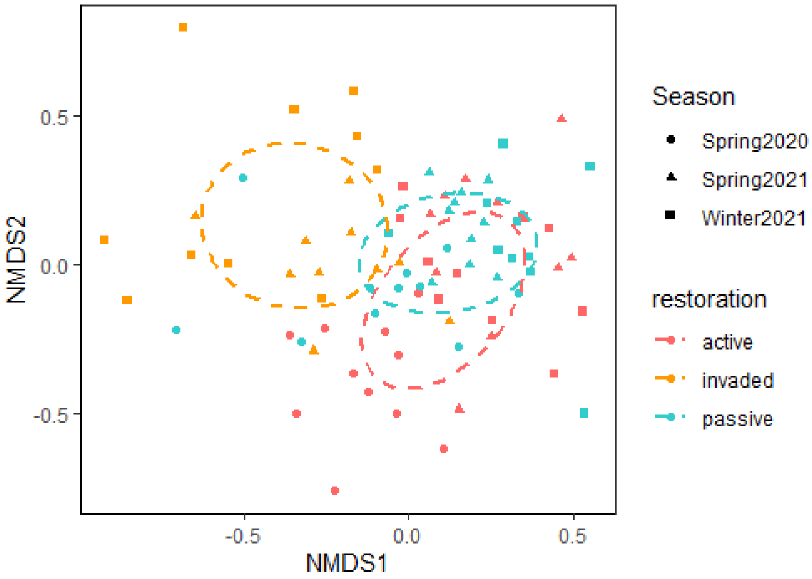


Fig. 19: NMDS of Collembola species densities at Bosplaas showing active and passive restoration sites and invaded sites over two years. Points represent relative densities, with different colours showing the restoration type and symbols the season of sampling. Ellipses show confidence for the different restoration groups. Stress: 0.2312, R: 0.2215, p<0.05.

Table 3: Indicator species and invasive status, using mean abundance data from all sites and types of restoration from 2020 and 2021 sampling (P < 0.05*, <0.01**, <0.001***).

	Indicator Species	**status**	**Stat(F-value)**	**p value**
2020				
Passive	*Entomobrya* sp. 2	Alien	0.427	0.004 **
	Sminthuridae sp.	Native	0.404	0.008 **
	Folsomides sp.	Native	0.335	0.041 *
Active+Passive	*Dicyrtomina* cf. *ornata*	Alien	0.504	0.001 ***
	Lepidocyrtus sp. 1	Alien	0.492	0.002 **
	Hypogastrura cf. *manubrialis*	Alien	0.481	0.002 **
	Isotomorus sp.	Alien	0.382	0.015 *
	Brachystomella sp.	Alien	0.366	0.024 *
	Lepidocyrtus sp. 2	Alien	0.333	0.035 *
2021				
Invasive	*Ceratophysella* sp.	Alien	0.346	0.004 **
	Sminthurinus sp. 1	Native	0.294	0.026 *
Passive	*Entomobrya* sp. 5	Alien	0.328	0.003 **
Active+Invasive	*Ceratophysella denticulata*	Alien	0.338	0.002 **
	Neanura muscorum	Alien	0.250	0.040 *
Active+Passive	*Brachystomella* sp.	Alien	0.603	0.001 ***
	Sminthurinus cf. *elegans*	Alien	0.569	0.001 ***
	Sphaeridia sp.	Alien	0.545	0.001 ***
	Hemisotoma sp.	Alien	0.531	0.001 ***

Table 4: Indicator species and invasive status, using mean replicate abundance data from Bosplaas winter 2021 and spring 2021 for all restoration types (P < 0.05*, <0.01**, <0.001***).

	Indicator Species	**status**	**Stat(F-value)**	**p value**
Winter (June)				
Invaded	*Hypogastrura* cf. *manubrialis*	Alien	0.537	0.001 ***
	Ceratophysella denticulata	Alien	0.527	0.001 ***
	Austrogastrura sp.	Native	0.374	0.029 *
passive	*Sminthuridae* sp.	Native	0.302	0.046 *
active+invaded	*Brachystomella* sp.	Alien	0.386	0.006 **
active+passive	*Sminthurinus* cf. *elegans*	Alien	0.778	0.001 ***
	Sphaeridia sp.	Alien	0.643	0.001 ***
	Hemisotoma sp.	Alien	0.532	0.001 ***
	Dicyrtomina cf. *ornata*	Alien	0.352	0.015 *
invaded+passive	*Tullbergia* sp.	Alien	0.395	0.009 **

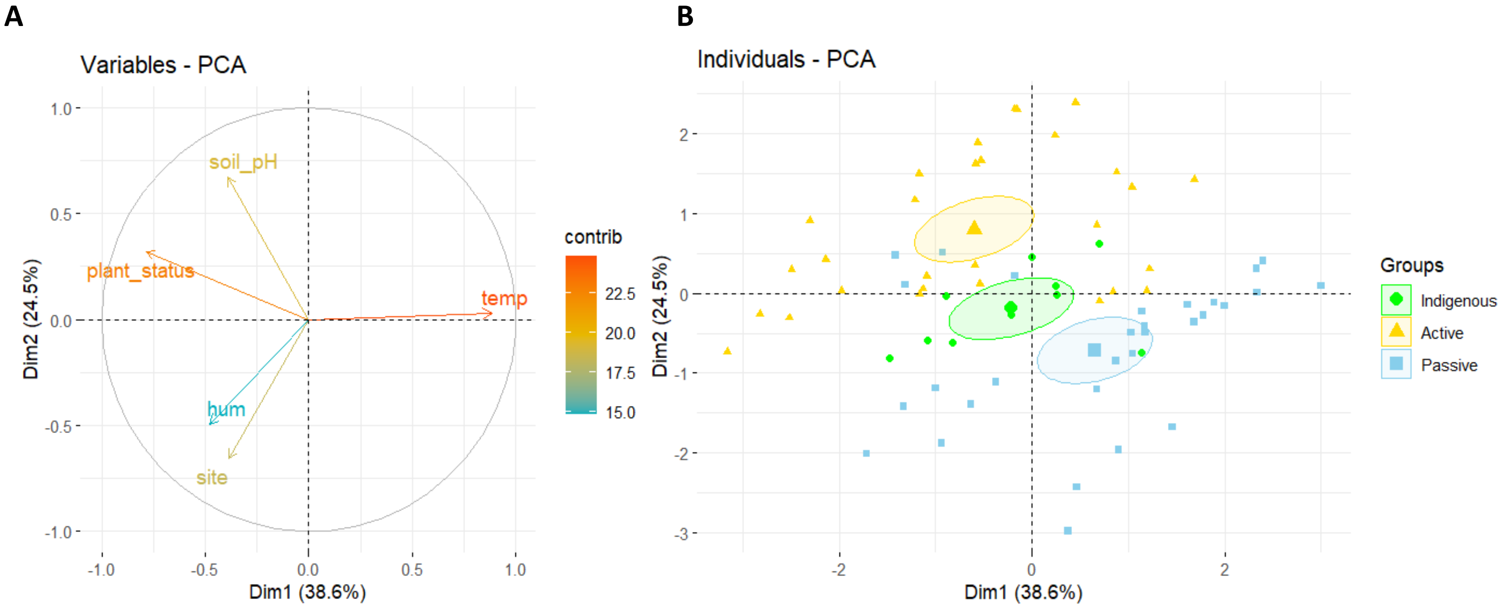


Fig 20: PCA on environmental variables from all farms during Spring 2020. A) Variables are represented by arrows and coloured according to their contribution. B) Ellipses represent 95% confidence estimates for the different restoration groups.

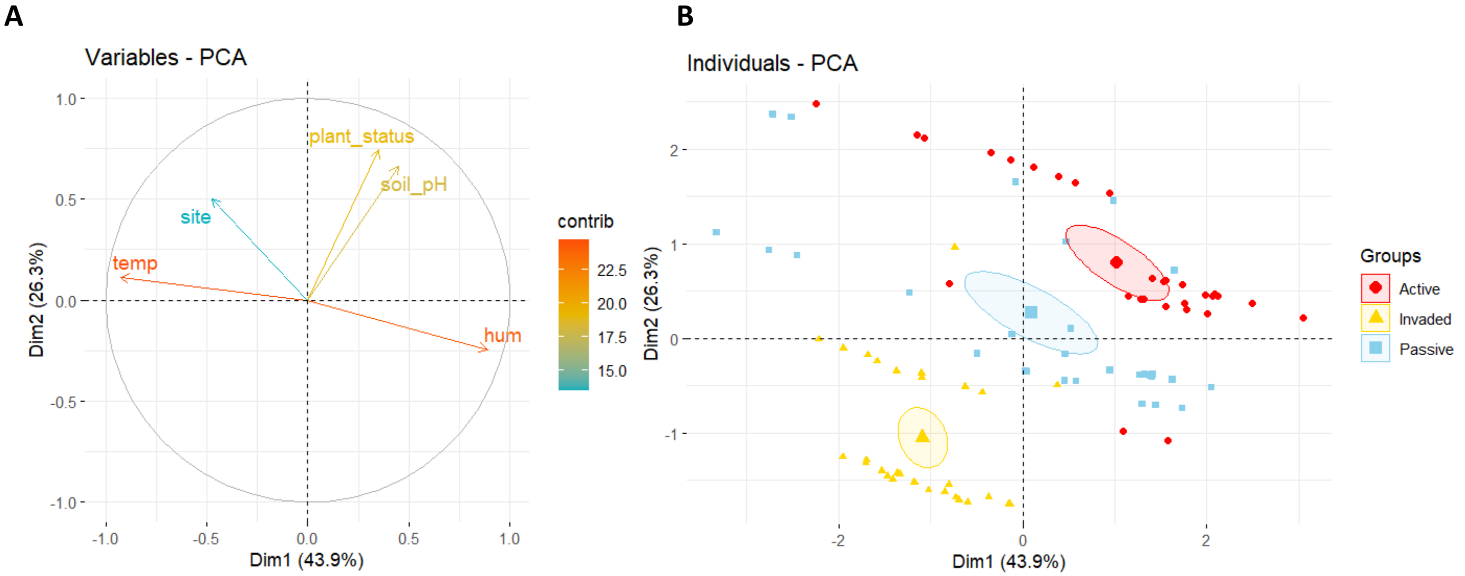


Fig. 21: PCA on environmental variables from all farms during Winter 2021. A) Variables are represented by arrows and coloured according to their contribution. B) Ellipses represent 95% confidence estimates for the different restoration groups.

Fig. 22: Spearman's correlation plot showing r values between different environmental parameters and Collembola species richness and abundance at all sites sampled during Spring 2020.

Fig. 23: Spearman's correlation plot showing r values between different environmental parameters and Collembola species richness and abundance at all sites sampled during Winter 2021.

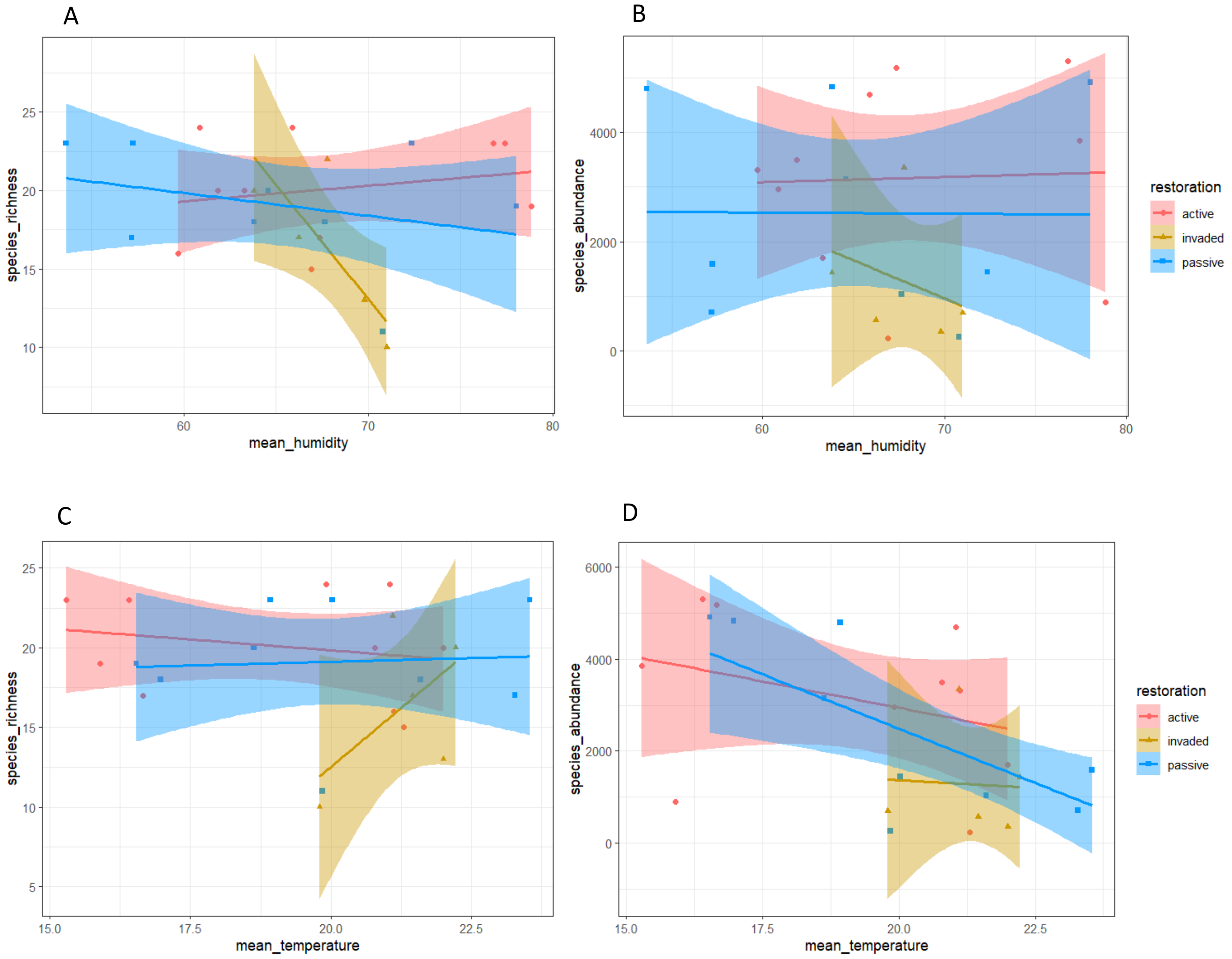


Fig. 24: Plot from GLM results, showing relationships between A) mean humidity and Collembola species richness (r = -0.082; P < 0.05) for different restoration treatments, B) mean humidity and Collembola abundance (r = -0.0359; 0.05 < P < 0.1) for different restoration treatments , C) between mean temperature and Collembola species richness (r = -0.0927; p> 0.05) for different restoration treatments, as well as D) mean temperature and Collembola species abundance (r =-0.3481, p< 0.05) under different restoration treatments.

Discussion

Effect of Restoration Practices on Collembola Diversity

This was the first study that investigated Collembola as belowground indicators within a restoration project in South Africa, and compared Collembola to aboveground plant data. Fourteen of the 34 Collembola species found in this study were native to South Africa. Native species were mainly found in undisturbed areas with little human activities, such as passive restoration sites and in unrestored alien-invaded sites. Results have also shown a possible seasonality in the occurrence of native Collembola within sites. Although seasonal data for this study was limited, the abundance of native Collembola was higher during Spring for two subsequent years at the Bosplaas passive restoration site. Other studies also described a direct linkage between seasons and soil invertebrates, which is due to seasonal reliance on plant community characteristics associated with springtail distributions (Petersen, 2011; Perera et al., 2022). In addition, Collembola abundances change during different seasons, due to their sensitivity to soil moisture and temperature (Petersen, 2011).

Soils in the study areas are generally coarsely textured, as the area of study is naturally dominated by infertile sandstone and quartzite soils of the Cape Supergroup (Midgley et al., 2003), here mostly consisting of sand and loam. Sandy soils are also associated with less carbon accrual (Baer et al., 2015), and are therefore also highly leeched soils. Periodic rainfall and aridity of certain areas in the country also affect soil carbon accumulation rates. In winter rainfall areas, such as the current study, conditions would be more favourable for decomposition and carbon storage due to lower evapotranspiration rates and higher surface soil moisture content (Baer et al., 2015).

Soils for invaded sites were found to have an overall slightly lower pH than restored sites, with pH for invaded sites ranging between 4.3 and 4.7, and between 4.9 and 5.4 for restored sites. Studies have indeed found that invasive plants alter soil pH, this is similar to results from Tererai et al. (2012) on *Eucalyptus* invasions in South Africa, who found that pH was significantly lower in riparian sites invaded by *E. camuldensis*. Other studies also found that invasion by *Eucalyptus* may increase soil pH in terrestrial grassy fynbos (Kerr et al., 2016; Jacobs et al., 2020), in contrast to riparian zones, where pH will be lower (Tererai et al., 2012; Jacobs et al., 2020). Soil pH can also be linked to humus type, although with a general low acidity across sites, differences in humus type may be low (Cassagne et al., 2003). It is also known that changes in soil pH, such as observed in the study by Tererai (2012), can cause significant changes in Collembola community structure (van Straalen and Verhoef, 1997; van Straalen, 1998; Cassagne et al., 2003; Harta et al., 2021). A study done by Rusterholz et al. (2014) in deciduous forests in Switzerland, found that invasive plants also caused shifts in species composition of Collembola in both leaf litter and soil. The study also confirmed that similar to findings in other studies (Hågvar, 1984; Maraun et al., 2003; Salomon et al., 2008) showing soil pH, soil moisture content and disturbance intensity influenced Collembola species compositions, rather than life-history traits (Rusterholz et al., 2014).

Although the differences in pH appear small, they did have some significant effects on Collembola distributions in this study, similar to what was found by Cassagne et al. (2003).

Soil pH in this study was especially significant for Active restoration sites in terms of community composition. Similarly, a study by Jacobs et al. (2020) also observed soil pH returning back to 'normal' (i.e. pre-invaded levels) following restoration efforts, which was paralleled by recovery of native soil microbial populations. Soil pH is also a very important driver of soil microbial community structure and diversity (Jacobs et al., 2020). Alterations of soil chemistry by *Acacia* and *Eucalyptus* trees also have profound impacts on native soil microbial communities by negatively affecting natural rhizosphere microbes and beneficial symbionts through pathogen accumulation, as well as allelopathic suppression of the natural microbial communities through invasive symbionts (Callaway et al., 2008; Coats et al., 2014).

Invasive alien plant species also alter the quantity, quality and timing of litter production, altering nutrient inputs into the soil. This can further alter linkages between above and below-ground organisms and their associated functions such as mineralization, further inducing feedback to the structure and dynamic of plant assemblages (Wardle and Peltzer, 2017; De Almeida et al., 2022). For example, litter from certain invasive plants such as knotweed in Normandy, France had a negative effect on Collembola communities, due to the extended decomposition rate and slower nutrient release compared to native plant species (De Almeida et al., 2022). Plant litter therefore also impacts Collembola communities. Litter by invasive plants may produce novel habitats for epedaphic (surface-dwelling) Collembola species in invaded open habitats, whereas in forest and closed habitat areas these effects could not be as pervasive due to pre-existing plant litter prior to invasion (De Almeida et al., 2022). Soil organisms such as Collembola, nematodes and mites are very responsive to the origin and thickness of plant litter (Wissuwa et al., 2012; De Almeida et al., 2022). For invasive plants in the current study such as *Eucalyptus* and *Acacia* species, not only can the amount of litter inputs influence species distributions of microbial communities and Collembola, but also the effect of litter from these plants on soil chemical properties and pH will have significant effects (Jacobs et al., 2020).

Both *Acacia* and *Eucalyptus* invasive species produce litter that alter soil and biogeochemical processes, ultimately altering soil microbial communities and ecosystem functioning (Slabbert et al.,2014; Jacobs et al., 2020). *Acacia* species can also be differentiated by their ability to fix atmospheric nitrogen through nodulating bacteria (Constantinides et al 1994; Corbin and D'Antonio, 2004; Jacobs et al., 2020), therefore *Acacia* litter will be enriched with nitrogen. *Acacia mearnsii* will reabsorb phosphorus rather than nitrogen from dying leaves, as the plant already has an abundance of nitrogen in its foliage (Tye et al., 2012; Jacobs et al., 2020). This creates a snowball effect in riparian zones, as litter now rich in nitrogen compared to carbon in already previously nutrient poor, sandy soils, increases the availability of nitrogen compared to carbon by ten-fold through accelerated decomposition and soil mineralization rates (Simaika et al., 2018; Jacobs et al., 2020), which further increases the abundance and growth rate of Acacia species (Werner et al., 2008; Jacobs et al., 2020).

During this study, alien invasive Collembola were found in extreme abundances in comparison to native Collembola, even in restored sites. This could be attributed to long term effects of invasive plants on plant litter and soil chemical composition. In the Western Cape, South Africa, Leinaas et al. (2015) also found that indirect effects from disturbances, such as changes in litter composition by *Galenia africana* (yellowbush), a shrub favoured by disturbances such as overgrazing (Leinaas et al., 2015), also facilitate invasion by non-native Collembola species such as *Hypogastrura manubrialis*. Invasive Collembola species in South Africa are typically associated with nutrient-rich organic soils (see Fjelberg, 1998), such as from yellowbush litter (Leinaas et al., 2015) and also invasive woody plants which provide higher litter inputs (Harta et al., 2021; De Almeida et al., 2022), and very rarely occur in nutrient poor sites across the Western- Cape (Janion, 2012; Liu et al., 2012; Janion-Scheepers et al., 2015; Leinaas et al., 2015). Indigenous Collembola, which are able to utilize low-quality litter in natural areas, have in some cases been found to be completely displaced in these nutrient-rich areas (Leinaas et al., 2015), similar to observations in the current study.

In this study, there were weak differences in Collembola assemblages between restoration treatments and stronger differences between restored and non-restored sites. Results from this study indicate that the belowground restoration process could take much longer than overserved above ground, as has been found in other studies (Liu et al. 2012). Following NMDS analysis on all sites over two years, Kleinplasie had the most dissimilarity between restoration types, this could also be due to the area of restoration being much bigger than the other two sites, expanding over 98 773 m^2 compared to Grootverlangen (5 023 m^2) and Bosplaas (8 080 m^2) (Van Zitters, 2021), therefore restoration areas at Kleinplasie were also situated further apart. Kleinplasie was also found to have the lowest abundances of Collembola and this could be indicative that this site has not yet been restored to the same degree as other sites have. Bosplaas had the least dissimilarity, instead of this being due to size, it could be due to Bosplaas also having the highest abundances of native Collembola, therefore microclimate variables and plant status in Bosplaas could probably have been more favourable among the different restoration treatments. Other factors may include Bosplaas being the 'oldest' site, where previous restoration attempts have already started prior to the 2016 restoration period (Tererai, 2012; Van Zitters, 2021).

Impact of Alien Vegetation on Belowground Diversity

Transformer alien species such as *A. mearnsii* are found to alter soil chemistry due to increased soil nitrogen levels (Gwate et al., 2021), causing soil to be unfavourable for native plant species (Leinaas et al., 2015; Tererai, 2012; Witkowski, 1991). Grasses such as kikuyu (*Cenchrus clandestinus*) often follow invasions and have an enhanced competitive advantage, often becoming dominant after alien clearance (Holmes et al., 2005), as seen at Kleinplasie and Grootverlangen passive restoration sites (Fig. A11). Although active restoration is better in this regard where weed removal is encouraged, it may be possible that human disturbance such as the planting of native plants, continuous removal and monitoring of invasive plants and additional (non-seasonal) irrigation contrary to natural cycles may cause native Collembola to take even longer to re-colonise. High abundances of alien Collembola were found at all restoration sites, even in comparison to alien-invaded, non-restored sites.

Studies found that collembolan species community structure are affected to various degrees by soil tillage, and are sensitive to different soil use practices (de Filho et al., 2016; Potapov et al., 2022). Artificial irrigation, also depending on the season that irrigation is applied, can also affect native Collembola communities (Lindberg et al., 2002). As native Collembola are more accustomed to the natural climate, frequent tillage will cause more humid and more favourable conditions for invasive species which may displace indigenous Collembola species (Fjelberg.,1998; Leinaas et al., 2015). Responses of native Collembola communities to certain habitat changes may also depend on their life forms, for example epidaphic, hemiedaphic and euedaphic species may react differently, as epidaphic (surface-dwelling) Collembola are more adapted to fluctuating environments (Peterson, 2002; De Almeida et al., 2022). Contrastingly, euedaphic species living deep in the soil may also respond less to aboveground changes that could more readily affect surface dwellers (De Almeida et al., 2022). Similarly, in the Western Cape, invasive Collembola species and life forms may also be less adapted to natural conditions such as soil nutrient status, temperature and humidity conditions as well as low nutrient litter composition in native habitats (Janion, 2012; Liu et al., 2012; Janion-Scheepers et al., 2015; Leinaas et al., 2015).

Alien grasses and plants were still found abundantly, especially at passive restoration sites (Fig. A11). Studies have suggested that nutrient enrichment of naturally infertile soils following disturbances can facilitate successful invasions by exotic species such as these alien grasses (Lake and Leishman, 2004). Indeed, invasive Collembola are often more associated with invasive plant species (Liu et al., 2012; Li et al., 2023), which could explain the high abundance of invasive springtail species in these sites. This could also be due to different physical, chemical and biotic characteristics still remaining among these novel grassy ecosystems, presenting possibly hostile conditions that remain for native species and leaving an open niche for invasive Collembola to thrive (Greenslade, 2018). Invasive Collembola may also be better adapted to colonise these grassy ecosystems from elsewhere than their native counterparts, and the soil chemical microhabitat these grasses create.

Areas dominated by invasive grasses have also been shown to contain more fine (low quality) remnant roots, which may lead to a higher C:N ratio of root tissue. High inputs of these low-quality roots have negative effects such as limiting decomposition (Silver and Miya, 2001; Baer et al., 2015), and also possibly limiting the growth and turnover of microbial communities in the soil. These communities play a role in facilitating the protection of soil carbon through formation of soil aggregates and stabilization through association with soil minerals (Six et al., 2002; Baer et al., 2015). Higher quality of root inputs, such as uncultivated highveld areas dominated by woody plant species partly also lead to better recovery of soil microbial communities and soil carbon content, as well as C:N ratios (Craine et al., 2002). Soil microbes play a crucial role in the functioning of different ecosystems, for example by influencing the performance of plants by maximizing nutrient uptake and reducing impacts of environmental and other stressors (le Roux et al., 2023; Trivedi et al., 2020). Microbes have a heterogenous distribution in soils, this is mostly due to variations in abiotic properties such as pH and organic carbon (Fierer et al., 2009; le Roux et al., 2023).

High leaf litter input from dense invasive thickets also lead to the deposition of organic material that differs in chemistry, quantity and quality from litter inputs in native areas (Ehrenfeld and Scott, 2017; Keet et al., 2021). In addition, soil may undergo other chemical changes resulting from the excretion of chemical compounds released by aboveground plant tissues and/or root exudes (Coats and Rumpho, 2014; Tharayil et al., 2009; Weidenhamer and

Callaway, 2010). This could also have distinct impacts on Collembola, similar to the other invasive plants that were removed from the area. Invasion by certain plant functional groups indeed has distinct impacts on soil organisms.

When comparing different restoration treatments and plant status (invasive or native), differences were found between Collembola communities within the different restoration treatments as well as between native or invasive aboveground plant status. Thus, it appears that the non-native vegetation may promote the success of other alien species, in this case alien Collembola, an interaction which elsewhere has been known to develop into a substantial synergy known as invasional meltdown (Simberloff, 2006). Although this might not turn out to be the case here, the changes in Collembola assemblages found, including the increase in the abundance and richness of invasive species, could also suggest that the invasion of natural Fynbos areas, by *Acacia* trees, *Eucalyptus* trees*,* pines or other invasive plant species, is also occurring elsewhere in the region (van Wilgen, 2009), which may also promote invasion by Collembola species into niches left open. Interestingly, restoration data also had significantly strong dissimilarities between groups. The effects of temperature and humidity on Collembola richness and abundance aligned more with results from other studies such as Liu et al. (2012), after adding plant status in as a factor. In addition, results from the PCA analysis showed that plant status had some significant effects on the variance within communities. From NMDs analyses and GLM, restoration treatment had the most significant effects compared to plant status. This effect may be attributed to different litter types, plant density and microclimate under or around the plants. When ruling out these environmental variables, and by looking only at Collembola abundances and densities, plant status had no significant effects on the Collembola abundances and only significantly differed among different restoration types. This could indicate a lag phase in the recovery of native Collembola.

Invasive plants such as *Eucalyptus camuldensis* and *Acacia saligna* can have lasting impacts even after restoration efforts (Nsikani et al., 2017). The recovery of ecosystems following alien plant removal may also depend on the alien plant in question, for example *Eucalyptus* species may cause lasting shifts in microbial communities following removal, which has also been found to slow down natural plant recovery (Jacobs et al., 2020). This may be attributed to the release of toxins and chemicals by *Eucalyptus* litter into the soil during decomposition, which is also made worse by slash and burning restoration methods (Ruwanza et al., 2015; Jacobs et al., 2020). Nitrogen-fixing plants such as *Acacia* species may modify soil nitrogen accumulation and cycling rates (Corbin and D'Antonio, 2004; Rice et al., 2004), further leading to positive invader-soil feedbacks, as well as higher competitiveness and invasiveness (Keet et al., 2021). The invasion of woody N-fixing species into habitats that previously lacked woody nitrogen fixers, such as *Acacia* species in South Africa (Witkowski, 1991), has been shown to result in substantial increases in soil nitrogen pools (Corbin and D'Antonio, 2004). Litter from nitrogen-fixating species could have significantly higher nitrogen content than litter from dominant native plants (Corbin and D'Antonio, 2004).

These invaders can leave legacy effects in the soil (Nsikani et al., 2017). These effects may include altered soil chemical or physical characteristics, as well as altered microbial communities that may persist even after the removal of these invasives (Corbin and D'Antonio, 2004; Corbin and D’Antonio, 2012). In these cases, the removal of alien plants alone, such as with passive restoration, will not be sufficient to return the ecosystem to its original state (Macdonald, 2004; Nsikani et al., 2017). Therefore, to restore and re-establish a fully functional native ecosystem, it is also important to take these effects into account, as well as how long they may persist. As found in a study done by Nsikani et al. (2017), this can take up to 10 years. These effects, as seen in plants and microbial communities (Corbin and D’Antonio, 2004; Corbin and D’Antonio 2012; Van Zitters, 2021), may also occur in belowground Collembola communities.

Alien vs Native Collembola species

The success of invasive arthropod species is often mediated by previous habitat disturbances, be it natural or man-made such as plant invasions that have other indirect chain effects on the ecology of the disturbed area. Invasive species establishment is often brought about by these effects (Leinaas et al., 2015). Invertebrates living in the soil may also take longer to respond to these changes and subsequently, they may also take longer to recover due to the extra disturbances caused by aboveground restoration, as well as legacy effects left by invasive plants, as discussed further in the following section.

Grootverlangen, was found to have the highest abundance of the invasive springtail *Hypogastrura manubrialis* (2,576 individuals) within its active restoration area. Similarly, *H. manubrialis* was found to be dominant in the nutrient-rich litter of *Galenia africana* (Leinaas et al., 2015). *Hypogastrura manubrialis* is known for high mobility and group migration, making it highly invasive (Leinaas et al., 2015).

In this study, there was also a strong association with *Sminthurinus* cf. *elegans* in both actively and passively restored sites during winter and spring at the Bosplaas study site. These high abundances can be due to the microclimate in the invaded sites, usually providing a more humid environment, preferred by invasive species (Liu et al., 2012). In contrast, the native species are more prevalent in the warmer and drier native vegetation, possibly due to their higher thermal tolerances or desiccation resistance (Liu et al. 2020; 2021). For example, a native *Parisotoma* species was more abundant in the fynbos (Liu et al., 2012) and Renosterveld (Leinaas et al., 2015) sites, while *Austrogastrura* sp. was the most abundant native species in the oldest restoration site in this study. Native Collembola were only found at passive non-disturbed restoration areas at the Bosplaas farm. These invasive species may have a negative impact on the native ones through competition or displacement, as has been found in sub-Antarctic South Georgia (Convey and Greenslade, 1991; Convey et al.,1998). Further work is needed to determine the functional impact of these invasive species.

Interestingly, in this study, alien Collembola such as *Brachystomella* sp. were significantly present amongst all sites restored both actively or passively (Table 3 and 4). *Dicyrtomina* cf. *ornata* was found to be present at all restored sites during spring, but not during winter sampling of 2021. *Sphaeridia* sp. and *Hemisotoma* sp. were indicative of restored areas in both winter and spring 2021, although these are all invasive Collembola species. These changes found in Collembola assemblages, including the pervasive presence of invasive

Collembola in restored areas may be due to invasions such as pine or *Eucalyptus* occurring elsewhere may promote consistent invasion by Collembola species (Van Wilgen, 2009; Liu et al., 2012), which could in this case be from the invaded sites across the stream or in upstream areas (Van Wilgen, 2009). As most Collembola are "superhydrophobic" (Gunderson et al., 2014), they are also easily transported on water and may raft along rivers to downstream areas (Potapov et al., 2020). Another explanation is also due to the small size of these restoration sites, they may also be experiencing an influx of invasive species from nearby agricultural areas or invaded sites, although Collembola in agricultural soils may have substantially lower abundances than invasive riparian forests in upstream areas. Relatively low densities of Collembola are also commonly observed in agricultural soils within both temperate regions and tropical environments (Culik et al., 2002). Collembola also have less dispersal ability across terrestrial areas, due to their small size (Heiniger et al., 2014). Habitat patches are never too small to fit their ecological needs, and collembolan populations can survive in very small patches when isolated (Schneider et al., 2007). Collembola may however readily disperse into nearby niches left open by newly established habitats (Greenslade, 2018). Whether this is taking place deserves investigation given the demonstration both in the Fynbos Biome and elsewhere that Collembola may substantially affect soil system functioning (Brussaard et al., 1997; Bengtsson, 1998; Wardle et al., 2004; Bengtsson et al., 2011, 2012).

Abiotic effects on Collembola diversity

Results from PCA analysis showed that active restoration sites were characterized by less acidic soil with higher soil pH, although this change is probably small, it had a significant effect, as has also been found in other studies (van Straalen and Verhoef, 1997; van Straalen, 1998; Cassagne et al., 2003; Harta et al., 2021). Collembola distributions in active sites were also shown to be strongly affected by plant status, which is likely due to active sites having the most native plants and litter, which causes soil structure as well as Collembola community composition to change. Thus, conditions become less favourable for invasive Collembola species, which are more adapted to high quality litter as well as soil environments associated with invasive plants, similar to findings by Fjelberg (1998) and Leinaas et al. (2015).

Contrastingly, passive sites are characterized by lower pH, as well as higher temperature, while plant status also had a strong, but different and more negative effect within these sites, probably due to only pioneer native plants persisting here. In addition, the soil had not been changed back to a natural state with alien plants still sprouting up, as well as some remnant litter left behind. Passive sites were also less covered, more barren and this could explain higher temperatures affecting Collembola communities. Interestingly, Collembola in the natural site is characterized by slightly higher humidity and lower temperature and the communities found here are localized within this one site.

Active sites are also characterized by higher soil pH and are also strongly distinguished due to plant status, communities in passive sites now lie closer and within a more neutral spot once alien invaded sites have been included in analysis. Passive sites are now characterized by only slightly lower pH, with varying temperature and humidity. Communities in active and passive restored sites overall are characterized by higher soil pH and are strongly affected by plant status in comparison to invaded sites, which are characterized by lower soil pH and higher humidity. Both active and passive sites can also be seen across a wide range of temperature and humidity conditions, as characterized by the riparian fynbos. Communities in invaded sites are exposed to a much narrower range of temperature and humidity, which is more favourable for invasive Collembola to thrive. In addition, these invasive communities are not as well adapted to dryer areas with nutrient poor litter, as has also been highlighted in other studies conducted the Western-Cape (Janion, 2012; Liu et al., 2012; Janion-Scheepers et al., 2015; Leinaas et al., 2015).

From PCA analysis from both years, Collembola communities are indeed separated across a gradient, with active sites and the one native site being distinguished by plant status and higher soil pH, higher humidity and lower temperatures, as well as some seasonal differences, as they are closer to a natural state. Passive sites are also strongly influenced by temperature and humidity conditions. Invasive sites are less influenced by plant status and characterized by lower pH, higher humidity and lower temperatures. The lesser effect of plant status here can be attributed to the even cover of invasive plants, mostly of the same species, as observed within these sites. The “site,” variable which in this case referred to farms/sampling locality, caused a negligible difference; effects of different farms and their associated differences can therefore be ruled out in comparison to environmental effects. For example, although Grootverlangen differed in terms of alien plant removal practices and didn’t have the additional “stack and burn” treatment as the other two farms, it didn’t cause any significant effects on Collembola species distributions. Using Pearson’s correlation methods (Touhidul Islam, 2021), there was a correlation between species abundance and mean humidity. There was found to be a moderate negative correlation between mean temperature and pH in all sites sampled during spring in 2020, and a positive correlation between mean temperature and species richness, as well as a strong negative correlation between species richness and humidity. Data from winter 2021 also had a moderate negative correlation between species abundance and mean temperature – this could be due to colder winter temperatures, and most 2021 data being sampled during winter, with colder areas probably being more humid or wetter, and the hotter places being drier. This was expected as Collembola have been found to be prone to desiccation (Choi et al., 2002; Kærsgaard et al., 2004; Chown et al., 2007; Parry et al., 2007), and therefore also prefer high humidity and lower temperatures, both acting to reduce differences between water activity between the organism and their environment (Chown et al., 2011; Hopkin, 1997; Liu et al., 2012). Alien-invaded areas, such as sites dominated by pine trees have higher humidity and this is likely associated with substantial litter layers (Scholes and Nowicki, 1998), where pine litter caused higher humidity and more favourable conditions for invasive Collembola species such as *Sminthurinus* cf. *elegans*, *Entomobrya* cf. *multifasciata* and *Ceratophysella* sp. found in Liu et al. (2012). Similar results were found in this study. In alien-invaded sites, *Hypogastrura* cf. *manubrialis*, *Ceratophysella denticulata* and *Sminthurinus* sp. are indicative of these areas (Table 3 and 4), except for *Austrogastrura* sp., a species native to South Africa (Janion-Scheepers et al., 2015), which was also found to be somewhat indicative of invaded areas during both winter and spring. Alien invasive plants, such as *E. camuldensis*, *A. mearnsii* and *Pinus sp.* also lead to increased height of the vegetation community, due to the novel invasives being mostly trees that grow up to several metres, and a reduction in understory shrubs (van Wilgen and Richardson, 1985), as they also create allelopathic conditions where native plants are unable to grow (Callaway et al., 2008; Weidenhamer and Callaway, 2010; Coats et al., 2014; De Almeida et al, 2022), and block out sunlight (Ruwanza et al., 2013; Holmes et al., 2020). These changes, as also observed during this study, are especially drastic when considering native vegetation which consist out of a diverse array of indigenous shrubs, and are also described as ‘closed scrub fynbos’, ’hygrophilous mountain fynbos’, as well as ‘broad sclerophyllous closed scrub’ (Holmes et al., 2005; Mucina and Rutherford, 2006).

Changes in vegetation structure affect the thermal heterogeneity available to organisms (Garcia and Clusella-Trullas, 2019). For example, Garcia and Clusella-Trullas (2019) showed these changes were have implications for thermoregulation. Optimal microclimates may therefore become less abundant or accessible, leading to increased costs for ectotherms (Basson et al., 2017; Garcia and Clusella-Trullas, 2019). These effects may cause constrained activity and also affect performance (Garcia and Clusella-Trullas, 2019). Collembola have been found to show strong relationships between trait variation of their thermal tolerance and community composition (Ellers et al., 2018; Hoskins et al., 2020). Many studies (for example Allen et al., 2016; Bahrndorff et al., 2006; Janion-Scheepers et al., 2018) have investigated thermal tolerance traits in Collembola and in particular their critical limits. It has typically been found that CTmin and CTmax can both show substantial phenotypic plasticity, unlike some other arthropods (Jensen et al., 2019). Significant differences can also be found in traits based on geography, climate, habitat and whether or not species are native to a given area (Bahrndorff et al., 2006; Janion-Scheepers et al., 2018; Hoskins et al., 2020; Jensen et al., 2019). Riparian zones, such as the area of this study, are also more prone to periodic flooding with nutrient-rich water. This can especially happen with heavy rains after a long dry period (Lake and Leishman, 2004). In this study, this is a frequent occurrence, especially during the time that the study was conducted after heavy winter rainfall that followed a long drought period. Native riparian species should therefore be more resilient as they are more adapted to these conditions. However, high nutrient runoff from nearby agricultural areas would still have a significant impact on native riparian springtail communities. Sudden nutrient enrichment of soils following heavy rains could have higher native plant mortality rates as well as higher establishment of invasive species (Lake and Leishman, 2004). In addition, although Collembola can survive and recover after flooding events, the long-term impacts of this are unknown (González-Macé and Scheu, 2018).

Invasive plants have been found to alter litter thickness, soil nutrient content, as well as soil chemical properties and soil pH, which in turn affect microbial communities for a longer period of time following restoration (Jacobs et al., 2020), and so too, as found in this study, it affects Collembola. This study confirmed that Collembola communities, as a part of the belowground system, take longer to recover than the observed environment above the soil and soil litter. Many aspects may affect this process, most of the reasons behind which has not been fully explored in this study alone. For example, different restoration types, when applied inappropriately or targeting the wrong invasive species, may be causing more lasting damage due to the presence of another invasive species, and may also have significant effects on how below-ground biota respond, as well as affecting their ability to recover (Jacobs et al., 2020). It is also worth noting that re-invasions may consistently occur from nearby invaded habitats (Simberloff, 2006; van Wilgen, 2009; Greenslade, 2018). The study also found that active restoration methods are the most effective way for restoring these riparian habitats back to a more natural state, as most of these systems have already crossed an environmental threshold and have been transformed into novel invaded habitats. These systems will be difficult to fully recover and will also require larger areas to be targeted to possibly prevent re-invasions. More collaboration with landowners will be needed, as these invasive systems are not just detrimental for the environment, but also for human and animal health along these riparian farms, which is another subject that could be explored in future studies.

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Chapter 3

General Conclusions

Intact riparian ecosystems support biodiversity and crucial ecosystem functions (i.e., climate regulation, soil retention, building seed banks and propagule dispersal, and energy transfer) central to both aquatic and terrestrial landscapes (Capon et al., 2013; Capon and Pettit, 2018). Human impacts such as soil erosion, alien species invasions, losses in local biodiversity and increasing rates of nitrogen deposition due to agricultural activities lead to both above and belowground biodiversity loss and also have negative effects on soil-dwelling organisms (Bardgett and Wardle, 2010). In regions with semi-arid climates like South Africa (van Zyl, 2003), the proliferation of invasive tree species can exacerbate the strain on the country's already scarce water resources by impeding runoff (Holmes et al., 2005). Within the Berg and Breede catchments, the prevalence of woody alien invasive plants is a pressing environmental concern due to their aggressive nature and ability to thrive (Rebelo et al., 2022).

The establishment of invasive plants can have dramatic effects on vegetation community structure within an ecosystem (Richardson et al., 2007; Liu et al., 2012). Alien species like *Acacia mearnsii*, commonly known as black wattle, as well as *Eucalyptus camaldulensis* (red river gum) into riparian habitats are major threats to conserving natural biodiversity in the Western Cape. The species can change soil chemistry by boosting nitrogen levels and has been found to transform native riparian habitats in both the Western Cape as well as other parts of the world, by changing the composition, structure and functioning of these ecosystems (Tererai et al., 2013). This alteration can pave the way for the establishment of non-native native plants or secondary alien species (Gwate et al., 2021). Particularly, ecophysiological traits exhibited by invasive nitrogen-fixing woody species, such as rapid growth rates and seeds with prolonged dormancy periods, contribute significantly to these soil legacy effects even after clearing efforts. In places where nitrogen levels in the soil rise, grasses might gain a competitive advantage, potentially becoming the dominant species following the removal of aliens (Holmes et al., 2005). This transformation of natural vegetation may also significantly affect soil communities. These changes alter the fundamental factors influencing soil biodiversity, such as vegetation and soil microclimate (Decaëns and Jiménez, 2002; Decaëns et al., 2006).

In the context of alien plant invasions and efforts to clear them thoroughly, one significant factor contributing to their persistent regrowth lies in the phenomenon of soil legacy effects, coupled with the dynamics of native soil seed banks. Research by Vosse et al. (2008) has highlighted a decrease in native species seed bank abundance following invasions, underscoring the impact of these interactions. Soil legacy effects encompass discernible alterations in soil biological, chemical, or physical conditions, as noted by Corbin and D'Antonio (2012) and Nsikani et al. (2018). Soil legacy effects from plants refer to the long-lasting impacts that invasive plants such as *Eucalyptus* sp. and *Acacia* sp. have on the soil properties and belowground communities even after they are no longer present by altering soil chemical properties through allelopathic compounds or secondary metabolites as well as toxic chemicals (in the case of *Eucalyptus* sp.) Remnant invasive plant litter can also still release compounds into the environment, also creating conditions that remain favourable for further invasions. These compounds can influence the soil's chemical composition, nutrient availability, and have significant impacts on soil microbial communities and the rhizosphere (Dakora and Phillips, 2002; Jacobs et al., 2020). *Acacia* species are also known for building up vast seedbanks, and are able to re-sprout after fire, making management even more difficult. *Pinus* species, which are less aggressive invaders, do not resprout after felling, burning of invaded sites two years after tree removal can successfully reduce invasive pine tree cover (Fill et al., 2016).

The recognition of the landscape history's influence on contemporary biodiversity patterns is gaining recognition as a critical determinant shaping current species assemblages (Hermy and Verheyen, 2007). Considering that human activities have significantly impacted most terrestrial ecosystems worldwide (Newbold et al., 2015), comprehending the legacy effects can enhance our understanding of current biodiversity patterns. Moreover, it enables us to anticipate the future ecological consequences of ongoing human practices on ecosystem services and functions (Vellend, Brown, Kharouba, McCune and Myers-Smith, 2013). A growing body of literature, including studies by Corbin and D’Antonio (2012), Rodriguez-Echeverria et al. (2013), and Nsikani et al. (2018), has demonstrated how these soil legacy effects pose formidable obstacles to restoration efforts following the removal of invasive nitrogen-fixing woody species. Moreover, several studies have proposed potential management strategies aimed at mitigating these barriers to restoration (Nsikani et al., 2018).

Active restoration involves replanting and re-establishing native plant communities after removing alien invasive vegetation. Conversely, passive restoration, also known as "natural regeneration," omits additional treatment following alien plant removal (Atkinson and Bonser, 2020; Holmes et al., 2020). Passive restoration, often a cost-effective approach, is viable when the natural recovery potential is high (Gann et al., 2019). This method is particularly effective in headwater and transitional riparian ecosystems with intact surrounding vegetation and persistent native propagules in their seedbanks (Vosse et al., 2008), or dispersed from upstream areas via water (Galatowitsch and Richardson, 2005; Holmes et al., 2020). Riparian seed dispersal occurs through floodwaters, sediment burial, animal burrowing, and seed dormancy until suitable germination conditions arise (Holmes et al., 2005), making seedbanks vital for ecosystem recovery via passive restoration.

Research has demonstrated that the composition and functionality of plant communities can exert notable influence on the distribution of soil-dwelling invertebrate communities (Wardle et al., 2004; Bardgett and Wardle, 2010). Although much attention has been devoted to aboveground biodiversity and its ecological functions, underground systems have received comparatively less attention, despite their vital role in environmental processes (Eisenhauer et al., 2011). The successful removal of invasive plants represents a significant challenge in ecosystem restoration efforts, of which active restoration can be argued as being the most effective, as has been found in various studies. Although it is a significantly more costly method (Fill et al., 2016), in this study it was found to be more effective for restoring both above-ground and belowground habitats, although below-ground invasive Collembola species remained present. When looking at belowground effects, there are still some significant challenges arising with active and passive restoration practices. This comes along with the effects of different methods of alien plant removal such as burning, as well as use of herbicides.

The complexities of post-removal habitat restoration arise from the intricate web of interactions within these ecosystems (Richardson et al., 2007). Invasive plants can drastically change habitat structure, species composition, and ecological processes (Catford et al., 2012). Thus, restoring these habitats to their original state requires a careful approach that considers the diverse interactions among organisms and environmental factors (Hobbs and Norton, 1996). Restoration practices such as slash and burn, or use of herbicides to inhibit further growth and resprouting of invasives, could worsen invasive effects, such as leaving permanent burn scars with little or no recovery of the native plants (Jacobs et al., 2020). Chemicals released by *Eucalyptus* trees, may for example only worsen below-ground effects followed by burning. Another important factor, which has been found to also have profound impacts on both microbes and Collembola inhabiting the soil, is the alteration of soil pH.

It is also of value to consider restoration methods used and consequences that these methods may also bring. Not only can invasive plants alter soil chemical properties, but so too can methods of invasive plant removal, such as burning methods or herbicide treatments, used to inhibit invasive *Acacia* trees (Holmes et al., 2020). In the fynbos environment, which is adapted to seasonal fire (Rebelo et al., 2022), it could at first glance seem harmless to the native ecosystem. However, due to native ecosystem properties having been altered by invasive plants, this could do more harm than good and cause natural areas to take even longer to recover, or not recover at all (Jacobs et al., 2020). Other effects such as the application of herbicide treatments during restoration efforts may also contribute to reducing soil pH when reaching soils (Weidenhamer and Callaway., 2010; Jacobs et al., 2020). All these aspects depend on the invasive plant that has been removed, although some sites could have several mixes of different invasive plants, as found in this study. Restoration practices could therefore also result in native microbial communities taking longer to recover, and so too Collembola as due to native species being intolerant to novel pH and chemical conditions (van Straalen and Verhoef, 1997; van Straalen, 1998; Cassagne et al., 2003; Harta et al., 2021). Secondary invaders such as kikuyu grasses are also favored by altered natural soil properties (Greenslade, 2018), letting novel soil chemical properties to remain and causing environments that are too hostile for native Collembola to re-colonise (Greenslade, 2018).

In addition to secondary invasive plants, altered soil pH and microbial communities as well as intensive restoration methods, slow re- colonization of Collembola into restoration sites may also be attributed to higher organic matter content in soils, left behind by invasive plants, as well as soil microbial communities that also remain altered for longer periods (Jacobs et al., 2020). Native Collembola in South Africa have been adapted to thrive in natural areas with low-quality litter. However, once soil chemical properties are changed by altered litter inputs from invasive plants, native Collembola have been documented to get entirely displaced by invasive species that thrive in these novel environments. Invasive Collembola species in South Africa are commonly associated with nutrient-rich organic soils (Fjelberg, 1998; Leinaas et al., 2015). Although they are much less abundant in natural, nutrient poor areas, they easily re-establish from invaded environments elsewhere into sites where invasive trees have been removed, and having left open niches and soil environments that are still too hostile for native Collembola to re-establish (Greenslade, 2018). Certain invasive Collembola species have also been known to be competitive within their environment and may displace native Collembola entirely (Leinaas et al., 2015). This thesis focused on using Collembola as bioindicators in monitoring the success of rehabilitation initiatives by the Berg River Improvement Programme within riparian ecosystems in the Berg and Breede catchments. Active and passive restoration practices was sampled and compared to one remnant native forest, as well as to alien-invaded systems.

Here I provide a summary of the key findings, limitations, and recommendations from the study, offering insights for future projects aimed at rejuvenating riparian zones. This research adds to the knowledge base on conservation strategies, especially within the Fynbos biome.

Conclusions

From hypotheses (Chapter 1):

1) There was no difference between springtail assemblages in active and passive restoration areas. However, there was a significant difference between springtail assemblages between restored and invaded areas.
2) Alien Collembola was equally present in invaded, non-restored areas; they were found to be more evenly spread, probably due to soil legacy effects. Native Collembola were also equally present in restored areas, and alien invaded sites, as they are evenly distributed and their presence may be site specific, or respond to soil legacy effects.
3) Effects of restoration appear to take longer in belowground communities (with Collembola as indicators) than in aboveground plant communities.
4) Aboveground plant invasive status does directly or indirectly influence Collembola by altering their species distribution and densities to a significant extent.

From Aims of the study:

1) Collembola communities differed significantly between active restoration sites, passive restoration sites, one native site and invaded sites where no clearing attempts have been made, these results are possibly due to effects of vegetation and litter types.
2) Collembola species richness and abundance were found to vary with plant status (i.e., native vs invasive plants) and abiotic environmental factors such as temperature, humidity and pH.
3) There were some seasonal effects on Collembola species richness and abundance (Although seasonal data was limited to one site), especially among native Collembola.

Results from this study showed that despite aboveground native plants being actively or passively restored, native Collembola species have been largely displaced by alien species, possibly altering their community composition completely. Native Collembola were not significant to a certain treatment, but densities were most significant for Bosplaas in Spring

2021 compared to other farms and sampling times. Native species were mainly found in undisturbed areas with little human activities, even in alien-invaded sites, and their abundance was highest during Spring, followed by heavy rain. It can also be argued that the density of native species found in this one site could be habitat-dependent (Smith et al., 2015), or due to soil legacy effects (Jones and Brown, 2018). Due to Bosplaas restoration efforts possibly being older, and having already taken place before the Berg River Improvement programme, the high densities at this site relative to more recently restored sites point to possible lag effects as observed above the ground. Certain species were found to be indicative of different treatments, such as *Sminthurinus* cf. *elegans,* which was found to be indicative for actively and passively restored areas in spring 2021 for all sites.

Overall, Collembola assemblages differed significantly among actively restored and invaded sites. Despite this pattern, smaller differences were found between active and passive restoration practices. However, active restoration can be concluded as the most significantly different from invaded sites in terms of Collembola community composition, and may therefore be the most effective method in restoring these communities closer to a natural state. One explanation is the surprising finding that plant invasive status did have some significant effects on Collembola community structure and densities, especially when looking at actively restored compared to invaded sites, which have completely different plant communities and different distributions of invasive and/or native plants. Invasive plants continue to have substantial impacts on various edaphic variables, including soil composition and moisture levels, as well as humidity and temperature within the ecosystem (Jones et al., 2020). Invasive plants often alter soil properties and microbial communities, impacting nutrient cycling and ecosystem processes (Callaway et al., 2004). Certain invasive plant species release allelopathic compounds, change soil structure, and exhibit different root traits compared to native species (Chapin et al., 2000). These changes can lead to shifts in nutrient cycling dynamics and affect ecosystem resilience. Native plants support diverse soil biota, including beneficial microbes and soil organisms, contributing to soil health and stability (Wardle et al., 2004). They form symbiotic relationships with mycorrhizal fungi, enhancing nutrient uptake and promoting soil aggregation (Johnson et al., 2015). Understanding these interactions is crucial for effective invasive species management and ecosystem restoration. In summary, invasive plants can have detrimental effects on soil communities and ecosystem functioning, while native plants play a crucial role in maintaining soil health and stability.

Another significant factor influencing Collembola diversity, especially in a wetland habitat is the presence of plant litter. As this study has revealed significant variations in Collembola densities associated with native or invasive plant status, as identified through PCA analyses on environmental variables such as pH, humidity, and temperature. However, it is imperative to note that these differences cannot be solely ascribed to microhabitat effects. As the areas under study underwent rehabilitation through the removal of alien invasive Eucalyptus trees and other plants. This is attributed to the reduction in plant litter, particularly beneath Eucalyptus trees, leading to drier soil conditions. Plant litter serves as a crucial habitat and food source for soil organisms like Collembola, and its reduction can impact their abundance and diversity (Smith et al., 2020; Johnson et al., 2018).

In this study, similar effects were found in below-ground communities as for a study on aboveground plants by Van Zitters (2021), where soil and geomorphological variables showed no difference between active and passive sites. However, the study also revealed correlations between several soil properties (such as organic carbon content, depth, bulk density, and concentrations of calcium, magnesium, phosphorus, and sodium) as well as geomorphological factors (including bank slope and buffer zone length) and various indices related to both native and alien vegetation. The total plant species richness in the study by Van Zitters (2021), encompassed both alien and native plants species and exhibited a positive correlation with several climatic factors, including the number of dry days, isothermally, annual precipitation, precipitation levels during the driest and wettest months, as well as precipitation in the wettest, driest, warmest, and coldest quarters. Conversely, plant species richness demonstrated a negative correlation with certain climatic variables, such as the annual mean temperature, temperature seasonality, maximum temperature during the warmest month, and mean temperature during the driest and warmest quarters. Similarly, species diversity also showed correlations with the same climatic variables, except for the annual mean temperature.

It can therefore be concluded that environmental effects such as season, temperature and humidity may have only temporary effects on Collembola in terms of overall abundances. Conversely, effects on individual plant species and therefore plant species composition and invasive status as well as their effects on soil properties and soil-microclimate may have long-lasting effects on Collembola and be less predictable (Smith et al., 2020; Jones et al., 2021). These long-lasting effects are mirrored in belowground soil communities such as Collembola, which are therefore excellent long-term environmental indicators.

Recommendations for future studies

A monitoring and evaluation period lasting between five to ten years, on average, is necessary to adequately assess lag effects and observe changes in ecosystem functioning following rehabilitation efforts (Gann et al., 2019; González et al., 2015; Feld et al., 2011; Naiman et al., 2005). This study has still fallen short of ten years, and future studies may observe further succession in the recruitment of native Collembola into restored/rehabilitated sites. Long-term alterations to the ecosystem resulting from these interventions are unlikely to be apparent within shorter time frames (Van Zitters, 2021). This study offers a further glimpse and added knowledge into the below-ground impacts of various types of rehabilitation on riparian ecosystems within the Berg and Breede catchments.

Despite coming with its own complications, active removal of invasive species remains the best approach, when implemented correctly. In some or most instances, replanting of native plants are essential for restoring native riparian habitats, and without follow- up clearing and adequately monitoring invasive species, any efforts will be rendered ineffective in the long term. The key that needs to be considered is carefully tailoring restoration methods for a species-specific approach, for example only burning areas where a pine tree was settled. Hand pulling of resprouting seedlings by large groups of volunteers or workers, as well as taking into account soil properties and possible consequences for the belowground environment. This could mean phasing out burning methods in areas where soils have not yet sufficiently recovered to a natural pH or chemical composition, as well as in areas that were invaded by *Eucalyptus* trees and *Acacia* trees.

It can also be suggested that native species that have been found to grow and survive under invasive *Acacia* trees to be used as pioneer plant species in order to slowly re-establish native plants into an altered soil environment following alien plant removal, as they are able to persist under allelochemical conditions (Ruwanza et al., 2015). Another approach, although it could be costly, would be to remove invasive plants, litter and plant roots, and then transfer some native soil and litter from elsewhere into these environments. This approach could be costly, but perhaps justifiable in extremely disturbed environments such as thick established *Acacia* forest stands. This could also have advantages such being that the full soil seed bank will be re-introduced as well as accompanying native microorganisms and Collembola.

Management implications for alien Collembola, although possible, will require future research into their linkages with microfauna and whether or not they may pose significant threats or possibly worsen invasion processes. Follow-up studies will be needed to rule out possible threats towards native Collembola species, although most native species are able to find spatial refuge in areas with low-quality litter, which their invasive counterparts are not adapted to utilize. These areas may include native riparian zones near the invaded site (if available), allowing them to gradually re-colonize the sites after restoration. For future studies, more sampling that encompasses more farms and study sites, may lay further light on these interesting findings. However, this study had its limitations in terms of time, the sorting of ~78 000 Collembola as well as finding ways to analyse available data, with seasonal effects and same-season effects having been limited. Another future recommendation will be to further adequately sample different seasons as well as rainfall events, in order to assess effects of natural events such as climate. As the knowledge of Collembola in South Africa is growing, the potential use of image recognition software and the standardisation of sampling protocols may also aid in the faster processing of samples (Potapov et al., 2022; Schneider et al. 2021). Lastly, sampling to collect plant data in the same amount of invasive and native replicates within restored vs. invaded sites or between certain types of plants may give further results on how below-ground communities are influenced and to what extent they are influenced by aboveground plant types.

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Appendix A

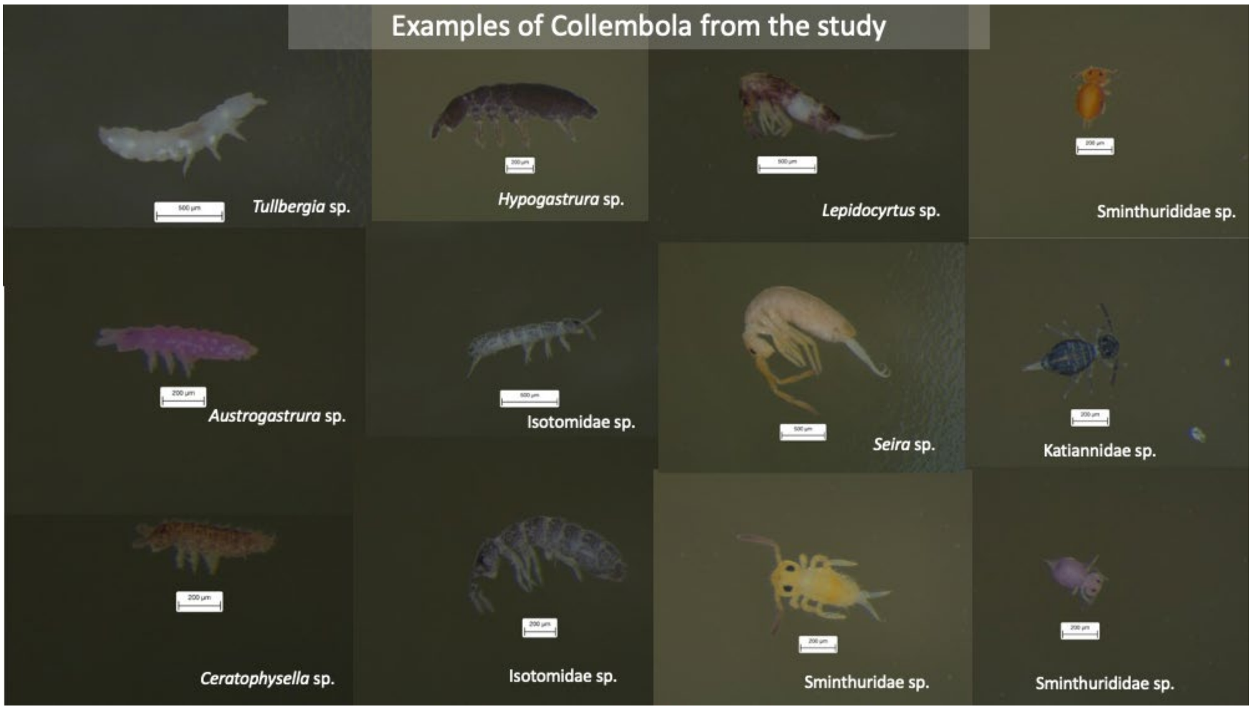

Fig. A1: Examples of successfully barcoded Collembola morphospecies.

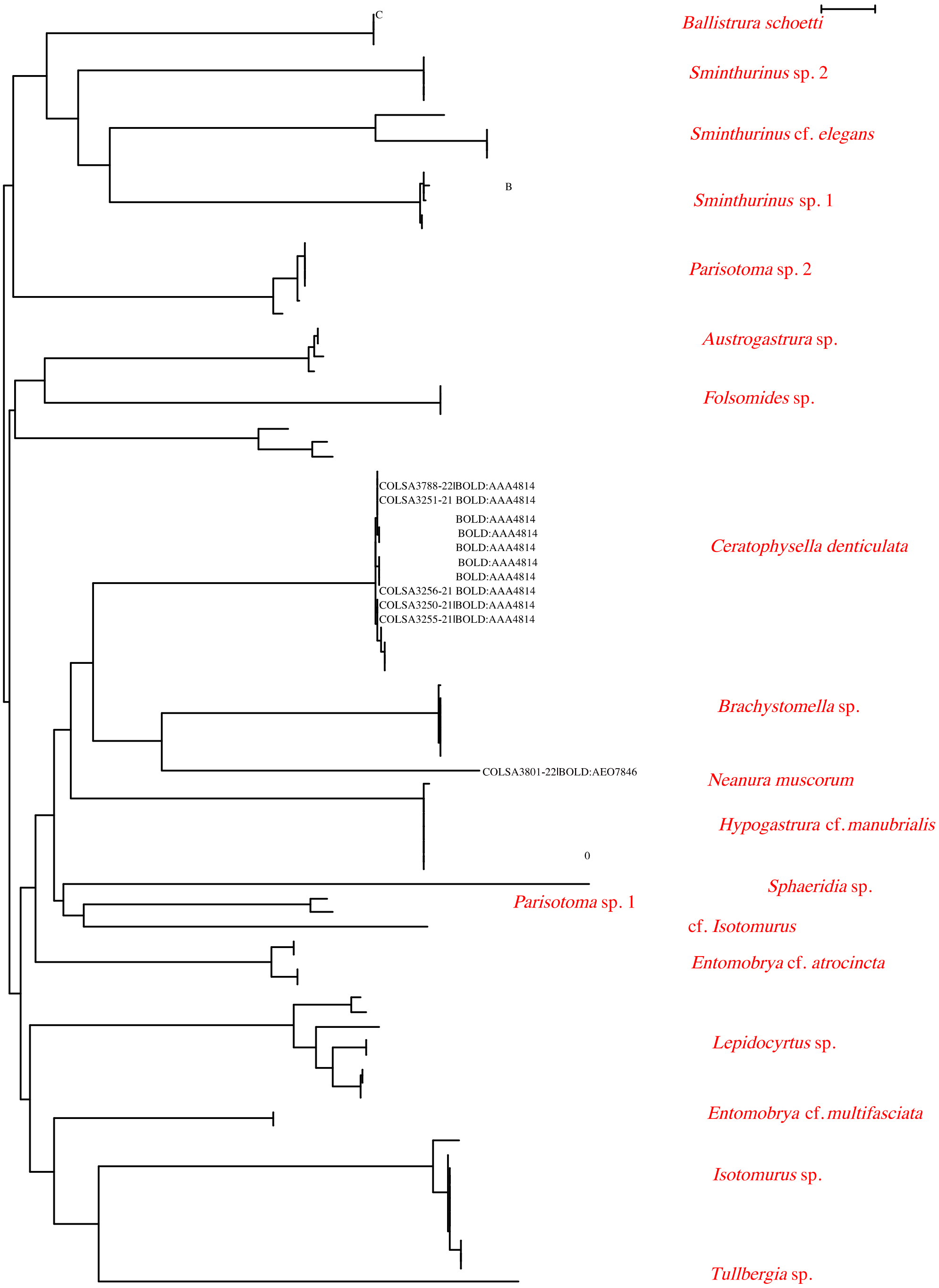

Fig. A2: Barcoding tree of species barcoded in this study.

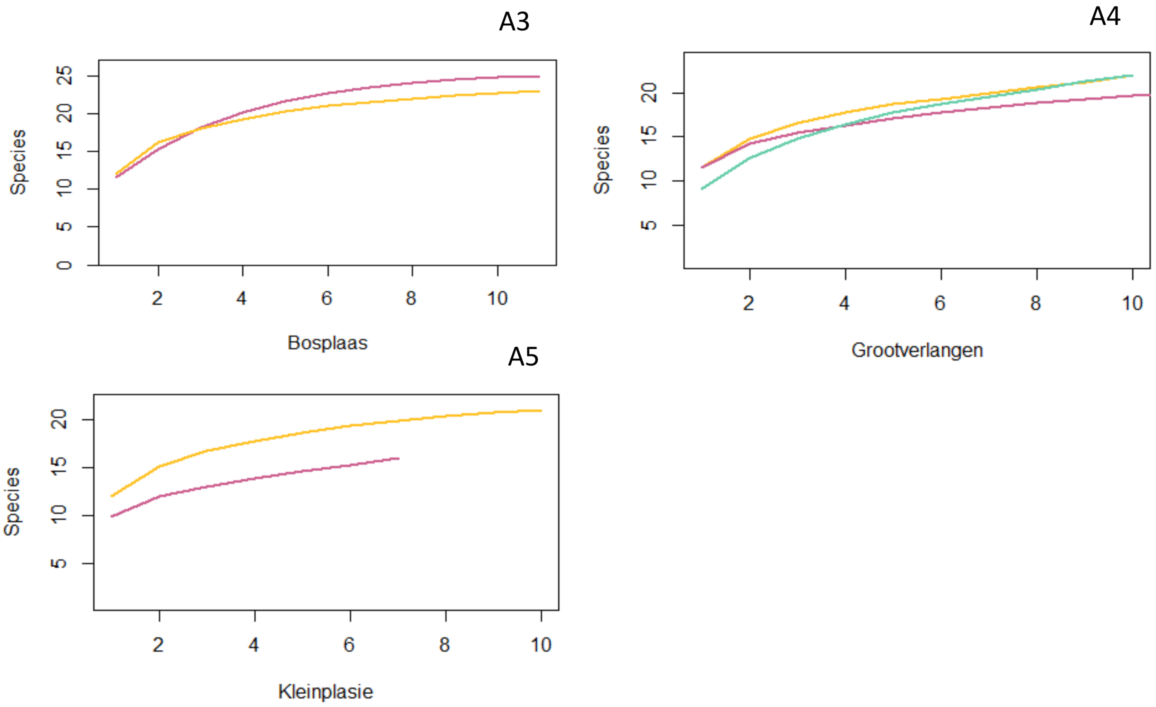

Fig. A3-A5: Species accumulation curves for active (pink) and passive restoration (yellow) sites and native restoration site (blue) at the three study sites sampled in Spring 2020, with Bosplaas top left (A3), Grootverlangen top right (A4) and Kleinplasie at bottom left (A5)

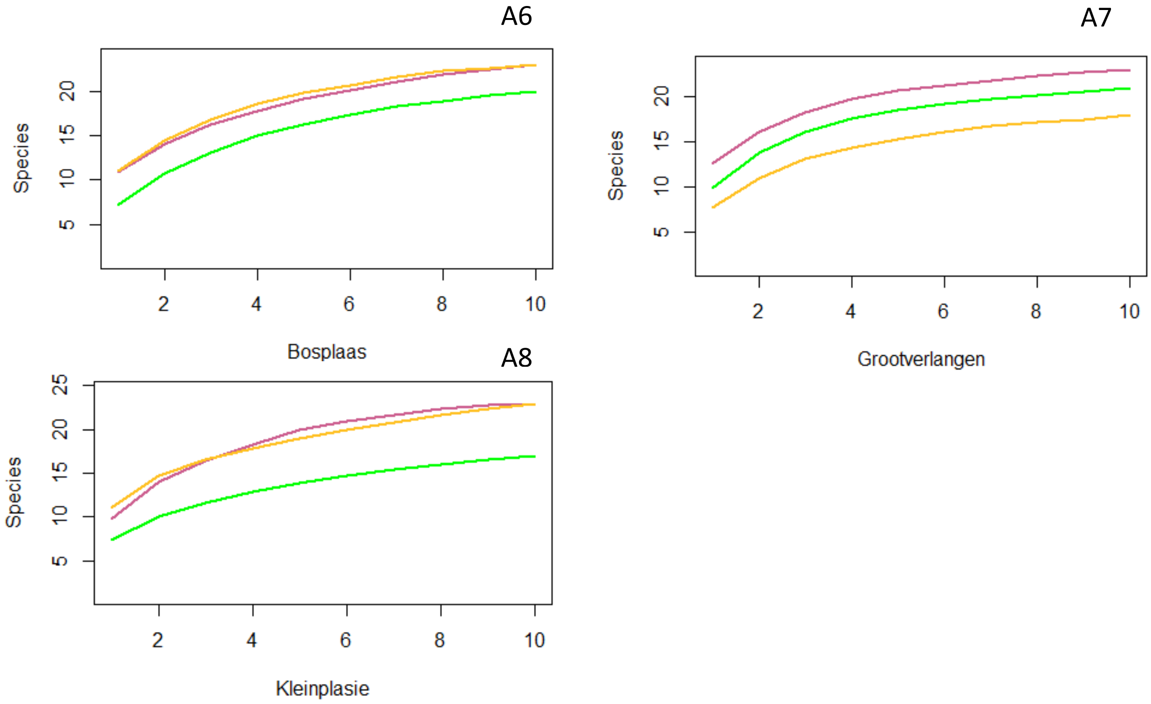

Fig. A6-A8: Species accumulation curves for active (pink), passive (yellow) and invaded (green) areas from Winter 2021 sampling, with Bosplaas top left (A6), Grootverlangen top right (A7) and Kleinplasie at bottom left (A8).

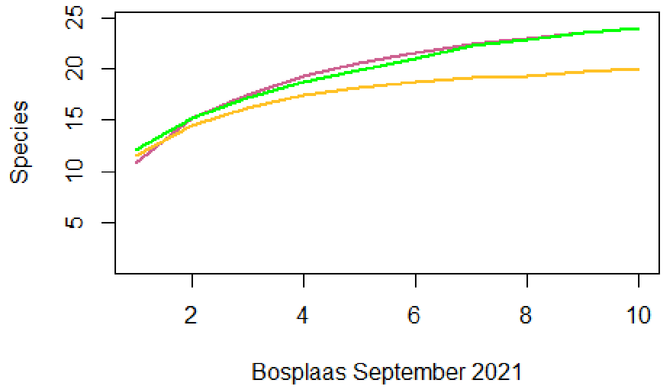

Fig. A9: Sample accumulation curve for Bosplaas site for active (pink), passive (yellow) and invaded (green) restoration areas from Spring 2021 sampling.

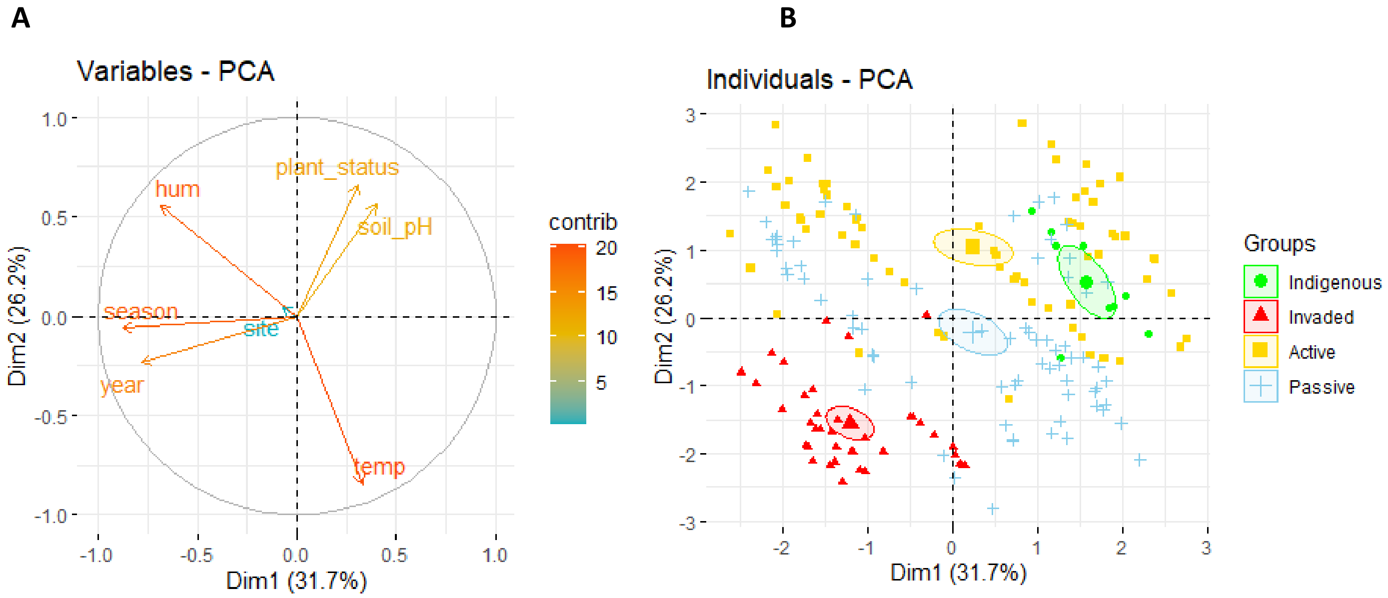

Fig. A10: PCA analysis from all sites and treatments sampled over two years, with A) contributions of environmental variables as well as effect of season and sampling year shown on the axis and (B) Collembola communities. This graph represents the variables included and their contribution to the different axis of the graph.

Fig. A11: Dense kikuyu grasses present at some of the active restoration areas, photo taken from the winter sampling in Grootverlangen.

Table A1: Bemlab soil results from each site sampled during the course of the study

	Type	pH	Resist.	H+	Stone	P (Bray ll)	K			
Site			**H+cmol/Kg**	**Vol %**	**P mg/kg**	**K mg/kg**	**Ca**	**Mg**	**K**	**Na**
Heph Alien	Loam	4.7	1.44	0.93	33.5	147	3.9	2.0	0.38	0.37
Bloekoms Alien	Loam	4.3	1.94	0.96	22.7	147	3.4	1.8	0.38	0.25
Lam Alien	Loam	4.5	1.99	1.02	41.5	273	5.8	3.4	0.70	0.57
Bos Active	Loam	5.3	0.61	1.11	30.7	109	4.1	1.7	0.28	0.19
Bos Passive	Loam	5.4	0.80	1.61	59.6	150	6.4	2.3	0.38	0.24
Groot Active	Sand	4.9	0.91	0.98	58.2	90.6	3.3	1.2	0.23	0.16
Groot Passive	Sand	5.3	0.53	0.93	44.3	57.1	2.2	0.76	0.15	0.11
Groot Native	Sand	5.0	0.72	0.36	57.3	61.8	2.2	0.16	0.12	0.12
Klein Active	Sand	5.3	0.49	0.93	27.3	93.2	2.7	1.0	0.24	0.14
Klein Passive	Loam	5.2	0.58	0.93	38.7	62.8	2.6	0.87	0.16	0.18

		C	S Am.acet	Na	K	Ca	Mg	T Value
Site	**%C**	**S Am.acet (mg/kg)**	**%Na**	**%**	**%**	**%**	**cmol/kg**	**%**
Heph Alien	1.71	8.6	4.58	4.70	48.23	24.74	8.09	17.76
Bloekoms Alien	1.84	10.4	3.22	4.89	43.76	23.17	7.77	24.97
Lam Alien	1.66	19.5	4.58	5.62	46.55	27.29	12.46	15.96
Bos Active	1.45	5.1	2.76	4.07	59.58	24.71	6.88	8.88
Bos Passive	2.20	7.4	2.37	3.76	63.27	22.74	10.12	7.86
Groot Active	1.27	6.6	2.76	3.96	56.88	20.68	5.80	15.72
Groot Passive	0.83	3.6	2.93	4.00	58.61	20.25	3.75	14.21
Groot Native	1.58	7.4	2.95	3.94	54.16	21.17	4.06	17.78
Klein Active	0.85	4.1	3.07	5.26	59.15	21.91	4.57	10.62
Klein Passive	0.93	4.0	4.10	3.64	59.20	19.81	4.39	13.25

Table A2: Details of successfully barcoded specimens from this study.

Species	**Process ID**	**BIN**	**Family**	**Order**
Entomobrya cf. *atrocincta*	COLSA3739-22	BOLD:AAC5785	Entomobryidae	Entomobryomorpha
Entomobrya cf. *atrocincta*	COLSA3740-22	BOLD:AAC5785	Entomobryidae	Entomobryomorpha
Entomobrya cf. *atrocincta*	COLSA3743-22	BOLD:AAC5785	Entomobryidae	Entomobryomorpha
Entomobrya cf. *atrocincta*	COLSA3744-22	BOLD:AAC5785	Entomobryidae	Entomobryomorpha
Entomobrya cf. *multifasciata*	COLSA3299-21	BOLD:ACM1156	Entomobryidae	Entomobryomorpha
Entomobrya cf. *multifasciata*	COLSA3300-21	BOLD:ACM1156	Entomobryidae	Entomobryomorpha
Lepidocyrtus sp.	COLSA3306-21	BOLD:AEK0204	Entomobryidae	Entomobryomorpha
Lepidocyrtus sp.	COLSA3309-21	BOLD:AEK0204	Entomobryidae	Entomobryomorpha
Lepidocyrtus sp.	COLSA3305-21	BOLD:AEK0205	Entomobryidae	Entomobryomorpha
Lepidocyrtus sp.	COLSA3307-21	BOLD:AEK0205	Entomobryidae	Entomobryomorpha
Lepidocyrtus sp.	COLSA3747-22	BOLD:AEK0205	Entomobryidae	Entomobryomorpha
Lepidocyrtus sp.	COLSA3308-21	BOLD:AEJ1494	Entomobryidae	Entomobryomorpha
Lepidocyrtus sp.	COLSA3310-21	BOLD:AEJ1494	Entomobryidae	Entomobryomorpha
Ballistura schoetti	COLSA3275-21	BOLD:AAN4915	Isotomidae	Entomobryomorpha
Ballistura schoetti	COLSA3279-21	BOLD:AAN4915	Isotomidae	Entomobryomorpha
Ballistura schoetti	COLSA3280-21	BOLD:AAN4915	Isotomidae	Entomobryomorpha
Folsomides sp.	COLSA3730-22	BOLD:AFB5639	Isotomidae	Entomobryomorpha
Folsomides sp.	COLSA3731-22	BOLD:AFB5639	Isotomidae	Entomobryomorpha
Folsomides sp.	COLSA3732-22	BOLD:AFB5639	Isotomidae	Entomobryomorpha
Isotomurus sp.	COLSA3733-22	BOLD:AAA5272	Isotomidae	Entomobryomorpha
Isotomurus sp.	COLSA3734-22	BOLD:AAA5272	Isotomidae	Entomobryomorpha
Isotomurus sp.	COLSA3735-22	BOLD:AAA5272	Isotomidae	Entomobryomorpha
Isotomurus sp.	COLSA3736-22	BOLD:AAA5272	Isotomidae	Entomobryomorpha
Isotomurus sp.	COLSA3737-22	BOLD:AAA5272	Isotomidae	Entomobryomorpha
Isotomurus sp.	COLSA3738-22	BOLD:AAA5272	Isotomidae	Entomobryomorpha
Folsomia candida	COLSA3727-22	BOLD:AFB5873	Isotomidae	Entomobryomorpha
Folsomia candida	COLSA3728-22	BOLD:AFB5873	Isotomidae	Entomobryomorpha
Parisotoma sp. 1	COLSA3271-21	BOLD:AEJ3867	Isotomidae	Entomobryomorpha
Parisotoma sp. 1	COLSA3273-21	BOLD:AEJ3867	Isotomidae	Entomobryomorpha
Parisotoma sp. 2	COLSA3714-22	BOLD:AFB6909	Isotomidae	Entomobryomorpha
Parisotoma sp. 2	COLSA3715-22	BOLD:AFB6909	Isotomidae	Entomobryomorpha
Parisotoma sp. 2	COLSA3716-22	BOLD:AFB6909	Isotomidae	Entomobryomorpha
Parisotoma sp. 2	COLSA3717-22	BOLD:AFB6909	Isotomidae	Entomobryomorpha
Parisotoma sp. 2	COLSA3718-22	BOLD:AFB6909	Isotomidae	Entomobryomorpha
Parisotoma sp. 2	COLSA3719-22	BOLD:AFB6909	Isotomidae	Entomobryomorpha
Isotomurus sp.	COLSA3293-21	BOLD:AAA5272	Isotominae	Entomobryomorpha
Isotomurus sp.	COLSA3294-21	BOLD:AAA5272	Isotominae	Entomobryomorpha
Isotomurus sp.	COLSA3296-21	BOLD:AAA5272	Isotominae	Entomobryomorpha
Isotomurus sp.	COLSA3298-21	BOLD:AAA5272	Isotominae	Entomobryomorpha
cf. *Isotomurus* sp.	COLSA3297-21	BOLD:AEK0616	Isotominae	Entomobryomorpha
Brachstomella sp.	COLSA3780-22	BOLD:AAC3250	Brachystomellidae	Poduromorpha
Brachstomella sp.	COLSA3781-22	BOLD:AAC3250	Brachystomellidae	Poduromorpha
Brachstomella sp.	COLSA3782-22	BOLD:AAC3250	Brachystomellidae	Poduromorpha

Brachstomella sp.	COLSA3783-22	BOLD:AAC3250	Brachystomellidae	Poduromorpha
Brachstomella sp.	COLSA3784-22	BOLD:AAC3250	Brachystomellidae	Poduromorpha
Brachstomella sp.	COLSA3785-22	BOLD:AAC3250	Brachystomellidae	Poduromorpha
Austrogastrura sp.	COLSA3260-21	BOLD:AEJ6537	Hypogastruridae	Poduromorpha
Austrogastrura sp.	COLSA3261-21	BOLD:AEJ6537	Hypogastruridae	Poduromorpha
Austrogastrura sp.	COLSA3262-21	BOLD:AEJ6537	Hypogastruridae	Poduromorpha
Austrogastrura sp.	COLSA3708-22	BOLD:AEJ6537	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3247-21	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3249-21	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3250-21	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3251-21	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3252-21	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3253-21	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3254-21	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3255-21	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3256-21	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3786-22	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3787-22	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3788-22	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3789-22	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3790-22	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Ceratophysella denticulata	COLSA3791-22	BOLD:AAA4814	Hypogastruridae	Poduromorpha
Hypogastrura cf. *manubrialis*	COLSA3239-21	BOLD:AAA9010	Hypogastruridae	Poduromorpha
Hypogastrura cf. *manubrialis*	COLSA3240-21	BOLD:AAA9010	Hypogastruridae	Poduromorpha
Hypogastrura cf. *manubrialis*	COLSA3241-21	BOLD:AAA9010	Hypogastruridae	Poduromorpha
Hypogastrura cf. *manubrialis*	COLSA3242-21	BOLD:AAA9010	Hypogastruridae	Poduromorpha
Hypogastrura cf. *manubrialis*	COLSA3243-21	BOLD:AAA9010	Hypogastruridae	Poduromorpha
Hypogastrura cf. *manubrialis*	COLSA3244-21	BOLD:AAA9010	Hypogastruridae	Poduromorpha
Hypogastrura cf. *manubrialis*	COLSA3248-21	BOLD:AAA9010	Hypogastruridae	Poduromorpha
Neanura muscorum	COLSA3801-22	BOLD:AEO7846	Neanuridae	Poduromorpha
Tullbergia sp.	COLSA3712-22	BOLD:AFB6698	Tullbergiidae	Poduromorpha
Sminthurinus cf. *elegans*	COLSA3764-22	BOLD:AAB3495	Katiannidae	Symphypleona
Sminthurinus cf. *elegans*	COLSA3765-22	BOLD:AAB3496	Katiannidae	Symphypleona
Sminthurinus cf. *elegans*	COLSA3766-22	BOLD:AAB3496	Katiannidae	Symphypleona
Sminthurinus cf. *elegans*	COLSA3767-22	BOLD:AAB3496	Katiannidae	Symphypleona
Sminthurinus sp. 1	COLSA3768-22	BOLD:AFB6019	Katiannidae	Symphypleona
Sminthurinus sp. 1	COLSA3769-22	BOLD:AFB6019	Katiannidae	Symphypleona
Sminthurinus sp. 1	COLSA3770-22	BOLD:AFB6019	Katiannidae	Symphypleona
Sminthurinus sp. 1	COLSA3771-22	BOLD:AFB6019	Katiannidae	Symphypleona
Sminthurinus sp. 1	COLSA3773-22	BOLD:AFB6019	Katiannidae	Symphypleona
Sminthurinus sp. 2	COLSA3323-21	BOLD:ABA9662	Katiannidae	Symphypleona
Sminthurinus sp. 2	COLSA3324-21	BOLD:ABA9662	Katiannidae	Symphypleona
Sminthurinus sp. 2	COLSA3325-21	BOLD:ABA9662	Katiannidae	Symphypleona
Sminthurinus sp. 2	COLSA3327-21	BOLD:ABA9662	Katiannidae	Symphypleona
Sphaeridia sp.	COLSA3322-21	BOLD:ABA9682	Sminthurididae	Symphypleona

Table A3: Collembola abundance and richness as well as environmental parameters for each site and sampling time, where N= total samples, Total = total Collembola sampled, sd= standard devation and se = standard error.

Samples 2020 summary:

Site	Type	N	Total	mean	Species richness	Mean temp	Mean humid	ph	sd	se
Groot_spring	Active	385	6801	17.665	21	20.909	61.064	5.3	63.350	3.229
Groot_spring	Passive	350	5746	16.426	23	22.16	59.680	5.1	93.913	5.020
Groot_spring	Native	350	2353	6.723	17	19,811	60,172	5.0	35.667	1.907
Bos_spring	Active	385	4673	12.138	26	20.300	61.291	5.5	92.796	4.729
Bos_spring	Passive	385	9633	25.021	24	19.900	63.420	5.0	159.895	8.149
Klein_spring	Active	245	5189	21.180	17	16.657	67.343	5.6	96.966	6.195
Klein_spring	Passive	350	4178	11.937	22	19.820	65.810	4.9	84.015	4.491

Samples 2021 summary:

Site	Type	N	Total	mean	Species richness	Mean temp	Mean humid	ph	sd	se
Groot_winter	Active	350	3352	13.529	24	15.47	77.85	4.9	53.630	2.867
Groot_winter	Passive	350	4735	14.031	19	16.54	78.03	5.3	69.938	3.738
Groot_winter	Invaded	350	4911	9.577	22	21.1	67.77	4.5	43.110	2.304
Bos_winter	Active	350	5307	15.163	23	16.41	76.77	5.3	103.038	5.508
Bos_winter	Passive	350	1695	4.843	23	19.99	72.05	5.4	15.771	0.843
Bos_winter	Invaded	350	2123	6.066	21	21.98	64.52	4.3	36.296	1.940
Klein_winter	Active	350	4923	14.066	24	21.07	65.97	5.3	67.552	3.611
Klein_winter	Passive	350	2289	6.540	24	23.41	57.21	5.2	26.828	1.434
Klein_winter	Invaded	350	911	2.603	18	21.51	66.58	4.7	9.738	0.521
Bos_spring	Active	350	2344	6.697	24	19.47	62.57	5.3	29.399	1.571
Bos_spring	Passive	350	2052	5.863	20	24.03	55.57	5.4	22.318	1.193
Bos_spring	Invaded	350	5121	14.631	24	22.16	64.12	4.5	101.450	5.423

Table A4: Dunn post-hoc test on different sampling periods between all Collembola densities as sampled over two years, adjusted with the Bonferroni method. Significant values are given in bold (P < 0.05*, <0.01**, <0.001***).

Comparison	Z	P.unadj	P.adj
Spring_2020 - Spring_2021	-2.781872	0.005	**0.016***
Spring_2020 - Winter_2021	-1.380447	0.167	0.502
Spring_2021 - Winter_2021	1.523510	0.127	0.382

Table A5: Dunn post-hoc test between native Collembola densities from all farms, as sampled over two years, adjusted with the Bonferroni method. Significant values are given in bold (P < 0.05*, <0.01**, <0.001***).

Comparison	Z	P.unadj	P.adj
Bosplaas – Grootverlangen	2.9846410	0.0028391135	**0.008****
Bosplaas – Kleinplasie	3.3742548	0.0007401583	**0.002****
Grootverlangen – Kleinplasie	0.5805705	0.5615299400	1.000

Table A6: Dunn post-hoc test on different sampling periods for only native Collembola as sampled in Bosplaas over two years, adjusted with the Bonferroni method. Significant values are given in bold (P < 0.05*, <0.01**, <0.001***).

Comparison	Z	P.unadj	P.adj
Spring2020 - Spring2021	-3.137117	0.001706179	**0.005****
Spring2020 - Winter2021	-1.521070	0.128242351	0.385
Spring2021 - Winter2021	1.780798	0.074945500	0.225

Table A7: Dunn test of native Collembola between all treatments sampled over 2 years.

Comparison	Z	P.unadj	P.adj
Active – Native	1.0487018	0.29431538	1.000
Active – Invaded	-2.1954330	0.02813255	0.169
Native – Invaded	-2.2376834	0.02524171	0.151
Active - Passive	-0.2060465	0.83675459	1.000
Native – Passive	-1.1537011	0.24862274	1.000
Invaded – Passive	2.0306824	0.04228722	0.254

Table A8: Dunn post-hoc test (of all collembola densities) between restoration treatments sampled in Spring 2020, adjusted with the Bonferroni method. Significant values are given in bold (P < 0.05*, <0.01**, <0.001***).

Comparison	Z	P.unadj	P.adj
Active- Native	3.211	0.001	**0.004****
Active-Passive	-0.843	0.399	1.000
Passive-Native	-3.837	< 0.001	**< 0.001*****

Table A9: Dunn test (of all Collembola densities) between restoration treatments sampled in Winter 2021, adjusted with the Bonferroni method. Significant values are given in bold (P < 0.05*, <0.01**, <0.001***).

Comparison	Z	P.unadj	P.adj
Active - Invaded	4.077	< 0.001	**< 0.001*****
Active – Passive	1.935	0.052	0.159
Invaded – Passive	-2.141	0.032	0.097

Table A10: Dunn test (all Collembola) between farms sampled in Spring 2020, adjusted with the Bonferroni method. Significant values are given in bold (P < 0.05*, <0.01**, <0.001***).

Comparison	Z	P.unadj	P.adj
Bosplaas - Grootverlangen	1.402	0.161	0.483
Bosplaas - Kleinplasie	1.035	0.301	0.902
Grootverlangen - Kleinplasie	-0.188	0.851	1.000

Table A11: Dunn test (all Collembola) between farms sampled in Winter 2021, adjusted with the Bonferroni method. Significant values are given in bold ($P < 0.05$*, <0.01**, <0.001***).

Comparison	**Z**	**P.unadj**	**P.adj**
Bosplaas – Grootverlangen	-0.552	0.581	1.000
Bosplaas – Kleinplasie	0.804	0.421	1.000
Grootverlangen – Kleinplasie	1.357	0.175	0.524

Table A12: GLM from species richness with restored (active and passive) as well as invaded sites as a factor of humidity. Significant values are given in bold ($P < 0.05$*, <0.01**, <0.001***).

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	2.661971	0.637064	4.178	0.000 ***
restorationinvaded	5.443042	2.697162	2.018	0.057
restorationpassive	0.527667	0.854956	0.617	0.544
mean_humidity	0.004982	0.009305	0.535	0.598
restorationinvaded:mean_humidity	-0.083663	0.040069	-2.088	**0.049 ***
restorationpassive:mean_humidity	-0.009201	0.012868	-0.715	0.483

Table A13a: GLM from species abundance with restoration as a factor of humidity.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	7.865311	2.151496	3.656	0.0016 **
restorationinvaded	4.508435	12.740684	0.354	0.727
restorationpassive	0.053032	2.962103	0.018	0.986
mean_humidity	0.002854	0.031471	0.091	0.929
restorationinvaded:mean_humidity	-0.080249	0.189352	-0.424	0.676
restorationpassive:mean_humidity	-0.003796	0.044636	-0.085	0.933

Table A13b: post hoc from species abundance with restoration as a factor of humidity.

	Estimate
(Intercept) == 0	7.865
restorationinvaded == 0	4.508
restorationpassive == 0	0.053
mean_humidity == 0	0.003
restorationinvaded:mean_humidity == 0	**-0.080***
restorationpassive:mean_humidity == 0	**-0.004****

Table A14a: GLM of species richness with plant status as a factor of humidity.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	3.224962	0.563002	5.728	9.22e-06 ***
plantstatusnative	-0.564664	0.897324	-0.629	0.536
mean_humidity	-0.003785	0.008533	-0.444	0.662
plantstatusnative:mean_humidity	0.007108	0.013474	0.528	0.603

Table A14b: Results from post-hoc test of species richness with plant status as a factor of humidity.

	Estimate	Std. Error	z value	Pr(>|z|)
native - invasive == 0	-0.2821	0.8652	-0.326	0.744

Table A15: GLM of species abundance with plant status as a factor of humidity.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	9.50709	2.04591	4.647	0.000 ***
Plantstatusnative	-3.46376	3.01460	-1.149	0.263
mean_humidity	-0.02675	0.03156	-0.847	0.406
plantstatusnative:mean_humidity	0.05471	0.04536	1.206	0.240

Table A16a: GLM from species richness with restoration as a factor of temperature.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	3.25537	0.51464	6.326	3.57e-06 ***
restorationinvaded	-4.05961	2.87588	-1.412	0.173
restorationpassive	-0.48968	0.79676	-0.615	0.546
mean_temperature	-0.01340	0.02691	-0.498	0.624
restorationinvaded:mean_temperature	0.18190	0.13465	1.351	0.192
restorationpassive:mean_temperature	0.02106	0.04025	0.523	0.607

Table A16b: Post hoc from species richness with restoration as a factor of temperature.

	Estimate
(Intercept) == 0	3.25537
restorationinvaded == 0	-4.05961
restorationpassive == 0	-0.48968
mean_temperature == 0	-**0.01340***
restorationinvaded:mean_temperature == 0	0.18190
restorationpassive:mean_temperature == 0	**0.02106***

Table A17a: GLM from species abundance with restoration as a factor of temperature.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	9.27563	1.53792	6.031	6.76e-06 ***
restorationinvaded	-1.40157	11.52237	-0.122	0.904
restorationpassive	1.82581	2.49505	0.732	0.473
mean_temperature	-0.06457	0.08179	-0.790	0.439
restorationinvaded:mean_temperature	0.03070	0.54212	0.057	0.955
restorationpassive:mean_temperature	-0.09964	0.12989	-0.767	0.452

Table A17b: Post hoc from species abundance with restoration as a factor of temperature.

	Estimate
(Intercept) == 0	9.276
restorationinvaded == 0	-1.402
restorationpassive == 0	1.826
mean_temperature == 0	-0.064
restorationinvaded:mean_temperature == 0	**0.031***
restorationpassive:mean_temperature == 0	-0.100

Table A18: Spearman’s correlation matrix comparing species richness and abundance with mean temperature, humidity and pH of all sites sampled in 2020.

	species_ abundance	**species_ richness**	**mean_ temperature**	**mean_humidity**	**pH**
species_abundance	1.000	0.162	0.136	-0.189	-0.289
species_richness	0.162	1.000	0.678	-0.639	-0.334
mean_temperature	0.136	0.678	1.000	-0.895	-0.502
mean_humidity	-0.189	-0.639	-0.895	1.000	0.099
pH	-0.289	-0.334	-0.502	0.099	1.000

Table A18: Spearman’s correlation matrix comparing species richness and abundance with mean temperature, humidity and pH of all sites sampled in 2021.

	species_abundance	**species_richness**	**mean_ temperature**	**mean_humidity**	**pH**
species_abundance	1.000	0.275	-0.480	0.424	0.263
species_richness	0.275	1.000	-0.026	-0.111	0.346
mean_temperature	-0.480	-0.026	1.000	-0.960	-0.334
mean_humidity	0.424	-0.111	-0.960	1.000	0.290
pH	0.263	0.346	-0.334	0.290	1.000