Investigation of interspersed shrubland patches along different topographical conditions within Afromontane grasslands, and their potential as conservation hotspots

By

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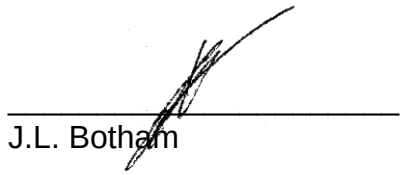
Declaration

I, Jason Lee Botham, declare that the thesis or publishable manuscripts that I herewith submit for the Doctoral Degree in Entomology at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.



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Abstract

Smaller, isolated shrubland patches are often overlooked in the context of their ecosystem functions and potential for conserving biodiversity, particularly in relation to small scale areas of diversity importance within a specific conservation area. The mountainous Golden Gate Highlands National Park (GGHNP) possesses a number of these smaller, isolated shrubland patches, providing several habitat types over a relatively small geographical area. While these shrubland patches have been acknowledged as areas of possible significance in the GGHNP, information on arthropod assemblages in these patches remains limited. This thesis investigates arthropod assemblages in these smaller shrubland patches, in response to various environmental factors, and provides a preliminary view of the current state of gene flow among five ubiquitous spider species. Three aims were selected to investigate environmental factors and their influence on arthropod assemblages, while a fourth addressed gene flow and genetic diversity of spider species.

In Chapter 2, we evaluate the effect of elevation and vertical stratification on arthropod species diversities and assemblages in shrubland patches present in the GGHNP. The results indicated differing environmental pressures between and within the sampled sites brought about by elevation and stratification. However, due to the relatively short elevational range and high heterogeneity of the localities, it is not conclusive as to whether elevation was the only factor responsible for differences in population diversities across the sites. Contrastingly, vertical habitat stratification influenced arthropod assemblage richness and composition despite the small vertical distance between strata. The study suggests that multiple contributing factors, together with stratification, affect arthropod assemblages.

Investigations into seasonal variation and temporal change on arthropod populations may provide insight into the protective nature of smaller shrubland patches against environmental change and disturbance. Chapter 3 aimed to determine how arthropod assemblages vary with seasonal changes in three vertical habitat strata in these smaller shrubland patches, representing isolated shrublands in a grassland-dominated montane environment subject to seasonal variation. Environmental variable changes temporally affected arthropod assemblages to differing degrees in each stratum, dependent on the level of disturbance. Beta-diversity was observed to gradually decrease for leaf litter across the localities. The study suggests that, depending on the level of protection of these patches, shrubland sites may act as high diversity zones during seasonal change as well as periods of disturbance.

Chapter 4 investigated the differences in composition of soil arthropod assemblages, and their species association, with a number of isolated shrubland patches and their immediate surrounding grasslands. The results indicated a higher association of soil biota with shrubland patches compared to the adjacent grasslands, while functional feeding groups were not discernibly different between the two habitat types. Results suggest that different soil arthropods are associated with shrubland patches of the GGHNP, hinting at their significance as areas of priority management for certain taxa and their importance in conservation strategies.

The final study (Chapter 5), analysed the state of gene flow and inferred migratory capability of five spider species between shrubland patches of the GGHNP. Relatively low nucleotide diversity, with a correspondingly high genetic diversity, was observed within populations for all species except one. Differing genetic differentiation indicated gene flow as being maintained, to a certain degree, between populations of the spider species, despite the mountainous terrain of the park. Additionally, the

presence of possible species complexes was inferred by phylogenetic analyses, highlighting a need for taxonomic revision of these species from a South African perspective.

The results of this thesis provide a unique insight into the state of arthropod diversity, and their association with shrubland patches of the GGHNP, by investigating arthropod assemblage responses, and determining the current state of gene flow and genetic diversity of spider species.

Keywords: Arthropods; Canopy; Conservation; Elevation; Gene Flow; Golden Gate Highlands National Park; Leaf Litter; Soil; Soil Biota; Spiders

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Chapter 1

General introduction

General introduction

The conservation of natural landscapes, in an effort to maintain liveable habitats and functional ecosystems for terrestrial biodiversity, remains an enduring endeavour. The alteration of natural ecosystems, due to a variety of anthropogenic disturbances, influences biodiversity composition and productivity (Bradshaw, 2012; de Lima et al., 2013; Hundera et al., 2013). Changes in biodiversity, in turn, impacts ecosystem composition and processes and services due to being intimately interrelated (Muchoney, 2008). Responses of arthropod communities to environmental change, both spatially and temporally, have been well studied (e.g. Didham and Springate 2003; Moretti et al., 2006; Basset et al., 2008a, b; Hirao et al., 2008; Cardoso et al., 2009; VanTassel et al., 2015), as knowledge of community responses to disturbance has allowed for more concerted efforts in the application of conservation strategies and environmental mitigation (Kremen et al., 1993; Bangert and Slobodchikoff, 2006; Hartley et al., 2007; Ulyshen, 2011; Leroy et al., 2014).

Spatial and temporal variation

Species diversity, being a key component of ecological systems, is fundamental in the process of most ecosystem services from a regulatory context (Hooper et al., 2002). This diversity is a vital component contributing to landscape complexity and structure, and is often one of the most commonly measured landscape attributes (Azevedo et al., 2014). However, determining how diversity is distributed over time and space is often one of the main challenges in conservation biology (Ramírez-Hernández et al., 2014).

Phenology and inter-annual variation of arthropod communities has become an important field of study, allowing for the determination and prediction of climate change

and early detection of major disturbances (Høye and Forchhammer, 2008; Hodgson et al., 2011; Pau et al., 2011; Cleland et al., 2012; Valtonen et al., 2013; Bowden et al., 2018). It is generally reasonable to assume that climate change would affect spatial and temporal association between species interacting at different trophic levels within an environment (Sparks and Carey, 1995; Stefanescu et al., 2003; Harrington et al., 2010). The short generation times, high species richness and abundance of arthropod communities make them well suited in addressing temporal dynamics and phenology in a number of environments (Smith and Smith, 2012; Valtonen et al., 2013).

While seasonal variation and abundance of arthropod assemblages is often linked to seasonal weather fluctuations, either directly or by available resources and predators (Azerefegne et al., 2001; Meltofte et al., 2007), variation may also arise due to species phenological adaptations to such environmental fluctuations (Tauber et al., 1986; Wolda, 1989; Roy and Sparks, 2000). For example, a study by Jönsson et al. (2009) described the temperature dependence of the spruce bark beetle, *Ips typographus*, a major insect pest of the mature Norway spruce forests. The study suggested that the shift in climate to warmer temperatures through the 20th century caused a shift in the beetle's activity periods, showing that monitoring of these populations in their respective geographical location was needed for adequate and sustainable forest management. As such, it is generally accepted that each species responds differently to changing environmental factors (e.g. Bale at al., 2002; Dingemanse and Kalkman, 2008; Kingsolver et al., 2011; Radchuk et al., 2013), highlighting the importance of monitoring arthropods on an ecological scale. The migratory ability of certain species will also impact their ability to track resources spatially, in turn affecting seasonal variation in abundance, often over several generations (Southwood, 1962; Brower, 1996; Larsen, 2005).

The inference of how assemblages are affected due to gradual changes in climatic conditions and disturbances are often investigated along gradients (Sheldon et al., 2011). While studies of temporal variability have increased, spatial studies still exceed them (de Juan and Hewitt, 2014). Elevational gradients across mountainous landscapes have been deemed key to understanding diversity patterns and their distribution, with increased focus placed on patterns of species richness (Botes et al., 2006; Sanders and Rahbek, 2012; Bishop et al., 2014; Foord and Dippenaar-Schoeman, 2016). Investigations pertaining to the effect of elevational gradients on species richness have demonstrated two distinct patterns, peak richness occurring at mid-elevations, and linear decline as elevation increases (Hebert, 1980; Rahbek, 1995; Foord and Dippenaar-Schoeman, 2016). A pattern in species richness is often observed along an elevational gradient, with this richness widely accepted to decline with increasing elevation as temperature decreases (Rahbek, 1995). However, whether this decline is monotonic or assumes varying shapes due to the investigated taxa or locality is still debatable. Various taxa and regions have reported mid-elevational peaks in species richness, with empirical support for small mammals (McCain, 2004), ants (Sanders, 2002; Chaladze, 2012), spiders (Chaladze et al., 2014), and plants (Grytnes and Vetaas, 2002; Grytnes, 2003). Causative factors for these mid-elevational peaks are thought to be related to elevational condensation zones (Rahbek, 1995), rainfall and productivity (Rosenzweig, 1992), area (Rahbek, 1997), and resource diversity (Gentry, 1988; Sánchez-Cordero, 2001), while other sources have attempted to explain this mid-domain effect with geometric theory (Colwell and Lees, 2000). Colonisation ability of taxa is also taken into consideration, with the dispersal ability of a species and the local ecological conditions acting as filters in colonisation dynamics, selecting those species with suitable traits for local

conditions (Guisan and Rahbek, 2011). However, while multiple hypotheses have been proposed to explain elevational diversity gradients, none of them accurately describe this phenomenon in full.

While there are many factors that may drive patterns of species richness across these elevational gradients (Beck et al., 2010), the interactivity of elevation with vertical stratification has warranted increased focus (Reynolds and Crossley, 1997; Ashton, 2013; Scheffers et al., 2013; Ashton et al., 2016). Vertical habitat stratification has long been an established concept in arthropod ecology, and is known to display habitat discontinuity between ground and canopy strata (Longino and Nadkarni, 1990; Basset et al., 2003). While relative diversity and endemism of species can be addressed across selected strata, results are often heavily dependent upon the behaviour and physiology of investigated taxa (Prinzing, 2005; Ulyshen 2011; van Dooremalen et al., 2013). The use of a wider range of arthropod taxa may, at times, provide a more holistic view of the mechanisms involved in spatial distribution and variation of arthropods across landscapes and elevations (Karr, 1991; Kremen et al., 1993; Kotze and Samways, 1999; Gerlach et al., 2013), as well as providing an assessment of biodiversity and ecological health.

Given that annual seasonal variation and elevation may alter entire populations of arthropods as a response to environmental change (Harrington and Stork, 1995), it is imperative that species populations be monitored as part of conservation initiatives. However, despite certain studies investigating temporal and spatial variation of arthropods across various strata and elevation (Oliveira and Scheffers, 2018), these investigations are often concentrated on large tropical forests (Chapin and Smith, 2019), with fewer studies conducted in smaller woody habitats in South Africa's temperate climate (Basset et al., 2003).

Soil quality and arthropod diversity

Soil ecosystems are among the most complex habitats on earth (Stork and Eggleton, 1992), consisting of many components, including macro- and mesofaunal, microbial and fungal life (Wall et al., 2012). It provides a protective habitat for at least part of the lifecycle of several faunal species (Stork and Eggleton, 1992), and essentially maintains natural ecosystems for most, if not all, terrestrial organisms as it plays a role in many food webs and developmental cycles (Stork and Eggleton, 1992; Yan et al., 2012). Soil quality can be defined as the ability of a soil to sustain biological productivity, maintain environmental quality, and promote plant, animal and human health (Doran and Parkin, 1994). It is an accepted approach to evaluate sustainability of ecosystems by assessing the fluctuations in soil quality (Schoenholtz et al., 2000).

Invertebrates are an essential component of soils, and are important as indicators for determining the state and suitability of soils for sustainable plant growth (Stork and Eggleton, 1992). Soil fauna has been found to play an important role in maintaining nutrient cycling and biological soil fertility (Wolters, 2000; Yan et al., 2012), as various groups are involved in vital soil functions and show sensitivity to soil environmental changes (e.g. Buckerfield et al., 1997; Paoletti and Hassal, 1999; Paolo et al., 2010).

Given the important role that soil arthropods play in the ultimate survival and continuation of ecosystems, it is justified to include soil arthropods as an active part of conservation considerations, especially in protected areas, in order to ensure the long-term sustainability of these ecosystems. However, despite the far-reaching effects that loss of soil biodiversity would cause, many conservation plans do not take soil fauna as a concerning factor when making conservation decisions. This may possibly be due

to the lack of information or studies on these faunal groups in parks and protected landscapes, with little known on their behaviour and precise soil functions in these areas.

Gene flow and genetic diversity

The development of natural landscapes into mosaics, either by anthropogenic influence or natural insularity, is known to have several ramifications on species diversity, population levels and distribution (Samways, 1996). Of major concern is the loss of gene flow between isolated faunal populations. A large number of species are known to comprise isolated populations which are subject to a loss of genetic diversity due to inbreeding, in turn elevating population extinction risks (Frankham et al., 2014; Frankham, 2015). While migrants are able to alter the distribution of genetic diversity within populations, ensuring increased homogeneity, the migratory ability of certain taxa can be ineffective to maintain adequate transfer of genetic variation between populations (Frankham et al., 2002), especially over large distances. This is particularly true of certain epigean species whose lifestyle has been noted to constrain gene flow (Caccone, 1985; Panaram and Borowsky, 2005; Porter, 2007; Osakabe et al., 2009; Smith et al., 2009).

Mountains are considered one of the major barriers to successful gene flow, with numerous studies investigating its effect on different taxa (Grobler et al., 2003; Vignieri, 2005; Measey et al., 2007; Lachmuth et al., 2010; Varudkar and Ramakrishnan, 2015). They often limit the extent of dispersal ability of species, increasing geographic isolation (Murphy et al., 2010; Qiong et al., 2017). However, it has also been suggested that mountains may act as more permeable filters over time, and not absolute barriers (Sánchez-Montes et al., 2018). The degree of movement

and flow of organisms through a landscape becomes critical for the long-term viability of metapopulations within a geographical area (Ovaskainen and Hanski, 2004; Taylor et al., 2006), increasing the prospect of recolonization after local extinction (Bouchy et al., 2005; Jiang et al., 2007). As such, ensuring functional connectivity, and understanding the factors that may influence it, has become a central theme in landscape ecology and conservation biology (Murphy et al., 2010). However, despite efforts to maintain gene flow between isolated populations in highly conserved areas, it is generally accepted that cessation or a decrease in gene flow is an important factor for speciation (Papadopulos et al., 2011; Feder et al., 2012; He et al., 2019). This warrants investigations as to the current condition of gene flow among populations of certain species in areas of conservation importance, in order to determine the status of evolutionary divergence among these populations, and the implications this holds for conservation.

Description and history of the study area

The Golden Gate Highlands National Park (GGHNP) is situated in the eastern part of the Free State Province, South Africa (28°30' S, 28°37' E). Proclaimed a National Park in 1962, and officially opened in 1963, the GGHNP originally covered a core area of 17.92 km^2, and was further enlarged to 116.30 km^2 over the next 26 years with the addition of surrounding farmlands to the park's conservation area (SANParks, 1989; Rademeyer and van Zyl, 2014). During this time, the park shared borders with the country of Lesotho and the adjacent Qwaqwa National Park (QNP). In 2004, the incorporation of the QNP into the GGHNP was announced as a means of increasing the viability and meaningful environmental management of the areas (Rademeyer and van Zyl, 2014). However, this amalgamation was not enacted until 2008 (SANParks,

2009), after which 211.28 km^2 of grassland was added to the GGHNP. A total area of approximately 340 km^2 is now designated as the GGHNP and encompasses a variety of terrestrial and wetland habitats with rich fauna and flora (SANParks, 2013). The topography of the park includes a number of sandstone outcroppings and high elevated areas ranging from approximately 1600–2900 m a.s.l., with the highest recorded point being the Ribbokkop peak at 2829 m a.s.l. (SANParks, 2013).

The GGHNP falls within a temperate climate and summer rainfall zone (Grab et al., 2011; Telfer et al., 2012). Descriptions of the climatic conditions experienced in the park are given in Chapters 2–4, and will therefore not be described here.

Vegetation in the park is dominated by montane grasslands with a variety of shrubland and forest patches (SANParks, 2019), leading to the GGHNP being labelled as the only grassland National Park in South Africa. The majority of plant species are designated under five main vegetative units as described by Mucina et al. (2006), with the two dominant veld types being Highland-Sourveld and *Themeda-Festuca* (SANParks, 2019). Encroachment between plant communities is a common occurrence (Kay et al., 1993). With the inclusion of the QNP, a marked difference between vegetation of the western and eastern sections of the park are evident. The flatter, generally lower lying, eastern section is largely dominated by natural grasslands with interspersed shrubland patches (Avenant, 1997; Mucina et al., 2006). While the higher elevated western part also carries a largely grassland vegetation, the more prominent gorges, valleys and clefts house a large number of shrubland and forest vegetation which, at times, is classified as high altitude Afromontane forests (Roberts, 1969; SANParks, 2019). Alien vegetation is also prominent throughout the park (Spear et al., 2011), with the removal of large quantities of invasive trees and

shrubs being the focus of management practices over the years (SANParks, 1987; SANParks, 2013).

As the focus of this thesis is shrubland patches located throughout the GGHNP, a general overview of the most dominant woody vegetation present in selected sites utilised in this study is given in table form in Chapters 2 and 3, along with site location and elevation. Shrubland patches throughout the park are associated with a variety of slope aspects, and these relatively small patches are embedded in a matrix of natural grassland that is subject to annual wildfires, which is a characteristic environmental factor preventing expansion of woody vegetation (Roberts, 1969; Trollope et al., 2002; Adie et al., 2017).

Apart from livestock, a variety of wildlife occurs throughout the boundaries of the park, the majority of which were re-introduced by the Parks Board (Labuschagne, 1969; Radmeyer and van Zyl, 2014). The different species were deemed to have originated from this area, and even before the GGHNP was proclaimed, a number of game were stocked into the region, including red hartebeest, blesbuck and black wildebeest (van Rensburg, 1968). In time, herds of these species, as well as herds of springbuck, eland and zebra, had settled within the surrounding landscape and have remained to this day (SANParks, 2012). Various animal censuses have deemed the majority of antelope species to have adapted well to this mountainous region (SANParks, 1974; SANParks, 1980; SANParks, 2012). Apart from ungulates, ornithological observations have identified up to 176 prominent bird species to occur in the park (SANParks, 2012), and a vulture restaurant was constructed in 1993 which operates as part of the international conservation programme in Southern Africa (SANParks, 2005). A number of horses and donkeys can also be found wandering the park due to their use in the past for traversing the mountainous terrain and transporting

material for fence construction (van Zyl, 1976; Radmeyer and van Zyl, 2014). These equines were, and still are, used by park rangers for patrol purposes, as well as by tourists for horse riding activities (SANParks, 1983; SANParks, 1985; Radmeyer and van Zyl, 2014). Despite the numerous reports of birds and vertebrates occurring in the GGHNP, very little information is available regarding invertebrates, with sporadic reports pertaining to small scale biodiversity, paleontological and taxonomic studies (Meyer, 1970; Louw, 1988; Bordy et al., 2009; Hugo-Coetzee, 2014). An exception is butterflies, where there is reasonable knowledge of the endemic and threatened butterflies of the GGHNP (Woodhall, 2005).

Thesis aims, objectives and overview

This thesis was conducted over a 24-month period in six shrubland patch localities of the GGHNP, and aimed at assessing four ecological aspects pertaining to arthropods associated with these shrubland patches. These aims are addressed and explored in their own individual chapters (Chapters 2–5), with multiple hypotheses put forward in the context of each study.

Due to the mountainous nature of the GGHNP, changes in elevation and slope aspect can provide varying environmental conditions in the shrubland patches of the park. Changes, such as patch structure, are able to impact faunal diversity and composition despite similar vertical stratification being present. In Chapter 2, I investigate how elevation and vertical stratification in shrubland patches of the GGHNP may affect arthropod species diversity and assemblage composition.

The role of smaller patches in maintaining diversity and species richness across montane mosaic environments during seasonal variation and periods of disturbance have been proposed (Kremen et al., 1993; Hunter, 2002; Adie et al., 2017). With this

in mind, in Chapter 3 I aim to evaluate how arthropod assemblages and communities vary across three vertical strata with seasonal change in these smaller shrubland patches, representing isolated shrublands in a grassland-dominated montane environment that is subject to considerable seasonal climatic variation. Seasonal variation and the impact of environmental variables on arthropod assemblages are the main premise of this study, alongside a preliminary look at species turnover responses in the three investigated strata.

Wintle et al. (2019) demonstrated the high conservation value of small patches, deeming them critical in their contributions to biodiversity conservation and systematic conservation plans. As many shrubland patches of the GGHNP occur within a montane grassland landscape, forming a patchwork of different habitats, their relation to the immediate surrounding environment, and the effect of this surrounding vegetation on arthropod assemblages, comes into question. Additionally, soil arthropod ecology is a sorely understudied field (Janion-Scheepers et al. 2016), particularly in the National Parks of South Africa. This is particularly true of the GGHNP as only small, taxonomically specialised studies (Meyer, 1970; Hugo-Coetzee, 2014) have provided any insight as to the current composition of soil biota that occurs in this mountainous environment. As such, the fourth chapter of this thesis attempts to determine soil arthropod assemblages associated with a number of isolated shrubland patches, and their immediate surrounding grasslands, within the GGHNP. Concurrently, a preliminary determination of soil biota that may be considered in future investigations as indicators in ecological studies of shrubland patches in the GGHNP is given.

As mentioned previously, the development of landscapes into mosaics has warranted consistent investigation as such occurrences are able to affect species

diversity, assemblage structure and distribution. Central to these investigations is the effect of isolation and population disconnection on genetic diversity, and maintaining connectivity between isolated patches in the form of gene flow. As mountains are considered a primary orographic barrier to maintaining genetic homogeneity, the GGHNP offers an opportunity to study gene flow and genetic diversity of faunal populations across a highly variable landscape. To this end, the aim of Chapter 5 is to identify the current state of gene flow and inferred migratory capability of five ubiquitous spider species between isolated shrubland patch populations of this National Park. In addition to this, the phylogenetic relationship of the investigated taxa to closely related, homologous species are also considered, in part, to provide preliminary context for future taxonomic research of these species in South Africa.

The final chapter (Chapter 6), discusses the results obtained from the four investigated aims, with emphasis placed on the most significant results in the context of conservation impact and mitigation. Some recommendations for conservation management of arthropod species richness and diversity across these shrubland patches are provided, as well as suggestions for future investigations applicable to the GGHNP.

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Chapter 2

Responses of arthropod assemblages to vertical stratification over a short elevation gradient in interspersed shrubland patches in a grassland landscape

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Abstract

Strong zonation of vegetation with elevation is often expected to confound studies of elevational diversity gradients, impacting faunal responses. This increases the importance of monitoring elevational responses of fauna over shorter gradients. Such investigations are particularly applicable to areas with relatively low elevational change such as shrubland patches in the Golden Gate Highlands National Park (GGHNP). Additionally, investigating faunal stratification across isolated landscape units, such as these shrubland patches, may inform as to the significance of these sites as priority areas for mangement. The main purpose of this study was to evaluate the effect of elevation and vertical stratification on arthropod species diversities and assemblages in selected shrubland patches of the GGHNP. These sites were sampled over a 24-month period from three habitat strata (i.e. canopy, leaf litter and soil), producing a total of 62 699 arthropod individuals, comprising 28 orders and 1211 morphospecies. A clear pattern of vertical stratification was indicated across all localities, with a higher number of unique species observed in the canopy layer. Assemblage diversity and richness decreased successively from the canopy to the soil stratum across all localities, while leaf litter maintained the highest abundance. Differing responses of diversity and species richness were noted across the different strata, with canopy assemblages experiencing a decline as elevation increased. Although increasing elevation showed significant correlation with species richness and diversity, the heterogeneous nature of the sampled localities may have had a greater effect on arthropod assemblages. Considerable potential to investigate the impact of patch heterogeneity over a short elevation gradient is emphasised.

Keywords: Canopy; Diversity; Golden Gate Highlands National Park; Leaf Litter; Soil; Species Richness

Introduction

Elevational variation is often considered one of many determinate factors to changes and interactions in arthropod assemblages across a landscape (Grytnes and McCain, 2007; Arnan et al., 2015). Studies have reported on the significant changes of arthropod diversities and species richness along elevational gradients, either to a positive or negative correlation, and their impact for future conservation, particularly with regards to climate change (Foord et al., 2015; Foord and Dippenaar-Schoeman, 2016; González-Reyes et al., 2017; Röder et al., 2017; Høye et al., 2018). Different diversity patterns emerge along elevational gradients, including mid-elevational peaks, linear decline, low-elevational plateaus, or certain combinations, which are often closely related to the ecology of a taxon (e.g. McCain and Grytnes, 2010; Ashton, 2013; Lee and Chun, 2015). Variation in species richness along an elevational gradient is driven through climate, space, biotic processes and evolutionary history (McCain and Grytnes, 2010). However, while investigations into these drivers often take place over large elevational ranges due to temperature variation giving rise to vegetation zonation, the investigation of their responses over smaller elevation ranges may provide insight for more specific drivers.

Locally (on small scales), elevation can be correlated to certain environmental attributes, such as biogeochemical soil properties (Behrens et al., 2014), which are usually not strongly related to elevation on a larger scale (Körner, 2007; Barry, 2008). Additionally, elevation is known to shape habitats in multiple ways. One such occurrence is by elevation zonation of vegetation types, whereby areas across a large elevation range differ in vegetation composition due to varying environmental conditions (Halpern and Spies, 1995). The strong zonation of vegetation with elevation is often expected to confound studies of elevational diversity gradients. In this sense,

relatively short gradients, containing similar vegetation types, may become valuable in investigating species responses to elevation.

Concurrently, while there are many factors that may drive spatial variation of species richness across elevational gradients, the impact of elevation on arthropod assemblages across specific vertical strata remains speculative. As such, the interactivity of elevation with vertical stratification has warranted increased focus (e.g. Reynolds and Crossley, 1997; Ashton, 2013; Scheffers et al., 2013; Ashton et al., 2016). Vertical habitat stratification has long been an established concept in arthropod ecology, and is known to display habitat discontinuity between ground and canopy strata (Longino and Nadkarni, 1990; Basset et al., 2003). Studies of vertical stratification have attributed a number of factors towards its effect on arthropod assemblages, including arthropod behaviour, inter- and intra-specific competition, availability of resources, and a variety of abiotic factors (Stork et al., 1997; Basset et al., 2003; Floren and Schmidl, 2008). These factors alter the degree to which arthropod communities are vertically stratified, influencing their relative diversity and endemism. This, in turn, influences the response of these communities to elevational change as differing responses of faunal richness have been documented along elevational gradients in various vertical strata (e.g. Olson, 1994; Reynolds and Crossley, 1997; Brühl et al., 1999; Jing et al., 2005; Hasegawa et al., 2006; Röder et al., 2010).

In this study we provide an evaluation on the effect of elevation and vertical stratification on arthropod species diversities and assemblages in selected woody shrubland patches present in the South African Golden Gate Highlands National Park (GGHNP). In particular, we investigate the response of arthropod assemblage

diversity and richness, in the various selected strata, to elevational change across a relatively short gradient.

Materials and methods

Study site and period

The GGHNP is situated in the Rooiberge of the eastern Free State Province, in the foothills of the Maluti Mountain range (28°30' S, 28°37' E), covering an area of approximately 340 km^2 (Fig. 2.1). It is the Free State Province's only National Park, better known for its landscape than its wildlife, and is labelled as a montane grassland landscape (Taru et al., 2013).

The park is located in the eastern Highveld region of South Africa, with elevation ranging from approximately 1600 to 2900 m a.s.l. (SANParks, 2013). Rainfall in the park occurs during warmer months of October to April, with relatively high rainfall of approximately 800 mm per annum (Groenewald, 1986). Heavy snowfalls are also known to occur during the winter months (Grab et al., 2011). The southern boundary of the park is formed by the Caledon River, which additionally forms the border between the Free State Province and the country of Lesotho (SANParks, 2013).

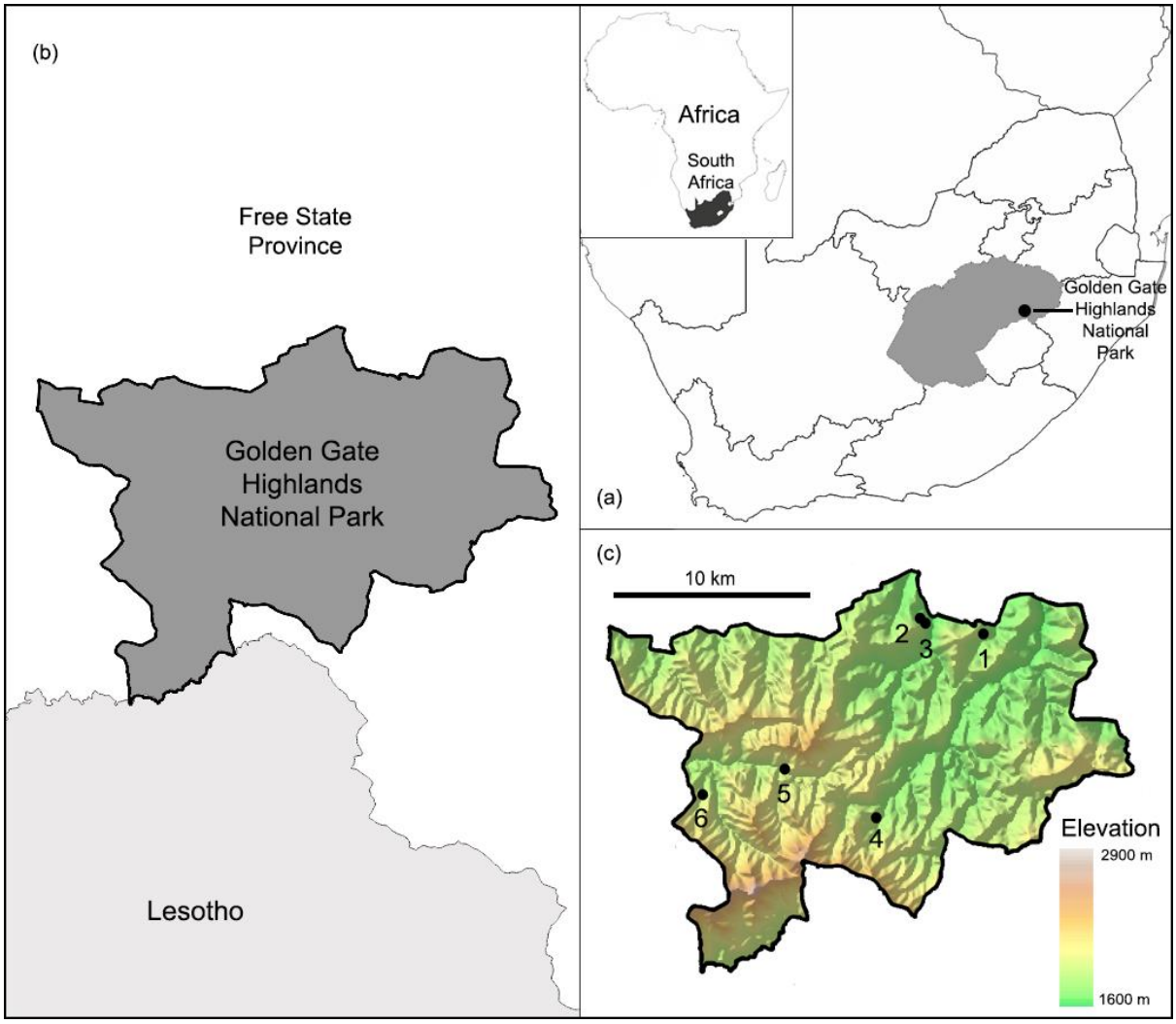

Fig. 2.1 Location of study area. (a) Location of Golden Gate Highlands National Park in the eastern section of the Free State Province, South Africa. (b) Golden Gate Highlands National Park on the border between the Free State and the country of Lesotho. (c) Location of selected study sites (1-6) at differing elevations (Elevation data obtained from Web GIS (http://www.webgis.com/srtm3.html), and developed using QGIS version 2.18.15).

Vegetation in the park comprises mostly rich montane grassland flora, with sporadic shrublands occurring in sheltered ravines and gorges, where the required moisture levels are maintained and protection is more favourable (Roberts, 1969; SANParks, 2019). The vegetative units of Northern Drakensburg Highland, Eastern Free State Sandy and Lesotho Highland Basalt Grasslands occur throughout the park (Mucina et al., 2006) (Supp. Fig. S2.1a).

The most common plant species in the park is the evergreen “Ouhout” (*Leucosidea sericea* Eckl. and Zeyh.), generally occurring in the valleys and along

streams in dense stands (Louw, 1988; Bissett et al., 2017; SANParks, 2019) (Supp. Fig. S2.1b). Groupings of vegetation occur more sporadically and in smaller areas throughout the mountainous regions of the park, leading to contrasting patches in the area. These shrubland patches commonly comprise the vegetative units of Basotho Montane Shrubland and Drakensberg-Amathole Afromontane Fynbos (Mucina et al., 2006) (Supp. Fig. S2.1a).

A total of six sites were selected for this study, focussing on shrubland patches over a short elevational range. Distance between these sites ranged from a minimum of 390 m to a maximum of 17 km. Due to the isolated nature and size of shrubland patches in the GGHNP, uniform stands were not available to measure the effects of elevation over a single area and slope aspect. Furthermore, accessibility to certain localities limited sites available for selection. In an attempt to overcome limited site selection, high sampling effort was adopted to ensure adequate representation of arthropod assemblages in the different strata. Each of the selected sites comprised a variety of woody vegetation, ranging from trees to shrub, providing vertical habitat stratification for sampling. The most common plant species were recorded for each site (Table 2.1).

Table 2.1 Table indicating site GPS location, elevation and major slope aspect, with most common plant species present at the time of sampling and their general phenology.

Site		1	2	3	4	5	6
Elevation (m a.s.l.)		1771	1711	1703	1830	1936	1880
GPS Coordinates		28°26'09" S 28°43'33" E	28°25'42" S 28°41'31" E	28°25'49" S 28°41'42" E	28°31'46" S 28°40'06" E	28°30'16" S 28°37'10" E	28°31'04" S 28°34'31" E
Slope Aspect		South East	North East	North	East	South West	East
Common plant species present	*Cussonia paniculata#*	x			x		
	Diospyros whyteana#	x			x	x	
	Euclea crispa#	x	x	x	x		
	Gymnosporia buxifolia#		x				
	Olinia emarginata#	x	x	x	x	x	x
	Pittosporum viridiflorum#	x	x	x	x		
	Leucosidea sericea#				x	x	x
	*Searsia dentata**	x	x	x	x		
	Olea europaea africana#	x	x	x			
	Widdringtonia nodiflora#					x	x
	Cliffortia linearifolia#					x	x
	Protea subvestita#	x			x		

Plant Phenology: # - Evergreen, * - Deciduous

As no uniform stands were available, each site was structured differently in terms of canopy coverage and spacing between trees and shrubs. Site 1 was composed of highly interspersed trees and increased grass-covered gaps (Supp. Fig. S2.2: 1). This formed more concentrated clusters of trees and shrubs along with discontinuous leaf litter coverage (ca. 8–10 m distance between tree clusters). Sites 2 and 3 were in close proximity to each other (ca. 400 m distance), with similar tree/shrub densities to site 1 along a slope (Supp. Fig. S2.2: 2–3). Both sites were smaller in their relative patch size to site 1. Sites 1–3 were located in the far northern reaches of the park. Vegetation composition in sites 4–6 provided a more condensed clustering (ca. 1–3 m between tree/shrub bases) and near continuous canopy coverage (Supp. Fig. S2.2: 4–6). Leaf litter of these sites resembled canopy coverage with little to no gaps in between trees/shrubs. Sites 5 and 6 contained hiking trails through the patch, which was utilised by tourists and site 6 was located next to a non-perennial river. Sites 5 and 6 were geographically located in the western reaches of the park, while site 4 was more isolated in the southern section.

During this study, a number of wildfires occurred throughout the GGHNP. Some of these fires impacted the sampling sites to varying degrees, with different levels of vegetation coverage loss. Notable wildfires occurred during September 2017, November 2017 and July 2018. Despite these burnings, regrowth of grasses and vegetation occurred relatively quickly in a month to two-months post-fire.

Sampling methodology

Sampling was conducted on a monthly basis over a period of 24-months, from April 2017 until March 2019. To determine arthropod diversity in each of the selected sites, sampling was conducted in three strata, consisting of lower canopy, leaf litter and soil. Each stratum was sampled using different methods, with vegetation beating used for canopies, hand collecting and Berlese-Tullgren-funnel extraction for leaf litter, and Berlese-Tullgren-funnel extraction for the soil.

Canopy samples were collected randomly, with a total of ten beat samples comprising 50 beats per sample. Branches, at a height of 1–3 m above the ground, were beat with a one-meter metal rod, directly above a 60 cm width catchment net. All collected specimens were then extracted from the catchment net by an aspirator and placed in 70% ethyl alcohol.

Leaf litter samples were obtained from directly underneath trees and shrubs, near to, and up to a maximum of 1 m away from their base, in each patch. A total of five samples were collected at random. Each sample consisted of a litre bag filled to the top with leaf litter. Hand collecting was performed in the field on two of the five samples by emptying the bags onto a white sheet and collecting specimens for a maximum of 30 minutes. These specimens were then placed in 70% ethyl alcohol. The remaining three litter samples were placed into a single Berlese-Tullgren-funnel,

with a connected storage bottle containing 70% ethyl alcohol, for seven days. Samples from both sampling methods were pooled.

Soil samples were collected in a scatter plot formation within the boundaries of each patch. A total of five soil samples were collected, with a single soil sample comprising approximately 500 grams. These soil samples were taken within the first 10 cm of topsoil of a designated spot. As the soil samples were taken randomly, certain samples fell near to the base of trees and shrubs while others were in their peripheral and the gaps between. All 5 samples were placed into a single Berlese-Tullgren-funnel, with a connected storage bottle containing 70% ethyl alcohol, for seven days. Soil moisture and temperature, at the spot where each soil sample was taken, was also recorded and averaged per month.

All collected specimens were identified to morphospecies level at the laboratory using available literature (Theron and Ryke, 1969; Olivier and Theron, 1989; Eggleton and Gaston, 1990; Triplehorn and Johnson, 2005; Dunger and Schlitt, 2011; Badenhorst, 2016; Slingsby, 2017), and counted monthly. All adult Arachnida specimens were deposited in the National Museum in Bloemfontein (NMBA).

Statistical analysis

To determine differences in species diversity between the sites and their elevation, as well as stratification, alpha diversity using Shannon-Wiener diversity and Simpson’s index of diversity of monthly and total assemblages were calculated, with corresponding Buzas & Gibson's evenness. Sample rarefaction (Mao tau) and richness estimators (Chao 1), along with sample completeness and coverage, were performed to provide extrapolation of species richness from the sampled data and to

assess resultant species richness from the sampling methodology (Chao, 1987; Chao and Jost, 2012; Chiu et al., 2014), using EstimateS version 9.1.0 (Colwell, 2013).

One-way analysis of similarity (ANOSIM) with Bray-Curtis index was then used to determine whether there was evidence for differences between stratum assemblages both within and between sites. ANOSIMs were performed with log-transformed abundance data (log10(n + 1)) using 9999 permutations. Nonmetric multidimensional scaling ordination (3D) (NMDS) with a Bray-Curtis similarity index was performed to distinguish between total arthropod assemblages between sites and strata based on morphospecies abundances. Similarities between total assemblages per stratum were calculated using cluster analysis of the Bray-Curtis similarity matrix with the paired group algorithm. ANOSIM, NMDS, cluster analyses and graphing of sample rarefaction curves were performed using Paleontological Statistics version 3.25 (Hammer et al., 2001).

The impact of elevation and stratification was analysed using linear mixed models (LMM), with month of sampling as a random factor, and elevation and stratum as fixed effects. Rank Shannon-Wiener diversity indices and rank observed species richness were utilised in this analysis, and performed using the lmerTest package in R, version 3.5.3 (Kuznetsova et al., 2017).

Results

A total of 62 699 arthropod individuals were sampled over the 24-month period, comprising 28 orders and 1211 morphospecies. Of these, Hymenoptera was the most species rich order, comprising 316 morphospecies, followed by Coleoptera (208 morphospecies), Hemiptera (150 morphospecies), Diptera (112 morphospecies) and Araneae (94 morphospecies). All of the remaining orders contained fewer than 65

morphospecies each. All sampled canopies showed higher species richness in comparison to leaf litter and soil (Table 2.2), although abundances were generally higher in the leaf litter.

Table 2.2 Assemblage characteristics of arthropods sampled from three habitat strata at six sites in the Golden Gate Highlands National Park from April 2017 to March 2019.

	Stratum	N	S_{obs}	Singletons	Doubletons	Unique species
Site 1	Canopy	3358	362	174	40	70
	Leaf litter	3990	156	52	23	18
	Soil	539	75	28	15	0
Site 2	Canopy	2965	316	128	54	40
	Leaf litter	5525	170	52	21	7
	Soil	722	70	27	7	5
Site 3	Canopy	3508	348	162	54	63
	Leaf litter	6669	193	70	24	26
	Soil	1328	64	21	15	2
Site 4	Canopy	6502	359	140	59	79
	Leaf litter	5114	175	66	17	18
	Soil	2913	111	35	15	10
Site 5	Canopy	3423	294	138	32	53
	Leaf litter	6178	178	57	15	16
	Soil	870	94	37	13	5
Site 6	Canopy	3361	308	128	51	49
	Leaf litter	5226	176	54	16	8
	Soil	508	52	20	7	0

Non-parametric species richness estimator Chao1 indicated a total species richness of 467–740 for site canopies, 214–303 for leaf litter and 78–151 for soil (Table 2.3). While completeness values showed relatively low sample completeness (average 65%), coverage estimators indicated a high sample coverage from the sampling methodology utilised (average 97%) (Table 2.3, Supp. Fig. S2.3). This suggests that the species sampled during the study period provides an adequate representation of individuals present in these areas.

Table 2.3 Total diversity and richness statistics of arthropods per site stratum. H - Shannon-Wiener diversity index, Simpson - Simpson's index of diversity, Evenness - Buzas & Gibson's evenness, S_{Chao1} ± SD - species richness estimate with 1 standard deviation, Completeness - Sample completion (S_{obs}/S_{chao1}), Coverage - Sample coverage. All arthropods were sampled between April 2017 and March 2019.

	Stratum	H	Simpson	Evenness	S_{Chao1} ± SD	Completeness	Coverage
Site 1	Canopy	4.230	0.949	0.189	740.34 ± 85.13	0.489	0.948
	Leaf litter	3.550	0.950	0.224	214.77 ± 21.79	0.726	0.987
	Soil	3.150	0.896	0.311	101.08 ± 12.99	0.742	0.948
Site 2	Canopy	4.280	0.962	0.228	467.65 ± 36	0.676	0.957
	Leaf litter	3.340	0.923	0.166	234.37 ± 24.09	0.725	0.991
	Soil	2.790	0.849	0.233	122 ± 28.96	0.574	0.963
Site 3	Canopy	4.230	0.959	0.197	590.93 ± 52.85	0.589	0.954
	Leaf litter	3.030	0.902	0.107	295.07 ± 33.64	0.654	0.990
	Soil	1.640	0.506	0.080	78.69 ± 8.38	0.813	0.984
Site 4	Canopy	3.280	0.855	0.074	525.08 ± 37.70	0.684	0.978
	Leaf litter	3.260	0.916	0.149	303.09 ± 45.69	0.577	0.987
	Soil	2.480	0.734	0.107	151.82 ± 18.50	0.731	0.988
Site 5	Canopy	3.780	0.920	0.149	591.48 ± 75.02	0.497	0.960
	Leaf litter	3.440	0.931	0.176	286.28 ± 41.39	0.622	0.991
	Soil	3.420	0.938	0.324	146.59 ± 23.76	0.641	0.958
Site 6	Canopy	3.730	0.909	0.135	468.58 ± 38.37	0.657	0.962
	Leaf litter	3.590	0.945	0.206	267.11 ± 35	0.659	0.990
	Soil	2.70	0.851	0.287	80.52 ± 17.53	0.646	0.961

Shannon-Wiener diversity index showed a consistent decline from the canopy to the soil at all of the sites (Table 2.3). Simpson's index of diversity and Buzas & Gibson's evenness varied between strata. Differences between assemblages in each stratum were shown to be highly significant globally (ANOSIM, Global $R = 0.794$, $p < 0.001$), as well as within each site (Table 2.4a). Similar results were found between the different elevations for each patch stratum (ANOSIM, Global $R = 0.275$, $p < 0.001$), with the soil strata having more similar assemblages between sites while still being significantly different (Table 2.4b).

Table 2.4 ANOSIM results of differences between assemblages of (a) the three habitat strata in each site, and (b) elevations per stratum.

(a)

Site	R	p-value
1	0.761	<0.001
2	0.755	<0.001
3	0.757	<0.001
4	0.798	<0.001
5	0.844	<0.001
6	0.864	<0.001

(b)

Stratum		
Canopy	0.547	<0.001
Leaf litter	0.322	<0.001
Soil	0.093	<0.001

This was confirmed by ordination of stratum-site totals, which showed a clear separation of assemblages by vertical stratification, with the epigeic assemblages showing more condensed clustering (Fig. 2.2a). The cluster analysis between the strata assemblages of each site showed higher similarity between the leaf litter and soil assemblages for all sites (although low, ~0.14) (Fig. 2.2b). Canopy assemblages were shown to cluster, to some extent, according to geographical locality, with sites 1–3, situated in the northern section of the park, possessing higher similarity, while sites 5–6 were more similar and situated in the western section. While somewhat similar results were indicated for leaf litter, the assemblages of sites 1 and 4 were noted to differ to that of canopy groupings.

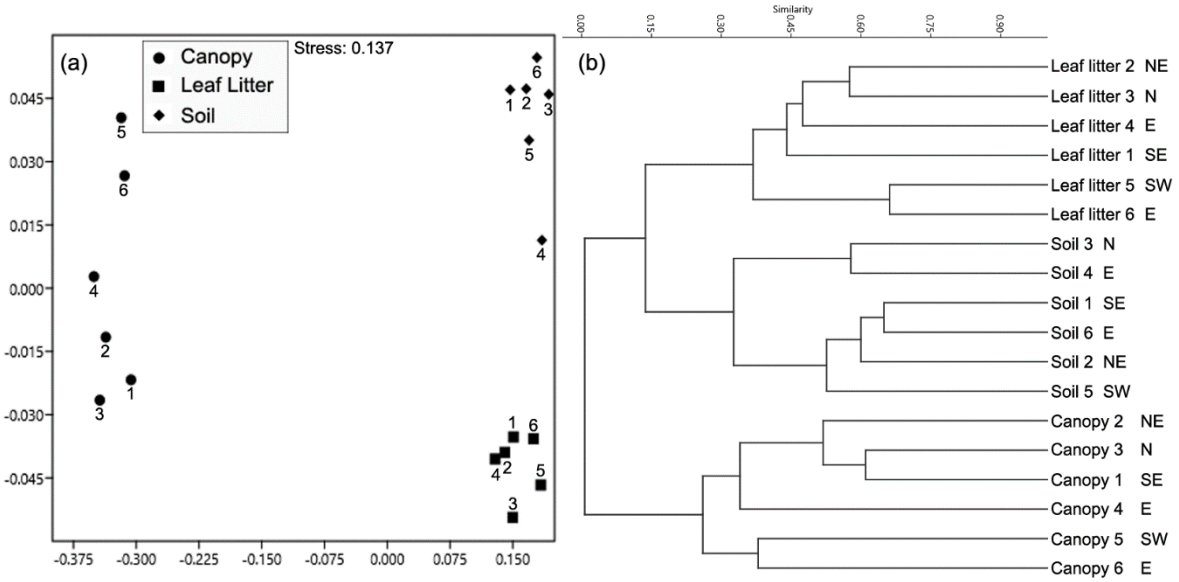

Fig. 2.2 Differences in arthropod assemblages from three habitat strata at six sites in the Golden Gate Highlands National Park based on total assemblage abundances and a Bray-Curtis similarity matrix: (a) NMDS (3D) ordination, and (b) cluster dendrogram using paired group algorithm. All assemblages were sampled during the period of April 2017 to March 2019.

Interaction of elevation with strata showed significant differences in arthropod diversity (LMM, Global $F_{2,403} = 18.232$, $p < 0.001$), and observed species richness (LMM, Global $F_{2,403} = 6.639$, $p = 0.002$), as elevation increased. Canopy diversity and richness was noted to decrease with an increase in elevation, while the equivalent of both leaf litter and soil showed an increase (Fig. 2.3). However, while diversity in all strata was significantly affected by elevation, canopy species richness was not affected in contrast to leaf litter and soil.

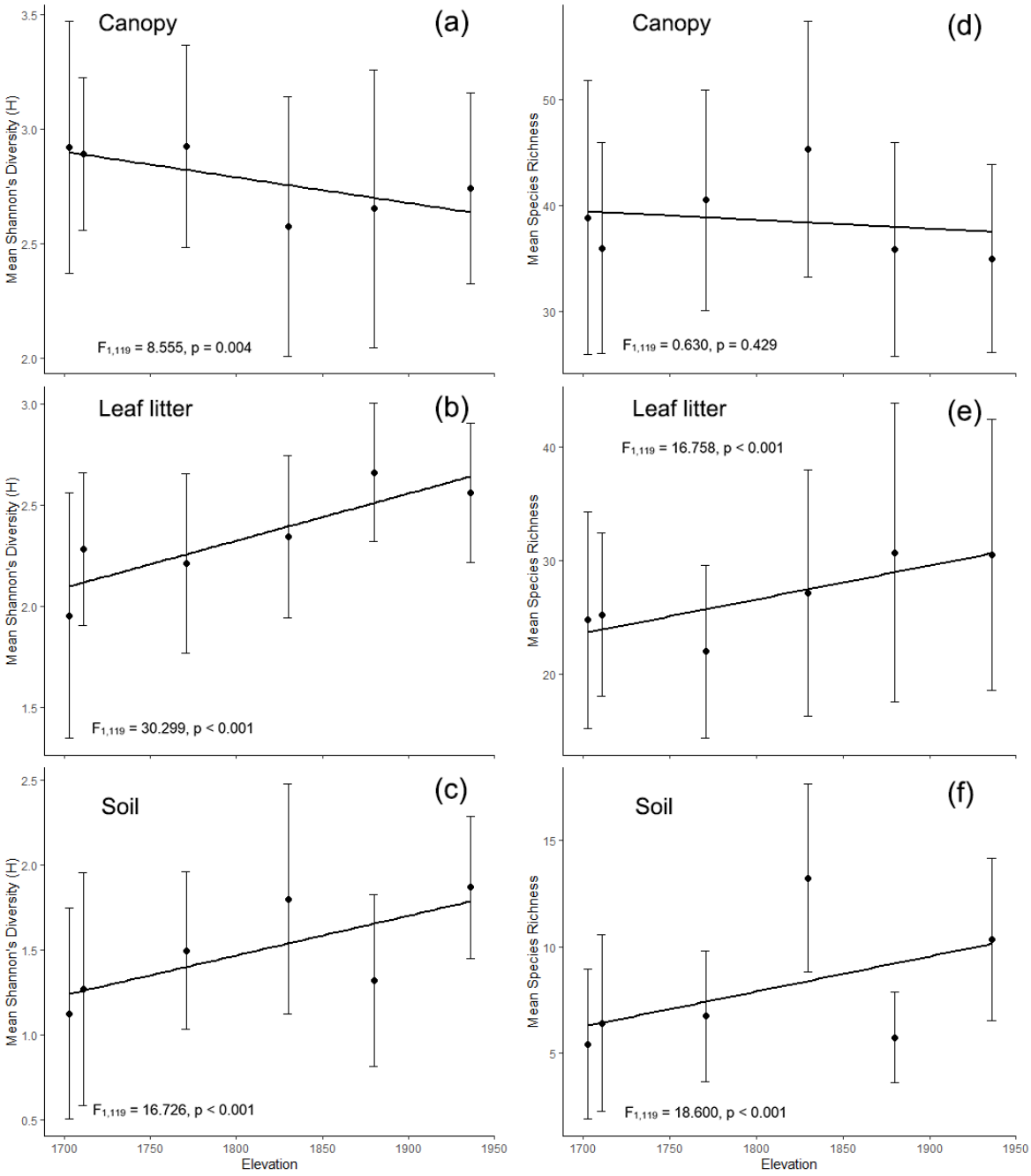

Fig. 2.3 Linear mixed effects model of mean ± SD monthly (a–c) Shannon-Wiener diversity index, and (d–f) species richness per stratum across the different site elevations during the period of April 2017 to March 2019.

Discussion

This was the first study conducted in a South African National Park attempting to determine how elevation and vertical stratification affects arthropod species diversity and richness along a relatively short elevational gradient. The results indicated that arthropod diversity and richness differed between the selected strata in relation to elevation, with canopy assemblages experiencing a decline in diversity and richness, and the opposite occurring in the lower strata. While this was unexpected, as an elevational gradient of approximately 200 m may be considered limiting in its effect of elevation-dependent processes (i.e. decrease in temperature and space), changes in community compositions of certain taxa and spatial variability have been noted along short elevation gradients (Lavoie and Bradley, 2003; Jarvis et al., 2015). Furthermore, while species richness of the strata followed the same pattern as diversity, elevation was indicated as not having a significant impact on canopy richness despite its pattern of decline.

Vegetation zones usually decline in plant richness with increasing altitude, producing varying, and often contrasting, habitats across a gradient (Lomolino, 2001; Jin et al., 2008). Such occurrences have been documented across large elevational ranges (Miyajima and Takahashi, 2007; Homeier et al., 2010; Eisenlohr et al., 2013), and over smaller ranges in more extreme climate zones (Mata-González et al., 2002). However, areas in a relatively small elevation range are more likely to possess similar vegetation (Halpern and Spies, 1995). In this sense, the short elevation gradient experienced during our study may not have drastically altered environmental conditions of canopy strata, and could be considered to occur in relatively the same elevational range. Contrastingly, soil moisture and temperature differed significantly, with a trend of increased moisture content present at the higher elevations alongside

decreased soil temperature (Supp. Fig. S2.4). Precipitation and moisture promote soil and leaf litter arthropod communities both directly and indirectly (Lensing et al., 2005; Chikoski et al., 2006), with increased species richness and diversity as a result. This corresponds to the results of the lower strata, with sampled assemblages displaying increased diversity and species richness at the higher elevations alongside increased moisture. However, as the monthly soil samples were pooled during sampling and analysis, the total diversity recorded may not be an accurate reflection of the soil parameters recorded during the different times of the year (Reinhart and Rinella, 2016; Kuznetsova and Saraeva, 2018).

While our results indicated elevation influencing arthropod diversity and species richness, the heterogeneous nature of the sampled localities may be a more likely causative factor. The highly heterogeneous topography of the GGHNP provides a highly heterogeneous landscape, influencing spatial structure and connectivity of patches (Pe'er et al., 2006). Variations in vegetation patterns and landscape features influence many natural phenomena, alongside ecological processes, affecting microclimatic and soil factors (Fu et al., 2000; Fu et al., 2004). Vegetation composition did differ between site localities in the GGHNP, with *L. sericea* being more dominant in the higher elevated areas situated on the south and western parts of the park (sites 4-6) (Roberts, 1969; Louw, 1988; Bissett et al., 2017). While sampling by Louw (1988) identified a total of 117 beetle species occurring on *L. sericea* in the GGHNP, with an estimation that the total richness of associates would be considerable, this was not compared with other tree and shrub species occurring in the park.

As a whole, faunal diversity and community composition are impacted by increased habitat diversity and heterogeneity (Tews et al., 2004), increasing respective abundance and diversity at the landscape scale (Benton et al., 2003). While

our study did not focus on slope aspect or spatial variation of the patches as impacting factors, it remains important for consideration. The relatively small size and isolated nature of the shrubland patches in the GGHNP do not provide uniform stands in which to adequately measure continuous elevation gradients along a single aspect. As such, the selected localities in our study differed in terms of slope aspect, as well as vegetation density and patch size. Slope aspect often plays a role in woody vegetative diversity and abundance (Malan et al., 1998), due to the effect of solar radiation exposure (Sternberg and Shoshany, 2001). This, in turn, can create differing environmental conditions related to temperature, humidity and evapotranspiration (Kutiel and Lavee, 1999), and the effect it has on arthropod assemblage structure (Foord et al., 2015; Haddad and Butler, 2018). In this sense, the selected localities in our study may have possessed unique microclimatic conditions, influencing arthropod assemblage diversity to a greater extent than elevation.

The occurrence of unique species in most site strata supports the notion of definite vertical stratification in these shrubland patches despite their relatively small size (Munyai and Foord, 2015; Cui and Zheng, 2016; Foord and Dippenaar-Schoeman, 2016; Adie et al., 2017; Haddad and Butler, 2018). Findings on distinct stratification of arthropod assemblages in the shrubland patches of the GGHNP is consistent with results from other studies around the world (e.g. Basset et al., 2001; Basset et al., 2003; Aikens and Buddle, 2012), suggesting that similar patterns of arthropod assemblage exclusivity are present in each stratum. This is especially true for the canopy of each locality in our study, showing the highest number of unique species present, in comparison to the lower strata. However, upper canopies may not have been sufficiently sampled using beat sampling during this study, it is therefore

recommended that more extensive sampling methods be used to thoroughly determine the unique species present in each patch.

The corresponding finding of distinct stratification has significance for the GGHNP for several reasons. The shrubland patches of the GGHNP are, in fact, few and far between over the landscape of the park, resulting in unique habitats within the grasslands (Adie et al., 2017). Presence of unique species and distinct separation between assemblage populations strongly hints at these patches providing variable habitats for species across the park. In addition, the heterogeneous composition of these small patches across the park further implies that each patch could ultimately play a varying role in sourcing species for their needed ecosystem functions (Lawes et al., 2005; Jiang et al., 2007; Ruiz et al., 2008). Despite the small size of these patches (the largest patch in this study being approximately 5731 m^2), the shrubland zones of the GGHNP should be considered as areas of priority management due to the species rich arthropod communities contained in the investigated strata.

This study provided the first insights into the effect of elevation and stratification on arthropod assemblages in the GGHNP. Our findings have indicated considerable potential for additional studies to investigate the impact of patch heterogeneity across an area with a relatively small elevation range. As species assemblage composition can depend heavily on floral diversity and habitat structure (e.g. Gardner et al., 1995; Bennett and Gratton, 2013), we would recommend that future studies include investigation into the effects of how structural variations, both on a local and landscape scale, could impact faunal assemblages over a short elevational gradient in this National Park.

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Chapter 3

Seasonal changes of arthropod richness, diversity and community composition in shrubland patch strata in a grassland landscape

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Abstract

Arthropod assemblage activity and composition patterns are often associated with seasonal and temporal changes. This phenomenon must be considered when investigating areas of conservation importance to ensure adequate monitoring, particularly in grassland areas prone to annual disturbances such as wildfires. This study aimed to determine seasonal changes of arthropod assemblages across interspersed shrubland patches in the Golden Gate Highlands National Park, South Africa (GGHNP). Monthly sampling was conducted over a 24-month period from three habitat strata, i.e. canopy, leaf litter and soil, in six shrubland patch localities. A total of 62 699 arthropod individuals were sampled, comprising 1211 morphospecies. Stratum assemblage changes followed the general trend of wet and dry seasonal shifts, but the extent of these changes differed between the two years of the study. The impact of recorded monthly ambient temperatures and rainfall on assemblage richness differed depending on the stratum and year of study, with the strongest correlation in the leaf litter of the first year. Intra-annual β-diversity patterns differed between strata, with a temporal decline seen in the leaf litter, while canopy and soil showed no variation and a slight increase, respectively. Differences in inter-annual β-diversities between the two years were found in both the leaf litter and soil stratum, with a higher species turnover in the first year. The results of this study provide a general overview of yearly seasonal arthropod assemblages changes in shrubland patches of the GGHNP, contributing useful and comparative data on species abundance and distribution patterns across these isolated patches. However, opportunity still remains to investigate these arthropod assemblage changes at a finer taxonomic resolution.

Keywords: Canopy; Environment; Leaf Litter; Soil; Species Turnover; Temporal

Introduction

Shrubland patches provide a myriad of environmental conditions for arthropod assemblages, due to the structural complexity and richness of plant species (Sanchez and Parmenter, 2002; Wagner et al., 2003; Harris et al., 2004; Pendleton et al., 2011; Adie et al., 2017). These conditions are often compounded due to the geographical locality and elevation of these patches, producing a spectrum of microclimatic conditions that impact arthropod succession and their assemblage composition patterns in temperate and tropical areas (Madigosky, 2004; Blaum et al., 2009; Checa et al., 2014). Stratification in a woodland scenario creates variation in arthropod assemblage compositions and how they change over time and space (Gruppe et al., 2008; Jacquemin et al., 2016). Such stratification is conducive to the lasting preservation of certain species (Basset et al., 1992; Lassau et al., 2005), but also impacts assemblage shifts during seasonal and habitat changes to differing degrees (Wagner, 2001; Kendrick et al., 2015; Beng et al., 2018).

Arthropod assemblage activity and composition patterns are often associated with seasonal and temporal changes (Richards and Windsor, 2007; Plant et al., 2018; Claflin et al., 2019), with behaviour and lifestyle, while generally taxon-specific, being driven by said changes in conjunction with vegetative coverage and diversity (Elith and Leathwick, 2009; Rose et al., 2016). The short generation times, high species richness and abundance of arthropod communities also make them well suited in addressing temporal dynamics and phenology in a number of environments (Smith and Smith, 2012; Valtonen et al., 2013).

Refugia, being habitable areas that provide a protective environment during unfavourable conditions, are important factors in the context of conserving species assemblages, due to their availability as stepping-stones in a variable landscape

mosaic, and their association with relict populations during periods of unfavourable climate or disturbance (Hampe and Petit, 2005; Ashcroft, 2010). While topographical variation produces smaller isolated patches in a mosaic system (Debinski and Holt, 2000; Williams et al., 2008; Ashcroft et al., 2009; Adie et al., 2017), these smaller patches are able to sustain rich and diverse communities despite their size (Jokimäki et al., 1998; Maynou et al., 2017; Adie et al., 2017; Prevedello et al., 2017), making them important in protected areas. Change in species assemblages between sites and over time often differ through the substitution of species from one site to another (turnover), and loss (or gain) of species in a single site, leading to the poorest assemblage as being a subset of the richest (nestedness) (Baselga and Orme, 2012). Ultimately, before efforts to conserve faunal communities can be undertaken, it is necessary to investigate how assemblages change over seasons and during periods of disturbance.

In this study we aimed to determine how arthropod assemblages vary with seasonal change in three vertical habitat strata in smaller shrubland patches of the Golden Gate Highlands National Park (GGHNP), representing isolated shrublands in a grassland-dominated montane environment subject to considerable seasonal climatic variation. In particular, we investigate the responses of arthropod assemblage abundance and species richness to environmental factors and the resultant species turnover.

Materials and methods

Study site and period

This study was conducted in the GGHNP, located on the eastern side of the Free State Province, South Africa (28°30′ S, 28°37′ E). The park covers an area of approximately 340 km^2. Designated as a montane grassland, the park falls in the vegetative units of Lesotho Highland Basalt, Eastern Free State Sandy and Northern Drakensburg Highland Grasslands (Mucina et al., 2006) (Supp. Fig. S3.1a).

The park is well known for its landscape, with the exceptional eroded sandstone cliffs being a primary tourist attraction (Taru et al., 2013). With its location being in the eastern Highveld region of South Africa, the park has an elevation range of approximately 1600–2900 m a.s.l. (SANParks, 2013) (Supp. Fig. S3.1b). The climate of the park is often dry and sunny during peak winter conditions from June to August, with average minimum temperatures ranging between 1–15°C (Grab et al., 2011), and heavy snowfalls have been recorded during winter months. Rainfall typically occurs during summer months of October to April, with approximately 800 mm per annum, and peak summer temperatures have been noted to range between 20–30°C (Groenewald, 1986).

While the park is primarily classified as a montane grassland, a number of sporadic shrubland patches occur at localities throughout the area, some in sheltered ravines and gorges while others are in more open landscapes (Grab et al., 2011; SANParks, 2019a). While these shrubland patches have been classified as either Basotho Montane Shrubland and Drakensberg-Amathole Afromontane Fynbos (Mucina et al., 2006), differences are noted between these vegetative units with encroachment of species between the two being a regular occurrence, especially in the ravines and gorges throughout the GGHNP.

The Basotho Montane Shrubland encompasses steep talus slopes and mountain flanks, supporting tall, and often dense, shrubland that is commonly dominated by evergreen plant species of “Ouhout” (*Leucosidea sericea* Eckl. & Zeyh.), “Blue Guarri” (*Euclea crispa* (Thunb.) Gürke), “Mountain Hard Pear” (*Olinia emarginata Burtt* Davy), “Wild Olive” (*Olea europaea* L. ssp. *africana* (Mill.) P.S. Green), and “Cape Cheesewood” (*Pittosporum viridiflorum* Sims) (Mucina et al., 2006; SANParks, 2019b). Common deciduous species also include “Nana-Berry” (*Searsia dentata* Thunb.) and “Parsley Tree” (*Heteromorpha trifoliata* (Hochst. ex A. Rich) H. Wolff).

Drakensberg-Amathole Afromontane Fynbos is more commonly present on steep valleys and escarpment slopes in low stands (1–3 m tall) at the heads of rivers in many of the gullies and depressions in the park. These patches are also commonly composed of evergreen tree and shrub species such as “Mountain Cedar” (*Widdringtonia nodiflora* (L.) Powrie), “River Rice-bush” (*Cliffortia linearifolia* Eckl. & Zeyh) and “Waterlily Sugarbush” (*Protea subvestita* N.E.Br.) (Mucina et al., 2006). Other common genera comprise *Passerina* L., *Eirca* L., *Euryops* (Cass.) Cass., and *Helichrysum* Mill. (Mucina et al., 2006).

Six sites were selected for this study encompassing shrubland patches at different localities of the park, comprising different dominant vegetation species from the previously described vegetative units (Table 3.1). Sites 1 and 4–6 were observed to contain elements of both the main vegetative units, while sites 2 and 3 were solely Basotho Montane Shrubland. This provided a range of patch types that occur in the GGHNP, which allowed for determination of general seasonal changes in arthropod assemblages. Monthly sampling, at the end of every month, was conducted over the period of April 2017 to March 2019, totalling 24-months.

Table 3.1 Table indicating the dominant plant species present at the time of sampling in each site, with the vegetative unit each species belongs to.

Dominant plant species	Site					
	1	2	3	4	5	6
Cussonia paniculata#	X			X		
Diospyros whyteana#	X			X	X	
Euclea crispa#	X	X	X	X		
Gymnosporia buxifolia#		X				
Olinia emarginata#	X	X	X	X	X	X
Pittosporum viridiflorum#	X	X	X	X		
Leucosidea sericea#				X	X	X
Searsia dentata#	X	X	X	X		
Olea europaea africana#	X	X	X			
Widdringtonia nodiflora$					X	X
Cliffortia linearifolia$					X	X
Protea subvestita$	X			X		
Vegetative Unit: # - Basotho Montane Shrubland, *$* - Drakensberg-Amathole Afromontane Fynbos						

During this study, a number of wildfires occurred throughout the GGHNP. Some of these fires impacted the sampling sites to varying degrees, with different levels of vegetation coverage loss (Supp. Fig. S3.2a–g). Notable wildfires occurred during September 2017, November 2017 and July 2018. Despite these burnings, regrowth of grasses and vegetation occurred relatively quickly in a month to two-months post-fire (Supp. Fig. S3.2h–j).

Sampling methodology

In order to determine how arthropod assemblages responded to intra-annual seasonal changes and disturbances across the selected strata, three sampling/collection methods were employed for the canopy, leaf litter and soil strata on a monthly basis.

Canopy arthropod assemblages were sampled by vegetation beat-catching, with a total of ten beat samples consisting of 50 beats per sample. A metre metal rod was used to randomly beat branches, at a height of 1–3 m above the ground, above a 60 cm wide catchment net throughout each site. All ten beat samples were

performed consecutively, and all collected specimens were extracted from the catchment net by means of an aspirator and placed in 70% ethyl alcohol.

Leaf litter arthropods were collected by a combination of hand collecting and Berlese-Tullgren-funnel extraction. A total of five leaf litter samples were obtained at random from directly beneath trees and shrubs and up to a maximum of 1 m away from the nearest base in each site. Each sample was characterised by a one-litre bag filled to the top with leaf litter. Hand collecting was conducted in the field on two of the five samples by emptying the bags onto a white sheet and collecting arthropods for a maximum of 30 minutes. All remaining samples were placed into a single Berlese-Tullgren-funnel with a connected storage bottle containing 70% ethyl alcohol for seven days. All specimens from both sampling methods were pooled.

Soil arthropods were collected by the same Berlese-Tullgren-funnel extraction method. A total of five soil samples were collected randomly in the boundaries of each site. Each soil sample comprised 500 grams of soil obtained by a soil auger in the first 10 cm of topsoil of a designated spot. Due to the soil samples being taken randomly, some samples were located near to the base of trees and shrubs with others in their periphery, and even some in the gaps between canopy coverage. All collected soil arthropods were placed into 70% ethyl alcohol.

Collected specimens were identified to morphospecies level at the laboratory using available literature (Theron and Ryke, 1969; Olivier and Theron, 1989; Eggleton and Gaston, 1990; Triplehorn and Johnson, 2005; Dunger and Schlitt, 2011; Badenhorst, 2016; Slingsby, 2017), and recorded for each successive month. Adult Arachnida specimens were deposited in the National Museum in Bloemfontein (NMBA).

Daily temperature and rainfall were obtained from the South African Weather Service (SAWS) for the total sampling period. Both maximum and minimum ambient temperatures were averaged monthly, and rainfall was totalled for each month.

Statistical analysis

Due to the sampling of this study taking place over a two-year period (24-months), the obtained data was divided into separate 12-month sets and analysed. This was done to compare the effect of seasonal assemblage change over two separate periods (April 2017–March 2018 and April 2018–March 2019). To determine whether there were differences in average maximum and minimum monthly temperatures and total monthly rainfall between the two 12-month periods, unpaired t-tests were conducted.

Diversity and richness estimators (observed species richness, Shannon-Wiener diversity and Chao1) per total yearly stratum were examined, alongside sample rarefaction (Sample completeness, Sample coverage and Mao Tau), using EstimateS version 9.1.0 (Colwell, 2013). The Shannon-Wiener diversity index accounts for both abundance and evenness of the species present and is calculated as the proportion of a species relative to the total number of species, multiplied by its natural logarithm (Shannon, 1948; Tuomisto, 2010). The result is then summed across species and multiplied by -1. Sample completeness is given as a proportion of observed species richness (S_{obs}) to the species richness estimate (S_{Chao1}), while sample coverage was calculated as the proportion of the total number of individuals in a community belonging to the species represented in the sample (Chao and Jost, 2012)

Repeated measures permutational multivariate analysis of variance (PERMANOVA) was conducted to test for intra-annual variation in arthropod species compositions in the canopy, leaf litter and soil strata across the six sites. This type of analysis accounted for the replicated sampling in the six sites over each 12-month period. PERMANOVAs were conducted in R version 3.5.3, using the vegan package (Oksanen et al., 2018), with a Bray-Curtis distance matrix and the six sites as statistical strata. When significance was indicated, pairwise contrasts were computed in order to determine which of the month-to-month pairs differed significantly in species assemblage composition. All p-values were adjusted using the false discovery rate method to account for multiple comparisons (Benjamini and Hochberg, 1995).

Nonmetric multidimensional scaling ordination (3D) (NMDS) with Bray-Curtis index was performed to graphically represent arthropod community composition change, based on arthropod abundances, over each 12-month period in each stratum, as well as overall differences in stratum assemblages per year. The results of such analyses that produce a stress value greater than 0.2 must be interpreted with caution (Clarke, 1993). All unpaired t-tests, ordination analyses and graphical sample rarefaction curves were conducted using Paleontological Statistics (PAST) (Hammer et al., 2001).

The effect and association of recorded average monthly environmental variables on observed monthly species richness and abundance of each stratum per year was determined by generalised linear mixed models (GLMM), with site as a random factor, using Poisson error structures. Due to environmental variables indicating multicollinearity (Supp. Fig. 3.3), each factor was modelled independently. Additionally, all yearly environmental variables were standardised to one by dividing

by the maximum value for each variable. This analysis was conducted using the lme4 package in R, version 3.5.3 (Bates et al., 2015).

Intra-annual beta (β)-diversity of the stratum assemblages over the full 24-month period was calculated from a compositional matrix (between sites), obtained from Whittaker's species turnover, for each sampling month. The average of each matrix was then calculated to represent β-diversity in each month alongside its standard deviation and graphically represented per stratum. All matrices were calculated in PAST.

Yearly temporal β-diversity in each of the selected strata was computed as "broad-sense" turnover (Koleff et al., 2003), using month-to-month pairwise Whittaker's species turnover in PAST. This provided a total of 66 possible month-to-month pairs for each stratum of each year. Species turnover was then graphically represented as density plots based on the mean β-diversity of randomly selected subsamples of size 25% and 75% of the 66 possible pairs, and calculated from 1000 replicates. A significant difference in species turnover between the two years of study was indicated when equivalent subsample density plots did not overlap by more than 50%. This graphical representation and analysis was conducted using R statistical software version 3.5.3 (R Core Team, 2019).

Results

Arthropod assemblage composition, richness and diversity

A total of 62 699 individuals were sampled over the 24-month period, consisting of 209 families and 1211 morphospecies. Of the three habitat strata sampled across the six sites the canopy was the most species rich, with a total of 879 morphospecies, followed by leaf litter (428 morphospecies) and soil (215 morphospecies). Yearly stratum observed species richness and Shannon-Wiener diversity were noted to decrease from the canopy layer to soil, with a higher species richness occurring overall in the second year of study (Table 3.2). Non-parametric species richness estimator S_{Chao1} indicated differing richness values per stratum dependent on the year of sampling, with higher estimators for the second year of study in all three strata. Abundance was higher in all three strata in the first year, while leaf litter contained the highest abundance of all strata in both years. Additionally, completeness estimators indicated relatively low sample completeness (average 70%), while coverage estimators indicated high sample coverage obtained by sampling methodology (average 99%) (Table 3.2, Supp. Fig. S3.4).

Table 3.2 Total yearly abundance and richness statistics of arthropods per habitat stratum. N - number of individuals, S_{obs} - observed species richness, H - Shannon-Wiener diversity index, S_{Chao1} ± SD - species richness estimate with 1 standard deviation, Completeness - sample completion (S_{obs}/S_{Chao1}), Coverage - sample coverage.

	Stratum	N	S_{obs}	Singletons	Doubletons	H	S_{Chao1} ± SD	Completeness	Coverage
Year 1	Canopy	12040	539	194	72	4.036	795.43 ± 50.08	0.678	0.984
	Leaf litter	16994	289	89	36	3.626	394.83 ± 30.16	0.732	0.995
	Soil	4255	139	48	14	2.549	214.18 ± 30.13	0.649	0.989
Year 2	Canopy	11077	676	246	104	4.572	962.97 ± 49.08	0.702	0.978
	Leaf litter	15708	326	95	42	3.766	429.83 ± 28.42	0.758	0.994
	Soil	2628	156	60	22	3.038	232.93 ± 26.91	0.670	0.977

Distinct arthropod assemblages between the three sampled strata were indicated by ordination and repeated measures PERMANOVA for both the first ($F_{2,213}$ = 18.590, $p < 0.001$) and second year ($F_{2,213}$ = 27.570, $p < 0.001$), with clear clustering by stratum (Fig. 3.1). Each stratum was therefore considered separately for intra-annual assessment. Unexpectedly, greater variation in assemblages was observed during the first year of the study in all of the strata (Fig. 3.1), despite species richness being higher for all of the methods during the second year (Table 3.2).

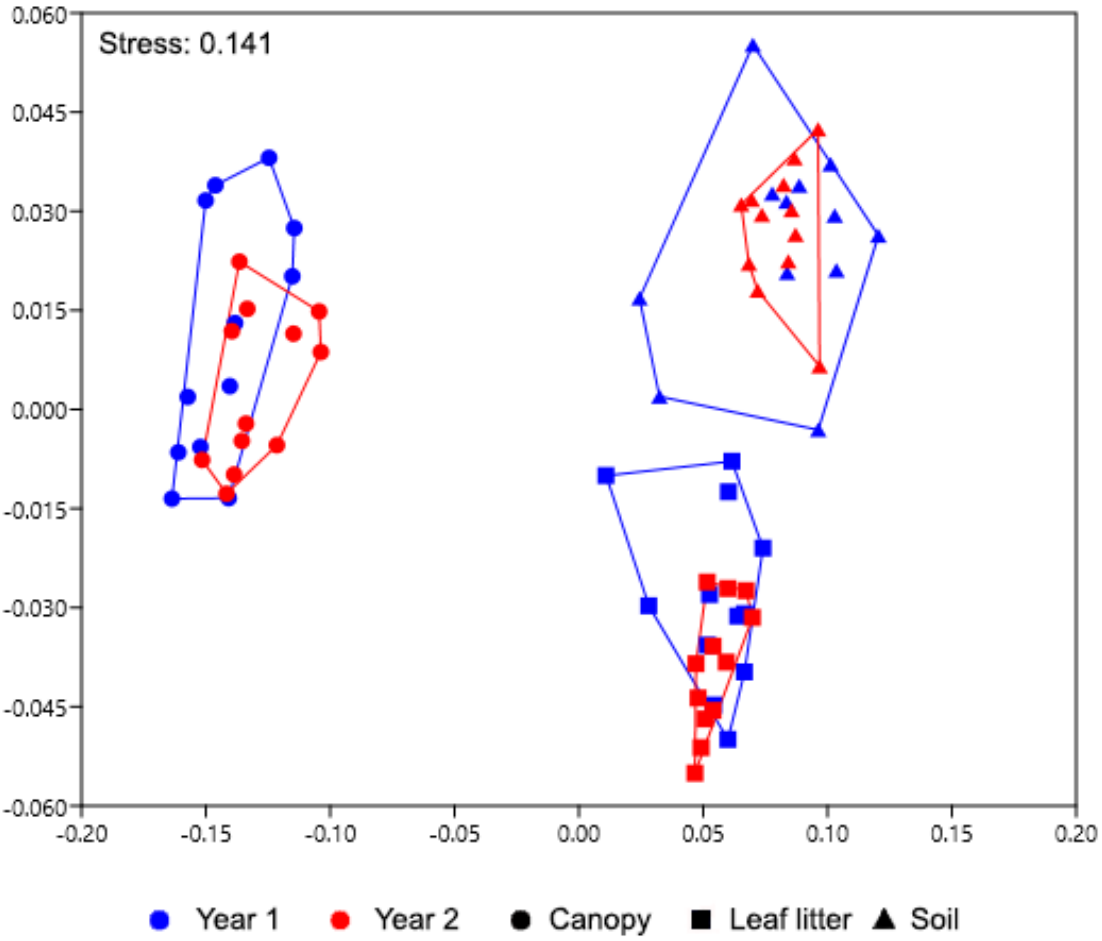

Fig. 3.1 Nonmetric multidimensional scaling (NMDS) (3D) ordination analysis of differences in arthropod assemblages between the three selected habitat strata for the two years of study.

Seasonal temperature and rainfall variability

Average monthly maximum temperatures were seen to decrease and increase during the typical autumn-winter and spring-summer months of the 24-month period respectively, ranging from a low of 16.45°C in July 2018 to a high of 29.26°C in December 2018 (Fig. 3.2). Average monthly minimum temperatures, during our study period, reached the lowest value in July 2018 at -1.90°C, with a peak during February 2018 at 13.46°C. Rainfall occurred during the typical period of October to April each year, with a maximum precipitation of 259 mm in March 2018 and a low of 1 mm in June and July 2018 (Fig. 3.2).

Neither average monthly maximum temperatures (unpaired t-test, $t_{22} = 0.752$, $p = 0.460$), minimum temperatures ($t_{22} = 0.214$, $p = 0.833$), or rainfall ($t_{22} = 1.281$, $p = 0.213$), differed significantly between the two 12-month periods of study. As a result, each 12-month period was considered separately for intra-annual assessments and determining the effect of environmental variables on stratum assemblages.

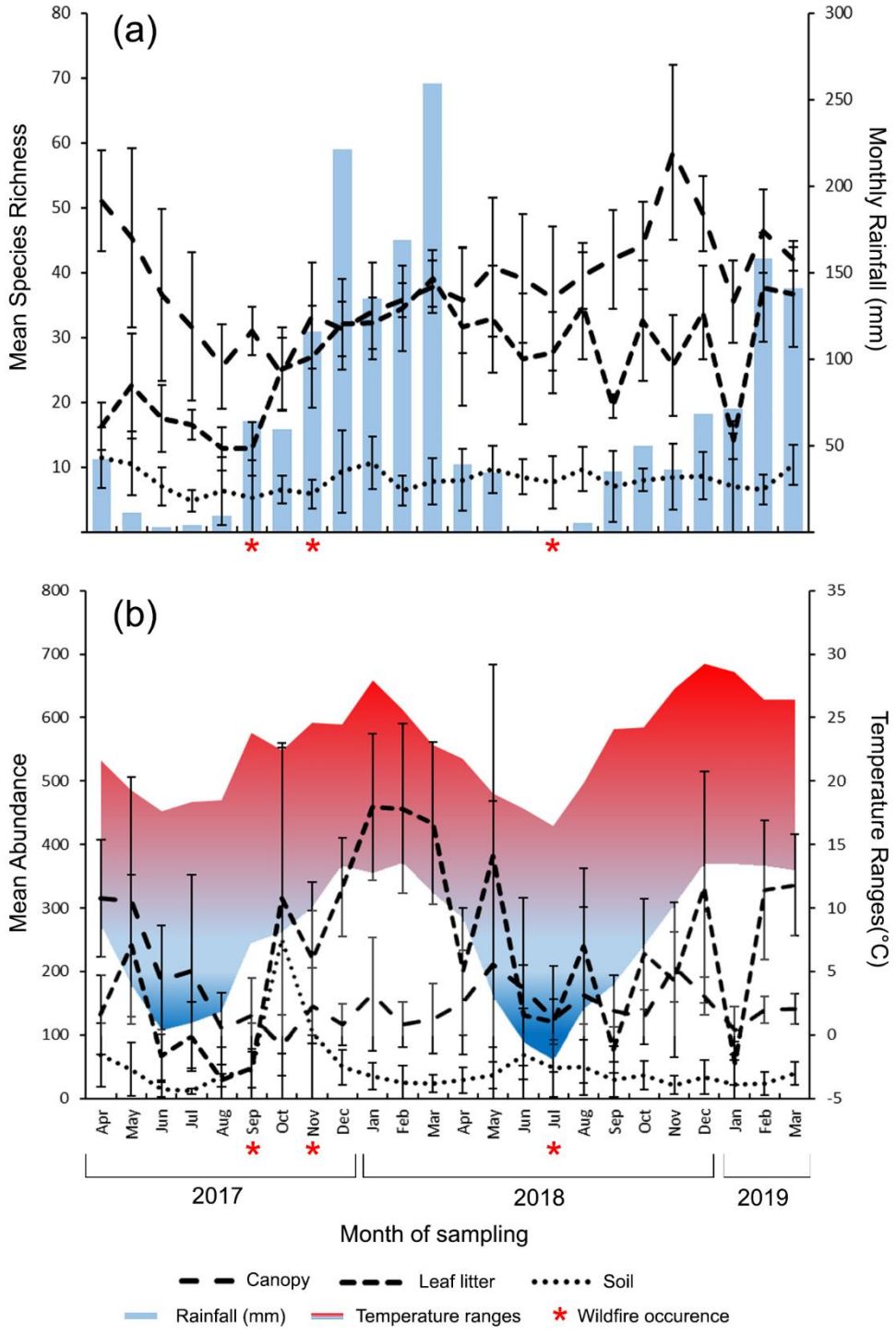

Fig. 3.2 Observed (a) mean species richness with monthly rainfall, and (b) mean abundance with average monthly temperature ranges of sampled arthropod assemblages per habitat stratum. Occurrence of wildfires during the study period indicated by asterisks.

Intra-annual arthropod assemblage variability patterns

Comparisons of arthropod assemblage responses to seasonal change for both years saw a general decrease in mean arthropod abundance and species richness across the strata during the winter months of June to August (Fig. 3.2). Increases in arthropod abundance and richness typically occurred during the spring and summer months with increased rainfall, during October to April, across most strata.

Significant intra-annual differences in species community composition were detected for all strata in both study years (Table 3.3). Ordination showed considerable overlaps of monthly assemblages for each stratum, with some compositions differing during certain seasons (Fig. 3.3). These differences mainly occurred between the dry winter months of June to August and the wetter months of November to January for both years (Supp. Table S3.1–S3.3). The extent to which stratum assemblages differed intra-annually varied between sites and year of study, with certain yearly assemblages overlapping considerably (Supp. Fig. S3.5–S3.7).

Table 3.3 Intra-annual species community differentiation in the three sampled strata. Significance codes: $p < 0.001$ ***, $p < 0.01$ **, $p < 0.05$ *.

		PERMANOVA
Stratum	Year	Community differentiation
Canopy	1	$F_{11,60} = 1.978$, $p < 0.001$ ***
	2	$F_{11,60} = 1.358$, $p < 0.001$ ***
Leaf litter	1	$F_{11,60} = 2.800$, $p < 0.001$ ***
	2	$F_{11,60} = 1.637$, $p < 0.001$ ***
Soil	1	$F_{11,60} = 1.976$, $p < 0.001$ ***
	2	$F_{11,60} = 1.482$, $p < 0.001$ ***

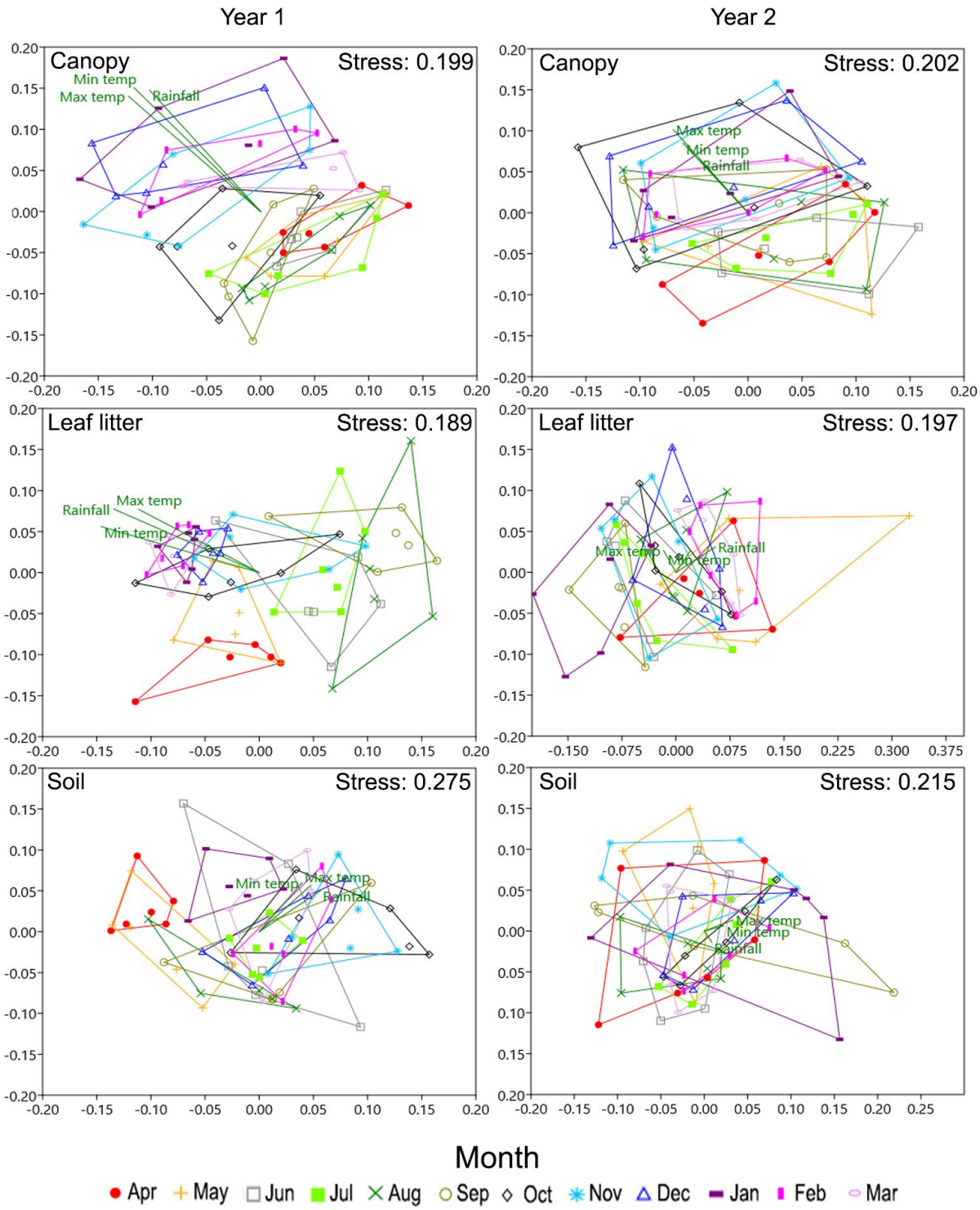

Fig. 3.3 Nonmetric multidimensional scaling (3D) (NMDS) ordination analysis of intra-annual arthropod assemblage changes for two years of study per selected stratum with recorded environmental factors. Each ordihull represents month of sampling and the lines connect sampled sites of that month.

Effect of environmental variables

Results of GLMMs showed varying effects of environmental factors on species richness and abundance across the selected strata in each year. During the first year of the study, canopy arthropod richness was not significantly affected by the recorded variables (Table 3.4). Leaf litter arthropod richness was affected by all of the recorded variables, while only minimum temperature affected soil arthropod richness. Temperature significantly affected arthropod richness in the canopy during the second year of the study, while only rainfall influenced leaf litter arthropod richness. Arthropod richness in the soil was not significantly affected by any of the recorded variables. The occurrence of more major grassland wildfires during the first year of study may have amplified the effect of environmental variables, due to vegetation coverage loss in some of the sampled sites. All environmental factors significantly influenced arthropod abundance across all strata for both years (Supp. Table S3.4). These results indicate that climate generally seems to play a role in the abundance and richness of arthropod assemblages.

Table 3.4 Results of generalised linear mixed-effects models of environmental factors influencing yearly arthropod species richness per stratum. Significance codes: p < 0.001 ***, p < 0.01 **, p < 0.05 *.

Random effects:		Year 1				Year 2			
Stratum	**Groups**	Variance	Std. Dev			Variance	Std. Dev		
Canopy	Site (n=6)	0.008	0.090			0.005	0.072		
Leaf litter	Site (n=6)	0.007	0.086			0.019	0.139		
Soil	Site (n=6)	0.118	0.344			0.095	0.309		
Stratum	**Fixed effects:**	Estimate	Std. Error	z-value	p-value	Estimate	Std. Error	z-value	p-value
Canopy	Max temperature	-0.144	0.179	-0.807	0.419	0.564	0.128	4.389	<0.001***
	Min temperature	0.035	0.058	0.596	0.551	0.144	0.045	3.209	0.001**
	Rainfall	-0.022	0.061	-0.366	0.715	0.111	0.058	1.913	0.056
Leaf litter	Max temperature	2.092	0.217	9.630	<0.001***	-0.168	0.152	-1.107	0.268
	Min temperature	0.875	0.076	11.580	<0.001***	0.057	0.053	1.072	0.284
	Rainfall	0.889	0.069	12.970	<0.001***	0.257	0.068	3.786	<0.001***
Soil	Max temperature	0.510	0.380	1.342	0.179	-0.189	0.286	-0.663	0.507
	Min temperature	0.264	0.126	2.098	0.036*	-0.042	0.100	-0.424	0.672
	Rainfall	0.126	0.128	0.987	0.324	-0.049	0.134	-0.365	0.715

Intra- and inter-annual species turnover

Intra-annual β-diversity per stratum indicated differing responses of species turnover as sampling progressed. Average monthly β-diversity of canopy arthropod assemblages showed higher turnover during the spring-summer periods, with slight decreases during the colder months (Fig. 3.4a). Leaf litter arthropod β-diversity increased during the autumn and early winter months when leaf abscission was highest, and decreased during and immediately after the occurrence of wildfires (Fig. 3.4b). Soil arthropod assemblages saw little change in monthly β-diversity throughout the study period, with decreases occurring at certain intervals which were not noted to correspond to seasonal changes. (Fig. 3.4c). Overall, temporal trends in intra-annual arthropod β-diversity saw little variation in the canopy stratum, and a slight increase in the soil stratum. However, leaf litter arthropod β-diversity showed a pattern of temporal decline, indicating a possible ongoing dynamic in the leaf litter assemblages.

Differences between yearly species turnover varied across the sampled strata (Fig. 3.5). Inter-annual β-diversity differed substantially between the two years of study in both the leaf litter and soil stratum, with the first year of both possessing a higher species turnover. No difference between yearly β-diversity was found for the canopy stratum assemblages.

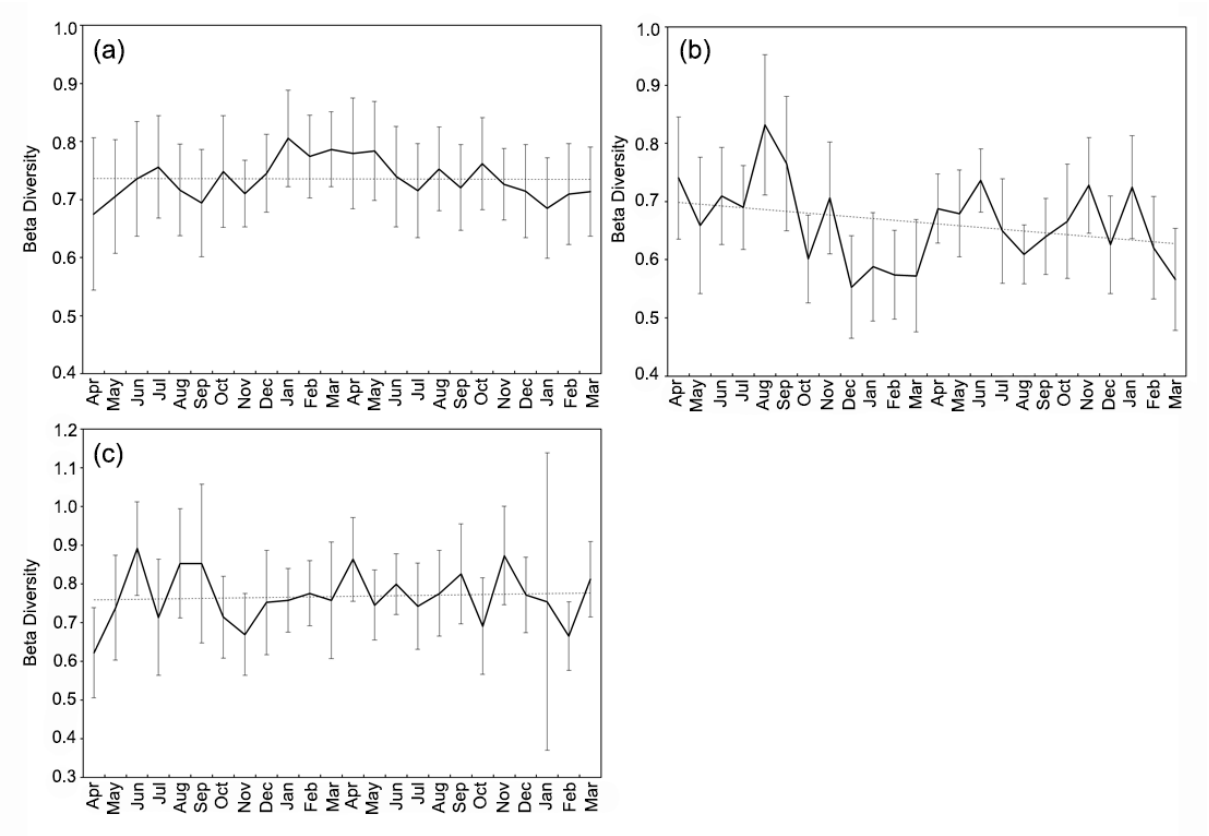

Fig. 3.4 Temporal variation of arthropod assemblage β-diversity in (a) canopy, (b) leaf litter, and (c) soil strata with trend lines.

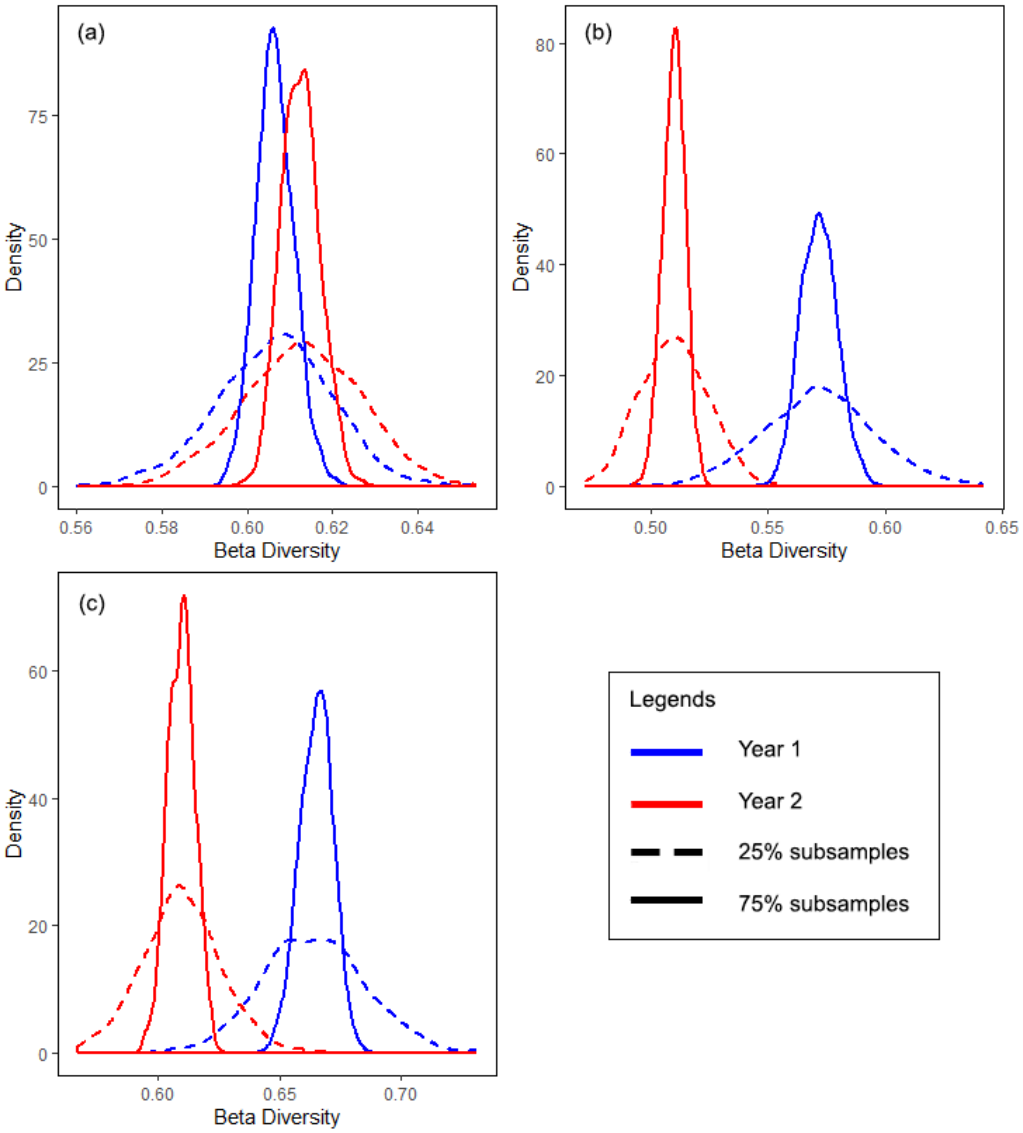

Fig. 3.5 Total intra-annual β-diversity for (a) canopy, (b) leaf litter, and (c) soil arthropod assemblages during both years of study.

Discussion

Arthropod assemblages showed a variety of seasonal and compositional responses during the two sampling years. The first year's assemblage variation corresponded more to patterns of rainfall, compared to that of the second, which displayed more erratic changes in species richness and abundance across the strata. Abundances in leaf litter showed strong correspondence to rainfall, with significant interaction between assemblages and rainfall of both years. General intra-annual assemblage changes primarily occurred between the wetter and drier months each year in the study, with shifts in species assemblages coinciding with seasonal changes. Shifts in species assemblages can often be attributed to changes in wet and dry seasons (Wagner, 2001; Pinheiro et al., 2002; Langlands et al., 2006; Doblas-Miranda et al., 2007; Liu et al., 2013), as well as changes in temperature ranges (Samways, 1996; Høye and Forchhammer, 2008). Despite environmental variables not being significantly different between the two years of study, patterns of seasonal assemblage variation were noted to differ inter-annually across all strata. This coincides with results of similar investigations of yearly seasonal and temporal assemblage changes (Schowalter and Ganio, 2003; Doblas-Miranda et al., 2007; Valtonen et al., 2013; Ramírez-Hernández et al., 2014).

While changes in shrubland canopy arthropod richness are often correlated with environmental fluctuation due to exposure (Basset et al., 2003), different annual responses were noted during our study. Species richness of canopies during the first year was seen to show a general decrease during the dry season with an increase observed during increased monthly rainfall. This trend was not necessarily seen during year two, as assemblage richness was observed to gradually increase throughout the year despite transitions of wet and dry seasons, with a major decrease recorded during

sampling periods with higher recorded ambient temperatures. The significant interaction between species richness of the canopies and minimum and maximum temperatures of the second year of study may indicate the possible effect of shifts in increased temperatures inter-annually on these assemblages. However, as canopy richness was shown to not be affected by environmental variables during the first year of sampling, this may also hint at the structural composition of the sampled shrubland patches providing a consistently sheltered environment (Gardner et al., 1995; Komposch and Hafellner, 2003; Kumar et al., 2009).

Leaf litter richness during the first year, exhibited a strong interaction with all environmental variables investigated. This differed in the subsequent year, with only rainfall showing a significant interaction with litter assemblages. Arthropod species richness of various leaf litter taxa is known to vary with seasonality, moisture, litter complexity, competition and temperature (Lensing et al., 2005; Butler and Haddad, 2011; Podgaiski and Rodrigues, 2017). Interception of light and precipitation by woody plant canopies, alongside plant species diversity, has often been a factor in the formation of lower strata conditions (Scholes and Archer, 1997). More closed canopy coverage is able to provide increased moisture and reduced temperature fluctuations suitable for sustained soil and leaf litter arthropod assemblages (Neumann, 1973). With the GGHNP experiencing a temperate climate, a pattern of reduced canopy coverage within certain sampled sites occurs seasonally. This abscission of leaf coverage increases exposure of lower strata while decreasing habitable canopy space. Additionally, the occurrence of grassland wildfires reduced vegetation coverage in the lower strata of four sites during the first year of study. The effect of fire on arthropod communities at the soil-litter layer has been well documented (Niwa and Peck, 2002; Apigian et al., 2006; Uys et al., 2006; Antunes et al., 2009; Vasconcelos

et al., 2009; Huebner et al., 2011), with the removal of litter coverage by burning having compounding effects on environmental variables on litter arthropod assemblages (Coleman and Rieske, 2006; Silveira et al., 2010; Ober and DeGroote, 2011).

Soil arthropod richness was largely unaffected by temperature and rainfall, with the exception of the first year of study, whereby minimum temperature showed variation from the expected. This may be attributed to the occurrence of wildfires decreasing vegetative ground coverage of certain sampled sites, further increasing temperature fluctuation and solar radiation exposure of the soil stratum during the cold winter months (Iverson and Hutchinson, 2002; Vermeire et al., 2005).

With regard to the effect of environmental variables, a strong correlation was indicated between abundance of assemblages and climate. While these results indicate generally that climate seems to play a role in arthropod abundance of all strata, it is unsure from these results alone how this relationship plays out functionally, particularly due to the multicollinearity experienced between the variables. The direct influence of physiological changes and the indirect influence of resource availability are likely explanative factors (Cammell and Knight, 1992; Cornelissen, 2011; Karuppaiah and Sujayanad, 2012). The possibility of certain groups of arthropods dominating the assemblages during seasonal change must also be taken into consideration, as abundance of certain species has been noted to respond differently to seasonal variation (Bale et al., 2002; Beng et al., 2018). Further investigation into seasonal responses of different arthropod orders may provide more insight into the intricate effects of temperature and rainfall in this region.

The most interesting aspect of this study was the differing rates of monthly and yearly seasonal species turnover across the sampled strata. The temporal trends

indicated by the monthly β-diversity of arthropod assemblages showed variation in dynamics occurring in the different strata. The occurrence of little variation in arthropod β-diversity in the canopy stratum, and a slight increase in this β-diversity in the soil stratum, indicates a more stabilised environment during seasonal variation. However, the temporal decline of arthropod β-diversity in the leaf litter stratum indicates a possible ongoing dynamic experienced by the leaf litter assemblages. This trend is indicative of a less stable environment, subject to increased disturbances and variation, such as wildfires. Such disturbances have an important role in explaining patterns of species diversity (Cadotte, 2007). The degree and frequency of disturbance is often an important factor affecting species turnover, with large-scale disturbances such as wildfires having a differing effect (Ferreira, 2018). Regeneration succession would then occur at the scale of disturbance, leading to species turnover being low (Maarel, 1993).

While species turnover did not significantly differ between both years in the canopy stratum, both leaf litter and soil showed a higher species turnover occurring in the first year of study. Such contrasting observations may once again be attributed to the occurrence of grassland wildfires. Two separate incidences of wildfires during the first year affected four out of the six sites sampled, decreasing lower strata vegetation. Conversely, during the second year, only a single wildfire occurrence affected a single site. However, the degree of burning was more extreme and caused sections of the site canopy stratum to be burnt. Despite this, the higher species turnover in the first year indicates higher levels of disturbance compared to the second, leading to different species turnover in the lower strata across the sampled sites. Similar instances of differing species turnover during periodical burnings have been documented, with certain taxa responding differently over short-term and long-term recovery

(Lussenhop, 1976; York, 2000; Fagan et al., 2006; Pinzon et al., 2013; Anjos et al., 2015).

The argument can be made that the β-diversity obtained during our study is generalised and does not provide partitioning of dissimilarity into the two components of turnover and nestedness (Baselga, 2010). By definition Whittaker's β-diversity yields a measure that equivalates turnover and nestedness (Whittaker, 1960; Tuomisto, 2010; Baselga and Orme, 2012). As such, the partitioning of β-diversity into two separate components, to account for the dissimilarity that is derived from only turnover and only nestedness, has become important to discern their contradictory processes underlying β-diversity (Baselga, 2010; Baselga, 2012). A more refined analysis of temporal species turnover, preferably a β-diversity partitioning that considers abundance, should therefore be considered for future investigations in the GGHNP to account for the use of multiple sites (Shimadzu et al., 2015).

The implications of differing inter-annual species turnover and the decreasing trend of β-diversity in the leaf litter assemblages must also be considered on the basis of conservation. Although increases and occurrences of little to no variation in species turnover occurred during this study, the overall yearly turnover was relatively low. As GGHNP acts as an important tourism destination and forms part of the Maloti-Drakensberg Catchment Complex (SANParks, 2011), the focus of ensuring adequate conservation of landscapes and ecosystems remains of importance. The monitoring of species turnover in this regard, both temporally and spatially, has often been of major consideration in regards to conservation efforts. Anderson and Ferree (2010), suggested that areas with low species turnover could be prioritised as refugia for the largest number of species, ensuring more diverse species assemblages. However, as the role of refugia is to provide a protective environment during unfavourable

conditions, they are often recorded as having a higher species diversity than the surrounding landscape (Médail and Diadema, 2009; Keppel et al., 2012). As this study focussed only on shrubland patches within a grassland matrix, the possibility of these patches to act as refugia, in relation to the surrounding landscapes, must still be investigated. This is particularly important due to the high occurrence of grassland wildfires preventing expansion of woody vegetation (Higgins et al., 2000; Trollope et al., 2002; Adie et al., 2017).

Seasonal monitoring of arthropod assemblages provides insight into the behavioural and physiological responses that may occur in populations across time and space. The results of our study provide a general overview of yearly seasonal arthropod assemblage changes in shrubland patch sites of the GGHNP, providing useful and comparative data on species abundance and distribution patterns across these isolated patches. Data on the responses of these assemblages to temporal variation, in the specific environmental conditions of the GGHNP, are necessary to predict effects of environmental changes on shrubland communities and the consequent effects on ecosystem structure and function (Schowalter and Ganio, 2003). However, as taxon specific responses differ during different environmental perturbations and seasonal changes, there is opportunity to investigate arthropod assemblage changes at a finer taxonomic resolution. Furthermore, the relation of these patchess to their surrounding environment and their role as potential refugia during seasonal changes and environmental disturbance must also be considered.

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Chapter 4

Soil arthropod assemblages associated with montane shrubland patches and their surrounding grasslands

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Abstract

Soil biota are often an important consideration regarding soil health and quality in ecology. However, a lack of information is often an impediment to utilising these biota effectively in conservation strategies. The investigation of soil biota may provide information on an area's health by utilizing a more holistic viewpoint of their functionality and endemism in the environment. This study evaluated soil arthropod assemblages associated with a number of isolated shrubland patches and their immediate surrounding grasslands in the Golden Gate Highlands National Park (GGHNP). Sampling was conducted over a 24-month period in six contrasting shrubland patch sites. A total of 14 728 individuals, comprising 317 morphospecies, were sampled. Soil arthropod assemblages were more species-rich inside the shrubland patches compared to their surrounding grasslands. After allocation of broad functional feeding groups to biota, omnivores, mycophages and predators were found to dominate both the shrubland patches and grasslands. Indicator and correlation analyses of sampled biota and the investigated localities showed greater association of soil biota with shrubland patches. However, there were no strong indicators (>0.70) for any of the habitat types investigated. Soil arthropod assemblage compositions differed between the isolated shrubland patches and their surrounding grasslands. Due to the isolated nature of these patches, the results suggest that different soil arthropods are often associated with shrubland patches of the GGHNP. This hints at the significance of these patches as areas for priority management. Further study is needed to determine the significance of different terrains in the GGHNP as preferential habitats for soil biota communities.

Keywords: Abundance; Conservation; Functional Feeding Groups; IndVal; Species Richness

Introduction

The role of soil biota in soil health and quality has often been regarded as an important factor in the ecological field (Karlen et al., 1997; Culliney, 2013). While a large portion of these studies are primarily focused upon the benefits of soil biota in an agricultural setting (e.g. Stork and Eggleton, 1992; Doran and Zeiss, 2000; Barrios, 2006; Brussaard et al., 2007; Kibblewhite, 2008; Louw et al., 2014), concerted efforts to expand our knowledge of these organisms within more natural ecosystems and processes have been pursued (e.g. Glanz, 1995; Dominati et al., 2010; Blankinship et al., 2011; Orgiazzi and Panagos, 2018).

In the past, a lack of research on soil fauna potentially limited the true understanding and functioning of the soil environment (Huhta, 2007; Janion-Scheepers et al., 2016). However, advances in ecology, sampling techniques and a general understanding of ecosystem processes, would potentially provide more insight into their biology and role in the environment (Phillipson, 1971; Cooper and Rao, 2006; Brevik et al., 2015; Coleman et al., 2017). Despite this progress, our understanding of soil ecosystems and their fauna remains limited, particularly in poorly studied habitats (Bradford et al., 2002; Wardle, 2002). This is particularly true within South Africa's highly unique and variable landscape, as research towards soil biota and their endemism remains limited (Louw et al., 2014; Janion-Scheepers et al., 2016).

The Golden Gate Highlands National Park (GGHNP) of South Africa exemplifies this problem, as very little information is available on its soil fauna and their functional diversity. Information that is available is restricted primarily to oribatid mite species (Meyer, 1970; Niedbała, 2006; Hugo-Coetzee, 2014), and no record of Collembola is present (Coates, 1970; Janion-Scheepers et al., 2015). This presents challenges when attempting to accurately monitor soil biota assemblages from an

ecological perspective. Functional feeding groups as a taxonomic surrogate have been suggested for rapid monitoring of grassland arthropod assemblages (Buschke and Seaman, 2011). However, its validity is still under debate (Mlambo, 2011). Nevertheless, to fully appreciate the diversity of soil-dwelling biota, understanding their functionality, specifically feeding habits, within an ecosystem provides insight into their role in ecosystem processes and responses to disturbance (Brussaard, 1997). As soil arthropods are linked to many food webs and ecological processes specific to their habitats (Coleman et al., 2017), investigating these assemblages in regards to their feeding could increase knowledge as to their role in different niches and their use during ecological integrity assessment (Brussaard, 1998; Rawer-Jost et al., 2000).

The GGHNP is a National Park in the Free State Province of South Africa, forming part of the greater Maluti-Drakensberg mountain system, with major importance as a conservation area. Its mountainous topography includes insular patches of varying complexity and size in a grassland landscape. Of particular interest is the occurrence of small isolated shrubland patches scattered throughout the park's grassland mosaic (Grab et al., 2011; SANParks, 2019a). Shrubland patches may possess a similar, and at times higher, faunal abundance and diversity compared to the grasslands surrounding them (Holmes and Richardson, 1999; De Wysiecki et al., 2000; Lambrinos, 2000; Procheş and Cowling, 2006). While faunal assemblage patterns may differ between these contrasting landscapes (Yekwayo et al., 2016a), increased heterogeneity and edge-effects of smaller patches in a landscape matrix often contribute to the conservation of faunal species (Pryke et al., 2013; Yekwayo et al., 2016b). Additionally, small patches may serve as areas of higher species diversity and richness for certain faunal groups (Whittington-Jones et al., 2008; Adie et al., 2017).

This study aimed to determine the composition of soil arthropod assemblages, and their species association, with a number of isolated shrubland patches and their immediate surrounding grasslands within the GGHNP. It was hypothesised that (1) the soil biota assemblages would be more species-rich inside the patches than the surrounding grasslands, (2) the soil diversity and richness would differ between sites due to locality, and (3) overall functional feeding groups would differ between the shrubland patches and their surrounding grasslands.

Materials and methods

Study site and period

The GGHNP is located in the Eastern part of the Free State Province, South Africa (28°30′ S, 28°37′ E), covering an area of approximately 340 km^2. Elevation ranges from approximately 1600 to 2900 m a.s.l. (SANParks, 2013). The geological composition of the park consists of three primary layers belonging to the Karoo Sequence that formed during different geological periods (Groenewald, 1986), i.e. a base of Elliot Mudstone, Clarens Sandstone and a capping of Drakensberg Basalt (SANParks, 2019b). Soils of the park are composed of a variety of deep and shallow basaltic soils, along with shallow sandy soils formed from eroded sandstone (Roberts, 1969; Kay et al., 1993). Six dominant soil types occur through out the park encompassing a variety of topsoil horizons and enrichment (Supp. Fig. S4.1).

The park has been designated as a montane grassland, falling in the vegetative units of Eastern Free State Sandy, Lesotho Highland Basalt and Northern Drakensburg Highland grasslands (Mucina et al., 2006). The most predominant grasses and herbs in the mountainous region belong to *Eragrostis*, *Themeda* and *Tristachya* (SANParks, 2019c). Sandstone cliffs and outcrops occur throughout the

park, forming ravines and gulleys that house shrubland patches (Grab et al., 2011, SANParks, 2019a). These patches are characterised by Basotho Montane Shrubland and Drakensberg-Amathole Afromontane Fynbos (Mucina et al., 2006).

Climate in the park is temperate, with summer rainfall periods occurring between October and April, and precipitation averaging between 700–800 mm annually (Groenewald, 1986). Winter months are cold and dry, with average minimum temperatures ranging between 1–15°C, and heavy snowfalls being recorded on the higher peaks of the park, while average summer temperatures are mild to warm, ranging between 13–26°C (Grab et al., 2011).

Grassland fires are a common occurrence in the GGHNP during the dry seasons. While the implementation of rotational burning for maintaining grazing areas throughout the park is an annual practice, several cases of arson have also been documented (Rademeyer and van Zyl, 2014). Particularly devastating wildfires have been recorded in which more than 6000 ha were destroyed (Smith, 1992; Muller, 2017; SANParks personnel, personal communication, 2017). A number of wildfires occurred during this particular study, impacting some of the already established sampling sites. The degree of vegetation loss differed between the sites. The most notable wildfires occurred during September 2017, November 2017 and July 2018. Due to the lifecycles of certain major grass species often adapted to regular fires in this grassland landscape (SANParks, 2019c), particularly *Themeda triandra* (Short et al., 2003), regrowth was seen to occur in one- to two-months post-fire.

A total of six sites were selected for this study (Fig. 4.1), with each shrubland patch surrounded by grassland. Monthly sampling was conducted over a 24-month period from April 2017 to March 2019.

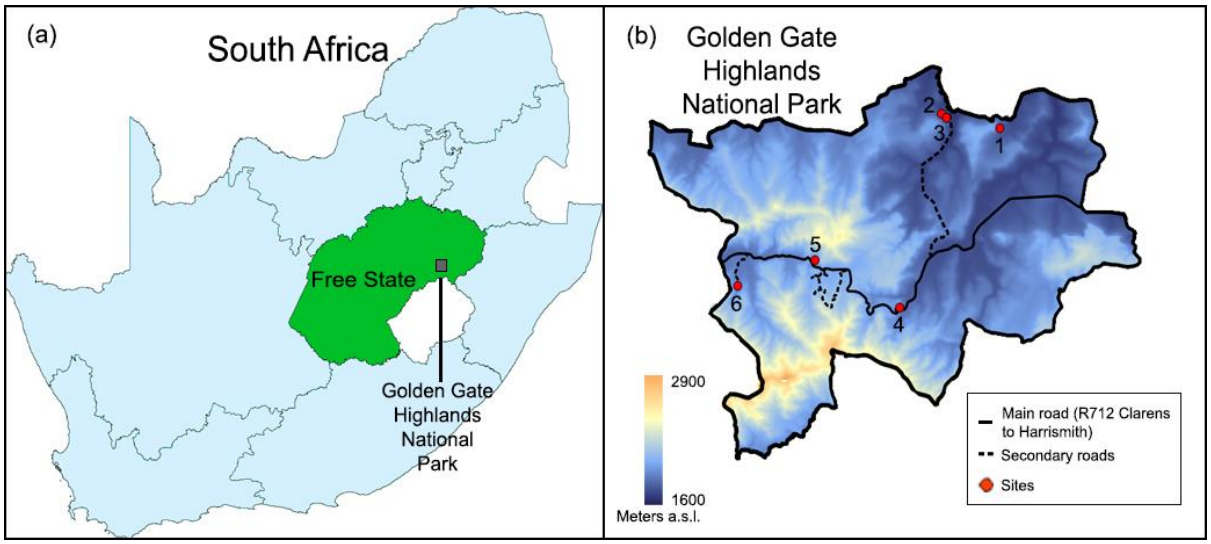

Fig. 4.1 Location of study area. (a) Location of Golden Gate Highlands National Park in the eastern part of the Free State Province, South Africa. (b) Location of study sites (1–6) (Elevation data obtained from Web GIS (http://www.webgis.com/srtm3.html), developed using QGIS version 2.18.15).

Sampling methodology and identification

Soil biota was obtained from three sub-sites at each of the six shrubland patches (hereafter localities). These localities consisted of soil present within the boundaries of the shrubland patch and two grassland sub-sites at measured distances of 10 and 25 m from the shrubland patch edge. Patch edges were designated based on where woody vegetation stopped and grassland began.

A total of five soil samples per sub-site were taken randomly per month. Individual soil samples consisted of 500 grams of soil sampled using a soil auger from the top 10 cm of soil of a designated spot. As soil samples were taken randomly, certain samples inside the shrubland patches were taken near to the base of trees and shrubs while others were in the periphery of the vertical canopy shadow. Soil samples from the grassland sub-sites were randomly sampled around each shrubland patch, encompassing the entire available perimeter at the demarcated distances.

Soil samples were pooled monthly for each locality sub-site and placed in individual Berlese-Tullgren funnels for seven days, with a connected storage bottle containing 70% ethyl alcohol, to extract soil biota. All collected specimens were identified to morphospecies level per monthly sample, using available literature (Theron and Ryke, 1969; Olivier and Theron, 1989; Eggleton and Gaston, 1990; Triplehorn and Johnson, 2005; Dunger and Schlitt, 2011; Badenhorst, 2016; Slingsby, 2017), and counted. Additionally, identified individuals were broadly categorised to a functional feeding group based on the same literature and the assistance of specialists. Indicator species were determined as described below. Taxa indicated to be associated with shrubland patches and grasslands, as indicator species, were identified to genus and species level with the assistance of specialists. All collected specimens, excluding Arachnida, were deposited with South African National Parks (SANParks) Scientific Services as reference specimens. Arachnida specimens were deposited in the National Museum in Bloemfontein (NMBA).

Statistical analysis

Total soil arthropod assemblage diversity (Shannon-Wiener diversity and Simpson's index of diversity) and richness estimators (Chao1 index) per sub-site of the six localities were calculated, alongside their corresponding total abundance and observed species richness, using EstimateS, version 9.1.0 (Colwell, 2013). Sample completeness was measured as sample coverage; being the proportion of the total number of individuals in a community belonging to the species represented in the sample (Chao and Jost, 2012). Due to the similarities in feeding behaviour of soil arthropods, the categorised functional feeding groups were incorporated into the calculated diversity index by means of species abundance, as described by

Badenhorst (2016). This provided a graphical representation of the total diversity of each locality sub-site with the functional feeding groups represented as a proportion of the diversity. Additionally, Pielou's evenness index was computed for each locality sub-site and compared with the functional feeding group proportions.

Permutational multivariate analysis of variance (PERMANOVA) was conducted to test for assemblage composition variation overall, as well as per locality, between sub-sites. When significance was indicated, pairwise contrasts of the three associated sub-sites were computed to determine which of the sub-sites differed significantly in terms of species composition. All PERMANOVAs were calculated with a Bray-Curtis distance matrix using the vegan package in R, version 3.5.3 (Oksanen et al., 2018). All p-values for pairwise contrasts were adjusted using the false discovery rate method (Benjamini and Hochberg, 1995).

Nonmetric multidimensional scaling ordination (3D) (NMDS) was conducted using the Bray-Curtis similarity index to graphically represent the associated soil arthropod community composition of the three sub-site types at the six localities. Additionally, similarities between the total assemblages of all 18 sub-sites were calculated by cluster analysis with the Bray-Curtis similarity matrix using a paired group algorithm. Both NMDS and cluster analysis were performed using Paleontological Statistics version 3.25 (Hammer et al., 2001).

Association of soil arthropod species patterns and combinations of overall sub-site type was established using the multi-level pattern analysis ('multipatt') function of the indicspecies package in R, version 3.5.3 (De Cáceres and Legendre, 2009). Determination of species associated with particular site types allows for the use of indicators in ecological studies by assigning an indicator value (IndVal) (Dufrêne and

Legendre, 1997). The original method of Dufrêne and Legendre (1997) calculated an IndVal index between recorded species and the designated site groups as an applicable rule for clustering, determining the indicator values through species fidelity (being predominantly found in a habitat type) and specificity (being abundant in a specific habitat type). This IndVal method is used by the 'multipatt' function with an extension of the original concept, as it examines indicator species of individual site groups as well as their combinations, as explained by De Cáceres et al. (2010).

Additionally, ecological preferences of species can be analysed with the use of several other indices (De Cáceres and Legendre, 2009). The use of diagnostic species can be used to characterise and indicate other biota assemblage types within an environment. Pearson's phi coefficient of association can be used in this regard to determine association in the form of fidelity (not to be confused with fidelity of the IndVal component), between species and habitat types (Chytrý et al., 2002). This coefficient was measured between two binary vectors in the form of presence and absence data to produce the correlation (De Cáceres, 2013). Furthermore, De Cáceres et al. (2010) advised caution when reporting the results of indicator species analyses for several species, due to multiple testing issues. Corrections for multiple testing is therefore necessary in this regard. All resultant p-values provided by the 'multipatt' function were adjusted by Bonferroni correction using the 'p.adjust' function in R, version 3.5.3 (R Core Team, 2019). After correction, only those species still indicated as being of significance were reported for association with the designated sub-site types and their groupings.

Results

A total of 14 728 individuals and 317 morphospecies were sampled over the 24-month period. On average, soil assemblages inside shrubland patches were more species-rich than the surrounding grassland sub-sites, while abundance differed depending on the locality (Table 4.1). Both Shannon-Wiener (H) and Simpson's (D) diversity indices also varied depending on locality, with certain soil assemblages being more diverse in the surrounding grasslands compared to those inside the shrubland patches.

Table 4.1 Total diversity and richness statistics of arthropods per locality sub-site. N – Number of individuals, S_{obs} – Observed species richness, H – Shannon-Wiener diversity index, D – Simpson's index of diversity, S_{Chao1} ± SD – Species richness estimate with 1 standard deviation, Coverage – Sample coverage. All arthropods were sampled between April 2017 and March 2019.

	Subsite	N	S_{obs}	H	D	S_{Chao1} ± SD	Coverage
Site 1	Inside	539	75	3.148	0.896	98.58 ± 11.77	0.948
	10 m	518	57	2.022	0.672	96.47 ± 19.57	0.942
	25 m	702	54	1.888	0.650	111.92 ± 30.79	0.959
Site 2	Inside	722	70	2.792	0.849	113.81 ± 23.44	0.963
	10 m	499	54	2.609	0.867	120.30 ± 34.51	0.938
	25 m	689	53	2.071	0.774	74.20 ± 11.46	0.965
Site 3	Inside	1328	64	1.636	0.506	77.12 ± 7.59	0.984
	10 m	303	46	2.713	0.868	74.78 ± 16.65	0.928
	25 m	325	53	2.847	0.885	99.29 ± 25.53	0.920
Site 4	Inside	2913	111	2.476	0.734	148.17 ± 16.76	0.988
	10 m	1045	77	2.911	0.898	99.21 ± 11.08	0.973
	25 m	1024	72	2.876	0.892	93.41 ± 11.32	0.976
Site 5	Inside	870	94	3.416	0.938	141.52 ± 21.17	0.958
	10 m	517	62	2.272	0.680	83.62 ± 11.21	0.950
	25 m	448	72	3.297	0.930	146.21 ± 36.39	0.922
Site 6	Inside	508	52	2.703	0.851	75.70 ± 14.27	0.961
	10 m	1320	64	1.583	0.491	80.49 ± 9.29	0.983
	25 m	458	54	2.799	0.873	90.06 ± 20.78	0.950

Functional feeding group proportions varied between localities, with omnivores and mycophages contributing the most towards the diversity across all sub-sites (Fig. 4.2). The proportion of predators and saprophages was observed to correlate with the higher diversity values, while the remaining feeding groups remained relatively minimal in abundance. Pielou's evenness index also corresponded to diversity values, as those sub-sites with a lower evenness were noted to mostly be dominated by ant species (Supp. Table S4.1–S4.6). Overall, soil arthropods in both shrubland patches and grasslands were dominated by taxa with an omnivorous, predatory and mycophagous feeding behaviour.

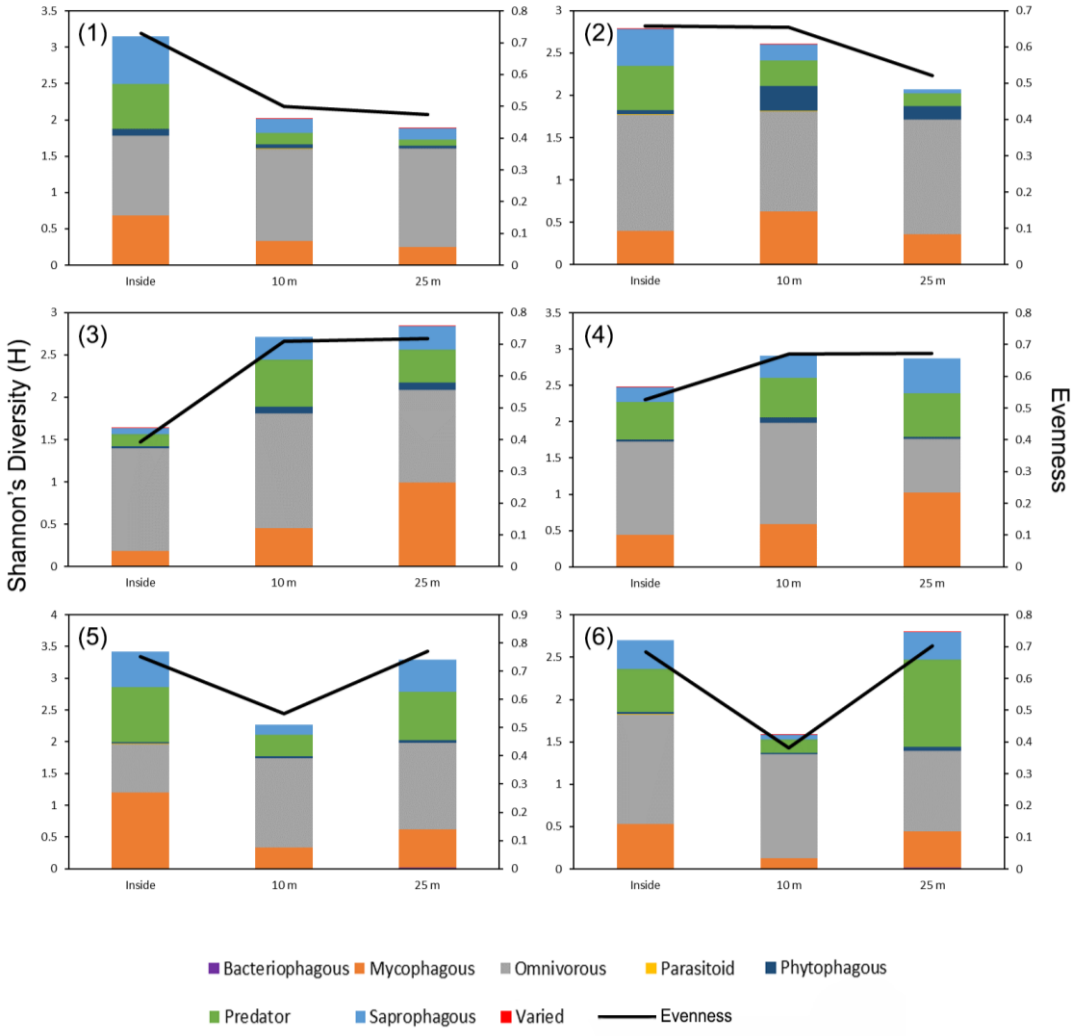

Fig. 4.2 Represented functional feeding groups of sub-sites from the six sampled localities (1-6), as a proportion of the total soil faunal diversity (Shannon-Wiener diversity), with Pielou's evenness index.

Global assemblage composition differed significantly between sub-sites (Table 4.2), with pairwise comparisons indicating different species compositions between assemblages inside the shrubland patches and the surrounding grasslands, but not between the two perimeter grassland sub-sites (Table 4.2). Locality-specific assemblage composition differences varied, with the majority of sites indicating significant differences between sub-sites (Table 4.2). Pairwise comparisons per locality also varied, with certain site species compositions differing between only the shrubland patch assemblages and the surrounding grasslands, while others showed significant difference between all sub-sites (Table 4.2).

Table 4.2 Soil arthropod community differentiation between sub-sites of the six sampled localities, with pairwise comparisons. Significance codes: 0.001 '***' 0.01 '**' 0.05 '*'.

PERMANOVA		Community Differentiation			
Global		$F_{2,429} = 1.980$, $p < 0.001$ ***			
Site 1		$F_{2,69} = 1.706$, $p < 0.001$ ***			
Site 2		$F_{2,69} = 1.207$, $p < 0.203$			
Site 3		$F_{2,69} = 1.034$, $p < 0.062$			
Site 4		$F_{2,69} = 2.931$, $p < 0.001$ ***			
Site 5		$F_{2,69} = 3.456$, $p < 0.001$ ***			
Site 6		$F_{2,69} = 1.689$, $p < 0.001$ ***			
Pairwise Comparison		F Model	R^2	p-value	p-adjusted
Global	Inside vs 10 m	2.712	0.009	0.001	0.002 **
	Inside vs 25 m	2.385	0.008	0.001	0.002 **
	10 m vs 25 m	0.869	0.003	0.621	0.621
Site 1	Inside vs 10 m	2.485	0.051	0.002	0.006 **
	Inside vs 25 m	1.421	0.030	0.117	0.176
	10 m vs 25 m	1.214	0.026	0.252	0.252
Site 4	Inside vs 10 m	2.662	0.055	0.002	0.002 **
	Inside vs 25 m	3.867	0.078	0.001	0.002 **
	10 m vs 25 m	2.269	0.047	0.001	0.002 **
Site 5	Inside vs 10 m	4.169	0.083	0.001	0.002 **
	Inside vs 25 m	3.834	0.077	0.001	0.002 **
	10 m vs 25 m	2.382	0.049	0.005	0.005 **
Site 6	Inside vs 10 m	1.862	0.039	0.020	0.030 *
	Inside vs 25 m	2.217	0.046	0.008	0.024 *
	10 m vs 25 m	1.040	0.022	0.395	0.395

NMDS analysis indicated some overlap between overall assemblages of the three sub-site types, with greater clustering of assemblages inside shrubland patches occurring (Fig. 4.3a). Cluster analysis did not indicate any particular pattern of similarity, as clustering of all sub-sites was observed to be mixed, with the grassland sub-sites of site 3 being the only ones to cluster based on locality (Fig. 4.3b). This would indicate that soil assemblages may be driven by more environmental factors than just locality. Species overlap between the sub-site types showed a higher number of unique soil-dwelling arthropods in the shrubland patches compared to the surrounding grasslands (Fig. 4.3c). This indicates soil biota may be more adapted to shrubland patches due to differences in environmental factors. However, the number of shared species between all sub-site types was also high, highlighting the survivability of these species across different habitat types in the GGHNP.

IndVal and correlation multi-level pattern analyses of soil arthropods in the three sub-site types provided association results for only the shrubland patch soil, along with a combination of the shrubland patch and 10 m soil (Table 4.3). The majority of associated species were noted to be the same for both IndVal and correlation analyses. However, indicator values were seen to differ between analysis type, indicating different species as being of highest indicator value. Sub-site grouping was also noted to differ, with no combination of sub-sites provided for correlation analysis. All indicator values were shown to be relatively low despite their significance. A number of mites and a single collembolan species were identified as occurring frequently in the shrubland patch sites, along with a single pseudoscorpion species. Those species associated with both the surrounding grasslands and the shrubland patch sites included a single mite, while no unique indicators were found for the grasslands alone. These results indicate a higher number of soil arthropod species as

often being associated with the shrubland patches compared to the surrounding grasslands.

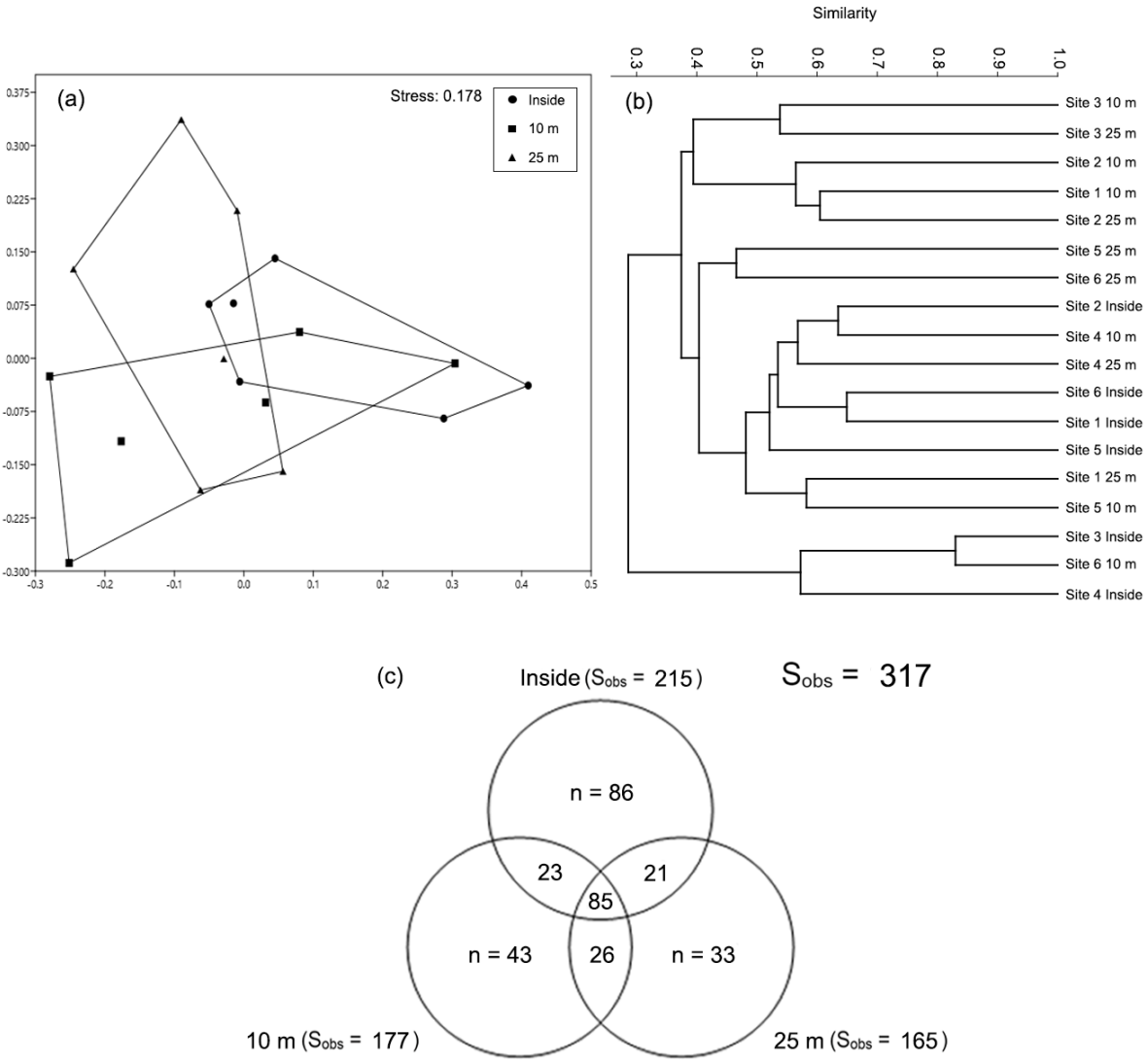

Fig. 4.3 Overall differences in soil arthropod assemblages from six shrubland patch sites and surrounding grasslands based on total assemblage abundances, species richness, and Bray-Curtis similarity matrix: (a) NMDS (3D) ordination of overall sub-site type, (b) cluster dendrogram of locality sub-sites using paired group algorithm, and (c) venn diagram indicating overlap in species from each overall sub-site type, with unique species shown. All assemblages were sampled during the period of April 2017 to March 2019.

Table 4.3 Results of IndVal and Pearson's phi coefficient multi-level pattern analysis for sub-site type and groupings. Only those soil arthropods indicated as being significant after p-value adjustment are shown.

Order	Family	Species/genus	IndVal Stat	Pearson's phi coefficient Stat
Inside				
Oribatida	Aleurodamaeidae	*Aleurodamaeus salvadordali*	0.454	0.276
Oribatida	Euphthiracaridae	*Acrotritia* sp.	0.389	0.322
Collembola	Hypogastruridae	*Hypogastrura* sp.	0.323	—
Trombidiformes	Tydeidae	*Pronematus* sp.	0.282	0.172
Pseudoscorpiones	Chthoniidae	*Austrochthonius* sp.	0.264	0.218
Inside & 10m				
Mesostigmata	Ologamasidae	*Gamaselliphis potchefstroomensis*	0.434	—

Discussion

The role of soil arthropods as tools of environmental assessment has established these biota as important indicators of long-term ecosystem sustainability (Parisi et al., 2005; Langor and Spence, 2006; Maleque et al., 2006; Gulvik, 2007). Determining the biota associated with habitat types and their functional roles in ecosystems contributes to the development of conservation monitoring strategies (Kremen et al., 1993; Longcore, 2003). Due to the ecological importance of the GGHNP, the benefit of monitoring soil biodiversity should be of consideration. The results obtained during our study provide a preliminary overview of current soil-arthropod biota assemblages, their association with shrubland patches and grasslands, and their functionality.

The occurrence of a higher species richness inside the sampled shrubland patches during this study correlated with other studies of woody vegetation soil fauna (Benito and Sánchez, 2000; Ferguson, 2001; Adolphson and Kinnear, 2008). However, the presence of a lower faunal richness in the surrounding grasslands contrasts with similar studies of grassland soil (Bardgett and Cook, 1998; Siemann, 1998; Sechi et al., 2018). The variability in locality of the investigated sites may explain these differences, as different slope aspects directly impact vegetative succession of both shrublands and grasslands (Malan et al., 1998; Sternberg and Shoshany, 2001;

Bennie et al., 2006; Gong et al., 2008). As leaf litter has been noted to increase faunal richness at the soil-litter layer (André et al., 1994; Ashford et al., 2013; Jiang et al., 2013), the higher species richness recorded inside the shrubland patches is supported by these studies. Additionally, soil composition throughout the grasslands of the GGHNP is considered to be relatively lacking in nutrients, due to the formation of shallow sandy soils from sandstone formations throughout the park (Roberts, 1966). The occurrence of grassland wildfires also should be considered, as the surrounding grasslands of five of the six investigated sites, as well as the inner shrubland soil of two sites, were burnt to differing degrees during this study. The impact of wildfires on soil mesofaunal diversity and richness has been well documented, with differing responses of soil fauna to burn type and severity (Neary et al., 1999; Blanco-Canqui and Lal, 2004; Doerr and Cerdá, 2005; Verma and Jayakumar, 2012). Overall the high overlap between sub-site types indicates possible adaptability of soil-dwelling arthropod species to certain habitats of the GGHNP.

The functional feeding groups observed during this study indicated a general trend of omnivorous, mycophagous and predatory feeding behaviour, which is often documented to predominate soil ecosystems (Ponsard and Arditi, 2000; Scheu, 2002; Cole et al., 2006; Coleman et al., 2017). The abundance of omnivores, predators, mycophages and saprophages varied between locality and sub-site type, indicating differences in environmental conditions, as well as different types of habitat filtering (Petchey and Gaston, 2002; Villéger et al., 2008). While the soil systems developed in grasslands are often different from those of shrublands and other vegetation types, due to a higher shoot and root biomass turnover (Bardgett and Cook, 1998), it is more likely that vegetation type in these areas is determined by soil structure. In this regard,

soil type may be more influential in determining artrhopod species diversity and functional feeding groups.

Increased activity of soil arthropod mycophages and saprophages is expected in woody shrubland patches, due to their variable effects on, and association with, leaf litter and nutrient mobilisation (Seastedt, 1984; Wardle et al., 1998). However, cases of lower nutrient availability within shrublands have been noted to enhance omnivory while inhibiting bacteriophages in certain taxa (Sechi et al., 2018). In fact, soil food webs of woody vegetation soil communities are often characterised by omnivorous and cannibalistic species (Digel et al., 2014). Grassland soil fauna have also been noted to occupy a variety of trophic feeding groups of below-ground detritus food webs (Moore et al., 1988), with a more scavenging lifestyle being adapted. The increased abundance of ant species noted during our study corresponds to these feeding behaviours, as ants have been documented as omnivores and scavengers in a number of habitats (Scott et al., 1987; Clark and Blom, 1991; Dean, 1992; Ryder Wilkie et al., 2010).

The benefit of investigating functional traits of soil arthropods contributes towards the determination of taxon-specific responses to environmental changes, as well as ecological processes (Violle et al., 2007; Díaz et al., 2013; Nevalainen and Luoto, 2017; Sechi et al., 2018). Gagic et al. (2015) suggested that functional diversity may be a better predictor of ecosystem function compared to commonly used diversity. Changes towards means and distributions of functional traits from a community niche perspective can often indicate disturbance and shifts in habitat functionality (Mulder et al., 2012; Mouillot et al., 2013).

Those species indicated as being associated with the shrubland patches represented a variety of soil biota groups. Of these, only *Aleurodamaeus salvadordali* Hugo-Coetzee, 2013 and species of *Acrotritia* Jacot, 1923 have been reported in the shrubland patches and grasslands of the GGHNP (Niedbała, 2006; Hugo-Coetzee, 2013). Additionally, of interest is the mite species *Gamaselliphis potchefstroomensis* (Ryke, 1961), which was first described in South Africa from humus in the Potchefstroom area in 1952 and 1959 (Ryke, 1961; Castilho et al., 2016). While this species was reported a second time by Halliday (2005) from clover plants in the George and Hermanus areas, it was not re-described due to a lack of female specimens. Therefore, the specimens collected during our study are only the second complete report of *G. potchefstroomensis* in South Africa after the type material.

Despite differences between IndVal and correlation analyses showing different species as being of higher indicator value, the association of the same species in both analyses points towards their relatively strong endemism in the sampled shrubland patches. The utilisation of occurrence or abundance to establish a smaller list of indicators is often more viable as opposed to the sampling of a whole community (De Cáceres, 2013), providing useful long-term environmental monitoring for conservation or ecological management. Additionally, the selection of species as indicators may provide a reflection of the biotic or abiotic state of the environment, evidence for impacts of environmental change, or predict the diversity of other species, taxa or communities in an area (De Cáceres et al., 2010; Podani and Csányi, 2010).

The results obtained during this study indicate shrubland patch soils of the GGHNP as containing richer and more diverse species assemblages in comparison to surrounding grassland soils, corresponding to our initial hypothesis. Additionally, differences in locality of the sampled sites possibly contributed to variability in

assemblage structure and diversity, further influenced by the impact of grassland wildfires. Despite being an overview, the results of broad functional feeding groups indicated a more omnivorous, mycophagous and predatory feeding behaviour in both shrubland patches and grasslands. However, further investigation is necessary to determine the specific feeding behaviour of soil arthropods throughout the GGHNP to provide a more in-depth analysis. Furthermore, while a small list of indicator species for shrubland patches of the GGHNP was determined based on sampled specimens, further sampling is necessary to fully ascertain the soil arthropod species associated with other patches present in the park. Increased focus should, therefore, be considered to gauge the significance of terrains in the GGHNP as preferential habitats for soil biota communities.

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Chapter 5

Gene flow and genetic diversity of five ubiquitous spider species from shrubland patches in a mountainous grassland landscape

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Abstract

Gene flow and genetic variation were examined within and among populations of five of the most common spider species in shrubland patches of the mountainous Golden Gate Highlands National Park (GGHNP), South Africa. These species included three active hunters, *Dendryphantes purcelli* Peckham & Peckham, 1903 (Salticidae), *Pherecydes tuberculatus* O.P.-Cambridge, 1883 (Thomisidae) and *Philodromus browningi* Lawrence, 1952 (Philodromidae), and two web-builders, *Neoscona subfusca* (C.L. Koch, 1837) (Araneidae) and a *Theridion* Walckenaer, 1802 species (Theridiidae). A total of 249 spiders (57 *D. purcelli*, 69 *N. subfusca*, 34 *P. browningi*, 56 *P. tuberculatus*, 33 *Theridion* sp.) were collected and analysed from six shrubland patch localities in the park. Analyses of sequence variation of the mitochondrial cytochrome oxidase c subunit I (COI) gene for each species revealed relatively low nucleotide diversity ($\pi < 0.0420$) but high genetic diversity ($Hd > 0.6500$) within populations for all species, except *P. tuberculatus*. Genetic differentiation was also noted to differ between species, with only *P. tuberculatus* indicating very large divergence ($Fst > 0.2500$). These results were reflected by gene flow, with *D. purcelli*, *N. subfusca* and the *Theridion* sp. estimated as experiencing more than one disperser per generation. Overall, highest gene flow was found in the two web-building species, indicating possible high dispersal ability of these spiders in the GGHNP. Additionally, constructed phylogenies indicated possible cryptic speciation occurring in the majority of the investigate species. Our current results indicate that the five investigated spider species were able to maintain gene flow between shrubland patch populations within the GGHNP to some degree, despite the mountainous landscape. However, further analyses incorporating additional molecular markers are needed to properly determine the extent of genetic diversity and gene flow of these species within the GGHNP.

Keywords: Araneidae; Ballooning; Genetic Differentiation; Mountains; Web-building

Introduction

Genetic diversity and gene flow between isolated faunal populations is warranting an ever-increasing focus from a conservation perspective (von der Heyden, 2009; Bertola et al., 2011). Ensuring phenotypic and genotypic similarities between populations of the same species relies upon essential connectivity, particularly across wide distribution ranges (Weiss and Fullerton, 2000). Habitat insularity, giving rise to isolated woody vegetation patches in a grassland landscape, produces variable and unique challenges to faunal lifestyles (Keyghobadi, 2007; Adie et al., 2017). Dispersal between such isolated patches is often dependent upon the distance of individual patches in relation to native vegetation (Ramirez and Haakonsen, 1999), with connectivity in and among populations being a direct consequence of taxon dispersal ability (Brown et al., 2001; Ricklefs, 2004; Jackson and Blois, 2015; Dimassi et al., 2016).

While mountainous regions provide a variety of habitats over a relatively small area, they often act as geographical barriers, limiting the extent of dispersal and gene flow (Smith, 1999; Tennessen and Zamudio, 2008; Murphy et al., 2010; Qiong et al., 2017). The Drakensberg mountain range of South Africa is one such example, with responses of gene flow and genetic diversity differing among certain taxa (Daniels et al., 2003; Grobler et al., 2003; Lachmuth et al., 2010; Phiri and Daniels, 2016). However, corresponding data pertaining to terrestrial species across this mountain range remains limited. Mountains, rivers and oceans are designated as the main topographical factors associated with gene flow and long-term barrier effects (Zalewski et al., 2009; Pagacz, 2016). In spite of this, while elevation and mountain ridges show an effect on terrestrial population connectivity (Riechert, 1993; Masta, 2000; Tennessen and Zamudio, 2008; Lagerholm et al., 2017), mountains may not act as

absolute barriers, instead forming more permeable filters (Sánchez-Montes et al., 2018). This, in turn, solicits different responses and variations in genetic structures spatially for different taxa.

Limitations to population homogeneity, in the form of incomplete or fragmented phenotypic and genetic variability, can occur due to physical and/or behavioural barriers (Parmesan, 2006; Kuntner et al., 2014). Those species with an aerial dispersal ability often possess a more homogenous genetic structure over a large geographical range compared to their substrate-bound counterparts, particularly spiders (Slatkin, 1993; Schäfer et al., 2001; Dimassi et al., 2016). While large numbers of spider species possess conserved morphological characteristics, highly conserved morphology in cryptic species is often associated with strong genetic differentiation (Bond et al., 2001). Additionally, phenotypic plasticity in the same spider species, due to the influence of local environmental factors as well as climate change, may be observed (Williams et al., 2008; Vilchis-Nestor et al., 2013). Investigating gene flow over a smaller geographical range, as to provide a basis for further genetic and taxonomic studies, becomes important in this regard. Concurrently, accurate delineation of species boundaries is fundamental in ecological and evolutionary studies, particularly in the assessment of biodiversity and the identification of areas of conservation priority (Phiri and Daniels, 2016). The presence of national parks and reserves across the Drakensberg provides areas to investigate such factors before application on a larger geographical scale.

Here we report on the first study to determine genetic diversity and gene flow of five of the most ubiquitous spider species in shrubland patches of the Golden Gate Highlands National Park (GGHNP) in the Free State Province, South Africa. The selected species include *Dendryphantes purcelli* Peckham & Peckham, 1903

(Salticidae), *Neoscona subfusca* (C.L. Koch, 1837) (Araneidae), *Pherecydes tuberculatus* O.P.-Cambridge, 1883 (Thomisidae), *Philodromus browningi* Lawrence, 1952 (Philodromidae), and a *Theridion* Walckenaer, 1802 species (Theridiidae). These taxa encompass a range of different dispersal behaviours and sedentary lifestyles, with aerial dispersion, via wind-mediated dispersal ('ballooning') of immatures, being a possible primary factor. While ballooning allows for long-distance dispersal of spider species (Greenstone et al., 1987; Greenstone, 1990), it is often random and dependant on environmental conditions and surrounding topography (Humphrey, 1987; Greenstone, 1990), alongside an individual's predisposition and motivation (Weyman, 1993). Consequently, while ballooning allows for quick dispersion and colonisation of new areas, its effectiveness in maintaining gene flow between relatively isolated populations may become limited due to its, largely, one-way movement (Vandergast et al., 2004; Reed et al., 2011).

In order to identify the state of gene flow and inferred dispersal capability of the five selected spider species, evaluation of genetic variability is required. Molecular approaches involving *mt*DNA sequences of cytochrome c oxidase subunit I (COI), to determine phylogeographic diversification fragments, were therefore applied on selected populations of these species in the GGHNP. It was hypothesised that (1) due to the mountainous topography of the GGHNP, gene flow would be limited in the majority of the investigated species. As a result, (2) genetic diversity would differ between populations, and (3), web-building spiders would experience higher gene flow compared to vegetation-dwelling active hunters with more mobile lifestyles, which tend to be more strongly substrate-dependent.

Materials and methods

Study species

Due to the differing lifestyles, behaviour and morphology associated with the five investigated spider species, a short taxonomic description of the genera and species, along with their general distribution and vegetation commonly inhabited in South Africa, is given.

Dendryphantes C.L. Koch, 1837 is a widely distributed genus of jumping spiders (Salticidae) with a near global distribution, with the exception of Australia and Southern Asia (World Spider Catalog, 2019). More than 50 species have been described, nine of which are known from the Afrotropical region. The general appearance of *D. purcelli* in both sexes (Supp. Fig. S5.1a and b) includes a flat, brown, oval carapace, and large eyes surrounded by black rings, with the eye fields dark in the centre and covered with silver spots of translucent guanine crystals (Haddad and Wesołowska, 2011). The abdomen is predominantly greyish-beige (slightly paler in females), with traces of a brown herringbone/chevron pattern and a pair of rounded brown spots at the midpoint. Mouthparts and sternum are brown, and the external margins of the endites are extended to form a triangular lobe. Legs are also brown, with darker femora. Individuals are relatively small, being on average 4.0–4.5 mm in length (Peckham and Peckham, 1903; Haddad and Wesołowska, 2011), and maintain a plant wandering, free-living lifestyle. This species has been collected from foliage of a variety of shrub and grass species across South Africa (Foord et al., 2011a; Haddad and Wesołowska, 2011; Haddad et al., 2013), and its distribution extends to the Western Cape Peninsula (Clark and Benoit, 1977; Dippenaar-Schoeman et al., 2010). While lack of records exists for *D. purcelli*, large numbers of jumping spider species are known to balloon as juveniles (Guarisco et al., 2001; Cumming and Wesołowska,

2004). Those species belonging to the genus *Dendryphantes* have also been postulated to make use of this dispersal method (Schmitt and Martini, 2014; Rubio et al., 2018).

The 123 species of *Neoscona* Simon, 1864 (Araneidae) occupy a mostly pantropical distribution (Grasshoff and van Harten, 2007; World Spider Catalog, 2019), with the investigated orb-weaver *N. subfusca* being widely distributed throughout the Afrotropical region (Grasshoff, 1986), particularly in the Savanna and Grassland biomes of South Africa (Dippenaar-Schoeman et al., 2010; Foord et al., 2011a; Haddad et al., 2013). Individuals have been collected from a variety of grass, herb and foliage types across a number of habitats (Dippenaar-Schoeman and Leroy, 2003; Fourie et al., 2013; Neethling and Haddad, 2013), where they maintain a preferential plant-dwelling lifestyle due to their construction of symmetrical orb-webs. *Neoscona subfusca* is often yellow to light brown in colouration, with a covering of white hairs and darker abdominal markings (Supp. Fig. S5.1c and d) (Grasshoff, 1986). Legs are often dark brown, possessing light annulations, and sternum colouration is darker along the margins with a pale centre. The abdominal dorsum is light, with a folium-like pattern, which is variable between individuals. Individuals range from 5 to 8 mm in length. Due to the web-building lifestyle of orb-weavers, ballooning is a common occurrence in *Neoscona* species (Plagens, 1986; Bell et al., 2005). Two infraspecific taxa of *N. subfusca* have been recorded, consisting of the subspecies *Neoscona s. alboplagiata* Caporiacco, 1947 and *Neoscona s. pallidior* (Thorell, 1899) (World Spider Catalog, 2019). However, literature is unclear why Grasshoff (1986) never synonymized them with *N. subfusca* in his revision of the Afrotropical fauna, but possibly he never examined their types and refrained from synonymizing them.

Pherecydes O.P.-Cambridge, 1883 is a genus of crab spiders (Thomisidae), comprised of seven species largely restricted to the Afrotropical region (World Spider Catalog, 2019), and a very wide distribution in South Africa (Dippenaar-Schoeman et al., 2010). Both sexes of *P. tuberculatus* are structurally similar, with differences only occurring in colouration (Supp. Fig. S5.1e and f) (Pickard-Cambridge, 1883; Dippenaar-Schoeman, 1980). Individuals of this species are dark grey to brown, with males possessing an often mottled, darker carapace suffused with yellowish-white. Legs are pale yellow, with marks and speckles of white and brown. The abdomen, pentagonal in shape, possesses a thin clothing of hairs and a few short bristles. Abdominal colouration is often a dull greyish-white with yellow tinges, and is marked with yellow-brown and dark brown (very dark ventrally for males). Eyes are situated in two recurved rows and are unequal in size. Lateral eyes are generally larger than the medians and situated on prominent tubercles. Body length of individuals range between 3–5 mm. While possessing a free-living, wandering lifestyle, and occurring on a variety of vegetation (Foord et al., 2011a; Haddad et al., 2013), individuals of *P. tuberculatus* were found to prefer “Wild Olive” (*Olea europaea* L. ssp. *africana* (Mill.) P.S. Green) above three other tree species studied (Neethling and Haddad, 2013). As many foliage-dwelling crab spider species are known to balloon during infancy (Gertsch, 1939; Evans, 1997), it can largely be assumed that *P. tuberculatus* employs the same method.

While superficially similar to the “true” crab spiders (Thomisidae), individuals of Philodromidae often have reduced true setae on their bodies and lack congruent eye tubercles (Homann, 1975). The genus *Philodromus* Walckenaer, 1826 is the most common of the philodromid crab spiders (over 250 species), and is cosmopolitan with a largely Holarctic distribution, with a few species occurring in Africa and Australia

(World Spider Catalog, 2019). Individuals of *P. browningi* are distinctly flattened and clothed in soft recumbent setae, and are generally large in size (carapace length average 8.5 mm) (Supp. Fig S5.1g and h) (Lawrence, 1952). Body colouration is often reddish-brown with cream markings, and legs are generally pale with dark spots and bands. Eyes arranged in two rows, with anterior medians larger than laterals and posteriors subequal. Abdominal dorsum possessing a large median yellow-white area that widens posteriorly. This is bordered on each side by a visibly dark band in the anterior two-thirds. This species is also widely distributed in South Africa, being commonly found wandering on bushes, tall grass, herbage and the trunks of trees (Dippenaar-Schoeman et al., 2010; Foord et al., 2011a; Haddad et al., 2013). It can largely be assumed that *Philodromus browningi* juveniles balloon, based on reports of its occurrence in other *Philodromus* species (Cokendolpher et al., 1979; Blandenier et al., 2013).

Species belonging to the tangle-web spider genus *Theridion* (Theridiidae) are often classified as gumfoot-web builders, whereby a three-dimensional web, consisting of a central area with or without a retreat, is constructed (Dippenaar-Schoeman and Leroy, 2003). This genus contains nearly 600 described species around the world, with approximately 14 species recorded in South Africa (World Spider Catalog, 2019). Unfortunately, most of these species have never been illustrated or redescribed, making the identification of *Theridion* extremely challenging. As our investigated species was not identified to species level, a general overview of the genus' morphology and its distribution is given. *Theridion* individuals often possess a globular abdomen with cream or greyish-brown colouration (Supp. Fig. S5.1i and j) (Levi and Levi, 1962). Additionally, a distinct, notched median pattern extends along the abdominal dorsum, ranging from grey-white to brownish-red. The legs are long,

with the first pair longest and third pair shortest, and are often banded. Eyes arranged in two transverse rows of four, with anterior row straight or procurved from the front and posterior row straight when viewed from above; all eyes generally subequal in size (Levi, 1957). Individuals small, ranging in size from 3 to 5 mm. Although the genus is widely distributed throughout South Africa, confirmed species records remain incomplete for most taxa (Dippenaar-Schoeman et al., 2010; Foord et al., 2011a; Haddad et al. 2013), due to the poor state of taxonomic knowledge. The records of *Theridion* species ballooning (Blandenier and Fürst, 1998; Larrivée and Buddle, 2011) make it highly likely that individuals of our investigated species also balloon while young.

Sampling

All sampled spider species were collected by beating foliage from canopies of six shrubland patch sites in the GGHNP between April 2018 and March 2019 (Fig. 5.1). A maximum of 12 sampled individuals per species per site were intended to accurately estimate genetic diversity and differentiation (Nazareno et al., 2017), however, certain sites did not yield the desired quantity for certain species (Supp. Tables S5.1, S5.3, S5.5, S5.7 and S5.9). All collected specimens (268 total) were confirmed to belong to the five selected spider species based on morphological characteristics defined from available literature (World Spider Catalog, 2019). Of those collected, a total of 249 adult and immature individuals (57 *D. purcelli*, 69 *N. subfusca*, 34 *P. browningi*, 56 *P. tuberculatus*, 33 *Theridion* sp.) were successfully utilised in genetic analyses. All specimens were stored in 96% ethyl alcohol under refrigerated conditions until genetic analysis. Collected adult spider specimens were deposited in the National Museum in Bloemfontein (NMBA).

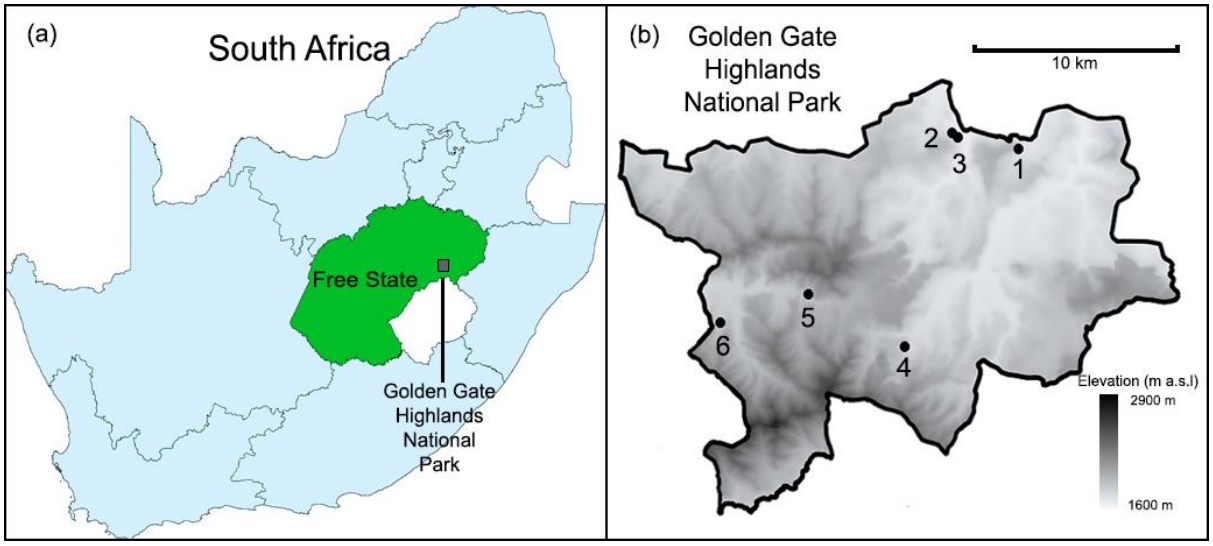

Fig. 5.1 Location of study area. (a) Location of Golden Gate Highlands National Park in the Free State Province, South Africa. (b) Map of the six shrubland patch sampling localities. Elevation data obtained from Web GIS (http://www.webgis.com/srtm3.html), developed using QGIS version 2.18.15.

DNA sequence comparisons

DNA extraction, PCR and Sanger sequencing for each selected individual was performed at the Canadian Centre of DNA Barcoding (CCDB) using standard protocols (CCDB, 2019a). One to two legs per individual were removed using sterile forceps, rinsed in 96% ethyl alcohol to limit residual contamination, and transferred into a 96-well microplate pre-filled with approximately 30µl of 96% ethyl alcohol. PCR amplification of the COI-5' barcode region was conducted using the primer pair C_LepFolF and C_LepFolR (CCDB, 2019b). All obtained sequences, collection data, specimen photographs and taxonomic identification of each specimen were deposited on the Barcode of Life Database (BOLD) system under the assigned project "Spiders of South Africa" (SPIZA): *D. purcelli* [SPIZA001-19–SPIZA059-19], *N. subfusca* [SPIAZ060-19–SPIZA131-19], *P. browningi* [SPIZA132-19–SPIZA174-19], *P. tuberculatus* [SPIZA175-19–SPIZA230-19], *Theridion* sp. [SPIZA231-19–SPIZA268-19].

For comparative analyses of phylogeny, sequences of our selected species were blasted using the BLASTN algorithm in the NCBI database to identify most homologous species. Voucher sequences of three to six of the most closely related species for each investigated genus were then downloaded (three sequences per species). Due to a lack of voucher sequences of species in the thomisid genus *Pherecydes*, sequences from three species in a closely related genus, namely *Tmarus* Simon, 1875, were obtained. All sequences were aligned per species using MAFFT Multiple sequence alignment version 7 (Kuraku et al., 2013; Katoh et al., 2019). Investigated taxa and voucher sequences were trimmed to a 658bp fragment for all species, with the exception of *P. browningi* and its related taxa, which were trimmed to 661bp.

Phylogenetic analyses, for the datasets per species, were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Model selection for both analyses was performed using the Akaike Information Criterion (AIC; Akaike, 1973) in jModelTest version 2.1.3 (Darriba et al., 2012). The ML analyses were conducted in PhyML version 3.1 (Guindon et al., 2010). Confidence values of branches were assessed via non-parametric bootstrapping with 1000 replicates (Felsenstein, 1985). Branch supports with bootstrap proportions of 80% or higher were regarded as sufficiently resolved nodes. Markov Chain Monte Carlo (MCMC) algorithm was used in the BI, and conducted in MrBayes version 3.2.7 (Ronquist et al., 2012). A total of two million generations were run for each species to ensure appropriate phylogenetic inference, with the exception of *P. browningi*, which was run for four million generations due to the increased number of base pairs. Four MCMC chains were run (3 hot, 1 cold), and sampling was performed every 1000 generations, with an adequate burn-in of 10% of the number of generations, determined from likelihood

scores and convergence of posterior probabilities (Dimassi et al., 2016), for each species. All trees sampled in the burn-in phase were discarded. Topologies with posterior probabilities (PP) of 80% or higher were regarded as sufficiently resolved nodes. In addition, inferred haplotypes and their relationships per sampled species were constructed by the Minimum Spanning Network (MSN) method (Bandelt et al., 1999), using PopART, version 1.7 (Leigh and Bryant, 2015).

Population genetic diversity and gene flow

After alignment and trimming of sequences of the investigated species as described above, the voucher sequences of all related species were removed from the datasets, and only the sequences of our five investigated spider species were used to analyse genetic diversity and gene flow. Investigated taxa sequences were assigned to the six localities from which the specimens were sampled. Levels of *mt*DNA genetic heterogeneity among populations were then quantified according to numbers of segregating sites (S), haplotype number (h), haplotype diversity (Hd), nucleotide diversity (π), and average number of pairwise nucleotide differences within a population (K), as calculated in DnaSP version 6.12.03 (Rozas et al., 2017). Pairwise genetic differentiation (Fst) between populations were calculated for each investigated species in DnaSP, alongside nucleotide substitution per site (Dxy), with higher values indicating greater genetic differentiation, while gene flow (Nm) was estimated as mean number of dispersers per generation among the populations.

Results

DNA sequence comparisons

Dendryphantes purcelli

A total of 13 haplotypes were identified for *D. purcelli* (Fig. 5.2a and b). The haplotypes H6 and H7 were shared among the northern populations (sites 1–3) of the park, and H8 and H9 were only found in site 2. Among the southern populations (sites 4–6), haplotypes H2 and H4 only occurred in site 4, and H3, H5 and H11 only in site 6. The remaining haplotypes were shared between the remaining localities, with the exception of H13, which was only found in site 3 and displayed a high number of mutations (50) in comparison to all other haplotypes. Haplotype H1 was the most widespread shared haplotype, occurring in all six localities.

Bayesian and ML phylogeny of the *Dendryphantes* species yielded a tree with strongly supported branches, with the sampled *D. purcelli* represented as a single clade (clade 1) by PP (100%), but not bootstrap values, against the other distinctive species (Fig. 5.2c). Sequences of *D. purcelli* clustered into two subclades (A and C) that were strongly supported by PP and bootstrap values (100% and 96%, respectively). The first subclade (C) was represented by only one individual, while the second (A) gathered the remaining sequences. Within subclade A, a smaller group (B) was robustly (95% bootstrap, 100% PP) separated from the other sequences. Derived clustering from the park populations showed that collected specimens were independently distributed from their geographic origin. Additionally, all specimens were grouped according to defined haplogroups in the MSN (Fig. 5.2c).

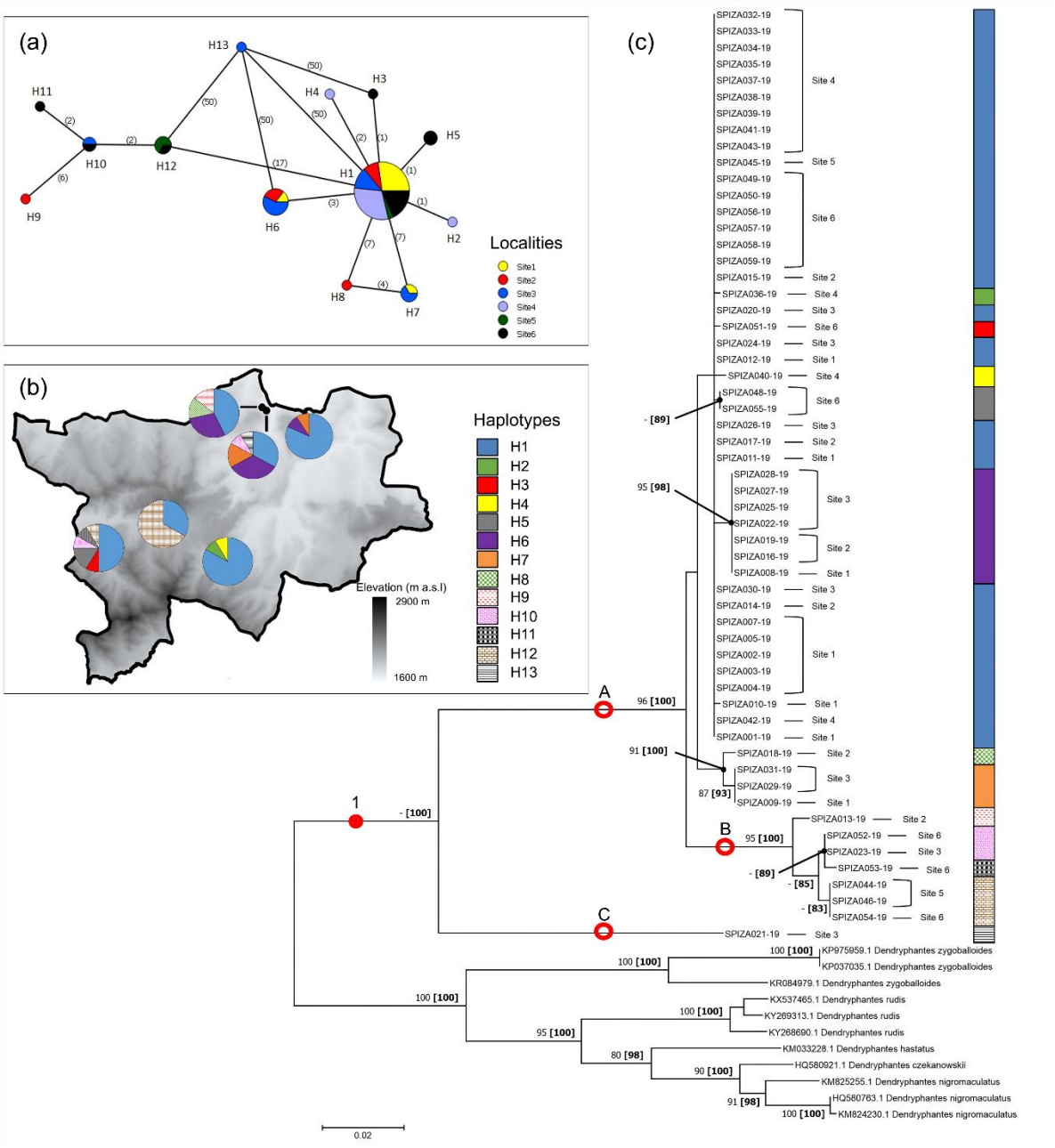

Fig. 5.2 Haplotype networks and phylogram of *Dendryphantes purcelli* populations of the Golden Gate Highlands National Park, based on a portion of mtDNA COI gene: (a) Minimum Spanning Network (MSN) connecting sampled sequences through putative mutational steps according to geographical locality of populations. Circles represent defined haplotypes, while circle diameters are proportional to frequency. Numbers in parentheses correspond to mutational steps. (b) Distribution of defined haplotypes within sampled localities. (c) Phylogenetic tree based on Maximum Likelihood (ML) showing relationship of D. purcelli with five sister species. Red circles and dots represent well supported and putative clades/groups respectively. Localities and haplotypes of individuals corresponding to the MSN are also indicated. Phylogram is rooted at midpoint. Numbers at nodes correspond to bootstrap percentages alongside posterior probability percentages in parentheses. Only values >80% were indicated.

Neoscona subfusca

Haplotypes of *N. subfusca* totalled 18, with two distinct groupings occurring, separated by a relatively high number of mutations (39) (Fig. 5.3a). The first group comprised haplotypes H1–H9, with H1, H2, H4, H5 and H9 occurring across localities, while H3, H6–H7, and H8 were only found in sites 5, 6 and 1, respectively. The second group contained haplotypes H10–17, with H12, H13 and H17 occurring in sites 3, 5 and 2, respectively, while the remainder occurred across the localities except site 6. Haplotype H18 remained separated from all other haplotypes by a high number of mutations (58) and was only found in site 3. Haplotypes from both groups were still widely distributed among the investigated localities (Fig. 5.3b).

The two groups of haplotypes constructed by the MSN for *N. subfusca* were similarly strongly supported by ML and Bayesian phylogeny (87% and 100%, respectively), with all sequences forming a clade (clade 1) containing two subclades (C and D), with the exception of one individual that was seen to group close to the voucher sequences of *N. vigilans* and *N. polyspinipes* (Fig. 5.3c). This individual was therefore considered a different species. Each of the two subclades (C and D) contained smaller groupings of sequences that were more strongly supported in subclade D (PP and bootstrap >80%), while subclade C contained more putative groupings (A and B) supported by PP values (>80%) but not bootstrap. The voucher *N. subfusca* sequence from Pakistan (Ashfaq et al., 2019), while not clustering strongly with any of the sequences retrieved from the park, was still seen to group within the clade representing *N. subfusca sensu stricto*.

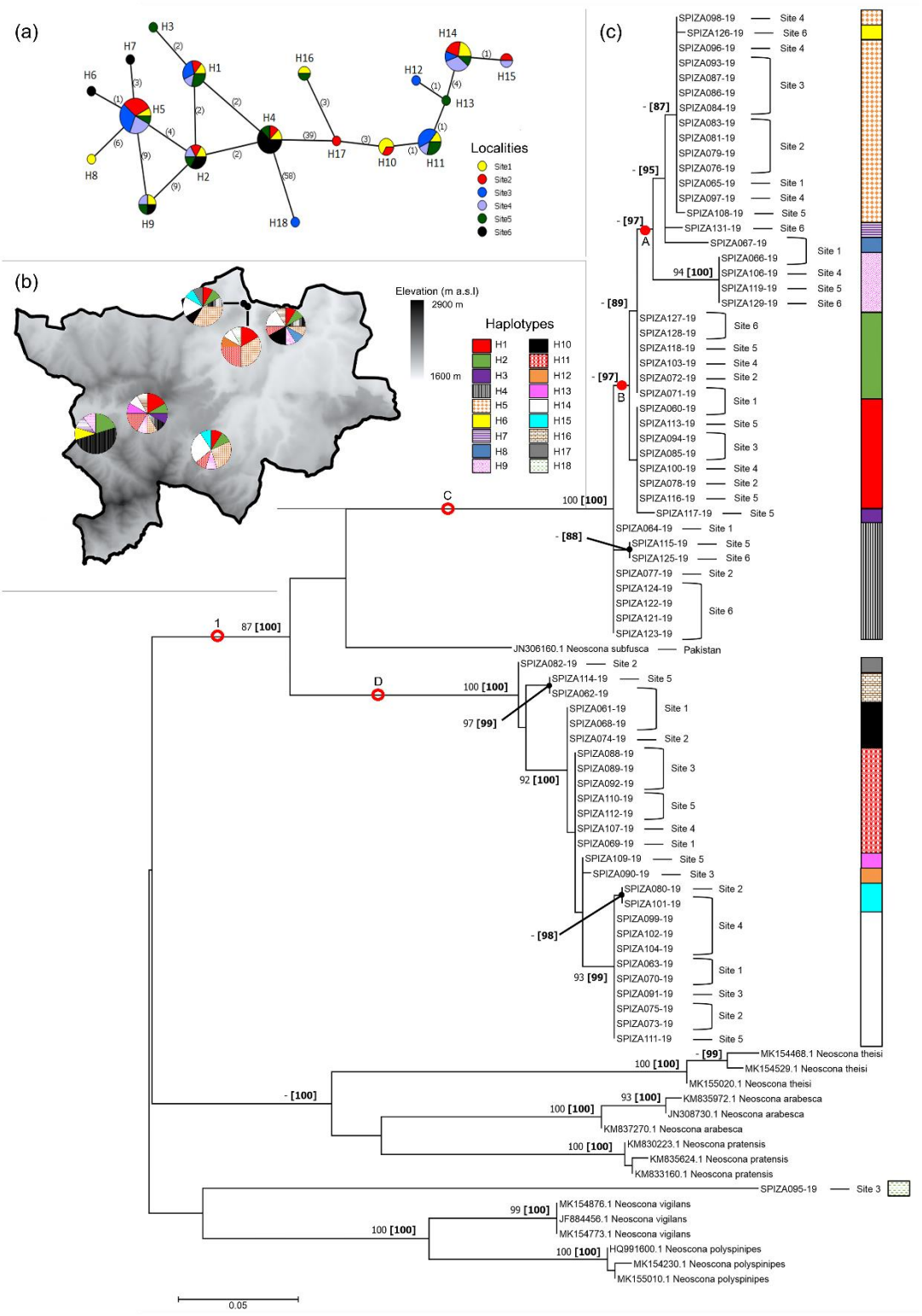

Fig. 5.3 Haplotype networks and phylogram of *Neoscona subfusca* populations of the Golden Gate Highlands National Park, based on a portion of mtDNA COI gene: (a) Minimum Spanning Network (MSN) connecting sampled sequences through putative mutational steps according to geographical locality of populations. Circles represent defined haplotypes, while circle diameters are proportional to frequency. Numbers in parentheses correspond to mutational steps. (b) Distribution of defined haplotypes within sampled localities. (c) Phylogenetic tree based on Maximum Likelihood (ML) showing relationship of N. subfusca with five sister species. Red circles and dots represent well supported and putative clades/groups respectively. Localities and haplotypes of individuals corresponding to the MSN are also indicated. Phylogram is rooted at midpoint. Numbers at nodes correspond to bootstrap percentages alongside posterior probability percentages in parentheses. Only values >80% were indicated.

Pherecydes tuberculatus

Only three haplotypes were identified for *P. tuberculatus*, with only a single mutation occurring between them (Fig. 5.4a). Haplotypes H2 and H3 only occurred in sites 4 and 3, respectively, while H1 was shared among all six populations (Fig. 5.4b).

All obtained sequences of *P. tuberculatus* formed a strongly supported species (100% bootstrap, 100% PP), clearly separate from the *Tmarus* species included in the analyses (Fig. 5.4c). Within the *Pherecydes* clade (clade 1), a single subclade (B), as well as numerous groupings of specimens were seen. All *P. tuberculatus* sequences in the phylogram were found to belong to haplotype H1, with the exception of two heterogeneous groups (A and C), one of which (C) was strongly supported by PP (94%), but not bootstrap values.

Philodromus browningi

The majority of the eight haplotypes of *P. browningi* were shared across most of the localities, with the exception of H4 and H7, which were only found in site 1, and H5 that was only found in site 2 (Fig. 5.5a). Haplotype H1 separated from the other haplogroups, due to a high number of mutations (53), and occurred in sites 1 and 5. The haplotypes H2 and H3 were the most commonly shared-haplotypes, occurring in sites 1–4, while H8 was only found in sites 2 and 3 (Fig.5.5b).

Sampled sequences of *P. browningi* formed a clade (clade 1) that was strongly supported by PP (83%), but not bootstrap values (Fig. 5.5c). These sequences formed two subclades (A and B), with subclade B containing only two individuals, and subclade A including the remainder. Strongly supported groupings (>90% for both bootstrap and PP) of specimens were noted in subclade A according to defined haplogroups. Due to weakly supported branches, certain specimens, initially

considered to be different haplogroups, were indicated as belonging to other haplotypes (Fig. 5.5c).

Theridion sp.

A total of 12 haplotypes were identified for the *Theridion* sp. investigated (Fig. 5.6a). Haplotype H3 was the most commonly shared haplotype, occurring in all populations (Fig. 5.6b). Haplotypes H9–H12 were exclusive to the northern populations of the park, with H10, H11 and H12 only occurring in sites 3, 1 and 2, respectively, while H9 was shared between sites 1 and 3. Populations on the south-western side of the park were seen to share haplotype H5, while H1 was shared between sites 1, 4 and 6. All other haplotypes were only found in site 4.

A strongly supported monophyletic clade (clade 1) of sampled *Theridion* sequences was represented by Bayesian and ML phylogenies (100% for both) (Fig. 5.6c). Within this single clade a number of groups (A–F) occurred, two of which (D and E) were strongly supported by PP and bootstrap values (>80%). The remaining four groups were strongly supported by PP values (>80%) but not bootstrap. Clustering from populations in the park indicated independent distribution of specimens from their geographic origin. Haplogroups defined by the MSN were represented by grouping of sequences in the phylogeny.

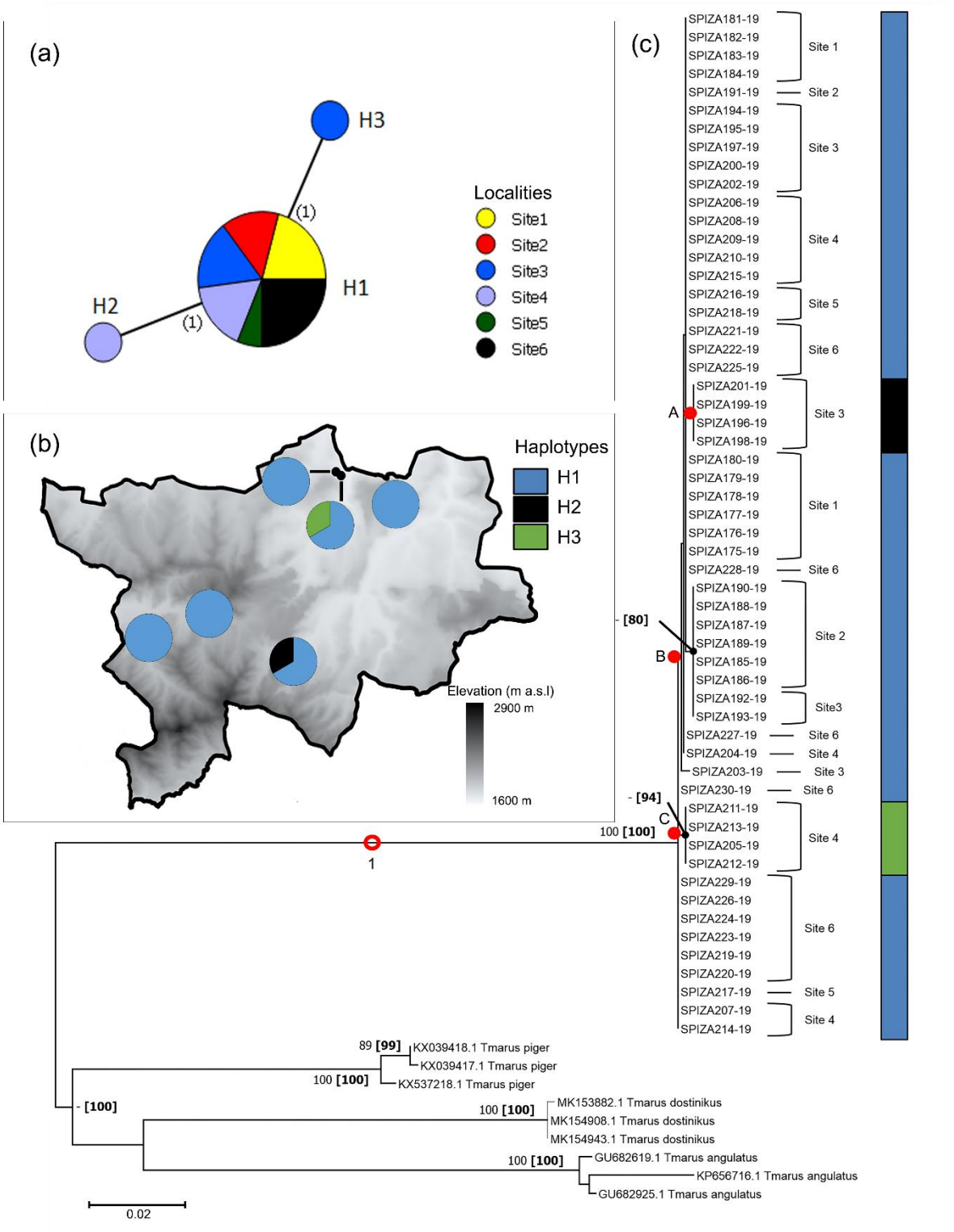

Fig. 5.4 Haplotype networks and phylogram of *Pherecydes tuberculatus* populations of the Golden Gate Highlands National Park, based on a portion of mtDNA COI gene: (a) Minimum Spanning Network (MSN) connecting sampled sequences through putative mutational steps according to geographical locality of populations. Circles represent defined haplotypes, while circle diameters are proportional to frequency. Numbers in parentheses correspond to mutational steps. (b) Distribution of defined haplotypes within sampled localities. (c) Phylogenetic tree based on Maximum Likelihood (ML) showing relationship of P. tuberculatus with three species from a related genus. Red circles and dots represent well supported and putative clades/groups respectively. Localities and haplotypes of individuals corresponding to the MSN are also indicated. Phylogram is rooted at midpoint. Numbers at nodes correspond to bootstrap percentages alongside posterior probability percentages in parentheses. Only values >80% were indicated.

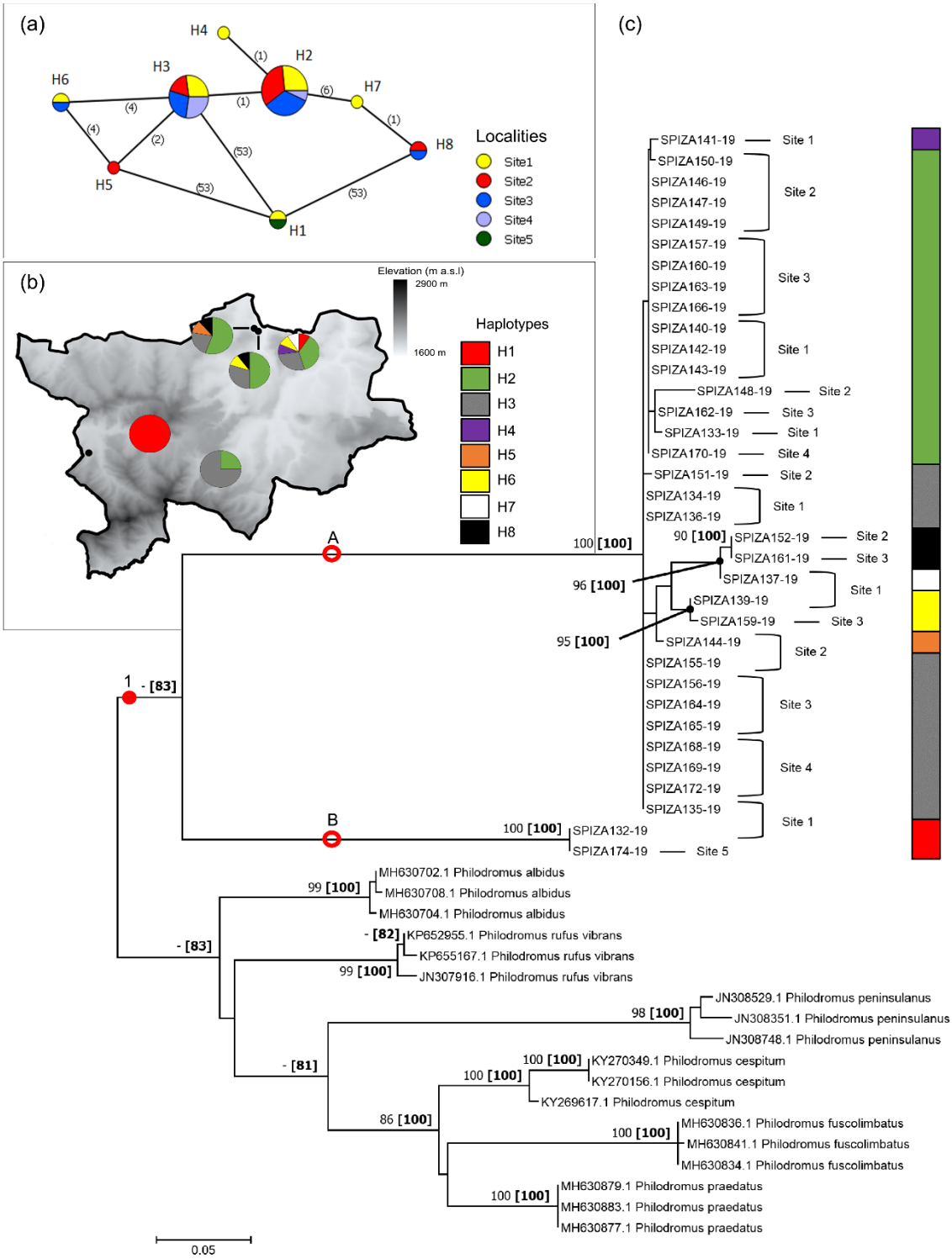

Fig. 5.5 Haplotype networks and phylogram of *Philodromus browningi* populations of the Golden Gate Highlands National Park, based on a portion of mtDNA COI gene: (a) Minimum Spanning Network (MSN) connecting sampled sequences through putative mutational steps according to geographical locality of populations. Circles represent defined haplotypes, while circle diameters are proportional to frequency. Numbers in parentheses correspond to mutational steps. (b) Distribution of defined haplotypes within sampled localities. (c) Phylogenetic tree based on Maximum Likelihood (ML) showing relationship of P. browningi with six sister species. Red circles and dots represent well supported and putative clades/groups respectively. Localities and haplotypes of individuals corresponding to the MSN are also indicated. Phylogram is rooted at midpoint. Numbers at nodes correspond to bootstrap percentages alongside posterior probability percentages in parentheses. Only values >80% were indicated.

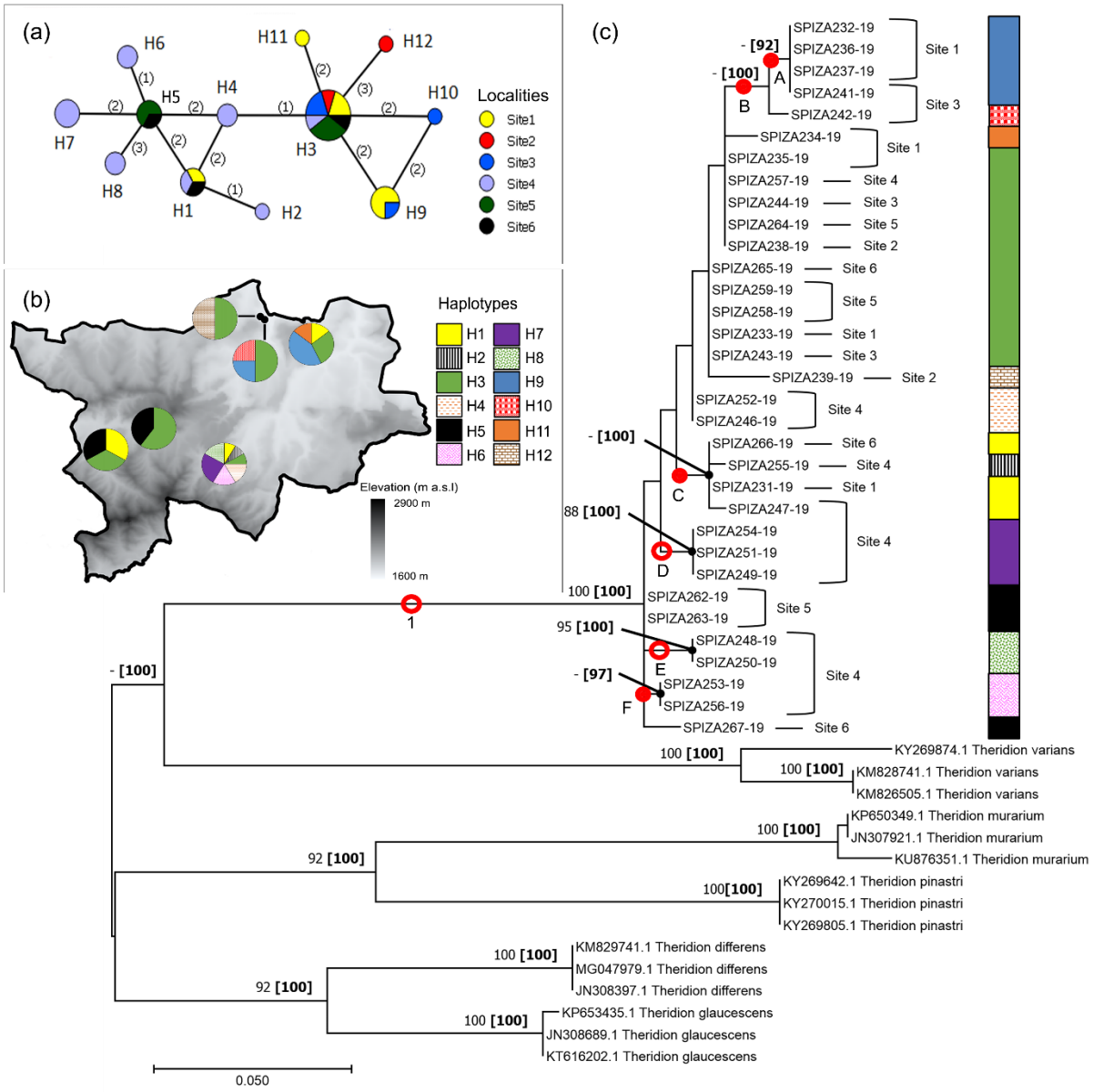

Fig. 5.6 Haplotype networks and phylogram of the *Theridion* sp. populations of the Golden Gate Highlands National Park, based on a portion of mtDNA COI gene: (a) Minimum Spanning Network (MSN) connecting sampled sequences through putative mutational steps according to geographical locality of populations. Circles represent defined haplotypes, while circle diameters are proportional to frequency. Numbers in parentheses correspond to mutational steps. (b) Distribution of defined haplotypes within sampled localities. (c) Phylogenetic tree based on Maximum Likelihood (ML) showing relationship of the Theridion sp. with five sister species. Red circles and dots represent well supported and putative clades/groups respectively. Localities and haplotypes of individuals corresponding to the MSN are also indicated. Phylogram is rooted at midpoint. Numbers at nodes correspond to bootstrap percentages alongside posterior probability percentages in parentheses. Only values >80% were indicated.

Population genetic diversity and gene flow

As a single individual of the sampled *N. subfusca* specimens (SPIZA095-19) was considered to be a different species based on the obtained phylogeny (Fig. 5.3c), this individual was removed from the dataset before genetic diversity and gene flow were calculated. Nevertheless, *N. subfusca* was observed to possess the most haplotypes (17 as opposed to the original 18 defined in the MSN and phylogeny), supporting a high haplotype (Hd = 0.9078) and nucleotide diversity (π = 0.0413) (Table 5.1). While the phylogenies of *D. purcelli* and *P. browningi* were also noted to contain individuals (*D. purcelli* - SPIZA021-19; *P. browningi* - SPIZA132-19 and SPIZA174-19) that may be interpreted as different species, these individuals were still represented in the major clade of their respective species (*D. purcelli* – Fig. 5.2c; *P. browningi* – Fig. 5.5c). In this regard, these individuals were retained during genetic diversity and gene flow calculations. As such, *D. purcelli* was seen to have the second highest number of haplotypes (h = 13), but did not possess the second highest haplotype diversity (Hd = 0.6510), which instead occurred in the *Theridion* sp. (Hd = 0.8807). Lowest haplotype diversity was seen in *P. tuberculatus* (Hd = 0.2597), with only three haplotypes being found across the six populations.

Table 5.1 Total diversity indices of the five investigated spider species from populations in the Golden Gate Highlands National Park, calculated from nucleotide sequence of mitochondrial COI gene.

Species	*Dendryphantes purcelli*	*Neoscona subfusca*	*Pherecydes tuberculatus*	*Philodromus browningi*	*Theridion* sp.
N	57	68	56	34	33
S	71	64	2	59	18
h	13	17	3	8	12
Hd	0.6510	0.9078	0.2597	0.7112	0.8807
Kt	7.4317	23.9723	0.2701	5.2513	3.1288
πT	0.0135	0.0413	0.0005	0.0136	0.0064
Fst	0.1503	0.0424	0.2727	0	0.1354
Nm	1.41	5.65	0.67	0	1.60

N: Number of sequences; S: Number of segregating (polymorphic/variable) sites; h: Number of haplotypes; Hd: Total haplotype diversity; Kt: Average number of nucleotide differences; πT: Total nucleotide diversity; Fst: Overall genetic differentiation; Nm: Gene flow.

Gene flow estimation was high among the populations of *D. purcelli* (Nm = 1.41), *N. subfusca* (Nm = 5.65) and the *Theridion* sp. (Nm = 1.60), while *P. tuberculatus* and *P. browningi* showed low levels of connectivity (Nm < 1.0) (Table 5.1). Genetic differentiation (Fst) mirrored these results, as those species with a high gene flow estimation were seen to possess a moderate to low genetic divergence (Fst < 0.16), while those with a lower rate of gene flow saw a higher divergence (Fst > 0.25) occurring among the populations. Unexpectedly, the gene flow of *P. browningi* was estimated to be zero, based on the current samples, with no genetic differentiation across the populations (Fst = 0). However, due to the lack of sufficient specimens from two of the sampled localities (sites 5 and 6) this result remains debatable.

The populations of *D. purcelli* indicated the highest haplotype diversity to occur in site 2 (Hd = 0.8095), while the highest nucleotide diversity and differences occurred in site 3 (π = 0.0260, K = 14.3333) (Supp. Table S5.1). Additionally, the population of site 6 contained the highest number of haplotypes (h = 6), while site 5 contained the lowest (h = 2). In terms of genetic differentiation, site 5 was seen to possess large divergence values (Fst > 0.15) compared to all other sites, with the exception of site 6 (Fst = 0.0767) (Supp. Table S5.2). It was also noted that a moderate genetic divergence (Fst = 0.1181) occurred between the two farthest populations of sites 1 and 6. Overall, genetic variability showed less genetic divergence (Fst < 0.1) occurring among the northern (sites 1–3) and eastern (site 4) populations of the park. Average number of nucleotide substitutions per site between all the populations (Dxy) varied from 0.0095 (sites 4 and 6) to 0.0285 (sites 3 and 5) (Supp. Table S5.2).

Haplotype diversity was relatively high across all populations of *N. subfusca* (Hd > 0.75), with the highest diversity shared between sites 1 and 5 (Hd = 0.9697) (Supp. Table S5.3). These two populations concurrently contained the highest number

of haplotypes (h = 10). Additionally, the highest nucleotide diversity and differences was seen in site 1 (π = 0.0473, K = 27.4546). Genetic differentiation showed no genetic divergence (Fst = 0) occurring between the populations, with the exception of site 6, in which very large divergences (Fst > 0.25) were noted against all other populations (Supp. Table S5.4). Dxy values between the populations were also noted to be relatively similar (0.0354–0.0437).

Due to only a single haplotype occurring across all populations of *P. tuberculatus*, with the exception of sites 3 and 4, haplotype and nucleotide diversity was indicated as zero (Supp. Table S5.5). Sites 3 and 4, on the other hand, each possessed an additional unique haplotype per locality, increasing diversity of these two populations (Hd = 0.4849, π = 0.0010). As only a single haplotype occurred in the majority of populations, genetic divergence was non-existent between sites 1, 2, 5 and 6, as both Fst and Dxy values were zero (Supp. Table S5.6), indicating no genetic variability between these populations. However, the same very large Fst value (0.2727) was noted for pairwise comparisons of sites 3 and 4 against all other populations, due to these two sites possessing their own unique additional haplotype. Furthermore, average number of nucleotide substitutions per site between sites 3 and 4 and the remaining populations were identical (Dxy = 0.0007), while the pairwise comparison between sites 3 and 4 portrayed a higher value (Dxy = 0.0013) (Supp. Table S5.6).

Despite a lack of sufficient specimens in sites 5 and 6, the remaining populations of *P. browningi* indicated site 1 as possessing the highest haplotype and nucleotide diversity (Hd = 0.8364, π = 0.0310), while site 4 possessed the lowest (Hd = 0.5000, π = 0.0013) (Supp. Table S5.7). Estimates of genetic differentiation showed no genetic divergence (Fst = 0) occurring between the northern populations (sites 1–

3), while moderate divergence (Fst average = 0.0458) was experienced between the eastern population (site 4) and those in the north (Supp. Table S5.8). Dxy values were also noted to differ between the populations, ranging from a minimum of 0.0060 between sites 2 and 3 to a maximum of 0.0183 between sites 1 and 3. However, further sampling is necessary from populations of sites 5 and 6 to properly gauge genetic differentiation and gene flow across the park.

While the *Theridion* sp. saw a maximum haplotype diversity occurring in sites 2 and 6 (Hd = 1) (Supp. Table S5.9), the fact that the number of individuals sampled was low (N < 4), and the number of haplotypes identified in these localities was equivalent to their sample sizes (site 2, h = 2; site 6, h = 3) may not represent true haplotype diversity in these populations. As such, the haplotype diversity indicated in site 4 (Hd = 0.9091) is instead regarded as the population with the highest diversity, due to a larger sample size (N = 12), with site 5 possessing the lowest diversity (Hd = 0.6000). Nucleotide diversity was also noted to follow the same trend (site 4, π = 0.0074), however, lowest nucleotide diversity was instead seen in site 3 (π = 0.0034) (Supp. Table S5.9). Genetic differentiation was observed to be relatively low (Fst < 0.1) among the majority of the localities, with sites 1 with 3, and 6 with 4 and 5, indicating a lack of barriers to gene flow (Fst = 0) (Supp. Table S5.10). Site 4 was also noted to possess a very large genetic divergence (Fst > 0.25) with the northern populations (sites 1–3). Average number of nucleotide substitutions per site were noted to follow the trends seen in the Fst values, with the highest number of substitutions occurring between sites 2 and 4 (Dxy = 0.0106).

Discussion

This was the first study conducted in a South African National Park that attempted to determine the state of gene flow of five common spider species, and their inferred dispersal capability, between selected shrubland patch populations. The results indicate that the majority of the investigated species were able to maintain gene flow between the selected populations despite the mountainous terrain. This occurrence was unexpected but not unwarranted, as previous cases of gene flow in spiders have noted smaller mountain ranges to not be a major geographic barrier to certain species (Masta, 2000; Lee et al., 2004). Consequently, genetic heterogeneity was relatively high among the selected populations of the investigated taxa, as quantified by haplotype diversity, with the exception of *P. tuberculatus*. It was also observed that the two web-building species, *N. subfusca* and *Theridion* sp., possessed the highest number of dispersers per generation, along with the lowest overall genetic divergence, of the five investigated taxa.

Subsequent analyses based on the COI gene indicated that the majority of the investigated spider species harbour high haplotype diversity among the sampled shrubland patch localities of the GGHNP, alongside moderate to low nucleotide diversity. Similar occurrences were reported in analyses of spider species from other countries (Peres et al., 2015; Dimassi et al., 2016; Tanikawa et al., 2017), albeit on a larger geographical scale. Unfortunately, few reports on the genetic diversity of South African araneid populations are available for comparison with our study (e.g. Franzini et al., 2013). While genetic diversity was relatively high among our investigated taxa, genetic differentiation was moderate to low, implying the possibility of such diversity being maintained by gene flow (Dimassi et al., 2016). Additionally, the current reported genetic variability experienced by the populations of the five investigated taxa may be

the result of unequal gene flow transfer between the selected localities (Fraser et al., 2004; Settepani et al., 2014).

A lower rate of gene flow was observed among the *P. tuberculatus* and *P. browningi* populations, where less than one individual per generation was estimated. Such decreased connectivity was exemplified by their genetic diversity, with the exception of *P. browningi* that indicated a relatively high diversity, despite a total of zero dispersers being estimated. Decreased gene flow has been reported for members of Thomisidae and Philodromidae (Morse, 1992; Gillespie et al., 1998; Garb and Gillespie, 2009), where substantial inbreeding within locales was observed and postulated. These occurrences have even been reported at relatively small scales (~100 m x 100 m) (Evans and Goodisman, 2002). Such poor dispersal abilities may lead to a build-up of genetic structure within populations, decreasing genetic heterogeneity over a period of generations, particularly among social spiders (Agnarsson et al., 2013). Certain species of *Philodromus* and Thomisidae have been reported to display forms of sociality (Kaston, 1965; Evans, 1998; Ruch et al., 2015), with the occurrence of winter quiescence forming so-called “pseudo-flocks” (Lowrie, 1942). However, before it can be speculated if the lower rate of gene flow and subsequent genetic diversity experienced by the populations of *P. tuberculatus* and *P. browningi* may be brought about by inbreeding, the occurrence of such social lifestyles must be investigated, as sociality is considered rare among spiders (Ruch et al., 2015). Additionally, the sample sizes of these two species obtained during this study must also be considered (Supp. Tables S5.5 and S5.7), as they may not have provided proper representation of genetic diversity and gene flow within the GGHNP, nor any evidence of sociality in these species.

Overall, higher genetic differentiation was observed to occur more commonly between the northern and western locales of the park for the majority of the investigated taxa. This was especially evident for *D. purcelli*, *N. subfusca* and *P. browningi*. Locales in the western section were more elevated than those in the north, and differences in the dominant vegetation between these localities were observed (Supp. Table S5.11). Habitat structure and isolation are well known to influence genetic exchange between populations, depending on species' behaviour and dispersal capabilities (Brandt and Lubin, 1998; Bailly et al., 2004; Bonte et al., 2004; Shochat et al., 2004). In certain instances, an agricultural area of 100 m can present a complete barrier to gene flow for certain spider species, due to removal of natural vegetation (Mader, 1984). Some spider species are also known to prefer certain types of vegetation over others (Rushton et al., 1987; Scheidler, 1990; Dippenaar-Schoeman at al., 2006; Foord et al., 2011b; Neethling and Haddad, 2013), which may impact their ability to colonise new habitats. Such preference is often documented in orb-weaver species, such as *N. subfusca*, in which vegetational complexity often determines site selection (Hodge and Storfer-Isser, 1997; McNett and Rypstra, 2000; Bilde et al., 2002). Additionally, when examining the topography of the park a distinct elevation range occurs across the north-western section of the park, extending further west and south, with a number of gulleys and depressions present. However, it is unclear at present whether this elevated area may represent a large orographic barrier within the confines of the park and whether it could be a major obstacle to aerial and terrestrial dispersal of spider species (Dimassi et al., 2016). Further studies investigating gene flow in localities across this elevated section must therefore be considered.

Obtained results indicated that the two web-building species, *N. subfusca* and *Theridion* sp., possessed the highest rates of gene flow among the investigated taxa. Wind-mediated dispersal of spiders via ballooning is frequently observed globally (Duffey, 1998; Blandenier, 2009). While ballooning is widely considered to be the primary mechanism of habitat colonisation by spiders (Dean and Sterling, 1985; Weyman, 1993; Bell et al., 2005), its role in maintaining genetic heterogeneity between populations has been considered to be more prevalent in web-building spider populations (Tolbert, 1977; Lee et al., 2015; Cho et al., 2018). Orb-web spider species may maintain genetic exchange over a distance of 23.6 km across a mosaic landscape (Ramirez and Fandino, 1996; Ramirez and Haakonsen, 1999). However, it was also speculated by Ramirez and Haakonsen (1999) that populations of araneids situated in a matrix of primarily unfavourable habitat may have little difficulty in maintaining genetic connectedness. These authors also noted that ballooning may not confer unlimited access among all populations in an area, suggesting this type of aerial dispersal may be less effective over long distances.

Opinion as to whether ballooning is an obligatory life history phase has also remained polarised (Tolbert, 1977; Weyman, 1993; Follner and Klarenberg, 1995), with certain reports indicating it as non-essential for certain species (Walter et al., 2005). This brings into question the use of ballooning in the five spider species we investigated. Therefore, while the high gene flow and increased connectivity between the majority of populations of *N. subfusca* and the *Theridion* sp. may be explained, at least in part, by the possibility of ballooning in these species, proper investigation as to the degree and commonality of this mechanism in South African spider species is still needed.

From the results obtained it can be considered that at least some dispersal and gene flow was occurring between all the populations sampled during this study, with certain populations experiencing fewer to no dispersal barriers, depending on distance and location. Such occurrences may allow for the amelioration of deleterious effects of inbreeding for the majority of the investigated populations (Frankham et al., 2002). As theorised by Wade and McCauley (1988), a good dispersal model is implied by high Nm values within populations that are associated with high levels of genetic diversity, given that random samples are recruited from previous living populations, or vice versa, across a geographic region. It could therefore be considered from our results that, out of the five investigated species, *N. subfusca* possesses a good dispersal model within the confines of the park, due to the high genetic diversity and dispersal rate seen among the selected populations. However, further studies are needed to determine the demographic history and full population expansion of all five investigated species in the GGHNP.

Another point of consideration regarding gene flow results of these spider species is the implication of uniparental inheritance and sex-biased dispersal. Due to only *mt*DNA being utilised in this study, there is the possibility that some of the haplotypes detected may have been from male lineages no longer existing, inflating the contemporary diversity seen in this study. Mated adult female social spiders are often responsible for gene flow, with little or no male gene flow among populations, resulting in highly inbred populations and large genetic differentiation between localities at both maternal and biparental inherited loci (Johannesen et al., 2009; Agnarsson et al., 2010). However, according to Greenstone et al. (1987) the dominant mode of dispersal in many spider species is pre-mating dispersal of juveniles, which results in an out-breeding mating system. Additionally, sub-social spider species have

been seen to exhibit male and female gene flow after the group phase, although a general reduction in gene flow for both sexes leads to sub-structured populations where the females are genetically related and this population is governed by female group-founding (Johannesen and Lubin, 1999; Ruch et al., 2009; Duncan et al., 2010; Johannesen et al., 2012). As such, the importance of determining sociality of the investigated spider species is emphasised to properly assess gene flow. Furthermore, the inclusion of additional microsatellites that are not subject to the evolutionary constraints of *mt*DNA would provide a clearer picture of the state of gene flow of these spider species in the GGHNP and beyond.

Incidentally, certain phylogenies indicated the possibility of more than one species among the taxa investigated here. Particularly, populations of *D. purcelli*, *N. subfusca* and *P. browningi* were highly heterogeneous in the GGHNP. Although *D. purcelli* and *P. browningi* comprised subclades that, while containing only a few individuals, were strongly supported, the sampled individuals of *N. subfusca* were unequally divided into two distinct subclades. As individuals of these three species were distributed in the same geographical area (the GGHNP), these clades may be considered as sympatric (Tanikawa et al., 2017). Additionally, due to the strength of the bootstrap and PP values of the two subclades of *N. subfusca* it can be reliably concluded that they are different species. However, while all sampled individuals were identified according to current morphological characteristics and descriptions, the reliability of identifications may have been detracted due to some of the sampled specimens being juveniles, as juveniles often cannot be reliably identified to species level for certain species, contributing to a possible source of error. Nevertheless, the observation of such heterogeneity over a relatively small geographical area not only brings into question the extent of species complexes, and the subsequent implications

for conservation, but also highlights issues regarding the reliability and extent of current taxonomic descriptions and identification of these species in South Africa.

In conclusion, molecular analyses were performed to identify the current state of gene flow and inferred dispersal capability of five ubiquitous spider species from six shrubland patch localities of the GGHNP. We found that the majority of the investigated species were able to maintain a high rate of gene flow despite the mountainous landscape. We also observed that all species, except *P. tuberculatus*, possessed relatively high genetic diversity, together with moderate to low genetic differentiation, which may be the result of overall maintained gene flow across the sampled sites. The inference of ballooning and its role in gene flow of the five investigated species is still under debate, due to a lack of information from South African studies. However, the two-web building species (*N. subfusca* and *Theridion* sp.) possessed higher rates of gene flow and less genetic differentiation compared to the other species. Unexpectedly, instances of high heterogeneity in phylogenies of *D. purcelli*, *N. subfusca* and *P. browningi* bring into question the extent of species complexes present in these taxa, considering current taxonomic descriptions and identification. Due to such genetic diversity and variability observed over a relatively small geographical range, further analyses using additional molecular markers and extended sampling are required to: (i) fully gauge the extent of genetic heterogeneity, gene flow and dispersal ability of spider species within the GGHNP and beyond in the Drakensberg Mountains, and (ii) identify factors that may affect and promote diversity in these selected spider species.

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Chapter 6

General discussion

General discussion

While the National Parks of South Africa have been established to ensure the conservation of large expanses of natural landscapes, many of these protected areas remain sorely understudied in terms of their habitat diversity and the viability of these habitats as priority areas for management. The results from this thesis provide a preliminary overview of the effect of isolated shrubland patches within the Golden Gate Highlands National Park (GGHNP), and how they may have the potential to attenuate environmental changes and disturbances on various arthropod assemblages (Chapters 3 and 4), while also providing evidence for their viability as priority areas for future research and management interventions (Chapters 2, 4 and 5).

Current management plans of the GGHNP

Conservation management of the GGHNP currently highlights several policies and frameworks for the 2013–2023 period (SANParks, 2013). Paramount to this is the designation and establishment of coherent spatial frameworks in and around the park to guide and co-ordinate conservation, tourism, and visitor experience initiatives. Zone planning of the GGHNP is currently based on analysis and sensitivity mapping of the biophysical, heritage, and scenic resources available (SANParks, 2013). Such zoning has highlighted several areas of special conservation importance, including insular *Olinea-Podocarpus* forests, Plateau grasslands, and Wetland/drainage line vegetation. Additionally, priority natural areas, required for the long term persistence of biodiversity in and around the park are considered, with certain areas in this zone also identified for future park expansion.

Ultimately, strategic plans for the GGHNP fall under five main categories, namely Bioregional, Biodiversity, Tourism, People and conservation, and Effective

park management (SANParks, 2013). Bioregional focuses on the position of the GGHNP to continue its role as part of the Maloti Drakensberg Transfrontier protected area process. Biodiversity effectively concentrates on water resource protection and sustainable and natural resource use, alongside maintaining ecological integrity through the management of invasive species, fire and wildlife management, and identifying species of special concern. Tourism strategies attempt to find a balance between providing activities and products for visitor enjoyment, while minimising impacts on conservation and biodiversity assets of the park. Managing and maintaining of cultural heritage remains the focus of People and conservation strategies, alongside contributing to the local economy, livelihoods, and education in an effort to contribute to local socio-economic development. Lastly, effective management and administrative support services become the focal point of Effective park management, with concentration on risk and financial management, alongside capital and infrastructure development and maintenance.

At present, biodiversity strategies implemented in the park apply ecosystem-based management with an assumption that this will also maintain arthropod habitats. As such, these conservation strategies do not take arthropods into explicit consideration, however, this does not imply they are not considered implicity. A few investigations have been undertaken in the park in an attempt to properly determine and provide an inventory of arthropod assemblages, while also attempting to determine species of special consideration (e.g. Meyer, 1970; Louw, 1988; Hugo-Coetzee, 2014). However, the incorporation and usage of arthropods in informing conservation strategies must still be considered.

Implications of significant findings and future research considerations

Inventory and monitoring are two essential, interrelated activities for conservation planning which differ in their objectives (Kremen et al., 1993). The use of inventory programs allows for the documentation of the spatial distribution of species populations, communities, guilds and ecosystems. This information, in turn, allows for the selection of reserves (McKenzie et al., 1989; Gjerde et al., 2018; van Schalkwyk et al., 2019), and assists in strengthening the case of habitat conservation by documenting the distribution of threatened or endangered species (e.g. Reinthal and Stiassny, 1991; Gjerde et al., 2018; Choe et al., 2019). Additionally, such inventory provides a basis for the selection of indicator species or those assemblages relevant for ecological monitoring (Kremen, 1992; Maslo, 2016; Costanza et al., 2018). Concurrently, the goal of monitoring programs is to assess changes that occur in ecosystem structure, composition, and function in response to natural factors, disturbance from anthropogenic influences, or management strategies over time (Kremen et al., 1993). However, during such monitoring it is necessary to separate variation in baseline conditions, due to natural disturbances and fluctuations, to those brought about by anthropogenic disturbances. As such, the monitoring of less disturbed or “pristine” haibtats, as well as those subject to disturbance factors, becomes necessary with the use of viable indicator species to monitor current management activites and suggest better management practices (Murphy and Noon, 1991; Underwood and Fisher, 2006; Medeiros et al., 2013).

In Chapter 2 it was found that vertical stratification influenced arthropod assemblage richness and composition. While many studies investigating vertical stratification are conducted in tropical forests, in which tree height exceeds 15 m (e.g. Lowman and Rinker, 2004; Basset et al., 2012), the shrubland patches sampled during

my study typically did not contain vegetation taller than 6 m. In this regard, the concept of small-scale vertical stratification between canopy and ground levels may be of consideration. A natural assumption of small-scale stratification would be that species populations would overlap greatly between the lower canopy and leaf litter strata due to the smaller distance, compared to larger forests. Species with higher mobility between these strata (e.g. ant species) may be shared between strata of a single site, or even show a general presence in all strata based on the species feeding behaviour (Basset et al., 2003). The findings support this theory (Chapter 2), with only a fraction of species being shared between strata. Therefore, the influence of habitat structure should be of consideration in this regard due to the major role it plays in diversity and abundance of arthropods (Stuntz et al., 2002). However, a high number of unique species were found specifically in the site canopies, in comparison to leaf litter and soil. Canopies in temperate systems are noted for their high arthropod diversity (Schroeder et al., 2009; Ulyshen, 2011), highlighting their importance for ecosystem stability (Naeem, 2002). This hints strongly at the canopies sampled during my study playing a major role in supporting populations that would otherwise be absent from these localities in the absence of trees and shrubs. Overall, the significant differences observed between strata of all sites suggests that species of each stratum should further be investigated in regards to their response and function, as there is a possibility that species groups in these strata play an integral role in ecosystem functions unique to their environmental niches.

The findings of Chapter 2 also showed a significant inverse interaction between canopy richness and diversity with incremental elevation changes in the GGHNP. However, the elevational differences experienced between the investigated sites may not be regarded as large-scale elevational range across vertices (i.e. more than 200m

altitudinal difference between sites). It is generally regarded that elevation would only have a significant effect on populations at large-scale ranges (Hodkinson, 2005), although, small-scale gradients have still been noted to produce monotonic declines (Ashton, 2013). Nevertheless, due to the variability of the localities selected in this study, it is still debatable as to whether elevational change was the only factor influencing arthropod species richness and diversity. The park itself exhibits unique environmental features, so it is highly likely that certain differences noted in this study may be related to other environmental features such as slope aspect (Malan et al., 1998; Foord et al., 2015).

The results obtained in Chapter 3 demonstrated the intricate changes brought about by seasonal variation. The short generation times, high species richness and abundance of arthropod communities make them well suited in addressing temporal dynamics and phenology in a number of environments (Smith and Smith, 2012; Valtonen et al., 2013). It can be suggested that changes on an annual basis affect arthropod assemblages to differing degrees in the GGHNP, with soil assemblage richness seemingly affected the least, and canopy and leaf litter following indistinct trends in response to seasonal change. While shrubland canopies are often considered a more variable environment due to exposure to abiotic factors compared to the lower strata (Maguire et al., 2014), canopy richness was seen to be largely unaffected by ambient temperature in this study (Chapter 3). It could be suggested that the structural composition of the sampled shrubland patches proved sufficient in buffering assemblages against certain environmental fluctuations, despite their relatively small size. When broken down into each year, significance tests for leaf litter assemblages emphasise significant environmental variable interactions, specifically during the first year of study with increased wildfires. As discussed in Chapter 3, it

could be argued that this may have been brought about by the removal of leaf litter cover by the frequent sweeping fires into the patches, exposing the remainder to the effects of environmental factors. Fires will also tend to remove habitats created by fallen dead trees present in the leaf litter stratum of these localities. Additionally, the current inhabitants utilise many of the dead trees and shrubs for firewood, causing disturbance and removal of vegetative matter. Leaf litter in these sites can be characterised as vastly different to that of larger forests, with litter areas being much smaller and coverage only limited to areas closer to isolated trees and shrubs. Nonetheless, their significance as important niches are somewhat emphasised in my findings, hinting at a highly dynamic group of arthropod populations residing within the study sites.

The importance of monitoring assemblage changes was especially emphasised by the patterns of species turnover experienced by the three investigated vertical strata (Chapter 3), whereby leaf litter was noted to experience a temporal decline of Beta-diversity (β-diversity). As β-diversity is known to increase, decrease or remain unchanged during periods of disturbance (Socolar et al., 2016), studies of β-diversity can aid in quantifying biodiversity loss (Karp et al., 2012), informing the placement of protected areas and management of landscapes for conservation (Gering et al., 2003; Wiersma and Urban, 2005). Of particular consideration is the importance of small dispersed habitats, such as the shrubland patches sampled in this thesis, which are considered to increase β-diversity across certain landscapes (Hunter, 2002). Tscharntke et al. (2002) has emphasised the importance of smaller patches, indicating that, for certain insect species, these smaller patches support more species in comparison to equivalent areas in larger patches. However, due to the sheer number of β-diversity metrics available today (Socolar et al., 2016), their use and suitability for

certain investigative questions remains a topic of debate (Anderson et al., 2011; Tuomisto, 2010). As such, the β-diversity utilised in this thesis may not be the most suitable as previously discussed in Chapter 3. Additionally, due to differences in phenological adaptations between species (Recher et al., 1996), further investigations are necessary to determine responses of different functional groups to seasonal variation. Nevertheless, the results obtained in Chapter 3 still provide a preliminary look at species turnover and seasonal responses of arthropods in the GGHNP, highlighting the sampled shrubland patches as areas of importance for future investigation.

As emphasised by Yekwayo (2016), conserving natural shrublands and grasslands as a unit together becomes important due to varying habitat preferences of arthropod functional groups and taxa. The results of Chapter 4 support this, as significant differences in species richness and assemblage composition of soil biota was observed between the sampled shrubland patches and their surrounding grasslands. Isolated shrubland patches in the GGHNP are generally interspersed across the landscape, with few patches aggregated closely in a single area. Arguably, the isolation of these patches and differences between the shrubland and grassland soils, and their respective leaf litter, have possibly resulted in soil assemblages becoming adapted to the shrubland soil ‘islands’ over many years. Based on the results, it can be deduced that soils within these patches provide a unique niche different to the grassland ecosystems (Ferreira et al., 2018), ultimately allowing for accommodation of a larger number of soil arthropod species in a given area. Additionally, while relatively high species richness of soil biota was noted across all the sampled shrubland patches, the association of different taxa with certain shrubland patches was observed. This greatly emphasises the importance of these shrubland

patches for priority mangement, not only on a canopy level as discussed previously, but in lower strata of patches in the GGHNP.

Monitoring of genetic diversity and the maintaining of population connectivity has remained an important area of consideration in conservation, highlighting areas of importance for future research and conservation strategies (e.g. Dimassi et al., 2016). However, while investigations of genetic diversity of certain faunal taxa have been undertaken in South Africa (e.g. O'Ryan et al., 1998; Whitehouse and Harley, 2001; Lesia et al., 2003; Tolley et al., 2010; Haworth et al., 2018), few have focussed on invertebrates (Wishart and Hughes, 2003; Timm et al., 2006), particularly in understudied localities. The results of Chapter 5 address an area of study that has not been investigated in the GGHNP, with genetic results obtained for five species of spiders sampled from the investigated shrubland patches. The study found a high genetic heterogeneity among populations of the spider taxa, despite the relatively small geographical size of the park. It is natural to assume that larger haplotype differences would occur over larger landscape distances, as opportunities for spiderlings to migrate long distances and establish new, isolated populations are theoretically more frequent during ballooning (Greenstone et al., 1987; Greenstone, 1990). However, the results suggest that even though populations among the patches of the GGHNP showed some isolation, certain species possessed a high rate of gene flow among populations. Although this implies that the mountainous slopes are not necessarily acting as impassable barriers to gene flow, the mechanisms which play a role in limiting gene flow of spider populations in the GGHNP are still unclear. The indication of increased heterogeneity within the phylogenies of the investigated species also highlight the possibility of species complexes being more prominent across the shrubland patches of the park, due to a highly variable landscape and the

epigean lifestyle of these spider species. This emphasises the importance of taxonomic identification and redescription of these taxa to ensure reliability. Overall, the results of this genetic investigation have shown that the GGHNP has potential as an area of future conservation research in relation to gene flow among a highly heterogeneous landscape, increasing its importance in South African conservation.

Of particular consideration throughout this entire thesis was the regularity and influence of grassland wildfires across the GGHNP. While many of the grass species in this area are relatively acclimatised to regular burning (Short et al., 2003; SANParks, 2019), woody vegetation is more susceptible to fire, constraining regeneration of these species and preventing expansion (Trollope et al., 2002; Adie et al., 2017). However, woody vegetation that is able to persist in a grassland matrix can be considered adequate colonisers, tolerant to disturbance and possessing a ruderal life-history while being light-tolerant and relatively fast growing (Tabarelli and Peres, 2002). Due to the common occurrence of wildfires throughout the GGHNP, disturbance among grassland arthropod assemblages can be considered relatively high. Current burning regimes in the GGHNP strive to emulate natural fires as faithfully as possible without endangering humans and infrastructure (SANParks, 2013). However, in this regard, consideration should also be given to ensure burning regimes also take into account arthropod assemblages that persist within the grassland matrix. The benefit of conserving smaller woody shrubland patches in a grassland landscape can allow for these areas to act as alternative habitats for matrix species, enabling these species to recolonise the surrounding environment post-disturbance (Lawes et al., 2005; Ruiz et al., 2008). This emphasises the benefits of monitoring smaller patches in relation to their surrounding environment, and should be of consideration for future conservation research within this National Park.

Recommendations to Park Management

The current GGHNP management plan has highlighted the importance of understanding and monitoring the species and habitats that contribute to the ecological integrity of the ecosystem (SANParks, 2013). The results of this thesis support this importance, particularly in the monitoring of smaller isolated shrubland patches within the GGHNP for the benefit of biodiversity. It is my recommendation that conservation of these smaller, isolated patches be continued, as these areas have the potential to maintain arthropod diversity present in the park, particularly when the impact of wildfires is considered. Yearly monitoring of shrubland patches is recommended for these reasons, (1) to provide a database for the status of shrubland patches across the park (i.e. Are these patches receding/expanding/remaining unchanged?) as bush-encroachment into the surrounding grasslands may affect species dynamics, particularly in areas prone to overgrazing (Turpie et al., 2019). (2) to identify changes in species assemblages, preferably species that potentially show sensitivity to change. And (3), to keep track of the ecological interactions occurring in the park as a whole (i.e. Are species moving into these patches from other localities? Are species disappearing from these areas? Are conditions in these patches changing over long-term?).

The information provided through consistent monitoring of arthropods, coupled with investigating protection methods of these patches against major disturbances such as wildfires, could provide vital information in the conservation of biodiversity in the GGHNP via comparative baseline conditions (Kremen et al., 1993). This could be achieved by providing an inventory of applicable assemblages, while clearly identifying possible indicators of disturbance; and ultimately investigating the full potential of these patches as important conservation areas for arthropods and indeed other

macro-, meso- and microfaunal groups. The significance of this monitoring strategy ultimately lies in the valuable information that it would provide to Park Management for the purpose of biodiversity management in the GGHNP, with monitoring data being integrated alongside an adaptive managment framework that will be triggered should the monitoring variable cross specific thresholds. Additionally, while the GGHNP management plan already distinguishes management zones based on broad elevational zones (SANParks, 2013), investigating the relation of these zones in regards to arthropod diversity and possible indicator species may be beneficial in the revision of areas of high sensitivity in conservation development frameworks.

Chapter 4 also highlighted that, while displaying a relatively high species richness, a lack of taxonomic research pertaining to soil biota is evident in the GGHNP, especially for mites and Collembola. Such a lack of information does, however, provide opportunities for future research concentrating on soil biota. Such an opportunity was exemplified by the identification of *Gamaselliphis potchefstroomensis* (Ryke, 1961) during this thesis, as it has not been recorded in this area before. Furthermore, its subsequent identification has provided the second full description of this species in South Africa. Due to the high habitat heterogeneity present in the park the possibility for further research pertaining to soil biota, and their ecosystem interaction, becomes evident. This is particulary true due to the highly erodible soils present throughout the GGHNP, causing increased disturbance and the formation of new habitats (van der Merwe et al., in press). Current management and conservation strategies involving soil focus on the impact and mitigation of erosion throughout the park, with particular emphasis on catchment protection areas (SANParks, 2013). However, current mitigation strategies and monitoring procedures utilised in erosion control in the park do not consider soil biota as potential indicators of rehabilitation. From the results

obtained in Chapter 4, the development of indicator species lists for a variety of habitats in the GGHNP is attainable. This, in turn, may further assist management in determining sensitivity of designated zones and the assessment of current conservation strategies.

Lastly, the high genetic heterogeneity observed across the shrubland patches of the GGHNP underlines the importance of monitoring genetic diversity in a conservation area. As the GGHNP forms part of the Maloti-Drakensberg Transfrontier Conservation Area (Peace Parks Foundation, 2019), it has become an essential area in maintaining diversity as a whole. Neel and Cummings (2003) suggested that it is necessary to conserve multiple populations to meet existing genetic conservation standards, which would in turn, provide a more successful chance in maintaining the existing among-population structure and processes (e.g. gene flow). Additionally, current wildlife management strategies of the GGHNP highlight the importance of recoding genetic integrity of species (SANParks, 2013). As such, consideration should still remain in monitoring current genetic diversity of various species within the GGHNP, particularly those most threatened by extinction, in an attempt to determine possible genetic bottlenecks and the risk of inbreeding. Adequate species delineation in and around the park may also arise from this monitoring process. Furthermore, the GGHNP remains one of the few National Parks situated along the Drakensberg, providing a prime area for population genetic research in a highly variable landscape, before application on a larger geographical scale.

Concluding remarks

While this thesis was the first of its kind to be conducted in the GGHNP, it has provided a unique insight into the diversity of arthropods across various strata of

shrubland patches and their responses to environmental changes. Valdés et al. (2020) has remarked on the potential of smaller vegetation areas to provide multiple services at higher performance levels per area, compared to their larger counterparts. While this is only one such study, other researchers have remarked on the effectiveness and potential of smaller, isolated patches to conserve and maintain ecosystem functions and diversity (e.g. Jokimäki et al., 1998; Maynou et al., 2017; Prevedello et al., 2017). The results of this thesis support this, as they indicate a need to conserve smaller natural shrubland patches to benefit the surrounding environment, both on a community and genetic diversity scale. As such, significance of these patches should not be dismissed solely due to their size, but rather regarded as vital areas of biodiversity conservation.

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Supplementary Material

Chapter 2

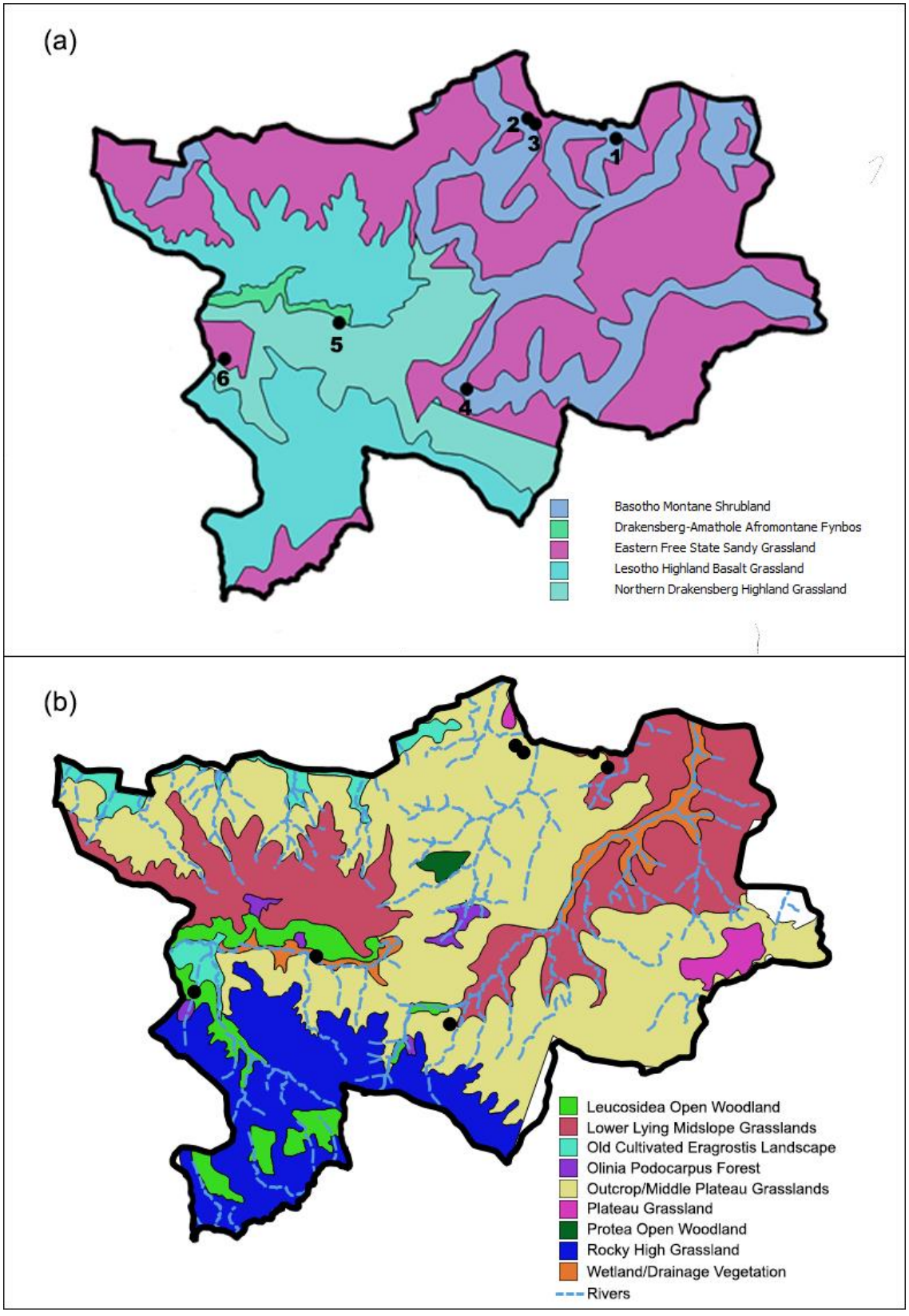

Fig. S2.1 Vegetation maps of the Golden Gate Highlands National Park with (a) vegetation units, and (b) specific plant distribution with site location. Maps developed using QGIS version 2.18.15. Vegetation unit vector geospatial dataset 2012 obtained from South African National Biodiversity Institute (SANBI) (http://bgis.sanbi.org/SpatialDataset/Detail/18). Specific plant distribution and major rivers geospatial map obtained from SANParks Geographic Information Systems and Remote Sensing Division.

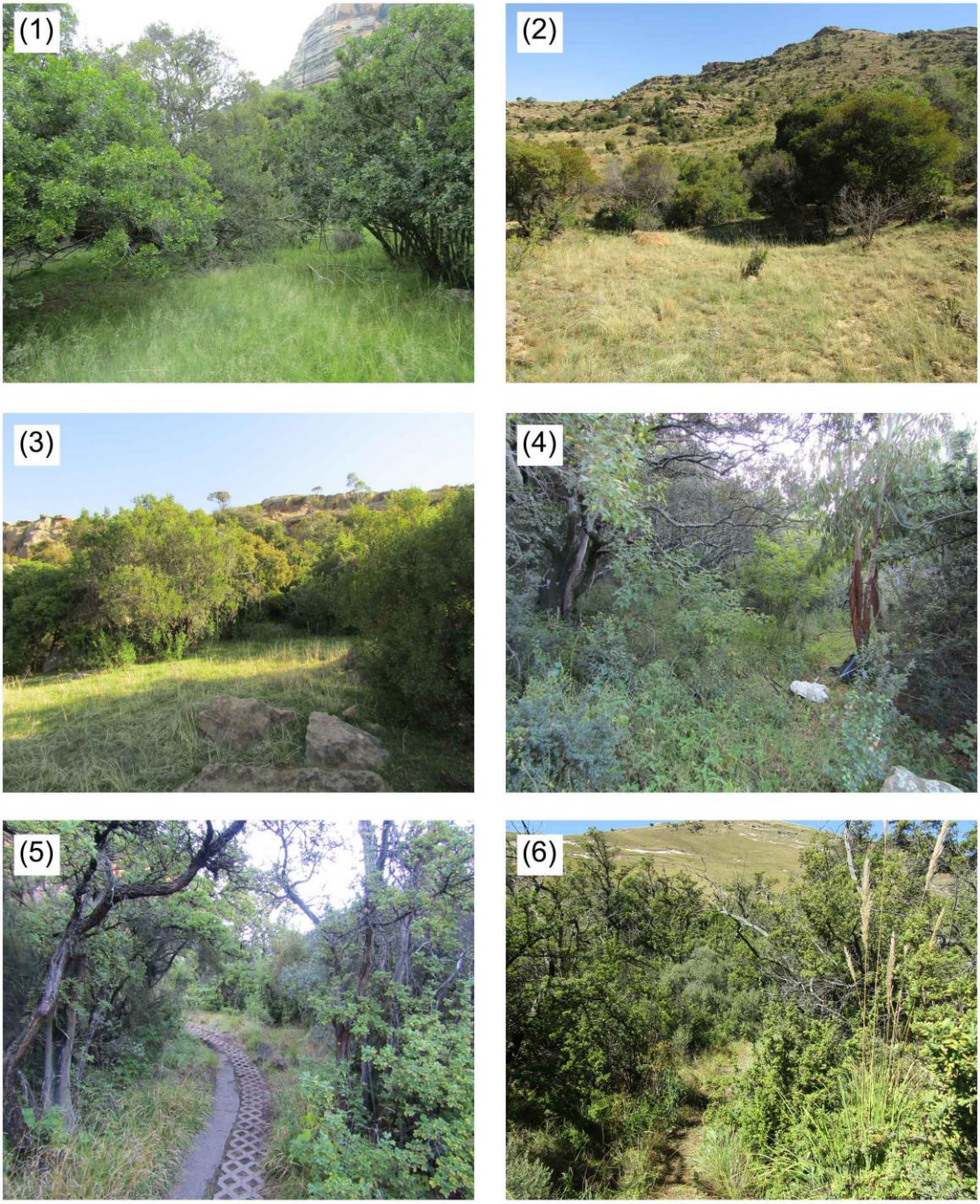

Fig. S2.2 Photographs showing general vegetation composition and spacing of sites 1–6.

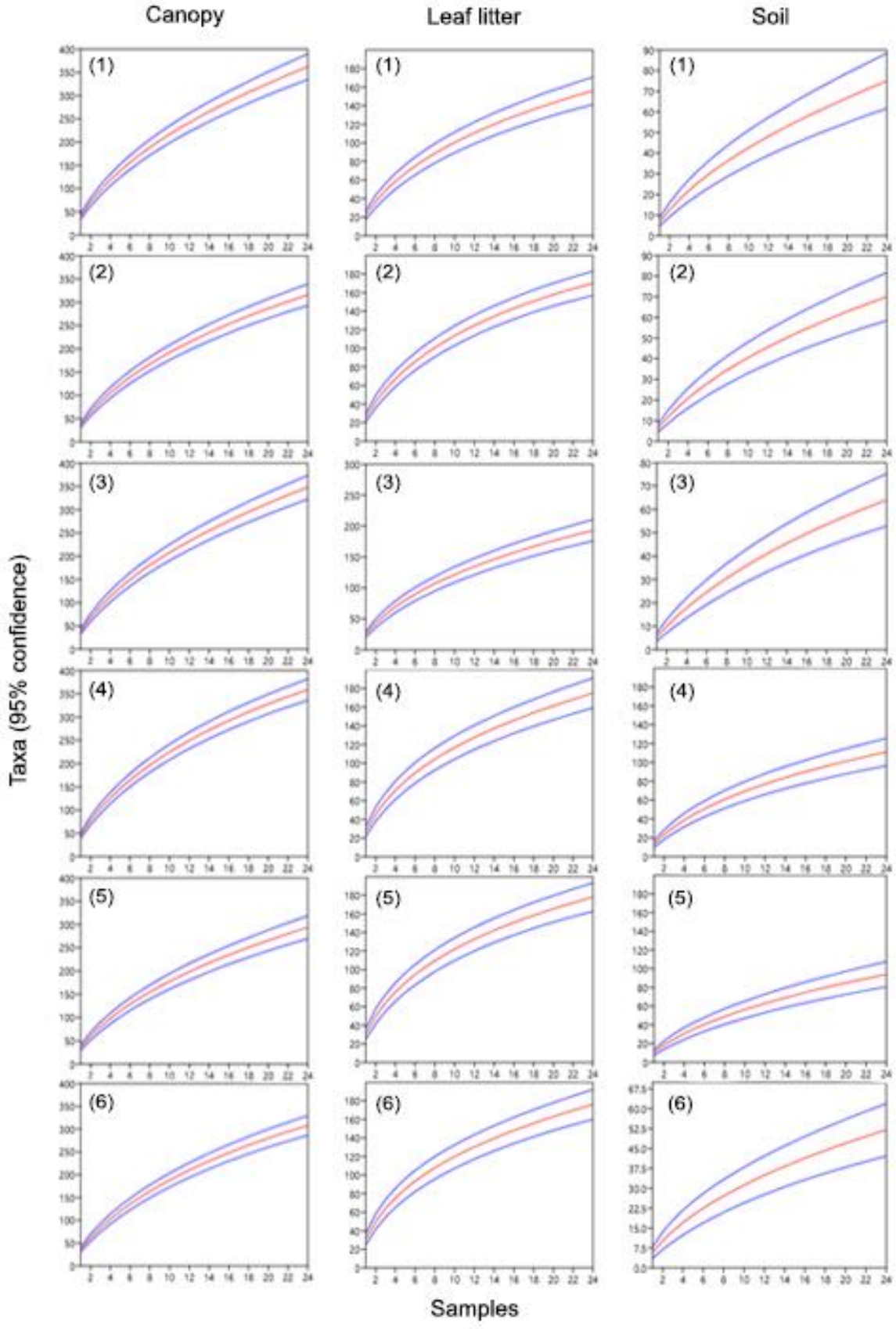

Fig. S2.3 Sample rarefaction curve (Mao tau) with 95% confidence intervals for monthly arthropod samples collected from three strata at each of the six sites (1–6).

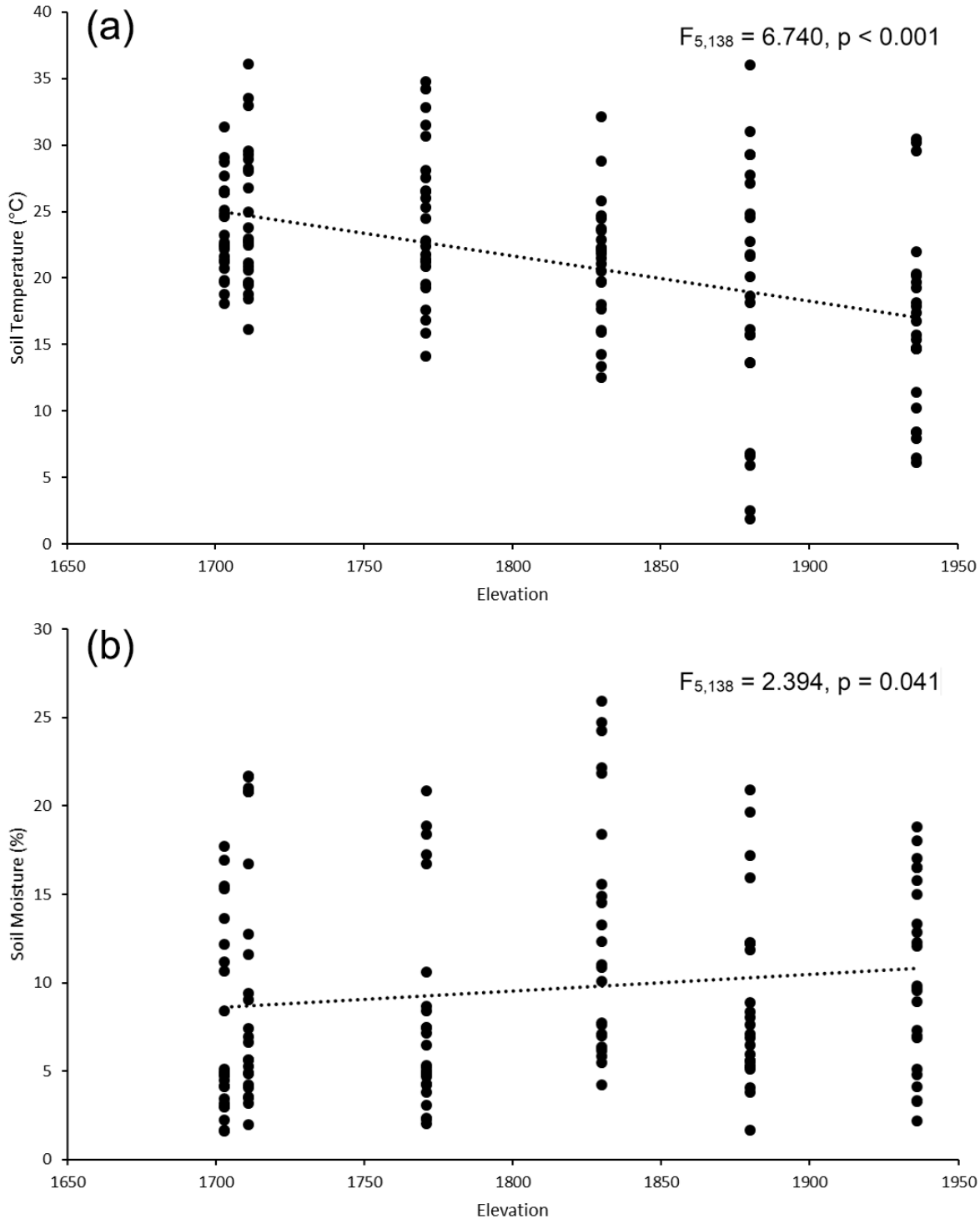

Fig. S2.4 Relationship between soil temperature (a) and soil moisture (b) and elevation across six shrubland patch localities in the Golden Gate Highlands National Park. Each point represents monthly reading taken at that elevation over the 24-month period. Lines indicate predicted linear relationships. Statistical p-values obtained via one-way ANOVA.

Supplementary Material

Chapter 3

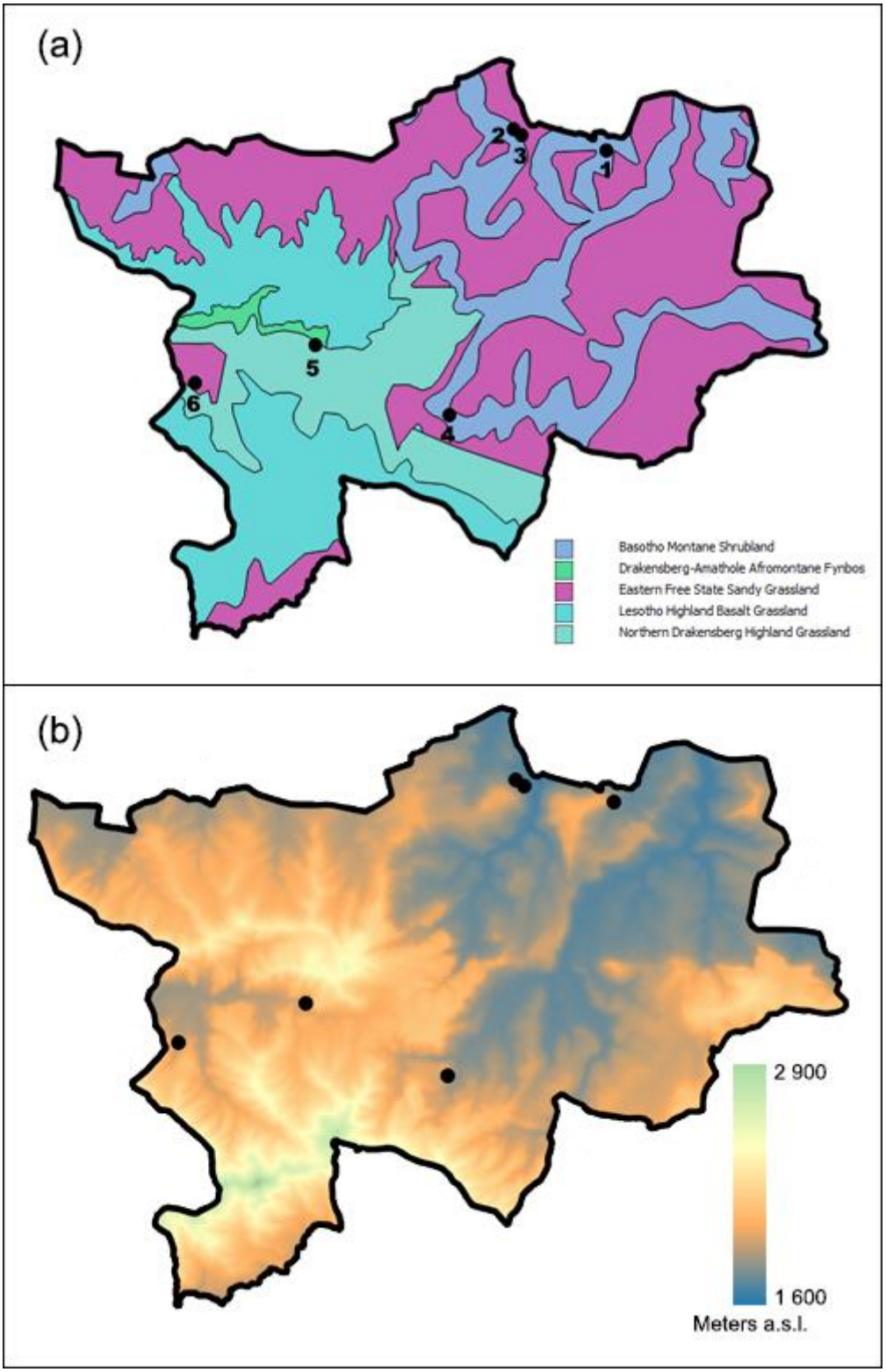

Fig. S3.1 Geographical maps of the Golden Gate Highlands National Park showing (a) vegetative units, and (b) general elevation throughout the park with site location. Vegetation unit and elevation map developed using QGIS version 2.18.15. Vegetation unit vector geospatial dataset 2012 obtained from South African National Biodiversity Institute (SANBI) (http://bgis.sanbi.org/SpatialDataset/Detail/18). Elevation data obtained from Web GIS (http://www.webgis.com/srtm3.html).

Fig. S3.2 Photographs showing extent of burning caused by grassland wildfires across sampled sites during the study as well as regrowth post-fire. Pictures indicate: (a) severe burn of grassland surrounding site 1, (b) degree of burn inside site 1 during September 2017, (c) degree of burn inside site 2 during November 2017, (d) minimal burn around site 3 during November 2017, (e) severe burn of grassland surrounding site 4, (f) severe burn directly around site 4 during September 2017, (g) severe burn around site 6 with woody vegetation also severely burnt, (h) regrowth of vegetation inside site 1 two-months after burning, (i) regrowth of surrounding vegetation of site 4 one-month after burning, and (j) regrowth of surrounding vegetation of site 6 two-months after burning with no regrowth of woody vegetation.

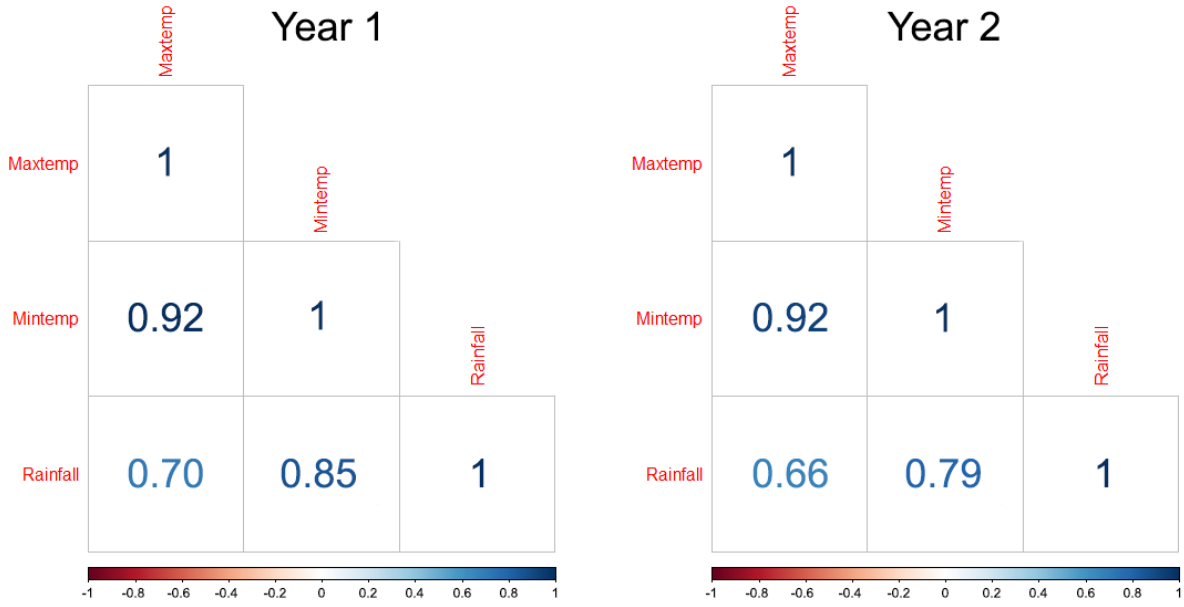

Fig. S3.3 Pearson correlation coefficient analysis between recorded environmental variables per year of study.

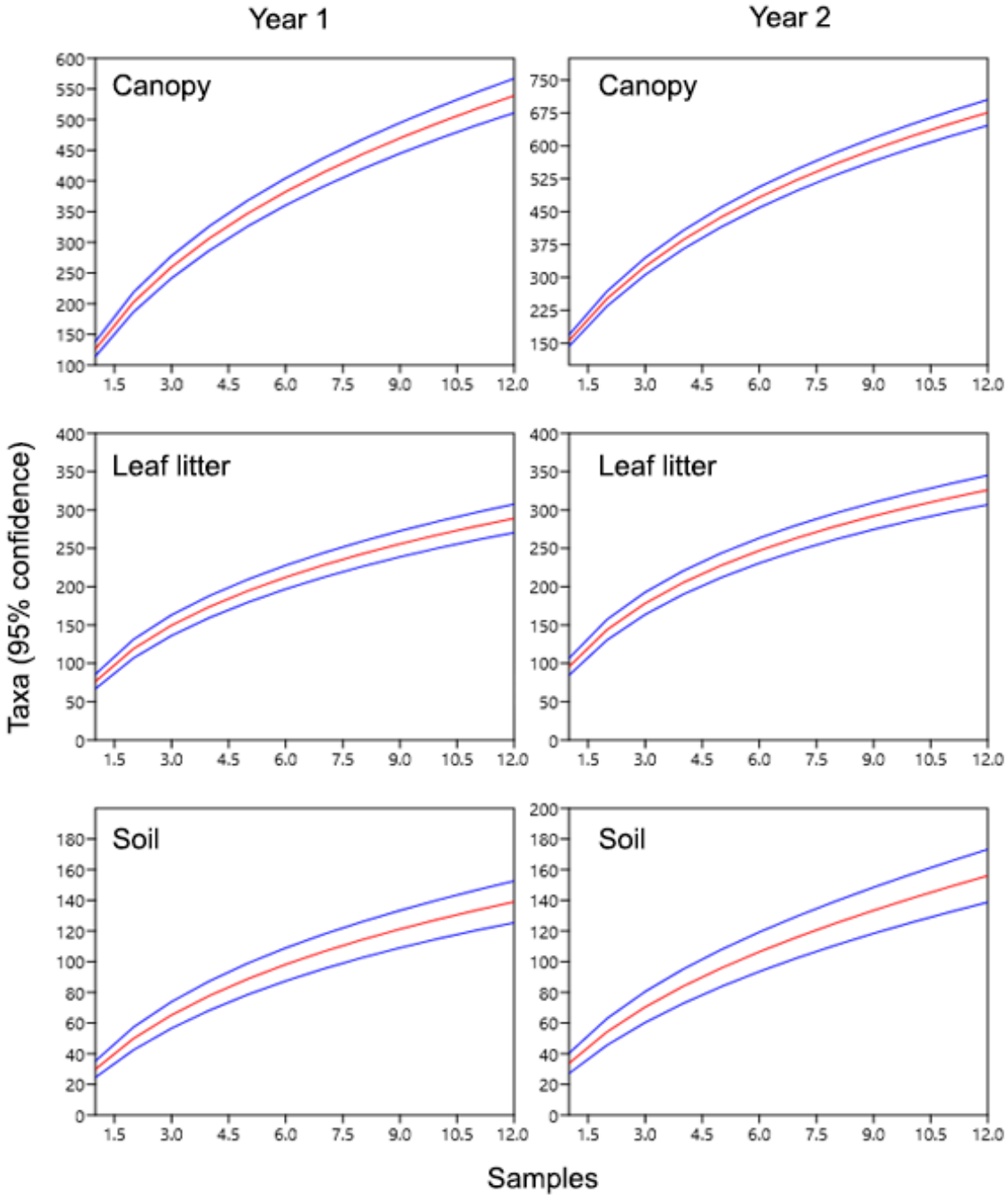

Fig. S3.4 Sample rarefaction curves (Mao Tau) with 95% confidence intervals of total yearly stratum assemblages.

Table S3.1 Pairwise comparison of arthropod assemblage composition between months (Apr-Mar) for canopy stratum during both years of study. Analysis performed by PERMANOVA with pairwise comparisons between group levels (months) with employed "fdr" method for multiple testing corrections. Significance codes: p < 0.05 *.

	Year 1				Year 2			
Pairs	F model	R^2	p value	p adjusted	F model	R^2	p value	p adjusted
Apr vs May	1.047	0.095	0.377	0.444	0.703	0.066	0.871	0.927
Apr vs Jun	1.251	0.111	0.267	0.346	0.837	0.077	0.654	0.785
Apr vs Jul	1.140	0.102	0.297	0.370	0.954	0.087	0.487	0.618
Apr vs Aug	2.191	0.180	0.026	**0.045** *	0.879	0.081	0.588	0.719
Apr vs Sep	2.952	0.228	0.002	**0.010** *	1.220	0.109	0.184	0.335
Apr vs Oct	3.209	0.243	0.006	**0.015** *	1.471	0.128	0.074	0.174
Apr vs Nov	3.387	0.253	0.005	**0.013** *	2.043	0.170	0.006	**0.040** *
Apr vs Dec	3.932	0.282	0.003	**0.010** *	1.926	0.162	0.019	0.078
Apr vs Jan	3.107	0.237	0.001	**0.010** *	1.610	0.139	0.056	0.154
Apr vs Feb	3.600	0.265	0.003	**0.010** *	1.423	0.125	0.096	0.204
Apr vs Mar	2.301	0.187	0.010	**0.021** *	1.142	0.102	0.225	0.362
May vs Jun	0.727	0.068	0.698	0.743	0.608	0.057	0.845	0.926
May vs Jul	0.596	0.056	0.825	0.838	0.746	0.069	0.770	0.876
May vs Aug	1.218	0.109	0.250	0.330	0.773	0.072	0.765	0.876
May vs Sep	1.205	0.108	0.246	0.330	1.044	0.094	0.406	0.547
May vs Oct	1.987	0.166	0.005	**0.013** *	1.178	0.105	0.163	0.307
May vs Nov	2.382	0.192	0.003	**0.010** *	2.005	0.167	0.002	**0.033** *
May vs Dec	2.868	0.223	0.001	**0.010** *	1.876	0.158	0.012	0.066
May vs Jan	2.258	0.184	0.002	**0.010** *	1.437	0.126	0.068	0.171
May vs Feb	2.494	0.200	0.003	**0.010** *	1.462	0.128	0.063	0.166
May vs Mar	1.705	0.146	0.058	0.089	1.096	0.099	0.304	0.456
Jun vs Jul	0.449	0.043	0.942	0.942	0.479	0.046	0.994	0.994
Jun vs Aug	1.065	0.096	0.339	0.407	0.626	0.059	0.856	0.926
Jun vs Sep	2.011	0.167	0.044	0.071	1.028	0.093	0.376	0.528
Jun vs Oct	2.611	0.207	0.004	**0.013** *	1.533	0.133	0.055	0.154
Jun vs Nov	3.011	0.231	0.001	**0.010** *	2.317	0.188	0.001	**0.033** *
Jun vs Dec	3.947	0.283	0.001	**0.010** *	2.262	0.184	0.003	**0.033** *
Jun vs Jan	3.137	0.239	0.001	**0.010** *	2.049	0.170	0.021	0.082
Jun vs Feb	3.389	0.253	0.002	**0.010** *	1.883	0.158	0.006	**0.040** *
Jun vs Mar	2.231	0.182	0.016	**0.029** *	1.610	0.139	0.049	0.147
Jul vs Aug	0.610	0.057	0.785	0.810	0.548	0.052	0.983	0.994
Jul vs Sep	1.162	0.104	0.277	0.352	0.915	0.084	0.574	0.715
Jul vs Oct	1.505	0.131	0.075	0.113	1.570	0.136	0.034	0.112
Jul vs Nov	1.894	0.159	0.014	**0.028** *	2.489	0.199	0.001	**0.033** *
Jul vs Dec	2.602	0.206	0.005	**0.013** *	2.318	0.188	0.004	**0.038** *
Jul vs Jan	2.207	0.181	0.002	**0.010** *	1.950	0.163	0.024	0.088
Jul vs Feb	2.356	0.191	0.003	**0.010** *	1.842	0.156	0.028	0.097
Jul vs Mar	1.741	0.148	0.032	0.053	1.623	0.140	0.042	0.132
Aug vs Sep	1.389	0.122	0.185	0.254	0.606	0.057	0.926	0.970
Aug vs Oct	2.047	0.170	0.029	**0.049** *	1.128	0.101	0.245	0.385
Aug vs Nov	2.598	0.206	0.005	**0.013** *	1.898	0.160	0.003	**0.033** *
Aug vs Dec	3.649	0.267	0.003	**0.010** *	1.790	0.152	0.013	0.066

Table S3.1 Continued.

	Year 1				Year 2			
Pairs	F model	R^2	p value	p adjusted	F model	R^2	p value	p adjusted
Aug vs Jan	2.955	0.228	0.002	**0.010** *	1.513	0.131	0.070	0.171
Aug vs Feb	3.180	0.241	0.003	**0.010** *	1.446	0.126	0.081	0.184
Aug vs Mar	2.259	0.184	0.022	**0.039** *	1.174	0.105	0.207	0.360
Sep vs Oct	0.900	0.083	0.471	0.545	1.135	0.102	0.275	0.422
Sep vs Nov	1.984	0.166	0.009	**0.020** *	2.085	0.173	0.006	**0.040** *
Sep vs Dec	2.174	0.179	0.007	**0.017** *	1.934	0.162	0.017	0.075
Sep vs Jan	2.185	0.179	0.003	**0.010** *	1.423	0.125	0.110	0.227
Sep vs Feb	2.237	0.183	0.011	**0.023** *	1.390	0.122	0.084	0.185
Sep vs Mar	2.185	0.179	0.007	**0.017** *	1.279	0.113	0.114	0.228
Oct vs Nov	1.343	0.118	0.099	0.142	1.002	0.091	0.481	0.618
Oct vs Dec	1.356	0.119	0.086	0.126	1.059	0.096	0.388	0.534
Oct vs Jan	1.805	0.153	0.002	**0.010** *	1.130	0.102	0.329	0.483
Oct vs Feb	1.958	0.164	0.008	**0.018** *	1.200	0.107	0.221	0.362
Oct vs Mar	2.027	0.169	0.001	**0.010** *	1.190	0.106	0.188	0.335
Nov vs Dec	0.925	0.085	0.547	0.612	1.010	0.092	0.465	0.614
Nov vs Jan	0.930	0.085	0.582	0.630	1.686	0.144	0.014	0.066
Nov vs Feb	1.399	0.123	0.154	0.216	1.853	0.156	0.003	**0.033** *
Nov vs Mar	1.775	0.151	0.015	**0.028** *	1.849	0.156	0.007	**0.042** *
Dec vs Jan	0.979	0.089	0.510	0.580	1.024	0.093	0.362	0.519
Dec vs Feb	1.670	0.143	0.045	0.071	1.198	0.107	0.218	0.362
Dec vs Mar	1.914	0.161	0.015	**0.028** *	1.306	0.115	0.119	0.231
Jan vs Feb	0.711	0.066	0.780	0.810	0.729	0.068	0.787	0.880
Jan vs Mar	1.084	0.098	0.322	0.394	0.863	0.079	0.677	0.798
Feb vs Mar	0.886	0.081	0.569	0.626	0.396	0.038	0.952	0.982

Table S3.2 Pairwise comparison of arthropod assemblage composition between months (Apr-Mar) for leaf litter stratum during both years of study. Analysis performed by PERMANOVA with pairwise comparisons between group levels (months) with employed "fdr" method for multiple testing corrections. Significance codes: p < 0.05 *.

	Year 1				Year 2			
Pairs	F model	R^2	p value	p adjusted	F model	R^2	p value	p adjusted
Apr vs May	1.979	0.165	0.032	**0.044** *	0.585	0.055	0.905	0.919
Apr vs Jun	2.936	0.227	0.003	**0.011** *	1.256	0.112	0.178	0.261
Apr vs Jul	3.553	0.262	0.003	**0.011** *	1.769	0.150	0.048	0.122
Apr vs Aug	3.324	0.250	0.005	**0.012** *	1.358	0.120	0.157	0.241
Apr vs Sep	3.676	0.269	0.002	**0.011** *	2.929	0.227	0.002	**0.026** *
Apr vs Oct	3.992	0.285	0.003	**0.011** *	1.148	0.103	0.257	0.333
Apr vs Nov	3.822	0.277	0.005	**0.012** *	1.788	0.152	0.041	0.122
Apr vs Dec	5.504	0.355	0.002	**0.011** *	1.490	0.130	0.079	0.157
Apr vs Jan	5.495	0.355	0.008	**0.014** *	1.955	0.164	0.007	**0.036** *
Apr vs Feb	4.937	0.331	0.005	**0.012** *	1.490	0.130	0.105	0.193
Apr vs Mar	5.096	0.338	0.001	**0.011** *	1.839	0.155	0.049	0.122
May vs Jun	2.556	0.204	0.008	**0.014** *	1.301	0.115	0.165	0.248
May vs Jul	2.986	0.230	0.011	**0.019** *	1.646	0.141	0.061	0.132
May vs Aug	3.159	0.240	0.002	**0.011** *	1.225	0.109	0.255	0.333
May vs Sep	3.545	0.262	0.002	**0.011** *	3.300	0.248	0.003	**0.033** *
May vs Oct	3.266	0.246	0.006	**0.012** *	1.514	0.131	0.091	0.172
May vs Nov	3.378	0.253	0.006	**0.012** *	1.954	0.163	0.024	0.083
May vs Dec	4.345	0.303	0.003	**0.011** *	1.580	0.136	0.062	0.132
May vs Jan	4.447	0.308	0.007	**0.013** *	2.220	0.182	0.006	**0.036** *
May vs Feb	3.992	0.285	0.006	**0.012** *	1.445	0.126	0.140	0.231
May vs Mar	3.999	0.286	0.005	**0.012** *	1.632	0.140	0.081	0.157
Jun vs Jul	0.937	0.086	0.564	0.573	0.488	0.047	0.939	0.939
Jun vs Aug	1.043	0.094	0.408	0.449	0.910	0.083	0.597	0.668
Jun vs Sep	1.822	0.154	0.007	**0.013** *	0.983	0.090	0.542	0.628
Jun vs Oct	1.922	0.161	0.023	**0.034** *	0.953	0.087	0.512	0.603
Jun vs Nov	1.805	0.153	0.029	**0.041** *	0.884	0.081	0.577	0.657
Jun vs Dec	3.463	0.257	0.005	**0.012** *	1.273	0.113	0.232	0.326
Jun vs Jan	3.573	0.263	0.001	**0.011** *	1.028	0.093	0.402	0.506
Jun vs Feb	3.406	0.254	0.001	**0.011** *	1.889	0.159	0.015	0.062
Jun vs Mar	3.595	0.264	0.004	**0.011** *	2.005	0.167	0.007	**0.036** *
Jul vs Aug	1.012	0.092	0.445	0.481	0.843	0.078	0.649	0.685
Jul vs Sep	1.598	0.138	0.036	**0.048** *	1.469	0.128	0.112	0.193
Jul vs Oct	1.880	0.158	0.027	**0.039** *	1.268	0.113	0.223	0.320
Jul vs Nov	1.689	0.144	0.035	**0.047** *	0.967	0.088	0.467	0.560
Jul vs Dec	3.535	0.261	0.003	**0.011** *	1.741	0.148	0.076	0.157
Jul vs Jan	3.955	0.283	0.003	**0.011** *	1.012	0.092	0.406	0.506
Jul vs Feb	3.923	0.282	0.004	**0.011** *	2.619	0.208	0.006	**0.036** *
Jul vs Mar	4.033	0.287	0.003	**0.011** *	2.652	0.210	0.005	**0.036** *
Aug vs Sep	1.206	0.108	0.211	0.249	2.065	0.171	0.009	**0.042** *
Aug vs Oct	2.191	0.180	0.013	**0.020** *	1.013	0.092	0.454	0.555
Aug vs Nov	2.068	0.171	0.013	**0.020** *	0.846	0.078	0.654	0.685
Aug vs Dec	3.780	0.274	0.003	**0.011** *	1.333	0.118	0.149	0.234

Table S3.2 Continued.

	Year 1				Year 2			
Pairs	F model	R^2	p value	p adjusted	F model	R^2	p value	p adjusted
Aug vs Jan	3.993	0.285	0.001	**0.011** *	1.638	0.141	0.025	0.083
Aug vs Feb	3.811	0.276	0.004	**0.011** *	1.638	0.141	0.045	0.122
Aug vs Mar	4.010	0.286	0.006	**0.012** *	1.803	0.153	0.048	0.122
Sep vs Oct	2.244	0.183	0.004	**0.011** *	2.142	0.176	0.012	0.053
Sep vs Nov	1.700	0.145	0.058	0.074	1.510	0.131	0.109	0.193
Sep vs Dec	3.869	0.279	0.003	**0.011** *	2.580	0.205	0.007	**0.036** *
Sep vs Jan	4.185	0.295	0.004	**0.011** *	1.410	0.124	0.050	0.122
Sep vs Feb	3.792	0.275	0.007	**0.013** *	3.862	0.279	0.001	**0.026** *
Sep vs Mar	4.317	0.302	0.002	**0.011** *	4.305	0.301	0.002	**0.026** *
Oct vs Nov	0.631	0.059	0.820	0.820	0.668	0.063	0.745	0.768
Oct vs Dec	1.229	0.109	0.241	0.279	0.773	0.072	0.653	0.685
Oct vs Jan	1.630	0.140	0.091	0.112	1.772	0.151	0.016	0.062
Oct vs Feb	1.916	0.161	0.023	**0.034** *	1.362	0.120	0.148	0.234
Oct vs Mar	2.285	0.186	0.014	**0.021** *	1.848	0.156	0.062	0.132
Nov vs Dec	1.669	0.143	0.092	0.112	0.743	0.069	0.634	0.685
Nov vs Jan	2.021	0.168	0.037	**0.048** *	1.212	0.108	0.255	0.333
Nov vs Feb	2.111	0.174	0.013	**0.020** *	1.858	0.157	0.034	0.107
Nov vs Mar	2.360	0.191	0.011	**0.019** *	2.137	0.176	0.022	0.081
Dec vs Jan	1.066	0.096	0.337	0.377	2.069	0.171	0.005	**0.036** *
Dec vs Feb	0.929	0.085	0.545	0.562	1.422	0.125	0.114	0.193
Dec vs Mar	1.318	0.116	0.200	0.240	1.953	0.163	0.057	0.132
Jan vs Feb	0.939	0.086	0.453	0.482	2.947	0.228	0.001	**0.026** *
Jan vs Mar	1.235	0.110	0.264	0.300	3.216	0.243	0.002	**0.026** *
Feb vs Mar	0.960	0.088	0.504	0.528	1.233	0.110	0.250	0.333

Table S3.3 Pairwise comparison of arthropod assemblage composition between months (Apr-Mar) for soil stratum during both years of study. Analysis performed by PERMANOVA with pairwise comparisons between group levels (months) with employed "fdr" method for multiple testing corrections. Significance codes: p < 0.05 *.

	Year 1				Year 2			
Pairs	F model	R^2	p value	p adjusted	F model	R^2	p value	p adjusted
Apr vs May	1.097	0.099	0.325	0.376	1.050	0.095	0.375	0.516
Apr vs Jun	2.366	0.191	0.003	**0.013** *	1.084	0.098	0.359	0.516
Apr vs Jul	4.176	0.295	0.002	**0.013** *	0.844	0.078	0.613	0.736
Apr vs Aug	2.786	0.218	0.003	**0.013** *	0.754	0.070	0.779	0.865
Apr vs Sep	3.154	0.240	0.003	**0.013** *	1.540	0.133	0.056	0.161
Apr vs Oct	3.607	0.265	0.004	**0.016** *	0.814	0.075	0.654	0.771
Apr vs Nov	3.375	0.252	0.003	**0.013** *	1.039	0.094	0.383	0.516
Apr vs Dec	4.221	0.297	0.001	**0.013** *	0.942	0.086	0.500	0.647
Apr vs Jan	3.190	0.242	0.001	**0.013** *	1.536	0.133	0.105	0.231
Apr vs Feb	3.291	0.248	0.003	**0.013** *	1.234	0.110	0.230	0.422
Apr vs Mar	3.151	0.240	0.003	**0.013** *	0.748	0.070	0.809	0.865
May vs Jun	1.545	0.134	0.012	**0.030** *	1.840	0.155	0.055	0.161
May vs Jul	3.180	0.241	0.002	**0.013** *	2.210	0.181	0.032	0.150
May vs Aug	1.939	0.162	0.004	**0.016** *	2.553	0.203	0.005	0.066
May vs Sep	2.545	0.203	0.003	**0.013** *	2.738	0.215	0.002	**0.044** *
May vs Oct	2.640	0.209	0.002	**0.013** *	2.457	0.197	0.015	0.099
May vs Nov	2.399	0.193	0.001	**0.013** *	1.491	0.130	0.099	0.231
May vs Dec	3.024	0.232	0.002	**0.013** *	2.420	0.195	0.008	0.075
May vs Jan	2.508	0.201	0.001	**0.013** *	2.810	0.219	0.002	**0.044** *
May vs Feb	2.209	0.181	0.005	**0.018** *	2.962	0.229	0.002	**0.044** *
May vs Mar	2.661	0.210	0.011	**0.029** *	1.722	0.147	0.063	0.173
Jun vs Jul	1.419	0.124	0.139	0.180	0.820	0.076	0.520	0.660
Jun vs Aug	1.121	0.101	0.262	0.314	1.229	0.109	0.265	0.448
Jun vs Sep	1.645	0.141	0.038	0.068	1.191	0.106	0.278	0.448
Jun vs Oct	1.556	0.135	0.078	0.117	1.034	0.094	0.392	0.517
Jun vs Nov	1.049	0.095	0.356	0.398	1.310	0.116	0.209	0.406
Jun vs Dec	1.597	0.138	0.070	0.110	1.023	0.093	0.373	0.516
Jun vs Jan	1.544	0.134	0.041	0.071	1.919	0.161	0.056	0.161
Jun vs Feb	0.821	0.076	0.706	0.728	1.180	0.106	0.298	0.468
Jun vs Mar	1.104	0.099	0.292	0.344	0.613	0.058	0.887	0.912
Jul vs Aug	1.631	0.140	0.065	0.107	1.163	0.104	0.278	0.448
Jul vs Sep	2.420	0.195	0.008	**0.025** *	2.101	0.174	0.010	0.081
Jul vs Oct	3.077	0.235	0.006	**0.020** *	0.298	0.029	0.988	0.988
Jul vs Nov	2.172	0.178	0.017	**0.037** *	1.605	0.138	0.104	0.231
Jul vs Dec	2.679	0.211	0.016	**0.037** *	0.505	0.048	0.898	0.912
Jul vs Jan	2.379	0.192	0.006	**0.020** *	2.103	0.174	0.032	0.150
Jul vs Feb	1.287	0.114	0.212	0.264	1.245	0.111	0.268	0.448
Jul vs Mar	2.504	0.200	0.019	**0.039** *	0.542	0.051	0.880	0.912
Aug vs Sep	0.714	0.067	0.820	0.833	1.949	0.163	0.042	0.161
Aug vs Oct	1.911	0.160	0.018	**0.038** *	0.634	0.060	0.793	0.865
Aug vs Nov	1.343	0.118	0.083	0.119	2.024	0.168	0.027	0.149
Aug vs Dec	1.847	0.156	0.050	0.085	1.530	0.133	0.085	0.216

Table S3.3 Continued.

	Year 1				Year 2			
Pairs	F model	R^2	p value	p adjusted	F model	R^2	p value	p adjusted
Aug vs Jan	1.870	0.158	0.011	**0.029** *	2.861	0.222	0.003	0.050
Aug vs Feb	1.371	0.121	0.073	0.112	1.142	0.102	0.333	0.500
Aug vs Mar	2.212	0.181	0.013	**0.032** *	0.733	0.068	0.791	0.865
Sep vs Oct	2.085	0.173	0.036	0.068	1.869	0.157	0.048	0.161
Sep vs Nov	1.696	0.145	0.067	0.108	1.484	0.129	0.091	0.222
Sep vs Dec	2.168	0.178	0.038	0.068	1.844	0.156	0.046	0.161
Sep vs Jan	2.079	0.172	0.017	**0.037** *	1.574	0.136	0.123	0.262
Sep vs Feb	1.692	0.145	0.082	0.119	2.539	0.203	0.006	0.066
Sep vs Mar	2.454	0.197	0.009	**0.027** *	1.527	0.132	0.080	0.211
Oct vs Nov	1.008	0.092	0.382	0.413	1.877	0.158	0.056	0.161
Oct vs Dec	1.440	0.126	0.202	0.256	0.862	0.079	0.551	0.686
Oct vs Jan	2.099	0.173	0.011	**0.029** *	2.470	0.198	0.020	0.120
Oct vs Feb	1.125	0.101	0.331	0.377	1.270	0.113	0.241	0.430
Oct vs Mar	1.717	0.147	0.098	0.135	0.617	0.058	0.813	0.865
Nov vs Dec	1.482	0.129	0.101	0.136	1.031	0.093	0.364	0.516
Nov vs Jan	1.561	0.135	0.035	0.068	1.056	0.095	0.325	0.499
Nov vs Feb	0.652	0.061	0.870	0.870	1.971	0.165	0.034	0.150
Nov vs Mar	1.049	0.095	0.382	0.413	1.301	0.115	0.227	0.422
Dec vs Jan	2.064	0.171	0.020	0.040	1.478	0.129	0.129	0.266
Dec vs Feb	1.018	0.092	0.395	0.420	1.355	0.119	0.196	0.392
Dec vs Mar	1.625	0.140	0.089	0.125	0.722	0.067	0.722	0.836
Jan vs Feb	1.467	0.128	0.116	0.153	2.619	0.208	0.011	0.081
Jan vs Mar	1.211	0.108	0.233	0.285	2.048	0.170	0.038	0.157
Feb vs Mar	0.911	0.083	0.500	0.524	0.841	0.078	0.563	0.688

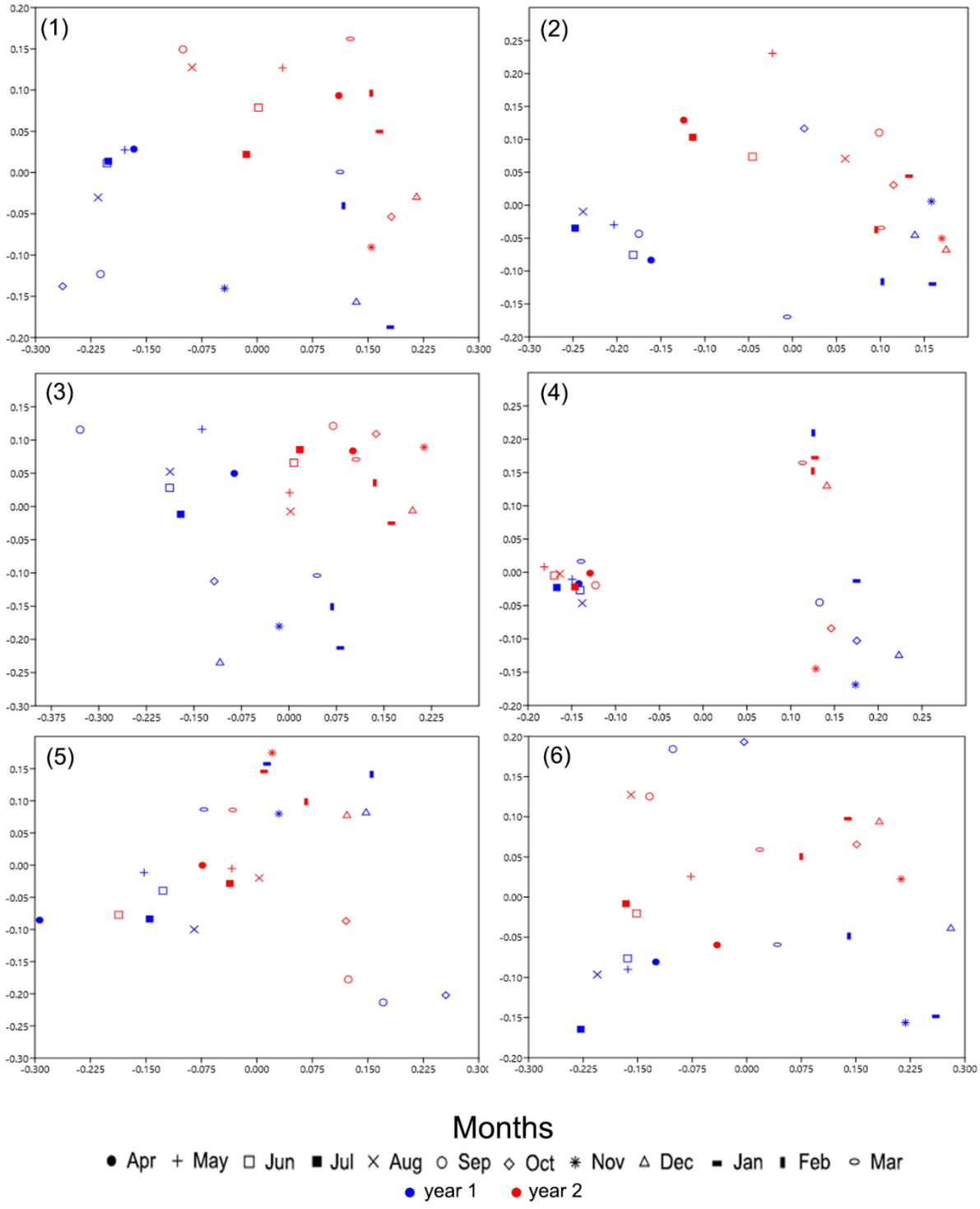

Fig. S3.5 Nonmetric multidimensional scaling (3D) (NMDS) ordination of intra-annual canopy arthropod assemblage changes during both years of study per sample site (1-6).

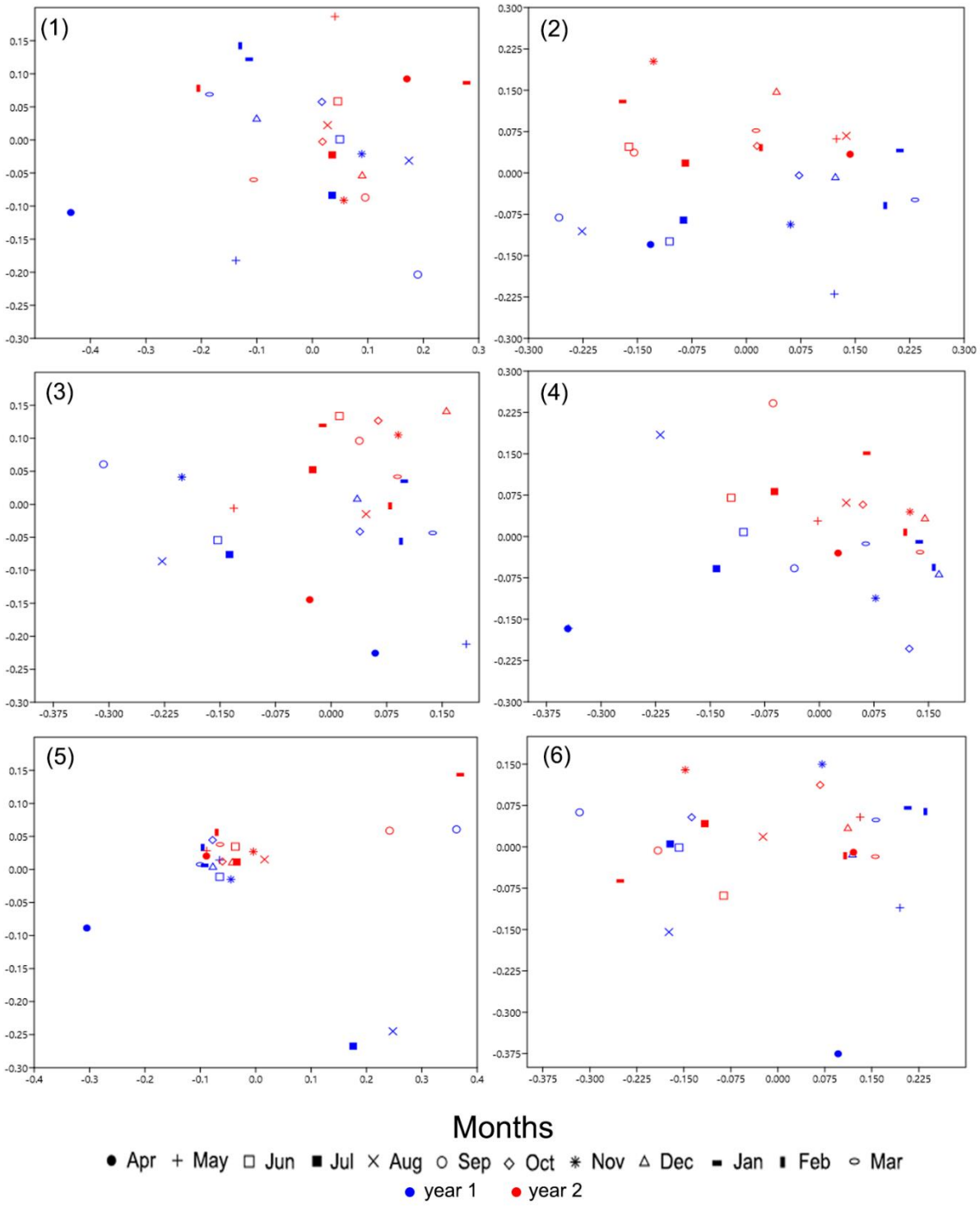

Fig. S3.6 Nonmetric multidimensional scaling (3D) (NMDS) ordination of intra-annual leaf litter arthropod assemblage changes during both years of study per sample site (1-6).

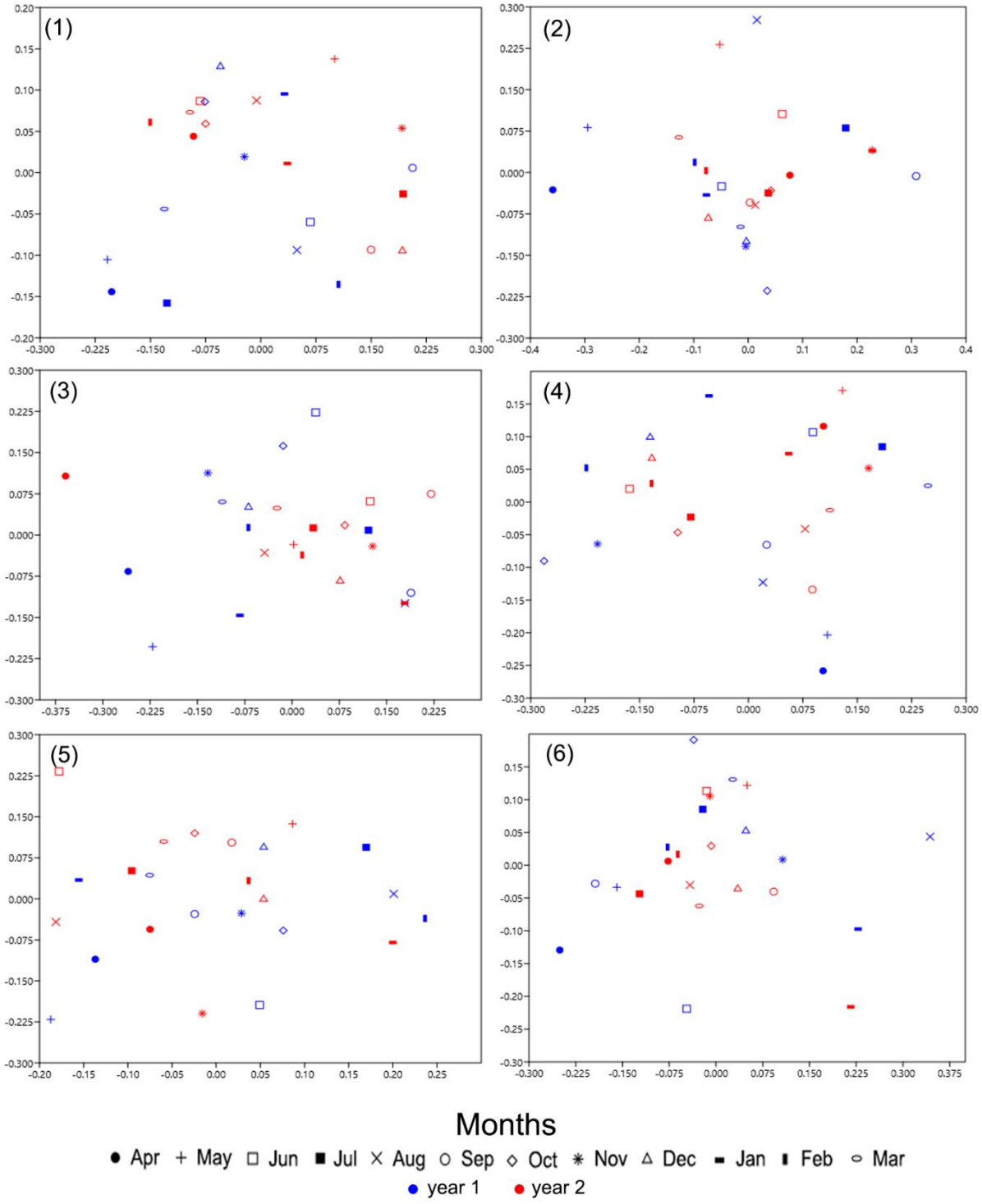

Fig. S3.7 Nonmetric multidimensional scaling (3D) (NMDS) ordination of intra-annual soil arthropod assemblage changes during both years of study per sample site (1-6).

Table S3.4 Results of generalised linear mixed-effects models of environmental factors influencing yearly arthropod abundance per stratum. Significance codes: p < 0.001 ***, p < 0.01 **, p < 0.05 *.

Random effects:		Year 1				Year 2			
Stratum	**Groups**	Variance	Std. Dev			Variance	Std. Dev		
Canopy	Site (n=6)	0.044	0.211			0.099	0.314		
Leaf litter	Site (n=6)	0.016	0.125			0.074	0.271		
Soil	Site (n=6)	0.524	0.724			0.235	0.485		
Stratum	**Fixed effects:**	Estimate	Std. Error	z-value	p-value	Estimate	Std. Error	z-value	p-value
Canopy	Max temperature	-1.392	0.082	-16.930	<0.001***	-0.264	0.066	-3.988	<0.001***
	Min temperature	-0.389	0.026	-14.810	<0.001***	-0.091	0.023	-3.920	<0.001***
	Rainfall	-0.646	0.030	-21.330	<0.001***	-0.186	0.032	-5.817	<0.001***
Leaf litter	Max temperature	4.369	0.072	60.580	<0.001***	0.456	0.056	8.069	<0.001***
	Min temperature	1.848	0.028	66.800	<0.001***	0.374	0.020	18.580	<0.001***
	Rainfall	1.412	0.022	65.110	<0.001***	0.685	0.024	28.960	<0.001***
Soil	Max temperature	1.326	0.138	9.635	<0.001***	-1.811	0.136	-13.350	<0.001***
	Min temperature	0.472	0.046	10.210	<0.001***	-0.636	0.048	-13.400	<0.001***
	Rainfall	-0.279	0.048	-5.774	<0.001***	-0.653	0.071	-9.191	<0.001***

Supplementary Material

Chapter 4

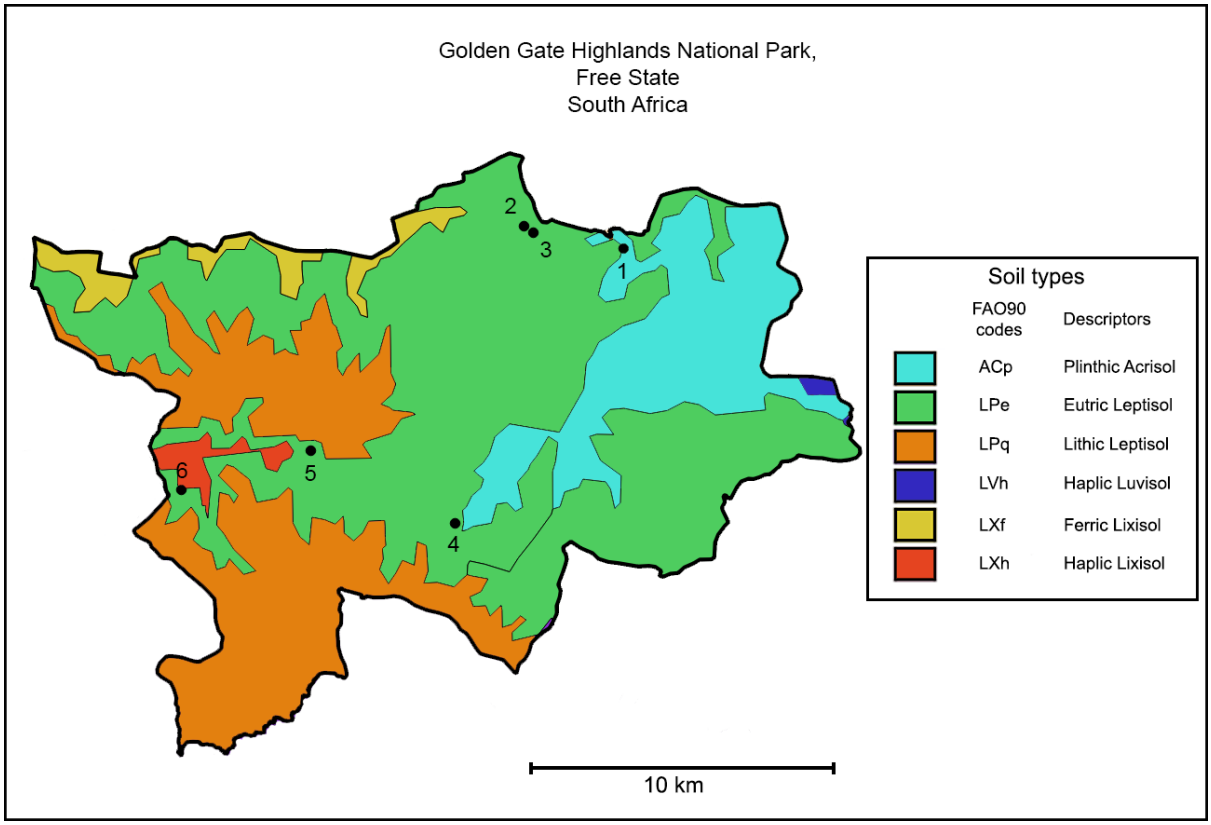

Fig. S4.1 Map of the Golden Gate Highlands National Park showing described soil types, in accordance with FAO90 major soil groups, soil type codes and descriptors, within the park territory alongside sampling site location. Soil types, codes and mapping zones obtained, with full permission, from SANParks Scientific Services, South Africa. Map processed using QGIS version 2.18.15.

Table S4.1 Abundance and observed species richness (S_{obs}) of major soil arthropod groups sampled from the three sub-sites of site 1. All individuals were sampled during the period of April 2017 to March 2019.

Site 1	Inside				10 m				25 m			
	Abundance	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**
Collembola	92	17.07	12	16.00	41	7.92	10	17.54	44	6.27	9	16.67
Acari - Mites												
Mesostigmata mites	22	4.08	3	4.00	11	2.12	3	5.26	4	0.57	3	5.56
Oribatida mites	102	18.92	13	17.33	16	3.09	5	8.77	40	5.70	7	12.96
Sarcoptiformes mites	21	3.90	1	1.33	4	0.77	1	1.75	5	0.71	1	1.85
Trombidiformes mites	77	14.29	15	20.00	96	18.53	13	22.81	86	12.25	10	18.52
Hymenoptera - Formicidae	189	35.06	12	16.00	321	61.97	4	7.02	503	71.65	10	18.52
Other Orders	36	6.68	19	25.33	29	5.60	21	36.84	20	2.85	14	25.93
Total	**539**		**75**		**518**		**57**		**702**		**54**	

Table S4.2 Abundance and observed species richness (S_{obs}) of major soil arthropod groups sampled from the three sub-sites of site 2. All individuals were sampled during the period of April 2017 to March 2019.

Site 2	Inside				10 m				25 m			
	Abundance	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**
Collembola	92	12.74	13	18.57	53	10.62	10	18.52	11	1.60	8	15.09
Acari - Mites												
Mesostigmata mites	29	4.02	4	5.71	14	2.81	2	3.70	7	1.02	3	5.66
Oribatida mites	146	20.22	13	18.57	39	7.82	8	14.81	29	4.21	9	16.98
Sarcoptiformes mites	10	1.39	1	1.43	5	1.00	1	1.85	0	0.00	0	0.00
Trombidiformes mites	55	7.62	11	15.71	101	20.24	12	22.22	136	19.74	15	28.30
Hymenoptera - Formicidae	355	49.17	8	11.43	226	45.29	4	7.41	450	65.31	8	15.09
Other Orders	35	4.85	20	28.57	61	12.22	17	31.48	56	8.13	10	18.87
Total	**722**		**70**		**499**		**54**		**689**		**53**	

Table S4.3 Abundance and observed species richness (S_{obs}) of major soil arthropod groups sampled from the three sub-sites of site 3. All individuals were sampled during the period of April 2017 to March 2019.

Site 3	Inside				10 m				25 m			
	Abundance	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**
Collembola	50	3.77	9	14.06	18	5.94	5	10.87	19	5.85	7	13.21
Acari - Mites												
Mesostigmata mites	32	2.41	6	9.38	13	4.29	4	8.70	15	4.62	6	11.32
Oribatida mites	109	8.21	12	18.75	51	16.83	12	26.09	63	19.38	10	18.87
Sarcoptiformes mites	25	1.88	2	3.13	15	4.95	1	2.17	66	20.31	2	3.77
Trombidiformes mites	89	6.70	8	12.50	43	14.19	5	10.87	20	6.15	5	9.43
Hymenoptera - Formicidae	978	73.64	7	10.94	151	49.83	11	23.91	125	38.46	11	20.75
Other Orders	45	3.39	20	31.25	12	3.96	8	17.39	17	5.23	12	22.64
Total	**1328**		**64**		**303**		**46**		**325**		**53**	

Table S4.4 Abundance and observed species richness (S_{obs}) of major soil arthropod groups sampled from the three sub-sites of site 4. All individuals were sampled during the period of April 2017 to March 2019.

Site 4	Inside				10 m				25 m			
	Abundance	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**
Collembola	152	5.22	16	14.41	154	14.74	17	22.08	163	15.92	13	18.06
Acari - Mites												
Mesostigmata mites	155	5.32	8	7.21	21	2.01	4	5.19	12	1.17	4	5.56
Oribatida mites	640	21.97	16	14.41	214	20.48	10	12.99	232	22.66	14	19.44
Sarcoptiformes mites	172	5.90	2	1.80	23	2.20	2	2.60	30	2.93	2	2.78
Trombidiformes mites	124	4.26	16	14.41	99	9.47	18	23.38	311	30.37	16	22.22
Hymenoptera - Formicidae	1517	52.08	5	4.50	500	47.85	8	10.39	259	25.29	10	13.89
Other Orders	153	5.25	48	43.24	34	3.25	18	23.38	17	1.66	13	18.06
Total	**2913**		**111**		**1045**		**77**		**1024**		**72**	

Table S4.5 Abundance and observed species richness (S_{obs}) of major soil arthropod groups sampled from the three sub-sites of site 5. All individuals were sampled during the period of April 2017 to March 2019.

Site 5	Inside				10 m				25 m			
	Abundance	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**
Collembola	133	15.29	19	20.21	9	1.74	5	8.06	52	11.61	9	12.50
Acari - Mites												
Mesostigmata mites	33	3.79	8	8.51	24	4.64	8	12.90	19	4.24	8	11.11
Oribatida mites	310	35.63	16	17.02	97	18.76	16	25.81	141	31.47	15	20.83
Sarcoptiformes mites	10	1.15	1	1.06	7	1.35	1	1.61	9	2.01	1	1.39
Trombidiformes mites	157	18.05	18	19.15	46	8.90	13	20.97	25	5.58	14	19.44
Hymenoptera - Formicidae	193	22.18	12	12.77	319	61.70	9	14.52	185	41.29	11	15.28
Other Orders	34	3.91	20	21.28	15	2.90	10	16.13	17	3.79	14	19.44
Total	**870**		**94**		**517**		**62**		**448**		**72**	

Table S4.6 Abundance and observed species richness (S_{obs}) of major soil arthropod groups sampled from the three sub-sites of site 6. All individuals were sampled during the period of April 2017 to March 2019.

Site 6	Inside				10 m				25 m			
	Abundance	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**	**Abundance**	**%**	**S_{obs}**	**%**
Collembola	53	10.43	8	15.38	27	2.05	9	14.06	21	4.59	9	16.67
Acari - Mites												
Mesostigmata mites	28	5.51	5	9.62	34	2.58	5	7.81	35	7.64	6	11.11
Oribatida mites	118	23.23	13	25.00	176	13.33	12	18.75	195	42.58	12	22.22
Sarcoptiformes mites	0	0.00	0	0.00	12	0.91	2	3.13	22	4.80	2	3.70
Trombidiformes mites	43	8.46	6	11.54	17	1.29	9	14.06	8	1.75	6	11.11
Hymenoptera - Formicidae	243	47.83	4	7.69	1027	77.80	11	17.19	155	33.84	6	11.11
Other Orders	23	4.53	16	30.77	27	2.05	16	25.00	22	4.80	13	24.07
Total	**508**		**52**		**1320**		**64**		**458**		**54**	

Supplementary Material

Chapter 5

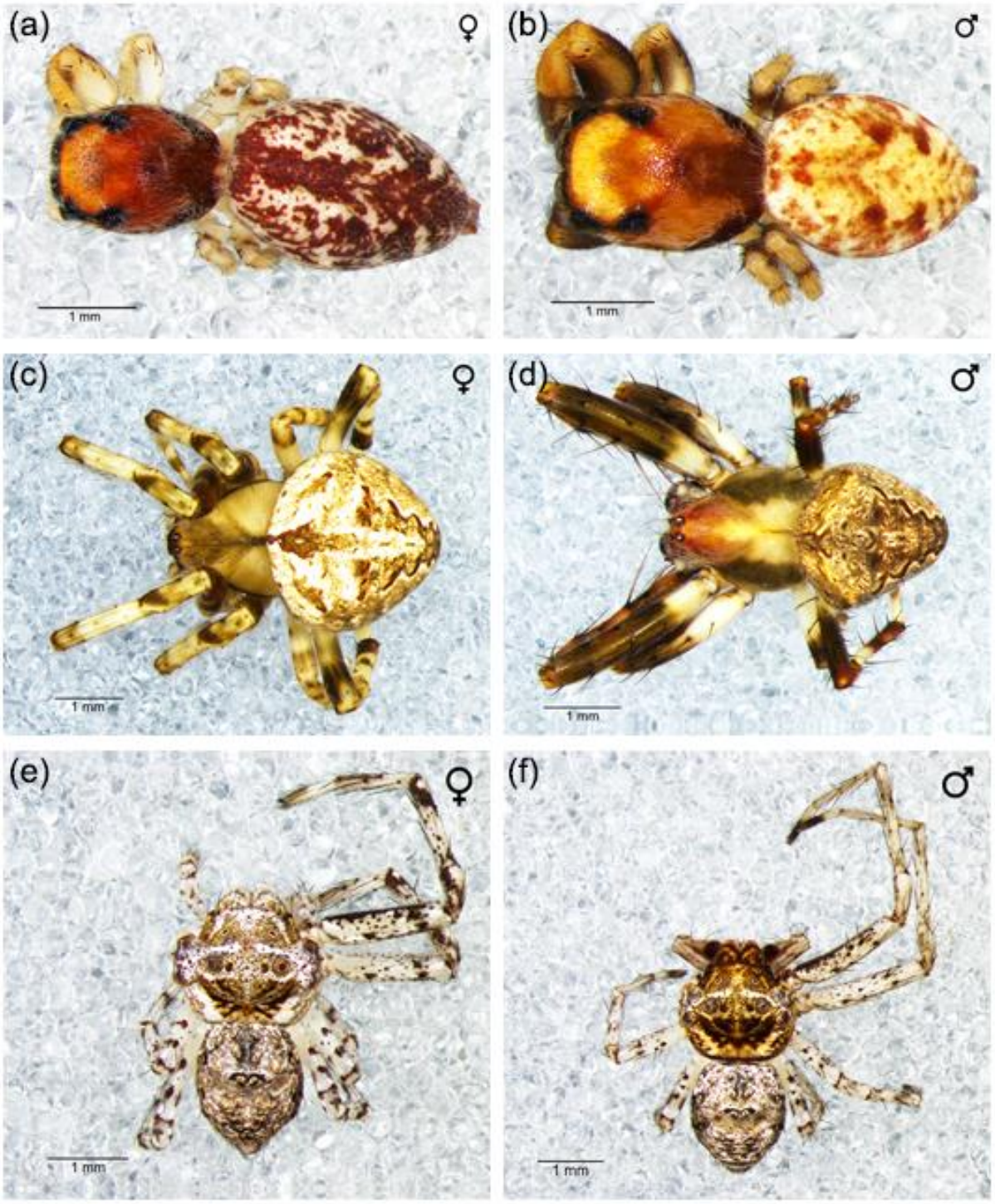

Fig. S5.1 Dorsal habitus of the five investigated spider species sampled from the Golden Gate Highlands National Park: *Dendryphantes purcelli* Peckham & Peckham, 1903 female and male (a and b), *Neoscona subfusca* (C.L. Koch, 1837) female and male (c and d) and *Pherecydes tuberculatus* O.P.-Cambridge, 1883 female and male (e and f).

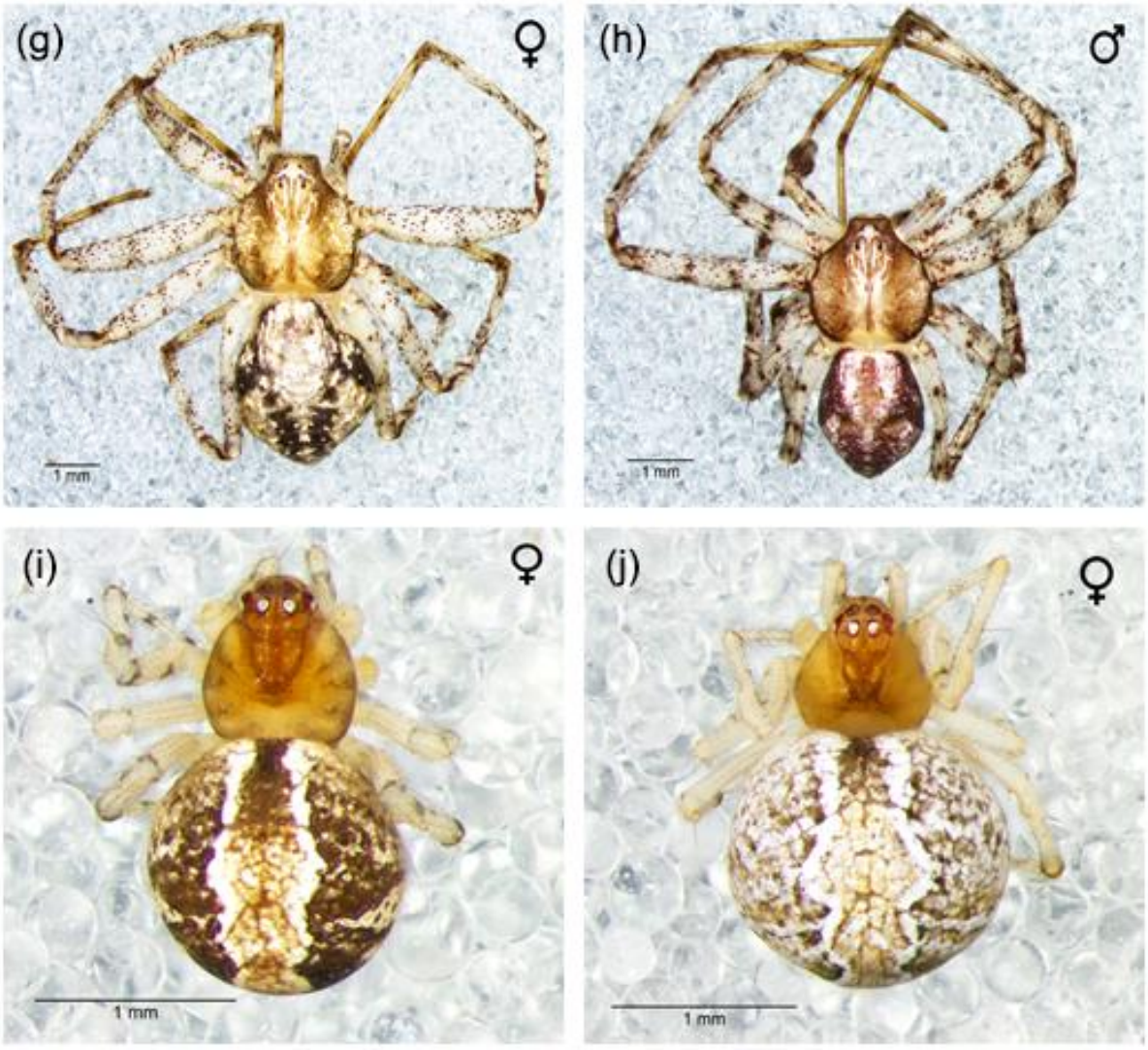

Fig. S5.1 cont. *Philodromus browningi* Lawrence, 1952 female and male (g and h) and *Theridion* sp. females, showing colour variation (i and j).

Table S5.1 Diversity indices of *Dendryphantes purcelli* populations in the Golden Gate Highlands National Park, calculated from nucleotide sequence of the mitochondrial COI gene

Site	N	S	h	Hd	K	π
Site 1	11	10	3	0.3455	1.8182	0.0033
Site 2	7	25	4	0.8095	8.3810	0.0152
Site 3	12	64	5	0.8030	14.3333	0.0260
Site 4	12	3	3	0.3182	0.5000	0.0009
Site 5	3	17	2	0.6667	11.3333	0.0205
Site 6	12	23	6	0.7576	8.3636	0.0152
Total	57	71	13	0.6510	7.4317	0.0135

N: Number of sequences; S: Number of segregating (polymorphic/variable) sites; h: Number of haplotypes; Hd: Haplotype diversity; K: Average number of nucleotide differences; π: Nucleotide diversity.

Table S5.2 Pairwise genetic differentiation (Fst) and nucleotide substitution per site (Dxy) among Golden Gate Highlands National Park populations of *Dendryphantes purcelli*. Fst values are represented in the bottom triangle of the matrix and Dxy values are represented in the top.

	1	2	3	4	5	6
1		0.0092	0.0151	0.0021	0.0215	0.0105
2	0		0.0194	0.0087	0.0230	0.0150
3	0.0309	0		0.0148	0.0285	0.0209
4	0	0.0790	0.0903		0.0210	0.0095
5	0.4464	0.2218	0.1852	0.4892		0.0193
6	0.1181	0	0.0167	0.1558	0.0767	

Table S5.3 Diversity indices of *Neoscona subfusca* populations in the Golden Gate Highlands National Park, calculated from nucleotide sequence of the mitochondrial COI gene

Site	N	S	h	Hd	K	π
Site 1	12	60	10	0.9697	27.4546	0.0473
Site 2	12	52	8	0.8939	25.5455	0.0440
Site 3	11	51	5	0.8182	26.6546	0.0459
Site 4	11	56	7	0.8909	27.0909	0.0466
Site 5	12	59	10	0.9697	26.1970	0.0451
Site 6	10	16	5	0.7556	4.6000	0.0079
Total	68	64	17	0.9078	23.9723	0.0413

N: Number of sequences; S: Number of segregating (polymorphic/variable) sites; h: Number of haplotypes; Hd: Haplotype diversity; K: Average number of nucleotide differences; π: Nucleotide diversity.

Table S5.4 Pairwise genetic differentiation (Fst) and nucleotide substitution per site (Dxy) among Golden Gate Highlands National Park populations of *Neoscona subfusca*. Fst values are represented in the bottom triangle of the matrix and Dxy values are represented in the top.

	1	2	3	4	5	6
1		0.0429	0.0433	0.0437	0.0431	0.0423
2	0		0.0416	0.0418	0.0416	0.0354
3	0	0		0.0428	0.0423	0.0389
4	0	0	0		0.0429	0.0394
5	0	0	0	0		0.0362
6	0.3476	0.2665	0.3088	0.3081	0.2685	

Table S5.5 Diversity indices of *Pherecydes tuberculatus* populations in the Golden Gate Highlands National Park, calculated from nucleotide sequence of the mitochondrial COI gene

Site	N	S	h	Hd	K	π
Site 1	10	0	1	0	0	0
Site 2	7	0	1	0	0	0
Site 3	12	1	2	0.4849	0.4849	0.0010
Site 4	12	1	2	0.4849	0.4849	0.0010
Site 5	3	0	1	0	0	0
Site 6	12	0	1	0	0	0
Total	56	2	3	0.2597	0.2701	0.0005

N: Number of sequences; S: Number of segregating (polymorphic/variable) sites; h: Number of haplotypes; Hd: Haplotype diversity; K: Average number of nucleotide differences; π: Nucleotide diversity.

Table S5.6 Pairwise genetic differentiation (Fst) and nucleotide substitution per site (Dxy) among Golden Gate Highlands National Park populations of *Pherecydes tuberculatus*. Fst values are represented in the bottom triangle of the matrix and Dxy values are represented in the top.

	1	2	3	4	5	6
1		0	0.0007	0.0007	0	0
2	0		0.0007	0.0007	0	0
3	0.2727	0.2727		0.0013	0.0007	0.0007
4	0.2727	0.2727	0.2727		0.0007	0.0007
5	0	0	0.2727	0.2727		0
6	0	0	0.2727	0.2727	0	

Table S5.7 Diversity indices of *Philodromus browningi* populations in the Golden Gate Highlands National Park, calculated from nucleotide sequence of the mitochondrial COI gene

Site	N	S	h	Hd	K	π
Site 1	11	58	6	0.8364	11.9273	0.0310
Site 2	9	9	4	0.6944	2.4444	0.0064
Site 3	10	10	4	0.7111	2.6444	0.0069
Site 4	4	1	2	0.5000	0.5000	0.0013
Site 5	1	–	–	–	–	–
Site 6	–	–	–	–	–	–
Total	35	59	8	0.7112	5.2513	0.0136

N: Number of sequences; S: Number of segregating (polymorphic/variable) sites; h: Number of haplotypes; Hd: Haplotype diversity; K: Average number of nucleotide differences; π: Nucleotide diversity.

Table S5.8 Pairwise genetic differentiation (Fst) and nucleotide substitution per site (Dxy) among Golden Gate Highlands National Park populations of *Philodromus browningi*. Fst values are represented in the bottom triangle of the matrix and Dxy values are represented in the top.

	1	2	3	4	5	6
1		0.0182	0.0183	0.0165	–	–
2	0		0.0060	0.0041	–	–
3	0	0		0.0043	–	–
4	0.0201	0.0702	0.0471		–	–
5	–	–	–	–		–
6	–	–	–	–	–	

Table S5.9 Diversity indices of the *Theridion* sp. populations in the Golden Gate Highlands National Park, calculated from nucleotide sequence of the mitochondrial COI gene

Site	N	S	h	Hd	K	π
Site 1	7	6	4	0.8095	2.2857	0.0047
Site 2	2	3	2	1	3.0000	0.0062
Site 3	4	3	3	0.8333	1.6667	0.0034
Site 4	12	11	7	0.9091	3.6061	0.0074
Site 5	5	3	2	0.6000	1.8000	0.0037
Site 6	3	4	3	1	2.6667	0.0055
Total	33	18	12	0.8807	3.1288	0.0064

N: Number of sequences; S: Number of segregating (polymorphic/variable) sites; h: Number of haplotypes; Hd: Haplotype diversity; K: Average number of nucleotide differences; π: Nucleotide diversity.

Table S5.10 Pairwise genetic differentiation (Fst) and nucleotide substitution per site (Dxy) among Golden Gate Highlands National Park populations of the *Theridion* sp. Fst values are represented in the bottom triangle of the matrix and Dxy values are represented in the top.

	1	2	3	4	5	6
1		0.0063	0.0037	0.0084	0.0045	0.0052
2	0.1395		0.0051	0.0106	0.0056	0.0072
3	0	0.0667		0.0081	0.0037	0.0048
4	0.2765	0.3607	0.3269		0.0063	0.0061
5	0.0714	0.1111	0.0370	0.1186		0.0038
6	0.0189	0.1905	0.0714	0	0	

Table S5.11 Table indicating the dominant plant species present at the time of sampling in each site.

Dominant plant species	Site					
	1	2	3	4	5	6
Cussonia paniculata	X			X		
Diospyros whyteana	X			X	X	
Euclea crispa	X	X	X	X		
Gymnosporia buxifolia		X				
Olinia emarginata	X	X	X	X	X	X
Pittosporum viridiflorum	X	X	X	X		
Leucosidea sericea				X	X	X
Searsia dentata	X	X	X	X		
Olea europaea africana	X	X	X			
Widdringtonia nodiflora					X	X
Cliffortia linearifolia					X	X
Protea subvestita	X			X		

Appendices

Official permits and agreements

South African National Parks – Research Permit (BOTJ1406)

South African
NATIONAL PARKS

RESEARCH AGREEMENT

BETWEEN

SOUTH AFRICAN NATIONAL PARKS
herein represented by **Dr IPJ Smit**
in his capacity as **Acting GM: Savanna and Arid Research Unit**
(hereinafter referred to as "SANParks")

AND

Mr J.L. Botham

9105206064188
Id no. _____
(hereinafter referred to as "the Researcher")

WHEREAS the Researcher submitted a research application to SANParks to conduct a research on **"Investigation of interspersed forest patches along different topographical conditions within Afromontane grasslands, and their potential as conservation hotspots"** ("Research") and to obtain a sample of a biological resource ("Material") in the **"Golden Gate Highlands National PArk"** ("the Park"); JLB

SIGNED at Skukuza on the 20 day of January 2017

WITNESSES

1. _____ I.R.J Smit
SANPARKS

2. _____

SIGNED at Bloemfontein on the 19th day of January 2017 ~~2016~~ JLB

WITNESSES:

1. _____

2. _____

_____
RESEARCHER

University of the Free State Ethics Board – Ethical Clearance

Faculty of Natural and Agricultural Sciences

20-Feb-2017

Dear **Mr Jason Botham**

Ethics Clearance: **Investigation of interspersed forest patches along different topographical conditions within Afromontane grasslands, and their potential as conservation hotspots**

Principal Investigator: **Mr Jason Botham**

Department: **Zoology and Entomology (Bloemfontein Campus)**

APPLICATION APPROVED

This letter confirms that a research proposal with tracking number: **UFS-HSD2017/0084** and title: **'Investigation of interspersed forest patches along different topographical conditions within Afromontane grasslands, and their potential as conservation hotspots'** was given ethical clearance by the Ethics Committee.

Your ethical clearance number, to be used in all correspondence is: **UFS-HSD2017/0084**

Please ensure that the Ethics Committee is notified should any substantive change(s) be made, for whatever reason, during the research process. This includes changes in investigators. Please also ensure that a brief report is submitted to the Ethics Committee on completion of the research.
The purpose of this report is to indicate whether or not the research was conducted successfully, if any aspects could not be completed, or if any problems arose that the Ethics Committee should be aware of.

Note:

1. This clearance is valid from the date on this letter to the time of completion of data collection.
2. Progress reports should be submitted annually unless otherwise specified.

Yours Sincerely

Prof. RR (Robert) Bragg

Chairperson: Ethics Committee
Faculty of Natural and Agricultural Sciences

Natural and Agricultural Sciences Research Ethics Committee
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