Patterns and drivers of forb diversity in South African grasslands

by

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Summary

Biodiversity forms the basis of ecosystem functioning. Quantifying diversity patterns and its drivers is needed to effectively conserve and maintain ecosystems. Diversity is scale-dependent and can be measured as richness at a local scale (alpha-diversity), compositional dissimilarity among sites (beta-diversity) and the accumulation of these two components as diversity across a landscape (gamma-diversity). The effect of the spatial scale of sampling on alpha- and beta-diversity patterns in the same highly diverse ecosystem has rarely been assessed. South African grasslands are both diverse and understudied, providing an ideal location to assess diversity patterns, their drivers, and how spatial scale impacts these. Despite being a grassland, the diversity of this biome is ascribed to herbaceous forb species, even though the majority of its biomass is comprised of grasses. The first aim of this dissertation was to assess patterns of forb alpha- and beta-diversity at species-, genus-, and family-level and the drivers thereof. The second aim was to assess the effect of spatial scale on the species-level diversity patterns and drivers. Forty-two grassland sites were sampled and the species, genus, and family alpha- and beta-diversity, calculated. A total of 487 species, belonging to 146 genera and 49 families were recorded. Beta-diversity was also examined at each taxonomic level via the use of the Jaccard distance index with the dissimilarity at species, genus and family level being 89.52 ± 5.93 %, 79.14 ± 8.82 % and 65.54 ± 9.85% respectively. Spatial grain had a pronounced effect on the non-parametric interpolation of gamma-diversity. Large grain alpha-diversity significantly increased with slope and certain soil and geology types and decreased with an increase in the degree of northness. Intermediate grain alpha-diversity was also significantly higher on certain soil types and textures. Small grain alpha-diversity significantly increased with longer fire return intervals and decreased with higher grazing pressure. The mean beta-diversity at large, intermediate and small grains was 89.52 ± 5.93%, 94.42 ± 5.12% and 97.34 ± 5.49% respectively. Turnover was the most important component of beta-diversity at all spatial grains. Geographic distance and the difference in the degree of northness between sites significantly increased beta-diversity across all spatial grains. In addition to this, the presence of the vertisol soil type and the difference in the days since the last fire increased beta-diversity at large, and intermediate grains respectively. Little variation in beta-diversity was explained at a small grain. The high levels of diversity of the South African grassland biome across all taxonomic levels was demonstrated by this study. Grassland sites were not only species rich, but demonstrated high compositional turnover, which indicated that unique suites of species can

be found in different regions. The pronounced effect of spatial scale on the patterns and drivers of diversity became apparent. Therefore, taking spatial scale and environmental gradients into account when making decisions with regards to systematic conservation planning or environmental impact assessments is critical, especially in highly diverse systems, such as the grassland biome. This will ensure the effective conservation of these systems and the associated ecosystem services.

List of abbreviations

AIC – Akaike information criterion

GDM – Generalized dissimilarity modelling

GLM - Generalized linear model

GPS – Global positioning system

ISRIC – International soil reference and information Centre

PCA – Principal component analyses

S – Species richness

SOTER – Soil and terrain

Table of Contents

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Declaration of originality

Full names of student:...… Annerine Myburgh ……………………………………

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Declaration

1. I understand what plagiarism is and am aware of the University's policy in this regard.
2. I declare that this ……………dissertation………………………… is my own original work. Where other people's work has been used (either from a printed source, Internet or any other source), this has been properly acknowledged and referenced in accordance with departmental requirements.
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Chapter 1

General introduction

Grassland diversity

As the dawn broke on the beginning of the Quaternary period, open grassland vegetation began to spread southwards from the equator (Bond, 2016; Carbutt et al., 2011; Osborne, 2008). The grassland biome is therefore relatively young in the geological time context and has consequently been described by some studies as an anthropogenically driven ecosystem state (Bastin et al., 2019; Nerlekar & Veldman, 2020; Veldman et al., 2015). However, grasslands represent mature ecological climax communities that existed under the same edaphic and climatic conditions as their forest counterparts, millennia before the existence of humans (Anderson, 2006; Bond & Parr, 2010; Uys et al., 2004). These ancient grasslands consequently evolved to house a vast range of endemic flora and fauna that are adapted to thrive in this open ecosystem (Anderson, 2006; Bond & Parr, 2010; Uys et al., 2004).

The grassland biome constitutes high altitude, treeless ecosystems, dominated in biomass by grasses (Mucina and Rutherford, 2006). The fire-adapted, shade intolerant grassland vegetation are adapted to lightning-induced cycles of fire to maintain the open structure of this system. Different positive environmental feedback loops maintain both the forest and the grassland ecosystems respectively, thereby ensuring a distinct boundary between the two systems (Bond, 2016; Bond & Parr, 2010; Harrison et al., 2003). However, in the event of anthropogenically driven changes to the fire regime, a shift may occur resulting in low intensity fires during the early dry season due to a lack of sufficient dry material to burn. Subsequently, fire intolerant, woody vegetation gradually invade the grasslands, expanding the forest margins (Bond & Parr, 2010; Uys et al., 2004). Therefore, the current anthropogenically driven expansion of forest backs the notion that grasslands may have had a more extensive, natural distribution across Africa between 33 and 26 thousand years ago (Anderson, 2006; Bond, 2016; Carbutt et al., 2017).

Presently, the southern African grassland biome covers approximately 28% of the terrestrial land surface area of the subcontinent that includes South Africa, Lesotho and Eswatini (*Figure 1 A*) (Carbutt et al., 2011; Muller et al., 2021). The grassland biome is the second largest South African biome and stretches across the inland plateau of southern Africa, extending to the mountainous regions below the escarpment and the high-lying regions of the eastern coast of the Eastern Cape, Mpumalanga and KwaZulu-Natal provinces (Carbutt et al., 2011).The majority of the biome's biomass is made up of C4 grass species, while the C3 grass species dominate the high lying, montane grasslands (*Figure 1 B*) (Anderson, 2006; Bond, 2016; Carbutt et al., 2017). However, the diversity of the southern African grasslands can be attributed to the vast range of herbaceous grassland forb species (Muller et al., 2021; Thuiller et al., 2006; Uys, 2006; Zaloumis & Bond, 2011). Comparatively little research has been conducted on South African grassland diversity; therefore, the ecological value of these systems has been underestimated for years (Muller et al., 2021; Neke and du Plessis, 2004). The predicted point species richness (within a 1 m^2 plot) being 1.4 times higher than other South African biomes, with the exception of the fynbos biome (Cowling et al., 2003). Extensive research has been done on the phytosociological structure of the grasslands. The classification of many distinct plant communities, often in small areas, in these grassland systems (Bezuidenhout et al., 1994; Bredenkamp and Bezuidenhout, 1990; Bredenkamp and Theron, 1976; Hoare and Bredenkamp, 2001; Grobler et al. 2006; Matthews et al., 1991; Smit et al., 1997) suggests that beta-diversity in these systems. Other uncertainties about forb diversity arise because forb flowering phenology, and the effect of fire intensity and fire return interval on these, remains unstudied (Fidelis and Blanco, 2014; Lamont and Downes, 2011; Pillips, 1920). Single-time-point diversity assessments in these systems may underestimate grassland diversity, as the timing of sampling will influence the number of flowering species observed.

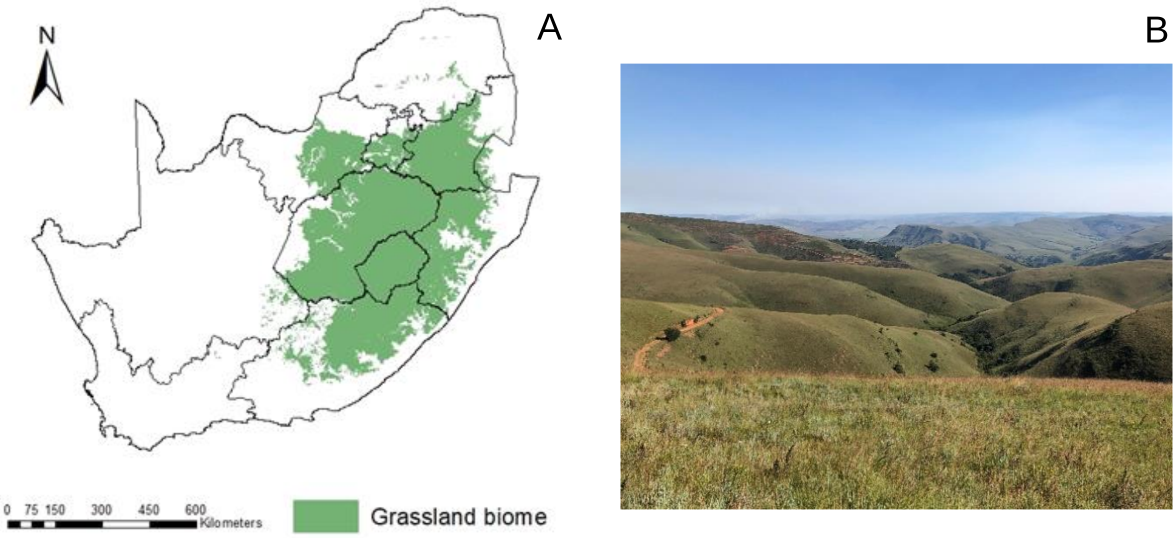

Figure 1: (A) A map depicting the location of the grassland biome of southern Africa. The biome level map was obtained from the South African National Biodiversity Institute's National Vegetation Map project (Mucina et al., 2018). (B) A photograph depicting a typical South African grassland.

Threats to grasslands

The cradle of humankind rests in the grasslands of Africa (Bobe and Behrensmeyer, 2004; Bond and Parr, 2010). Therefore, it is not surprising that humans and grasslands have been intricately linked for millennia (Bond and Parr, 2010; Carbutt et al., 2017; Egoh et al., 2011; Ellis, 2011). Vast coal and gold reserves are distributed throughout the grassland biome (Mentis, 2006; Neke & du Plessis, 2004; Olivier, 2020). The mineral resources, coupled with agricultural activities, have led to the transformation of large areas of this biome (Neke and du Plessis, 2004a; Olivier, 2020). Transformation poses a severe threat to the integrity of the grassland biome, as it has been demonstrated that grassland species richness is decreased in secondary, transformed grasslands when compared to untransformed grasslands (Siebert, 2011). Much of the natural grasslands were also transformed by plantation forestry, with 1.3 million ha of exotic tree species having been planted in South Africa, with most plantations being established in the grassland biome (Morris and Scott-Shaw, 2019; Neke and du Plessis, 2004a; Von Maltitz et al., 2019). The effect of overgrazing is pronounced in these systems with notable changes in the community composition being associated with overgrazing, as these systems are often only managed to suit agricultural purposes with no regard for the conservation of grassland diversity (Little et al., 2015; Morris and Scott-Shaw, 2019; Bredenkamp et al., 2006). Tree planting (afforestation), on the other hand, has relatively recently emerged as a growing threat to grassland integrity. Tree planting has been proposed as a silver bullet solution to mitigate climate change through carbon sequestration; however, large regions that have been put forward as suitable for afforestation, are in fact ancient open ecosystems. Therefore, the tree planting movement poses a severe threat to the diversity of grasslands (Bastin et al., 2019; Bond, 2016; Canadell and Raupach, 2008; Veldman et al., 2015b).

The remaining natural grasslands are often managed to suit the purposes of the agricultural industry, in terms of burning and grazing, and not to maintain diversity (Gordijn et al., 2018; Nerlekar and Veldman, 2020; O'Connor, 2005; Uys, 2006). These management regimes are often inappropriate to preserve the structure of these complex systems in terms of biomass, diversity and plant growth responses (Scott-Shaw and Morris, 2015; Uys et al., 2004; Venter et al., 2020). Appropriate burning and grazing regimes are important to maintain grassland diversity (Anderson, 2006; Gordijn et al., 2018; Morris et al., 2021; Solofondranohatra et al., 2020). However, the effect of burning and grazing are different for the herbaceous and grassy component (Little et al., 2015; Nerlekar and Veldman, 2020; Uys et al., 2004). Forb species are fairly resilient to the effect of burning; though relatively short fire return intervals yield the highest forb species richness, while high pyrodiversity generally increases compositional turnover (Gordijn et al., 2018; Gordijn & O'Connor, 2021; Morris et al., 2021). High intensity grazing, on the other hand, decreases forb species richness and also alters the forb species composition (Harrison et al., 2003; Scott-Shaw & Morris, 2015). Grass species richness increases at intermediate fire- return intervals, while compositional turnover increases with short fire- return intervals (Gordijn et al., 2018; Gordijn & O'Connor, 2021; Morris et al., 2021). Additionally, in the face of heavy overgrazing, unpalatable, tall grass species tend to increase, which often out shade the shade-intolerant forb species, altering the species composition and reducing forb abundance (Bond and Parr, 2010; Scott-Shaw and Morris, 2015; Wilcox et al., 2020). As forb species diversity underlies the majority of grassland diversity, maintaining and understanding the drivers of forb diversity is critical for grassland conservation and management (Bond, 2016; Carbutt et al., 2017; Nerlekar and Veldman, 2020; Zaloumis and Bond, 2011).

Understanding diversity and its measurement

The increased global awareness of open ecosystem value is starting to shift the focus from grassland management solely for agricultural purposes, to realizing the importance of managing grassland ecosystems to maintain diversity. This transition to a more sustainable approach to open ecosystem management is not simply important from an aesthetic perspective, but to maintain ecosystem functioning, provisioning of ecosystem services and overall ecosystem stability and resilience (Balvanera et al., 2006; Duffy, 2009; Tilman et al., 1997; Wortley et al., 2013). However, the first step to achieve this goal is to accurately quantify patterns of grassland floristic diversity and its environmental underpinnings.

Diversity can be understood at many scales. Local diversity is often quantified as species richness or alpha-diversity (Gaston, 2000; Moreno et al., 2009a; Whittaker, 1972a). Beta-diversity quantifies species compositional changes between sampled sites (Baselga, 2010; Bush et al., 2016; Jost, 2007a; Mori et al., 2018; Socolar et al., 2016). Together, alpha- and beta-diversity across a landscape is known as gamma-diversity (Gaston, 2000; Moreno et al., 2009a; Whittaker, 1972a). Both richness and compositional dissimilarity across a landscape are needed to inform conservation planning and management (Kukkala and Moilanen, 2013; Rondinini et al., 2006; Slootweg and Kolhoff, 2003; Socolar et al., 2016). Identifying areas of high species richness can be flagged using alpha-diversity measures, while beta-diversity among regions is useful to identify compositionally unique systems irrespective of richness, and also assists in conserving the maximum diversity in the smallest area, if limited space and funds are available.

Species-level presence-absence data is useful to quantify diversity patterns; however, quantifying diversity patterns at higher taxonomic levels, such as genus- and family-level is appropriate, as this can be used as a proxy for evolutionary and phylogenetic histories (Chao et al., 2014; Gaston, 2000; Neeson et al., 2013). In traditional methods of estimating diversity, all species are considered to be equally different from each other. However, the importance of considering evolutionary history and divergence between species has come to the foreground. Communities with high taxonomic and consequently, evolutionary and phylogenetic diversity are considered more diverse than communities with low taxonomic diversity. Therefore, communities that have species assemblages that belong to a wide range of genera and families are functionally and evolutionary more divergent (Chao et al., 2014; Gaston, 2000; Neeson et al., 2013).

Accurately quantifying diversity patterns is further complicated by the fact that diversity assessments are influenced by the spatial scale of the assessment (Barton et al., 2013; Talebi et al., 2021; Wills and Read, 2017). Spatial scale encompasses both spatial extent (the distance between sampled sites) and spatial grain (the area of the sampled sites). Each of these spatial components affect diversity pattern measures and drivers in a distinct manner (Barton et al., 2013; mac Nally et al., 2004; Wills and Read, 2017). Alpha-diversity increases with an increase in both spatial extent and grain (Barton et al., 2013; Palmer and White, 1994). An increase in the spatial grain leads to a larger portion of the total number of species per unit measurement being sampled by chance and due to microhabitat and niche specialization, where specific species occupy and are adapted to very distinct regions within a sampled site, such as around rocks or in shaded areas (Barton et al., 2013; Palmer and White, 1994; Talebi et al., 2021). Therefore, if a larger area is sampled, the chance of sampling more species that only occur in specific microhabitats, increases. A greater extent of sampling, on the other hand, increases the likelihood of sampling additional species. This is because due to increased habitat heterogeneity over larger distances and due to a decrease in spatial autocorrelation (Mistral et al., 2000). Habitat heterogeneity and isolation may increase species richness, as certain species are adapted to live under a specific set of environmental conditions or may be geographically isolated from the other communities. An increase in the extent of sampling will increase the probability of sampling more species that occupy different habitats that are geographically isolated from each other (Barton et al., 2013; Palmer and White, 1994; Talebi et al., 2021). An increase in the extent of sampling will also decrease spatial autocorrelation, where sites that are close to each other are more likely to have similar species assemblages, while sampling sites that are further apart will increase the possibility of sampling new species (Mistral et al., 2000).

Beta-diversity is influenced by both spatial grain and extent, as well as the interaction between these two spatial components (Barton et al., 2013; Steinbauer et al., 2012). Sampling larger spatial grain sizes often results in a decrease in the compositional dissimilarity between sites (Barton et al., 2013). When spatial extent is fixed, the likelihood of sampling very different species assemblages across different plots decreases with an increase in the area of the sampled plot. A larger portion of the total species pool and most widespread species may be sampled by chance and due to the sampling of species from a variety of microhabitats in a larger sampled area, thereby decreasing the compositional dissimilarity between sampled sites as the grain of sampling increases (Barton et al., 2013; Steinbauer et al., 2012). An increase in spatial extent, on the other hand, will generally lead to an increase in compositional dissimilarity when the spatial grain is fixed. Increased compositional dissimilarity is observed with an increase in the distance between sampled sites due to both an increase in environmental heterogeneity and a decrease in spatial autocorrelation. The chance of sampling different species assemblages, that are adapted to specific sets of environmental conditions, across a landscape will increase with the distance between sites, as distant sites are more likely to have different environmental conditions when compared to sites that are located closer together (Barton et al., 2013). Furthermore, sites that are located close to each other have a greater chance of having similar species assemblages due to spatial autocorrelation (Mistral et al., 2000). Therefore, more insight into diversity patterns and drivers, as well as the effect of the spatial scale on the observed diversity patterns and drivers is essential to understand how to effectively assess, manage and conserve these systems. Quantifying how spatial scale influences the results of diversity analyses in highly diverse ecosystems, such as the grassland biome, will be useful not only in the context of grassland conservation, but also in the application of diversity assessments in other highly diverse ecosystems.

Aims and objectives

The aim of this dissertation is to quantify diversity patterns and drivers and the effect of spatial extent and grain on the observed patterns and drivers in the under-sampled grassland regions of South Africa. This dissertation is presented as two studies built around data that was collected over three months during the spring and summer of 2020 and 2021 in the under-sampled grassland regions of South Africa across 42 sites. Chapter 2 of this dissertation quantifies patterns and drivers of alpha- and beta-diversity of grassland forbs at different taxonomic levels. The objectives of this chapter are to assess patterns of alpha- and beta-diversity at species-, genus- and family-level; and to determine which (if any) landscape, climatic and disturbance variables regulate the observed patterns of alpha- and beta-diversity at species-level. I hypothesise that alpha diversity of forbs is high in these systems and that the observed alpha-diversity is regulated by certain climatic (precipitation and temperature gradients), landscape (geology, soil type and soil texture) or disturbance variables (grazing pressure and fire) (Cowling et al., 2003; Mucina et al., 2018). I also hypothesise that beta-diversity of forbs is high in these systems and that the observed beta-diversity of forbs is driven by the difference in climatic variables such as mean annual precipitation and mean annual temperature (Cheng et al., 2011; Talebi et al., 2021), landscape variables such as slope or soil type (Dembicz et al., 2021; Filibeck et al. 2019; Talebi et al., 2021) and disturbance variables such as fire return interval and grazing pressure (Alrababah et al. 2007; Biondini et al., 1989), between sites. The aim of Chapter 3 was to determine the effect of spatial scale on estimates of diversity measures and drivers. The first objective of this chapter was to assess what the effect of spatial extent is on estimates of beta-diversity. The second objective was to assess the effect of spatial grain on the patterns of gamma- and beta-diversity. The third objective was to assess the effect of spatial grain on the drivers of both alpha- and beta-diversity. I hypothesise that an increase in the spatial extent of sampling will increase estimates of beta-diversity. Secondly, I hypothesise that increasing the spatial grain of sampling will increase the estimates of gamma-diversity but decrease the estimates of beta-diversity. Lastly, I hypothesise that the drivers of both alpha- and beta-diversity will change with the spatial grain of sampling.

This dissertation demonstrates that the grasslands of South Africa are exceptionally diverse across all taxonomic levels. The immense diversity of forb species across all taxonomic levels in the sampled grassland regions is highlighted as well as the importance of considering both richness and compositional dissimilarity in these systems when quantifying diversity patterns. Aspect, soil and geology type, as well as grazing and fire regimes regulate diversity patterns. Therefore, it is important to consider these landscape and disturbance gradients when selecting sites for conservation and when conducting environmental impact assessments, as diversity patterns vary along these gradients. However, the spatial extent and grain of sampling influence estimates of diversity patterns and drivers. The research findings presented here have implications for understanding diversity patterns in the under-sampled and threatened grassland biome of South Africa and how sampling extent and grain impact diversity assessments in highly diverse ecosystems.

Chapter 2

Quantifying alpha- and beta-diversity in the historically under-sampled grasslands of South Africa

Introduction

Ancient, open grassy systems were moulded by natural cycles of fire and herbivory, millennia before the existence of humans (Bond, 2016; Morris, 2021; Ratnam et al., 2016; Solofondranohatra et al., 2020). Nevertheless, grasslands and savannas are often regarded as anthropogenically degraded systems (Bastin et al., 2019; Bond and Parr, 2010; Muller et al., 2021; J. W. Veldman et al., 2015a). This can mainly be attributed to the Eurocentric notion that grassy systems are an early successional stage, with forests being the climax vegetation communities (Bastin et al., 2019; Bond, 2016; Solofondranohatra et al., 2020; Veldman et al., 2015b). Under this misguided paradigm, open vegetation is thought to exist as a result of anthropogenically driven deforestation and burning events (Bastin et al., 2019; Bond, 2016; Bond and Parr, 2010; Solofondranohatra et al., 2020). Consequently, little distinction has been made between ancient, old-growth grasslands (that have not been anthropogenically transformed in any way) and secondary grasslands (any anthropogenically altered grassland that have been left to recover) (Nerlekar and Veldman, 2020; Veldman et al., 2015). The fact that ancient and secondary grasslands are lumped together is problematic as secondary grasslands are generally deprived in terms of species richness and differ vastly from old-growth grasslands with regards to species composition (Nerlekar and Veldman, 2020; Veldman et al., 2015). Thus, grassland systems have been under-valued and under-studied in most parts of the world (Bond, 2016; Ratnam et al., 2016; Solofondranohatra et al., 2020; Uys, 2006).

In South Africa, where grasslands occupy approximately one third of the land area, these ecosystems are both the most transformed and least protected (Carbutt et al., 2011; Morris, 2021; Muller et al., 2021). Up to 60% of grasslands have been transformed, with 28.8% of the biome considered threatened and with only 3% being formally protected (Carbutt et al., 2011; Little et al., 2015; Skowno and Monyeki, 2021). Some unprotected grasslands remain intact and only lightly utilised, with approximately 15% of the biome used as rangelands for game and livestock (as of 2009) that have minimal impacts on grassland diversity at moderate grazing severities (O’Connor and Kuyler, 2009). However, other anthropogenically driven threats are ever-present and growing in the form of mining, forestry, agriculture and tree planting (Carbutt et al., 2011; Egoh et al., 2011; Muller et al., 2021; Neke and du Plessis, 2004b; O'Connor and Kuyler, 2009; Olivier, 2020).

The grassland biome is exceptionally species rich, as the point diversity of this biome is thought to be 1.4 times higher than other biomes, with the exception of the fynbos biome (Cowling et al., 2003). However, on a larger spatial scale (1000 m^2) the overall average richness of the grassland biome was higher than that of the fynbos biome (Cowling et al., 2003). The exceptional grassland floristic diversity is also showcased at higher taxonomic levels, as grassland family-level richness exceeds the diversity of the Cape floristic region by a factor of three (Bond and Parr, 2010; Cowling et al., 2003; Scott-Shaw and Morris, 2015; Uys, 2006). The grassland biome supports unique and often threatened or endangered plant communities (Carbutt et al., 2017, 2011; Neke and du Plessis, 2004) including three centres of plant endemism, namely the Wolkberg centre, the Drakensberg Alpine centre and the Midlands Putative centre (Carbutt et al., 2011; Mucina and Rutherford, 2006; O'Connor and Kuyler, 2008). The high diversity associated with this biome can largely be attributed to forb species, even though the majority of the biomass is made up of grass species (Morris, 2021; Muller et al., 2021).

High levels of diversity is associated with effective ecosystem functioning, which in turn translates into the provisioning of ecosystem services (De Groot et al., 2002; Egoh et al., 2011; O'Connor and Kuyler, 2009; Siebert and Dreber, 2019; Zuo et al., 2012). The grassland biome provides essential ecosystem services, such as carbon sequestration, the prevention of erosion, water purification and the regulation of the hydrological cycle (De Groot et al., 2002; Egoh et al., 2011; O'Connor and Kuyler, 2009). Diversity is also associated with functional redundancy in grassland ecosystems. This decreases the possibility of invasions by exotic species and provides ecosystem resilience to disturbances such as drought (Morris and Scott-Shaw, 2019). Therefore, understanding and conserving the diversity of the South African grassland biome is of paramount importance, not only from a biological perspective, but for the continued functioning of ecological processes across the globe. However, a poor understanding currently exists of the true diversity across the expanse of this biome, as large regions have been historically under-sampled (Bond and Parr, 2010).

Diversity is often measured in terms of species richness (alpha-diversity) (Gaston, 2000; Moreno et al., 2009a; Uys, 2006). However, increasing emphasis is being placed on estimating differences in the species composition between sites (beta-diversity), as beta-diversity is thought to contribute significantly to landscape-level diversity (gamma-diversity) (Barton et al., 2013; Jost, 2007; Sepkoski, 1988; Soininen et al., 2018). High alpha-diversity at local scales does not directly translate into high-gamma diversity, as gamma diversity is dependent on the independent alpha- and beta-diversity components across a landscape. However, alpha-diversity can be used to identify single grassland sites with exceptionally high richness which should be flagged for conservation and should ideally not be transformed (Wilson et al., 2005). Beta-diversity, on the other hand, is a useful tool to measure the compositional changes of species across a landscape, which indicates whether larger areas should be sampled to determine the true conservation value of a grassland region (Baselga, 2010; Sepkoski, 1988; Whittaker, 1972b; Wilson et al., 2005). Quantifying beta-diversity informs conservation planning efforts and aids in the construction of landscape-level interconnected conservation areas that represent the maximal diversity (Bush et al., 2016; Slootweg and Kolhoff, 2003).

Therefore, accurately quantifying grassland diversity patterns and drivers can be used as a tool to help limit the ongoing impact of the multiple threats to this biome (Carbutt et al., 2011; Holness et al., 2018; Uys, 2006). The importance of understanding what drives and regulates grassland diversity is critical to prioritise sites for conservation and to understand how to effectively manage these systems. Fire, climate and grazing are known drivers of grassland richness, community composition and productivity (Buitenwerf et al., 2011; Wilcox et al., 2020; Zuo et al., 2012). High structural diversity has also been shown to increase grassland richnessand turnover (Hoare and Bredenkamp, 2001). Forb diversity is dependent on a fairly regular burning intervals to maintain diversity (Biondini et al., 1989; Morris, 2017; Wilcox et al., 2020). South African grassland forb alpha- and beta-diversity increased at relatively short fire return intervals, while forb beta-diversity increased with higher pyrodiversity (Gordijn et al., 2018; Gordijn and O’Connor, 2021). However, it has been demonstrated that the seasonality of burning events has little to no effect on forb alpha-diversity, especially during the dormant season, as only dead, above-ground leaf material is removed (Fynn et al., 2004; Gordijn and O’Connor, 2021).

Forb diversity is also influenced by grazing intensity. Forb alpha- and beta-diversity diversity decreases significantly with high intensity grazing (Harrison et al., 2003; Scott-Shaw and Morris, 2015). In the face of overgrazing, unpalatable, tall grass species tend to increase, which often shade out the shade-intolerant forb species, altering the species composition, as well as reducing forb abundance (Bond and Parr, 2010; Scott-Shaw and Morris, 2015; Wilcox et al., 2020). High intensity grazing also decreases the above-ground photosynthetic material of forbs, which can eventually lead to a decrease the size of underground storage organs and the eventual death of the plant, thereby reducing overall forb abundance (Morris, 2021).

While the influence of grazing and fire on diversity patterns of South African grasslands has been explored, patterns and drivers of grassland diversity has not been assessed at large spatial scales and has mostly only been assessed at species level. This is problematic as conservation efforts are conducted at large spatial scales (Cowling et al., 2003; Morris, 2021; Muller et al., 2021; Uys et al., 2004). The most accurate method to quantify diversity patterns is through the use of species-level data, which forms the basis of systematic conservation planning. Understanding patterns of species diversity across a landscape is critical to determine the distribution of rare or endangered species and to aid in the identification of unique and sensitive grasslands in South Africa. However, obtaining true estimates of the overall species diversity in highly diverse ecosystems is a near impossible task. Estimating diversity patterns at higher taxonomic levels, especially at genus level, can alternatively be used as a proxy for species diversity and phylogenetic history (Moreno et al., 2009; Neeson et al., 2013). Furthermore, understanding what environmental, climatic and landscape factors drive diversity at large spatial scales will assist in effective conservation planning and will provide environmental impact assessments with increased insight into the patterns of species turnover and diversity at larger spatial scales (Kukkala and Moilanen, 2013; Rondinini et al., 2006).

A research gap exists with regards to quantifying the diversity of grassland forbs in terms of not only alpha-diversity, but also beta-diversity and the drivers thereof at a large spatial scale and at higher taxonomic levels. Assessing diversity patterns in the South African grassland biome is of essence, as this biome is thought to be exceptional in terms of diversity, especially when considering forb species (Carbutt et al., 2011; Morris and Scott-Shaw, 2019; Scott-Shaw and Morris, 2015; Uys, 2006). Subsequently, this study aimed to assess the diversity patterns of forbs and the drivers thereof in some of the under-sampled grasslands of South Africa. The objectives were to quantify patterns of alpha-diversity at species-, genus- and family- level; to assess drivers of alpha diversity; to quantify beta-diversity at species-, genus- and family-level; and to assess if there are significant drivers of the observed beta-diversity. Alpha diversity of forbs was anticipated to be high in these systems and was predicted to be regulated by certain climatic variables, such as temperature range, mean annual temperature and mean annual precipitation (Zhang et al., 2018; Talebi et al., 2021), certain landscape variables, such as soil type and or texture (Dembicz et al., 2021; Filibeck et al., 2019) and by certain disturbance variables, such as grazing pressure and fire return interval (Harrison et al., 2003; Koerner and Collins, 2014). Beta-diversity of forbs was also expected to be high in these systems and the observed beta-diversity driven by differences in climatic variables such as mean annual precipitation and mean annual temperature (Cheng et al., 2011; Talebi et al., 2021), landscape variables such as slope or soil type (Dembicz et al., 2021; Filibeck et al. 2019; Talebi et al., 2021) and disturbance variables such as fire return interval and grazing pressure (Alrababah et al. 2007; Biondini et al., 1989), between sites.

The results from this research can be used to further the abilities of environmental impact assessments to accurately quantify floristic diversity patterns in grassland ecosystems. A better understanding of the impact of grassland transformation on the loss of unique suites of grassland forbs will be obtained. This will further aid conservation initiatives in the preservation of the largest number of species in the smallest possible area. Furthermore, this study will also determine if a link exist between the observed alpha- and beta-diversity patterns and certain climatic, landscape and disturbance drivers. This information will be useful when determining which areas should be prioritized for conservation and which areas can be transformed with a minimal impact on grassland diversity. In addition to this, sampling was done in historically under-studied grasslands in regions that are otherwise heavily transformed; therefore, the results from this research will also contribute to an improved understanding of the diversity of these grasslands.

Methods

Site selection

The little-sampled north-eastern, moister grasslands of South Africa spanning northern KwaZulu-Natal, southern Mpumalanga, and Gauteng were examined (*Figure 2, Table S1*). Forty-two grassland sites with no history of ploughing, which were burned within six months of sampling, were identified to be sampled. Selected sites were not overgrazed or otherwise transformed. The landowner of each site was consulted about the land use histories of the sampled site in order to confirm that the sites were not previously transformed. There was some variation in the extent of grazing and type of grazers (cattle, sheep and game) at each site, as grazing is an inevitable part of these landscapes. Grazing intensity ranged from lightly grazed, moderately grazed to frequently grazed. The sites were situated on privately owned land, and permission was obtained from all landowners to sample on their land.

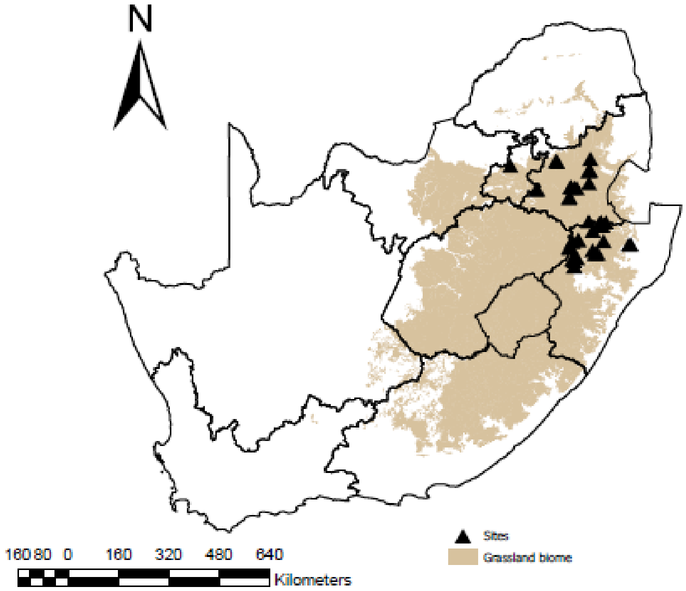

Figure 2: A map outlining the grassland biome of South Africa (in brown) and the location of the 42 sites that were sampled for this study. The biome level map was obtained from the South African National Biodiversity Institute's National Vegetation Map project (Mucina et al., 2018).

Data collection

Sampling was undertaken according to the *Global Grassy Group protocol* (globalgrassygroup.github.io). Forty-two sites with homogenous vegetation were selected. Where possible, sites were selected to represent some topographical variation. At each site, 21 circular plots with a diameter of 1 m were assessed for species richness and composition. The 21 plots (0.79 m^2 per plot) were established in a cross-shaped pattern, orientated along cardinal directions as north-south and east-west (total area 16.59 m^2) (*Figure S1*). A central plot was placed in the middle at the cross point of the two transects, and the latitude and longitude recorded in decimal degrees. Within each plot all the forb species were identified and recorded (which in the context of this study includes herbaceous species, woody forbs, dwarf shrubs and succulents). Exotic species were included in the species list, as they constituted less than 5% of all species. Additionally, a sample of unidentified specimen was often taken to the H.G.W.J. Schweickerdt herbarium (PRU) for identification. In the field, unidentified species were given descriptive pseudonyms to ensure consistent naming at future sites. At every site, herbarium vouchers were taken and *iNaturalist* observations were made of key flowering species. This was done to contribute to future diversity studies and the knowledge base of species distribution and phenology. Care was taken not to damage rare or endangered species. Herbarium and iNaturalist data obtained from this study were deposited into the Foundational Biodiversity Information Programme database (fbip.co.za). Further data were collected at a plot-level as structural characteristics of percentage grass aerial cover (visually estimated), percentage vegetation aerial cover (visually estimated) and percentage bare ground.

At each site, the following data were recorded:

1) Date of sampling

2) Altitude (in metres via a Garmin etrex 30 GPS)

3) Date of the last fire (as described by the landowner)

4) Aspect (with a compass, recorded as one of the four main cardinal directions)

5) Slope (scored on an ordinal scale from 1 to 3, with 1 representing flat to gentle slopes, 2 representing moderate slopes and 3 denoting steep slopes)

6) Average soil depth (recorded in four random plots with a soil auger)

7) Grazing pressure (the grazing pressure was scored on a scale from one to three, with one being slightly to not grazed at all; two being indicative of moderate grazing; and three representing strong evidence of frequent grazing)

8) Soil texture (using the field technique of hand texturing (Salley et al., 2018; Vos et al., 2016), soil texture was classified into one of the following broad groups: clay; clay-loam; loam; sandy-loam; and sand) and soil colour (with the use of a Munsell's soil colour chart) (Marqués-Mateu et al., 2018).

The following additional climatic, landscape and disturbance variables were obtained after sampling was completed with the use of ArcGIS (Environmental Systems Research Institute (ESRI), 2017) for each site after sampling in order to understand if diversity patterns varied with the following gradients:

9) Mean annual precipitation; mean annual temperature; minimum temperature of the coldest month; and temperature range (www.worldclim.com/version2) (Fick and Hijmans, 2017).

10) Northness, as a measure of aspect (recorded at each site with the use of a compass), was

calculated for each site using the following conversion: *cos(aspect in radians)* (Piedallu and Gégout, 2008; Pierce et al., 2005).

11) Soil type obtained from the ISRIC world soil information SOTER database (www.isric.org/explore/soter) (Dijkshoorn et al., 2008).

12) Geology obtained from the Council of Geoscience (www.geoscience.org.za) (Council for Geoscience, 2019).

13) Fire return interval and fire radiative power (measure of fire intensity) was obtained from (Archibald et al., 2013).

Statistical analyses

Alpha-diversity

The total number of species, genera and families were calculated for the 42 sampled sites. Subsequently, the overall number of species and genera per family, as well as the species:genus ratios were obtained. Alpha diversity at plot and site level was determined using the raw species, genus and family richness. To estimate if the sampling effort approached the true number of species, genera and families in these grassland regions, species, genus and family level rarefaction-curves were constructed with one thousand permutations per curve.

To assess environmental correlates of alpha diversity, environmental characteristics were grouped into categories related to landscape, climate and disturbance regimes. Landscape variables are those that describe the physical nature of the sites, namely soil texture, soil type, geology and slope. Climate variables are those that relate to the macro- and microclimate at the sites, namely mean annual precipitation, mean annual temperature, temperature range, minimum temperature of the coldest month and aspect. Disturbance variables are those that relate to disturbance regimes of sites, namely grazing pressure, time since the last fire, fire return interval and fire radiative power (Pélissier et al., 2003; Uys et al., 2004; Zuo et al., 2012).

PCA ordinations were done for each subset of predictor variables i.e., landscape, climate and disturbance variables, with all the variables scaled preceding the analyses. All variables were checked for normality with the use of histograms and a quantile-quantile plots. If the assumptions for normality were violated, the variables were transformed (Paliy and Shankar, 2016; Wigley et al., 2016). Fire radiative power and days since the last fire were square root transformed, while a quadratic transformation was used for mean annual precipitation.

For each PCA, the proportion of variation explained by every principal component axis was compared and the most important principal component axes (with a cut-off value of 15% for the climate PC axes, *Table S2* and 20% for the disturbance PC axes, *Table S3*) within each ordination was selected to be used as predictor variables in the best subset modelling analysis. The first three climate principal components and the first two disturbance principal components were selected to be included in the model selection process (*Table S2 & S3*). Mean annual precipitation and northness related most strongly to climate PC-1 (*Figure S4*), while mean annual temperature related most strongly to climate PC-2 and northness most strongly to climate PC-3 (*Figure S2*). Fire return interval and grazing pressure related most strongly to disturbance PC-1 (*Figure S3*), while days since the last fire related most strongly to disturbance PC-2. In the case of the landscape variables, little variation was explained by each principal component; therefore, the raw variables were incorporated into the best subset modelling analysis, rather than the principal components (*Table S4*).

A full generalized linear model (GLM) with a Poisson distribution, species richness as the response and the three climate principal components, two disturbance principal components and raw landscape variables as the predictor variables, was set up. Best subset modelling was used based on the Akaike Information Criterion (AIC), using the ‘leaps’ algorithm (Aho et al., 2014) to select the most parsimonious model. The GLM model was restricted to four predictor variables, based on the ten events per variable rule (Peduzzi et al., 1996). The results from the best subset modelling (AIC = 294.93), indicated that the best model included soil type, geology, slope and climate PC-3 (*Figure S2*). A dispersion test was also conducted to test for overdispersion. No evidence of overdispersion was found.

Beta-diversity

Beta-diversity was quantified with the use of the inverse Jaccard distance index (1- Jaccard similarity) (Koleff et al., 2003). The Jaccard distance index was selected, as it is robust in the assessment of presence-absence data and also accounts for numerical and geographic under-sampling (Koleff et al., 2003; Schroeder and Jenkins, 2018). Moreover, it has been demonstrated that presence-absence beta-diversity measures are more appropriate in diversity analyses that deal with many rare species (Socolar et al., 2016). The mean species, genus and family beta-diversity at plot and at site level was calculated. Ternary plots were constructed to visualize the dissimilarity in the species, genus and family compositions between sites. Rank-abundance curves were also constructed at species-, genus- and family-level to determine the evenness of species, genera and families.

Generalized dissimilarity modelling (GDM) with the Jaccard distance index was used to assess if certain landscape, climatic and disturbance variables had a significant effect on beta-diversity at site level (Fitzpatrick et al., 2021, 2013; Jewitt et al., 2016). GDM was used, as this method accounts for spatial autocorrelation and assumes a non-linear relationship of species compositional changes across different environmental and disturbance gradients, otherwise known as non-stationarity (Fitzpatrick et al., 2013; Jewitt et al., 2016). Prior to conducting the GDM analyses, an initial correlation matrix containing all the raw landscape, climate and disturbance predictor variables was constructed to eliminate highly correlated variables. The categorical variables, namely soil type and geology were subdivided into their constituent categories in order to construct presence-absence dummy variables for each site. A correlation matrix was subsequently constructed to remove highly correlated variables (*Table S5*). The following highly correlated variables (with a Pearson's correlation coefficient of $r > 0.6$) were eliminated: temperature range, fire radiative power and minimum temperature of the coldest month. The following variables were retained for the full model assessment: geographic distance between sites, time since the last fire, grazing pressure, fire return interval, mean annual precipitation, mean annual temperature, northness, slope, soil type, soil texture and geology. A curvilinear relationship between the compositional dissimilarity of forb species between sites and the dissimilarity in the above-mentioned environmental and disturbance predictor variables were assumed. The default of three I-splines values per predictor was used (partial regression fit of each predictor variable), with the maximum value of the I-spline curve representing the total amount of variation in compositional dissimilarity between sites explained by a particular correlate (Ferrier et al., 2007; Fitzpatrick et al., 2021, 2013; Jewitt et al., 2016). Variable importance was assessed with one thousand permutations in the full model in order to select the best subset of variables.

The percentage deviance change when the variable was included in the fitted model versus when the variable was excluded was used to assess variable importance. In turn, each variable was permuted, while the other variables were kept constant. This process was repeated for one thousand permutations, with the variable importance and significance reassessed with each permutation (Ferrier et al., 2007; Fitzpatrick et al., 2021). Backward selection allowed the model to drop the least important variable at each permutation, until only significant variables remained. A GDM analysis was subsequently conducted on the three most important predictor variables, that was retained by the model selection process to assess if these variables had significant effect on beta-diversity at site level and how much deviance was explained by each predictor variable. The model selection process indicated that the most important predictor variables to include was geographic distance, northness and the vertisol soil type (soils with a clay-like nature, Salley et al., 2018) with 28.95% deviance explained by this model (p-value < 0.05).

Diversity data analyses was done with the use of R (R Core Team, 2018), using the following packages: "psych" (Revelle, 2021a), the "psychTools" (Revelle, 2021b), the "BiodiversityR" (Kindt and Coe, 2005), "dplyr" (Wickham et al., 2021), "leaps" (Lumley and Miller, 2020) "vegan" (Oksanen et al., 2019), "bestglm" (A.I. McLeod et al., 2020), "MASS" (Ripley et al., 2013), "stats" (R Core Team, 2021), "geosphere" (Hijmans, 2019), "gdm" (Fitzpatrick et al., 2021).

Results

Alpha-diversity

Across the 42 sites, 487 forb species in 146 genera and 49 families were recorded (*Table 1*, *Table S8*). The mean number of species per site was 32.6 ±9.7, while the mean number of genera per site was 24.4 ± 8.14 and the mean number of families per site was 14.4 ± 3.91 (*Figure 3 A*). The maximum number of species, genera and families recorded at site-level were 58, 43 and 24 respectively (*Figure 3 A*). The mean number of species per plot was 6.1 ± 3.2, with a mean number of genera and families as 5.6 ± 3.01 and 4.5± 2.13 respectively (*Figure 3 B*). The maximum number of species, genera and families at plot-level was 21, 18 and 14 respectively (*Figure 3 B*).

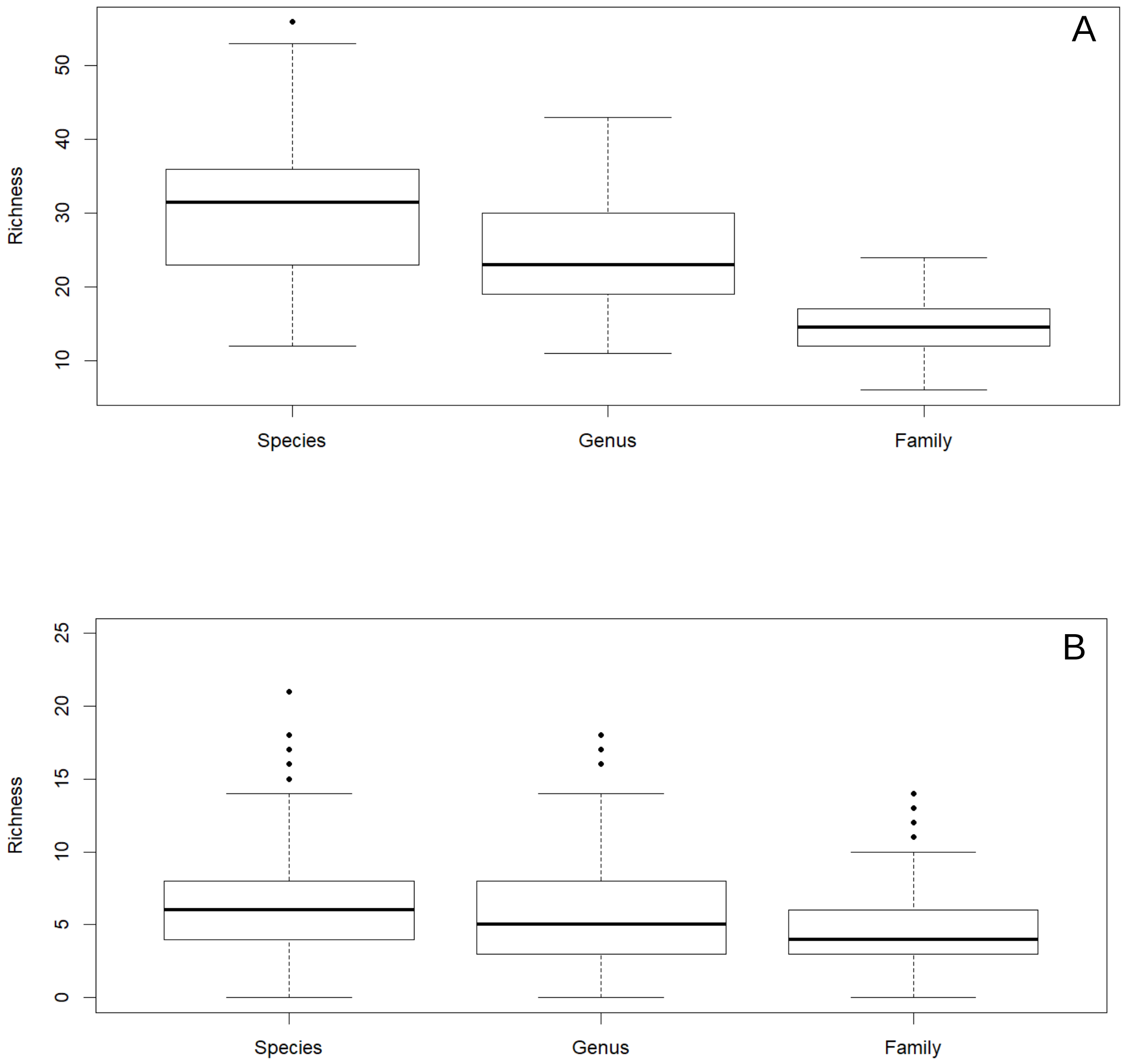

Figure 3: Boxplots of the species, genus and family richness of forbs in the grassland biome of South Africa at site- (A) and plot level (B).

Table 1: A table displaying the number of South African grassland forb species and genera per family, as well as the species:genus ratio per family.

	Families	Number of species	Number of genera	Species:Genus
1	Asteraceae	106	33	3.212121212
2	Fabaceae	62	18	3.444444444
3	Rubiaceae	18	8	2.25
4	Apocynaceae	17	4	4.25
5	Hyacinthaceae	17	4	4.25
6	Acanthaceae	12	5	2.4
7	Hypoxidaceae	12	3	4
8	Iridaceae	11	5	2.2
9	Malvaceae	11	3	3.666666667
10	Convolvulaceae	10	2	5
11	Lamiaceae	10	7	1.428571429
12	Asphodelaceae	8	3	2.666666667
13	Euphorbiaceae	8	3	2.666666667
14	Geraniaceae	7	2	3.5
15	Campanulaceae	6	2	3
16	Crassulaceae	6	2	3
17	Thymelaeaceae	6	2	3
18	Hypericaceae	5	1	5
19	Polygalaceae	5	1	5
20	Scrophulariaceae	5	4	1.25
21	Solanaceae	5	1	5
22	Santalaceae	5	1	5
23	Amaryllidaceae	4	2	2
24	Apiaceae	4	2	2
25	Commelinaceae	4	2	2
26	Lobeliaceae	3	2	1.5
27	Oxalidaceae	3	1	3
28	Plantaginaceae	3	1	3
29	Aizoaceae	2	1	2
30	Boranginaceae	2	1	2
31	Cleomaceae	2	1	2
32	Cucurbitaceae	2	1	2
33	Dipsacaceae	2	1	2
34	Onagraceae	2	1	2
35	Peraceae	2	1	2
36	Phyllanthaceae	2	1	2
37	Polygonaceae	2	1	2
38	Ranunculaceae	2	1	2
39	Verbenaceae	2	2	1

40	Agavaceae	1	1	1
41	Alliaceae	1	1	1
42	Gentianaceae	1	1	1
43	Linaceae	1	1	1
44	Molluginaceae	1	1	1
45	Orobanchaceae	1	1	1
46	Pteridaceae	1	1	1
47	Velloziaceae	1	1	1
48	Violaceae	1	1	1
49	Vitaceae	1	1	1

The family with the highest number of species and genera was Asteraceae, followed by Fabaceae and Rubiaceae, while the highest species:genus ratio were recorded for Hypericaceae, Polygalaceae, Santalaceae and Solanaceae (*Table 1*, *Figure 7 C*). Ten families were represented by a single species, while 26 families were represented by a single genus (*Table 1*), which contributes not only to overall taxonomic richness, but also to compositional dissimilarity between sites. However, the grassland species:genus ratios observed in this study do not vary substantially when compared to the species:genus of the fynbos biome (Goldblatt and Manning, 2002).

The species-level rarefaction curve indicated that the sampling effort did not approach the total number of forb species in the sampled grassland regions, as the rarefaction curve was still rising steadily after 42 sites were sampled (*Figure 4 A*). There was no indication of the curve starting to plateau, as would be the case if the number of species in the sampled grassland regions were exhausted by the sampling effort. Therefore, the true number of forb species in these systems will be much higher than found in this study. The genus-level rarefaction curve also did not flatten off (*Figure 4 B*). This indicated that the true number of genera is still higher than what was observed in this study. The rate at which the family-level rarefaction curve was rising decreased after approximately thirty sites were sampled but was still rising steadily after 42 sites were sampled (*Figure 4 C*).

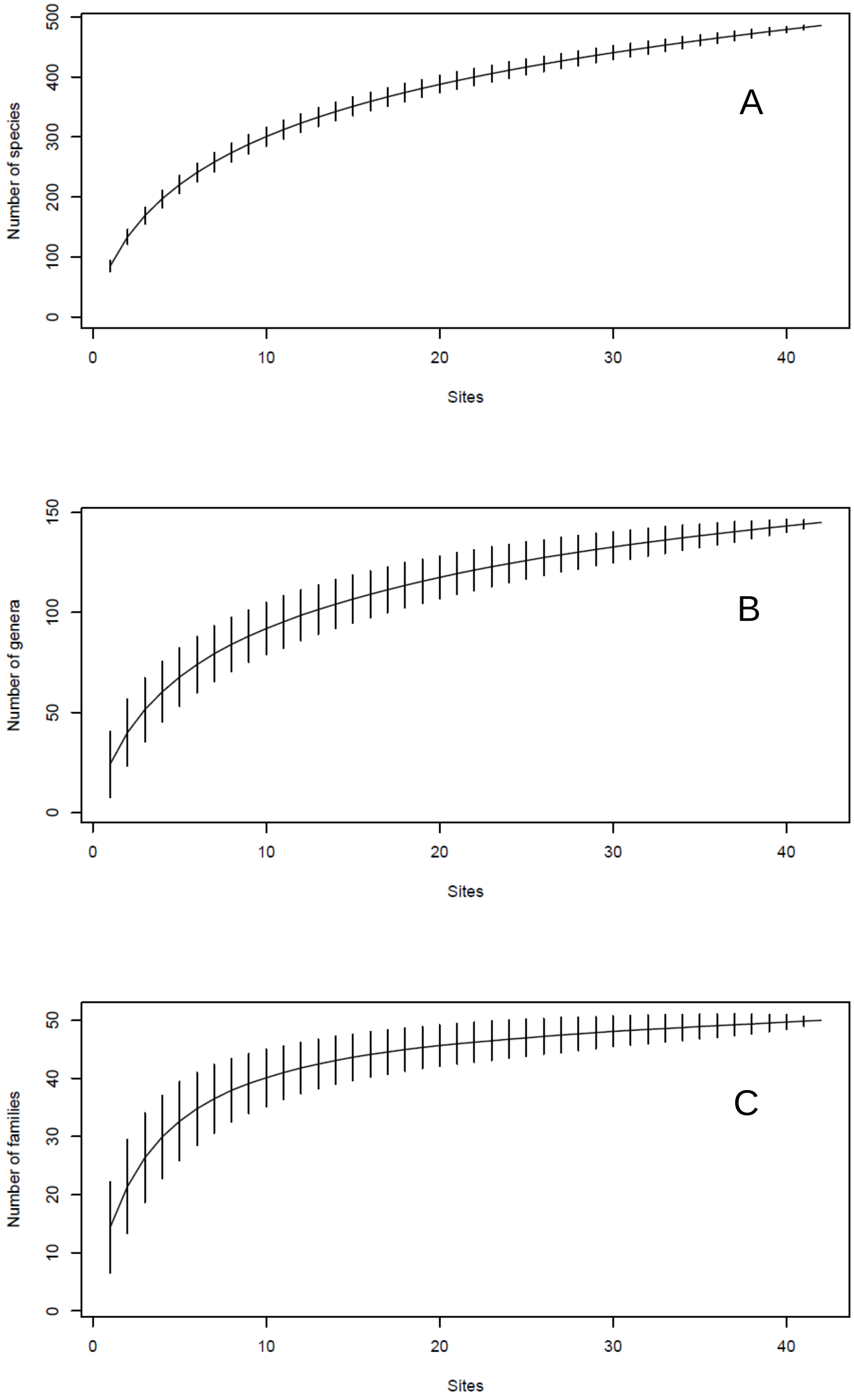

Figure 4: Rarefaction curves of the number of forb species (A), genera (B) and families (C) across 42 sampled sites in the grassland biome of South Africa.

Soil type, geology, slope and the third principal component of the climate variables had a significant effect on alpha-diversity ($p < 0.05$) (*Table 2, Figure S2*), with a pseudo Nagelkerke R^2 value of 0.83. Northness related most strongly to the third principal component of the climate variables (*Figure S2*). Higher species richness was associated with the acrisol soil type (soil with a clay-like nature and high bulk density) and igneous geology type (rocks formed through the solidification of magma) (Cavalli et al., 2020; Paltineanu et al., 2020). Richness increased with slope but decreased with an increase in the degree of northness.

Table 2: Best subset results from a generalized linear model used to assess the effect of climate, landscape and disturbance on forb alpha-diversity in the South African grassland biome. Significant drivers are highlighted in bold. The Tukey 'honest significance difference' method was used to determine if significant differences exist between soil types (Table S7).

Drivers:	**Estimate**	**Chi-square**	**Df**	**p-value**
Soil type	-	18.55	8	**1.75×10^{-2}**
Geology	-	9.73	3	**2.11×10^{-2}**
Slope	0.11	6.68	1	**9.75×10^{-3}**
Climate PC-3	-0.24	16.75	1	**4.27×10^{-5}**

Beta-diversity

The mean species, genus and family beta-diversity at site-level (Jaccard distance) were 89.52 ± 5.93 %, 79.14 ± 8.82 % and 65.54 ± 9.85% respectively (*Figure 5*). The high level of dissimilarity in the species-level beta-diversity between sites was further highlighted by the ternary plot (*Figure* 6 A), as very few species were shared between sites (a'), with the majority of the species belonging either to the focal site (c') or the neighbouring site (b'). The genus-level ternary plot, on the other hand, illustrated that the sites were less dissimilar in terms of genus composition, but that the majority of genera were still not shared between all sites (*Figure 6 B*). At family-level, more families were shared between sites than genera or species (*Figure 6 C*). However, many families were still not shared between sites with more than 50% compositional dissimilarity among many of the sites.

The species-level rank-abundance curve demonstrated that the most dominant species at site-level was the invasive *Richardia brasiliensis*, while the second and third most dominant species were *Ocimum obovatum* and *Helichrysum rugulosum* respectively (*Figure 7 A*). The species-level curve depicted high levels of dominance by a few species. On genus-level, the rank-abundance curve showed that the following genera were the most dominant, in a decreasing order of dominance: *Helichrysum*, *Hypoxis* and *Senecio* (*Figure 7 B*). The family-level rank-abundance curve indicated that Asteraceae was the most dominant, followed by the Fabaceae and Rubiaceae (*Figure 7 C*).

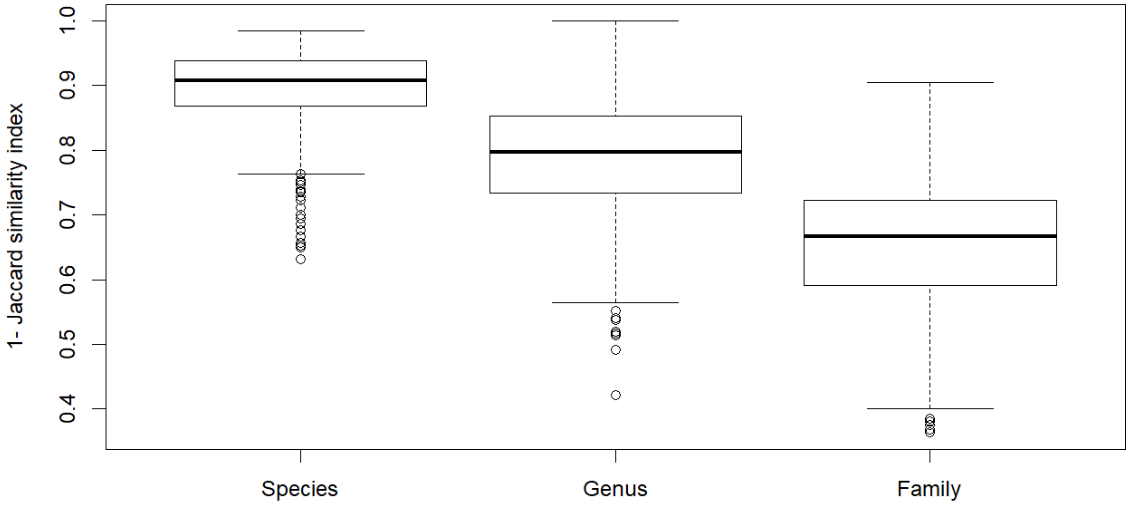

Figure 5: Boxplots of the beta-diversity (Jaccard distance) of forbs in the South African grassland biome at site level at the taxonomic levels of species, genus and family.

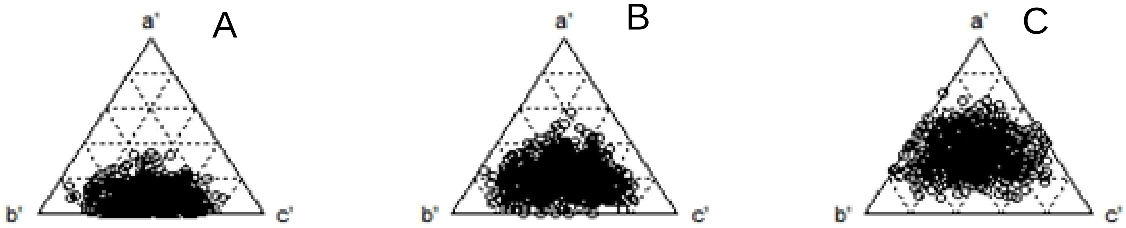

Figure 6: Ternary plots depicting the shared forbs between sites (a'), the forbs unique to the neighbouring site (b') and the forbs unique to the focal site (c') at species- (A), genus- (B) and family-level (C).

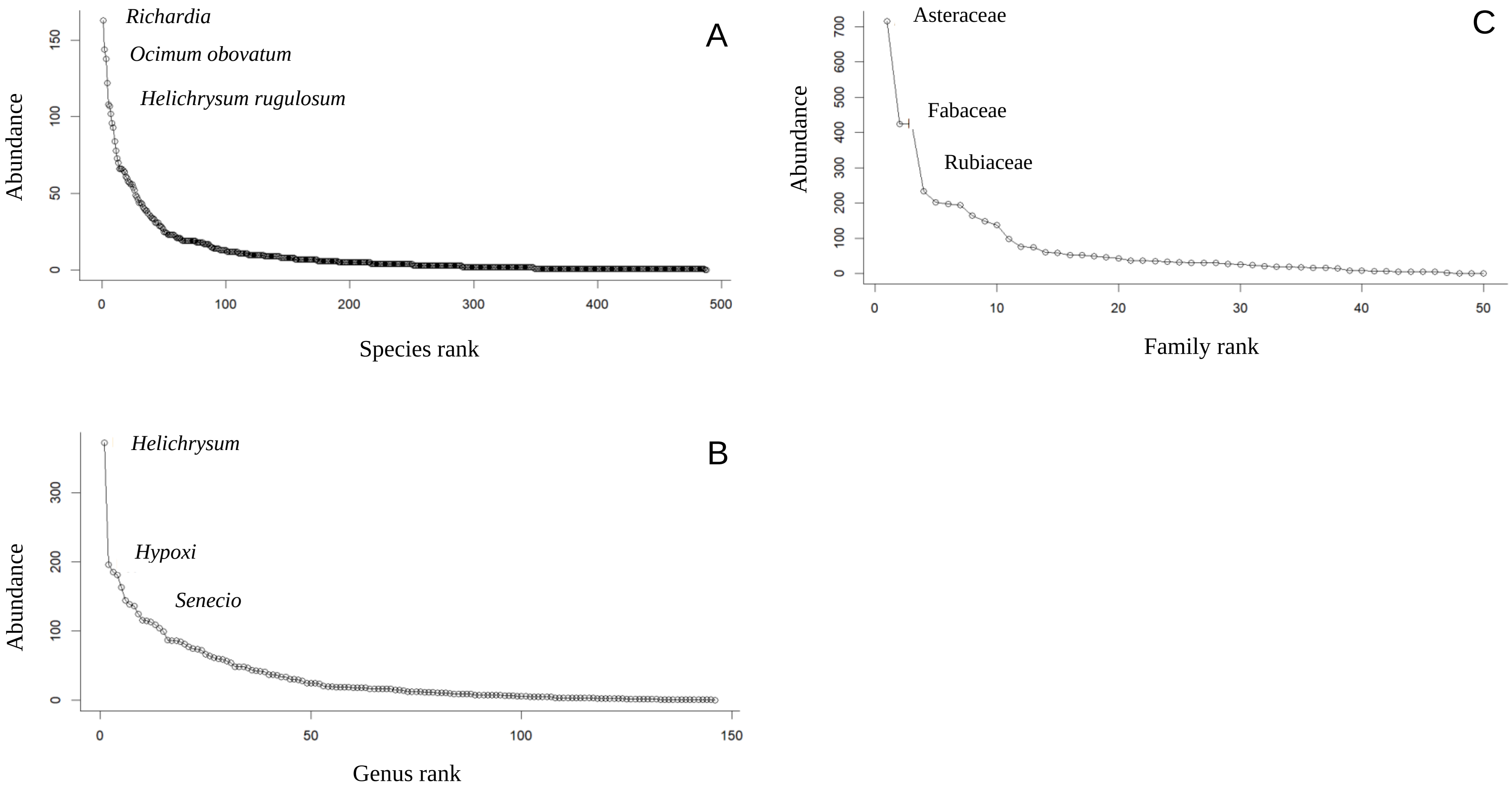

Figure 7: Rank-abundance curves at species- (A), genus- (B) and family-level (C) of forbs in the South African grassland biome. High dominance of a few species, genera and families are depicted, with the three most dominant species, genera and families named on the graphs (in order of dominance).

The results from the GDM analysis indicated that geographic distance, northness and the vertisol soil type had a significant effect on beta-diversity of forb species in the South African grassland biome (*Table 3, Figure S5*). The variation explained by this model was 28.95%.

Table 3: A table displaying the results from the best subset generalized dissimilarity model, constructed with 1000 permutations, to assess the effect of a number of predictor variables on beta-diversity of grassland forbs. The relative variable importance of each predictor, calculated as the sum of the three I-spline coefficients is displayed. The variables that had a significant effect are highlighted in bold.

Driver	**Variable importance**	**p-value**
Geographic distance	73.15	$\mathbf{< 1 \times 10^{-3}}$
Northness	8.65	$\mathbf{3 \times 10^{-2}}$
Vertisol	9.82	$\mathbf{4 \times 10^{-2}}$

Discussion

Comparatively high levels of grassland forb alpha-diversity (across all taxonomic levels) site level and moderately high levels of alpha-diversity at plot level were observed in this study (Cowling et al., 2003; Mucina and Rutherford, 2006). Four hundred and eighty-six species were sampled in 42 sites. The high richness observed in a comparatively small area of the grassland biome in this study suggest that the species richness estimations of 4000 species for the entire grassland biome might underestimate the true gamma diversity of this biome (Cowling et al., 2003; Uys, 2006). The alpha-diversity observed in this study at site level is relatively high compared to previous estimates of South African grassland diversity that predicted grassland species richness to be between 9 and 39 species in a 100 m^2 (Mucina and Rutherford, 2006), while the mean number of forb species observed at site-level (in an area of 16.59 m^2) was 32.6 ±9. The mean plot-level richness that was observed in this study (in a 0.79 m^2 area) was 6.1 ±3.2, which is nowhere near the global maxima of 89 species in a 1 m^2 area that was observed in the semi-natural temperate grasslands of Romania (Wilson et al., 2012). However, the species richness observed in this study is also relatively high when compared to other biomes, such as the drier parts of the savanna biome with 50 to 100 species in 1000 m^2 and the Nama Karoo with an average species richness of 47 species in a 1000 m^2 (Mucina and Rutherford, 2006).

The richness of the grassland biome is further exemplified by the fact that the sampling effort in this study still under-estimated the true number of species in the sampled sites. The accumulation curves, which illustrated that the sampling effort of this study did not approach the true number of species and genera in the sampled region, highlights this notion. It is important to note that the most widespread and abundant species that was observed in this study was the invasive *Richardia brasiliensis*. This species was present in 18 of the sampled sites, which indicates that this species has invaded many natural grassland regions. The family-level accumulation curve indicated that the sampling effort was starting to flatten somewhat at 49 families, though it did not reach an asymptote and kept on rising steadily after 42 sites were sampled, which indicated that the true number of families is likely to be higher than estimated in this study. The high alpha-diversity at all taxonomic levels can be used as a crude indicator of phylogenetic diversity and unique evolutionary histories, as well as the potential for future adaptation (Gaston, 2000; Moreno et al., 2009b; Quan et al., 2018).

The most species- and genus-rich families were the Asteraceae, Fabaceae and Rubiaceae, with 38% of all the recorded species belonging to these families, while ten families were only represented by one species and genus, which echoes the results from previous studies on South African grassland diversity (Botha et al., 2017; Muller et al., 2021). The exceptionally high number of species and genera that belong to the Asteraceae family, when compared to other families in this study, is indicative of the evolutionary success of this family, not only in the grassland regions, but also on a global scale (Panero and Crozier, 2016). The Asteraceae are not only an important component of ecological mature grasslands but are also some of the first species to colonize a disturbed area with the use of wind-dispersed and dormant seeds (Jakobsson and Eriksson, 2003; Muller et al., 2021). These seed traits increase resilience to disturbance (Jakobsson and Eriksson, 2003; Muller et al., 2021). The resprouting ability of the Fabaceae, in addition to the endozoochorous seed dispersal mechanism used by this family, also aids in the ability of this family to resist disturbance and rapidly occupy unpopulated areas (Laudia et al., 1997; Muller et al., 2021). The Rubiaceae on the other hand are wind-dispersed, like the Asteraceae, which indicates that this mode of dispersal may be key to the success of these two families in the grassland biome (Bremer and Eriksson, 1992; Jakobsson and Eriksson, 2003). The high species:genus ratios that were found in multiple families are also indicative of successful evolutionary diversification and radiation (Gaston, 2000; Krug et al., 2008). Low intra-generic competition is predicted where high species:genus ratios are observed as different species occupy distinct ecological niches (Gotelli and Colwell, 2001). The species:genus ratio was not particularly variable between families when compared to the impressively variable species:genus ratios of the Cape Floristic region, where diversification was mainly confined to a few genera (Goldblatt and Manning, 2002). This trend might change if the species:genus ratios were calculated for the entire grassland biome, as only a fraction of this biome was sampled during this study. However, the results from this study suggest that grassland diversity is not only linked to speciation within a few genera, but to diversification at higher taxonomic levels.

The presence of the igneous geology type and acrisol soils was associated with higher alpha-diversity. The weathering of different parent rock material will create the inorganic building blocks of different soil types and subsequently, the ecosystems that can be supported. Therefore, underlying geology and weathering patterns influence the formation of different soil types and textures, which in turn regulates the identity and number of plant species occurring in the soils (Paoli et al., 2006; Van Breemen and Buurman, 2002). Soil types vary in their pH, nutrient availability and water availability (Critchley et al., 2002; Pausas and Austin, 2001; Ratliff et al., 1983). The high species richness on the acrisol soil type may be linked to its clayey nature, high bulk density and the associated increased water holding capacity (Cavalli et al., 2020; Paltineanu et al., 2020). Alpha-diversity also increased at greater slope inclinations. More variable microhabitats on steep slopes may result in increased alpha-diversity as different microclimates may support different species (Stein et al., 2014). Livestock grazing resulted in a decline in species richness; in grasslands selective grazing by livestock decreases overall richness (Cheng et al., 2011; Dembicz et al., 2021b; O'Connor, 2005). An increase in the degree of northness was associated with lower forb alpha-diversity. North-facing slopes receive higher levels of insolation in the southern hemisphere, resulting in increased heat- and drought stress due to the higher levels of solar radiation (Coops et al., 2000; Zeng et al., 2005). This can act as microclimatic filters in regulating richness (Coops et al., 2000; Mahmoudi et al., 2018; Polyakova et al., 2016; Zeng et al., 2005).

It is surprising that macroclimate played no role in predicting alpha diversity across the area considered in this study. Previous studies have found strong links between precipitation and temperature gradients and alpha-diversity (Harrison et al., 2018; Talebi et al., 2021; Zhang et al., 2018). This study assessed diversity patterns only across some extent of the grassland biome; the driest and the wettest part of the biome were excluded. However, this study was conducted at a relatively large spatial scale, where the importance of precipitation and temperature gradients did not come out as important. The range in mean annual precipitation (250 mm) and mean annual temperature (4.32 °C) across all sites was not very large, which may also explain why precipitation and temperature gradients were not important drivers of alpha-diversity (*Table S1*). Findings may change if the effect of e.g. rainfall on the diversity across the entire grassland biome was assessed, as rainfall ranges between 400-2500 mm across the biome (Mucina and Rutherford, 2006). Therefore, using future climate change predictions as a tool for systematic conservation planning to conserve the maximum alpha-diversity in grasslands will be ineffective in this area of the grassland biome (Harrison et al., 2018; Midgley et al., 2002).

The results from this study suggest that, for effective conservation planning, maximising conserved areas on relatively steep slopes with southern aspects, on acrisol soils and igneous geologies, will maximise grassland alpha-diversity, regardless of macroclimatic precipitation and temperature gradients (Anderson, 2006; Liancourt et al., 2013; Nerlekar and Veldman, 2020; Silveira et al., 2020; Slootweg and Kolhoff, 2003; Uys et al., 2004). However, solely selecting grassland regions to conserve along these gradients will not maximize the total number of species conserved due to the high levels of beta-diversity between grassland sites. The grassland regions that were assessed during this study were characterized by high beta-diversity at all taxonomic levels. High compositional dissimilarity at species, but also at higher taxonomic levels indicate that sampled grassland sites do not only differ from one another in terms of species composition, but also in terms of phylogenetic and evolutionary histories (Chao et al., 2014; Gaston, 2000; Neeson et al., 2013). Higher taxonomic diversity between sites will consequently increase overall diversity further, as forbs at different grassland sites are not closely related, but phylogenetically diverse and thereby functionally diverse (Chao et al., 2014; Gaston, 2000; Neeson et al., 2013).

An increase in beta-diversity was observed with an increase in geographic distance and the difference in the degree of northness between sites. The species composition of vertisol soils were also different to those of other soils. Soil types that were not sampled frequently may not have come out as significant drivers of beta-diversity. An increase in compositional dissimilarity at greater distances may be linked to the increased stochastic occupation and loss of species in sites that are further apart (Conradi et al., 2017; Segre et al., 2014). Therefore, the possibility of losing species or having new species occupy a site by chance increases with the distance between sites. The limited dispersal abilities of some species may lead to a permanent loss of a species from a site if the site is located at a great distance from other sites where the species does occur (Bremer and Eriksson, 1992; Jakobsson and Eriksson, 2003; Soons and Heil, 2002). Niche-based exclusion might also result in increased beta-diversity at greater distances, as sites that are further apart are more likely to have distinct sets of environmental and ecological dynamics (Conradi et al., 2017; Segre et al., 2014). The effect of aspect in regulating beta-diversity may be related to the microclimatic effect of varying levels of insolation on north- and south-facing slopes (Coops et al., 2000; Gallardo-Cruz et al., 2009; Yang et al., 2020). Higher insolation levels on north-facing slopes in the southern hemisphere leads to increased heat and drought stress (Coops et al., 2000; Gallardo-Cruz et al., 2009; Yang et al., 2020). Forb species that are more tolerant to dry, hot conditions are expected to colonize northern-slopes compared to the drought intolerant species on southern slopes. The importance of aspect in regulating beta-diversity was greater than the macroclimatic gradients of precipitation and temperature across the 42 sampled sites. Other studies show that beta-diversity displayed a curvilinear relationship with temperature patterns, landscape features and geographic distance between sites (Conradi et al., 2017; Segre et al., 2014). However, no relationship was found between the beta-diversity and precipitation or temperature gradients, or any disturbance drivers, such grazing pressure, fire frequency and fire return interval in this study . The disproportionate importance of aspect may indicate that microclimate is key to create heterogeneity across a landscape, which in turn increases the deterministic process of niche- based exclusion and the associated increase in beta-diversity (Conradi et al., 2017; Opedal et al., 2015; Yan et al., 2020).

Only 28.95% of grassland beta-diversity was explained. This indicates that other deterministic and stochastic processes (dispersal, local extinction or speciation) that were not considered in this study may also be key in regulating compositional dissimilarity of forbs in South African grasslands. Differences in species composition between sites are often driven by stochastic processes, such as random extinction and establishments, or evolutionary radiation of species (Conradi et al., 2017; Opedal et al., 2015; Yan et al., 2020). However, deterministic environmental filters have also been shown to be critical in regulating beta-diversity (Conradi et al., 2017; Isbell et al., 2017; Mori et al., 2018). Co-existence of different species is promoted through the deterministic niche-based exclusion where the establishment of individuals with similar resource requirements is inhibited (Fargione et al., 2003; Harpole and Tilman, 2006; Weiher et al., 2011). As biotic interactions, such as competition and facilitation, were not considered, this might explain why a relatively small amount of variation in beta-diversity was explained by the model in this study.

Conclusion

The patterns of high richness and turnover observed in the sampled grasslands suggest that the anthropogenic transformation of comparatively small grassland areas may lead to the loss of unique suites of potentially restricted species and biotic homogenization where only disturbance tolerant species survive, while more sensitive species are lost. On the other hand, the strategic selection of large grassland areas with high measures of beta-diversity and richness could facilitate the effective conservation of floristic diversity in the face of increasing land use by mining, agriculture and forestry. This study has demonstrated that only conserving grasslands with high measures of alpha diversity will not lead to the conservation of landscape level diversity, due to the high compositional dissimilarity between sites. Therefore, current conservation efforts that are mostly focused on the preservation of relatively small grassland areas with high alpha-diversity will not effectively conserve the unique flora and functioning of South African grasslands (Carbutt et al., 2017, 2011). To most effectively conserve ecological functioning and provisioning of ecosystem services in grasslands, large areas of compositionally distinct grassland sites across a landscape need to be conserved, while taking aspect, as well as the slope, soil type and geology of a region into consideration. This being said, the value of conserving and maintaining small grassland conservation areas that are already in existence is notable, as unique suites of species, as the high beta diversity recorded between sites indicates that even under this scenario new species will be conserved with the addition of every site (Smit et al., 1997).

Chapter 3

The effect of spatial grain and extent on diversity patterns and drivers in a diverse South African grassland

Introduction

Understanding diversity patterns and drivers is central to community ecology where accurately quantifying diversity in different ecosystems remains an unresolved challenge (Marsh and Ewers, 2013; Palmer and White, 1994; Vellend, 2010). Just as diversity can be measured and understood at varied spatio-temporal scales, the methods used to quantify diversity are also influenced by the spatial and temporal scale of the study (Dembicz et al., 2021a; Marsh and Ewers, 2013; Steinbauer et al., 2012). Even though many studies have highlighted the dependence of diversity patterns and drivers on spatial scale (Barton et al., 2013; Dembicz et al., 2021b), the effect of spatial scale on diversity patterns and drivers is rarely considered (Chase et al., 2018). This knowledge gap is problematic, as spatial scale will influence the assessment of diversity patterns and drivers and will ultimately alter conservation efforts accordingly (Kukkala and Moilanen, 2013; Turner et al., 1989). Therefore, the effect of spatial scale on diversity assessments should be considered, as healthy ecosystem functioning and the provisioning of ecosystems services is dependent on the conservation of biodiverse systems (Duffy et al., 2017; Egoh et al., 2011; Mori et al., 2018).

Most commonly, diversity is calculated as the number of species in a site (alpha-diversity) (Gaston, 2000; Moreno et al., 2009a; Uys, 2006). However, estimating the temporal or spatial differences in species composition between sites (beta-diversity) has been receiving more attention, as, along with alpha-diversity, beta-diversity contributes significantly to diversity at a landscape scale (gamma-diversity) (Barton et al., 2013; Jost, 2007a; Sepkoski, 1988; Soininen et al., 2018). In addition to this, beta-diversity can be decomposed into turnover and nestedness (Baselga, 2010). Turnover highlights the dissimilarity between community assemblages due to replacement of species, while nestedness gives the dissimilarity due to the loss of species between community assemblages (Baselga, 2010). However, the individual contribution of alpha- and beta-diversity to gamma-diversity varies with the ecosystem (Colville et al., 2020; Mori et al., 2018; Olivier and van Aarde, 2014). Diversity patterns, in turn, are driven by a range of environmental, disturbance and biological processes (Dembicz et al., 2021c; Koerner and Collins, 2014; Talebi et al., 2021). Disentangling the relationship between the observed diversity patterns and the proposed drivers thereof is heavily dependent on the spatial scale at which the study is conducted, as both diversity patterns and drivers are influenced by spatial scale (Biondini et al., 1989; Dembicz et al., 2021a; Klanderud et al., 2015; Uys, 2006; Vellend, 2010; Zuo et al., 2012).

Spatial scale encompasses both spatial extent (area across which diversity is quantified) and spatial grain (the spatial resolution at which diversity is quantified) (Barton et al., 2013; Dembicz et al., 2021a; Wills and Read, 2017). Each of these components influence diversity patterns in a distinctive way. Alpha-diversity is known to increase overall with both spatial extent and grain (Barton et al., 2013; Palmer and White, 1994). This relationship can further be unravelled. In general alpha-diversity tends to increase rapidly at local scales with an increase in the spatial extent of sampling, while increasing more gradually at intermediate extents and again rapidly at large extents (Barton et al., 2013; Hortal et al., 2010; Hulme, 2008). Increasing the spatial extent of sampling over small areas will add large numbers of species due to microhabitat and niche specialization (Barton et al., 2013; Palmer and White, 1994; Stein et al., 2014a). At intermediate extents, the effect of microhabitat specialization falls away and fewer new species are sampled when compared to the overall number of species across the landscape (Barton et al., 2013; Hortal et al., 2010). Over large spatial extents, a sharp increase in the number of species sampled is observed. This may be due to macroecological landscape and climatic heterogeneity and isolation. Therefore, species assemblages will differ over large spatial extents, as different sets of environmental and biotic filters may lead to different evolutionary histories and radiation between geographically isolated communities (Barton et al., 2013; Dembicz et al., 2021a; Talebi et al., 2021). These patterns are only observed at relatively large spatial grains, as a large number of the local species at each site need to be sampled (Palmer and White, 1994). At small spatial grains, an increase in the extent of sampling will also increase the number of species, but more gradually when compared to larger spatial grains. A more gradual increase in the number of species is observed, as the chance of only sampling widespread species is high, while the chance of sampling range restricted species is lower at small grains (Barton et al., 2013; Dembicz et al., 2021a; Talebi et al., 2021). Therefore, an interaction exists between how spatial grain and extent affect measures of alpha-diversity (Hulme, 2008; Palmer and White, 1994; Steinbauer et al., 2012). It is also important to note that when diversity assessments are conducted, the spatial scale of the study should reflect both the biology of the target species and the research question (Barton et al., 2013; Wiens, 1989).

The relationship of beta-diversity with spatial scale is complex, as the compositional differences between sites are influenced by both the spatial grain and extent (Barton et al., 2013; Steinbauer et al., 2012). An increase in grain size is expected to result in a decrease in compositional dissimilarity between sites when spatial extent is fixed, as a proportional increase in the number of species sampled within a site is expected, as the likelihood of encountering more species that occupy specific niche and microhabitats increases (Barton et al., 2013; Keil et al., 2012; Stein et al., 2014a; Steinbauer et al., 2012). An increase in spatial extent will generally lead to an increase in compositional dissimilarity when the spatial grain is fixed. This effect can be linked to an increased probability of encountering unique species assemblages at greater spatial extents due to stochastic occupation and extinction of species, or due to variable niche-occupation between sites (Barton et al., 2013; Conradi et al., 2017; Segre et al., 2014). A small sampling grain, on the other hand, is likely to result in high compositional dissimilarity between sites, as only a small proportion of species that occupy a specific niche and microhabitat will be sampled, which will be very different even over relatively small spatial extents (Barton et al., 2013; Steinbauer et al., 2012).

Understanding what environmental gradients and disturbance factors drive the observed alpha- and beta-diversity patterns is invaluable for effective systematic conservation planning, as appropriate sites for conservation should be located in accordance with the environmental and landscape gradients associated with high diversity (Filibeck et al., 2019; Kukkala and Moilanen, 2013; Talebi et al., 2021). Different environmental and landscape drivers may affect diversity measures uniquely at different spatial extents and grains, which should be considered when areas are selected to be conserved (Dembicz et al., 2021b; Filibeck et al., 2019; Talebi et al., 2021). This spatial scale phenomenon may explain the variety of drivers that have been found to be important of the same natural ecosystems (Auestad et al., 2008; Filibeck et al., 2019).

The effect of spatial scale on the floristic diversity, as well as the drivers thereof, has been poorly explored, especially in highly diverse ecosystems. The grassland biome of southern Africa presents a prime opportunity to investigate the effect of spatial scale on the above-mentioned factors, as this biome is thought to be floristically diverse, but remains under-studied (Bond and Parr, 2010; Cowling et al., 2003; Thuiller et al., 2006; Uys, 2006). The biome makes up approximately 28% of the subcontinent and provides essential ecosystem services, such as the regulation of the hydrological cycle, carbon sequestration and the prevention of erosion (Carbutt et al., 2011; De Groot et al., 2002; Egoh et al., 2011). This biome is thought to be one of the richest biomes in South Africa, as the point species richness of the grassland biome is predicted to be 1.4 times higher than all other biomes, with the exception of the fynbos biome (Cowling et al., 2003; Muller et al., 2021). It's diversity can be ascribed to the range of herbaceous forb species that grow under the grass canopy that gave this biome its name and make up the majority of the biomass of this system (Morris, 2021; Muller et al., 2021). However, the diversity of large regions of the grasslands have been poorly quantified, and therefore little is known regarding its diversity patterns and drivers (Cowling et al., 2003; Mucina and Rutherford, 2006; Muller et al., 2021). Nearly 60% of this biome has already been transformed, mainly through mining, agriculture and forestry (Carbutt et al., 2017; Morris, 2021; Muller et al., 2021). It is poorly understood how land use changes affect species loss, as diversity patterns are largely unexplored in these systems (Carbutt et al., 2011; Muller et al., 2021). Therefore, ensuring that appropriate spatial scales of assessment are applied when conducting future environmental impact assessments are of essence for the conservation of this biome (Egoh et al., 2011; Glasson et al., 2019; Turner et al., 1989).

The aim of this study was to assess the effect of spatial scale (in terms of both spatial grain and extent) on diversity patterns and drivers in a South African grassland. The first objective was to test how gamma diversity estimates increased with an increase in the spatial grain of sampling (Barton et al., 2013; Polyakova et al., 2016; Talebi et al., 2021). It was hypothesised that an increase in the spatial grain of sampling will lead to significantly higher estimates of gamma-diversity across all non-parametric estimators, signifying high local beta-diversity. The second objective was to assess the effect of spatial grain on the drivers of alpha-diversity. It was hypothesised that the importance of the climatic, landscape and disturbance drivers of grassland alpha-diversity will change with the spatial grain of sampling. The third objective was to test the effect of the spatial extent of sampling on beta-diversity patterns. It was hypothesised that beta-diversity is higher across large spatial extents. The last objective was to assess the effect of spatial grain on beta-diversity patterns and drivers. It was hypothesised that beta-diversity drivers of beta diversity change with spatial grain (Dembicz et al., 2021b; Talebi et al., 2021). Changes in both alpha- and beta-diversity with changing grain provide insight into alpha- and beta-diversity at small extent, and how that compares with gamma-diversity.

Methods

Data collection

Grassland forb diversity data was collected in the under-sampled grassland regions of north-eastern regions of South Africa, spanning northern KwaZulu-Natal, southern Mpumalanga and Gauteng by sampling 42 grassland sites that contained 21 nested plots per site. Climate, landscape and disturbance variable data was also collected for each site as outlined in *Chapter 2: Methods - Site selection & Data collection*. Three different spatial grains of forb diversity data were constructed from the plot data: for the small spatial grain, only the data of the centre plot of each site was considered (with an area of 0.79 m^2); for the intermediate spatial grain, the data from the centre plot and four plots spaced five metres away from the centre plot were included (with a total area of 3.95 m^2); for the large spatial grain, the data from all the plots per site were considered (with a total area of 16.59 m^2).

Statistical analyses

Gamma-diversity

The process of estimating species richness is complex, especially in ecosystems that boast exceptionally high species richness, where a multitude of rare species will not be sampled even with extensive sampling efforts (Chao and Chiu, 2016). In these cases, non-parametric estimators can be used to estimate total species richness, as these estimators depend on the frequency occurrence of singletons and doubletons in the estimation of species richness (Supplementary Methods S1). The assumption is, that while abundant species are sampled frequently and suggest no information about the abundance of rare species, the frequency of sampling singletons and doubletons is used to infer the richness of rare species in the community. This leads to an improved estimation of species richness (Brose, 2002; Chao and Chiu, 2016). To test the effect of the spatial grain on gamma-diversity, the total species richness across all sites was estimated at three spatial grains (small, intermediate and large) using the non-parametric estimators Chao 2; Jacknife 1; Jacknife 2; and Bootstrap.

To assess if the true number of species were being approached by the sampling effort at each spatial grain, sample-based rarefaction curves were created for each spatial grain with 1000 permutations. The rarefaction curves gave an indication of how many new species were added as new sites were sampled. The slope of the rarefaction curves was calculated at each of the 42 sites for each grain size. The slope was plotted against sample size. This gave an indication of the rate of the addition of new species as new sites were sampled. This graph will therefore suggest how the rate of species addition changes at different spatial grains.

Alpha-diversity

To assess the drivers of alpha diversity at different spatial grains, three generalized linear models (one for each spatial grain) with Poisson distributions were utilized. Instead of using the raw climate, landscape and disturbance drivers for in the GLMs, PCA ordination was conducted to prevent overfitting the model for each subset of predictor variables, i.e. landscape (soil texture, soil type, geology and slope, *Table S4*), climate (mean annual precipitation, mean annual temperature, temperature range, minimum temperature of the coldest month and northness, *Table S2*) and disturbance (grazing pressure, time since the last fire, fire return interval and fire radiative power, *Table S3*) as described in *Chapter 2: Methods: Statistical analyses – Alpha-diversity*. The same principal component axes were used at each spatial grain, as only one set of drivers were recorded per site. The first three climate principal components and the first two disturbance principal components were selected to be included in the model. A cut-off value of 15% for the climate PC axes and 20% for the disturbance PC axes were used to select the most important PC axes. In the case of the landscape variables, little variation was explained by each principal component; therefore, the raw variables were incorporated into the best subset modelling analysis, rather than the principal components (*Chapter 2: Methods: Statistical analyses – Alpha-diversity*, *Table S4*).

A full generalized linear model (GLM) with species richness as the response and the selected climate principal components, disturbance principal components and raw landscape variables was set up for each spatial grain. Best subset modelling, based on the Akaike Information Criterion (AIC) using the 'leaps' algorithm (Aho et al., 2014) was used to select the most parsimonious model. A maximum of four variables was included in each spatial grain model, based on the ten events per variable rule (Peduzzi et al., 1996). The most parsimonious GLM for alpha-diversity at a large spatial grain included soil type, geology, slope and climate PC-3 as predictor variables (AIC = 294.93), which is the same model that was mentioned in *Chapter 2*. The most parsimonious GLM at an intermediate spatial grain retained climate PC-1, soil type, soil texture and slope (AIC = 289.6), while the most parsimonious GLM at a small spatial grain retained only climate PC-1 and disturbance PC-1 (AIC = 205.3). Dispersion tests were conducted to test for overdispersion of each GLM, no evidence of overdispersion was found.

Beta-diversity

To assess the effect of spatial extent on beta-diversity, the Jaccard distance index (1- Jaccard similarity) was used, while the spatial grain was kept constant at plot level (Koleff et al., 2003). To test the effect of spatial extent on beta-diversity, local extent beta-diversity (compositional dissimilarity between all the plots within a site) and regional extent beta-diversity (compositional dissimilarity between the centre plots of all sites) were compared with the use of the Jaccard distance index. A Welsch-two-sample t-test was used to test if a significant difference exists between the beta-diversity at local and regional spatial extents (Welsch, 1977). A GLM with a binomial distribution was constructed and the generalized linear hypothesis testing with multiple means comparisons and the Tukey contrasts method used to assess if significant differences in beta-diversity exists between small, intermediate and large spatial grains (Jaccard et al., 1984).

The mean dissimilarity at small, intermediate and large spatial grains were decomposed into the two components of beta-diversity, namely turnover and nestedness. Two generalized linear models with binomial distributions were used, along with the generalized linear hypothesis testing technique using the multiple means comparisons with the Tukey contrasts method, to assess if significant differences exist between the turnover component of different spatial grains and the nestedness component of the different spatial grains.

To test which climate, landscape and disturbance predictor variables had a significant effect on beta-diversity at different spatial grains, generalized dissimilarity modelling (GDM) with the Jaccard distance index was used. GDM accounts for the distance between sites and variation of species compositional change along environmental and disturbance gradients (Fitzpatrick et al., 2021, 2013; Jewitt et al., 2016). Spatial autocorrelation is considered with this method, as well as non-stationarity, which encompasses a non-linear relationship of species compositional change across different environmental and disturbance gradients (Fitzpatrick et al., 2013; Jewitt et al., 2016).

As outlined in *Chapter 2: Methods: Statistical analyses – Beta-diversity,* an initial correlation matrix was constructed (*Table* S5) to eliminate highly correlated variables (with a Pearson's correlation coefficient of $r > 0.6$), before conducting the GDM analyses at different spatial grains. The following predictor variables were included in the full model assessment: geographic distance between sites, time since the last fire, grazing pressure, fire return interval, fire radiative power, mean annual precipitation, mean annual temperature, temperature range, minimum temperature of the coldest month, northness, slope, soil type, soil texture and geology. A curvilinear relationship of the compositional dissimilarity of forb species between sites at large, intermediate and small spatial grains and the above-mentioned environmental and disturbance predictor variables were assumed. A default of three I-splines values per predictor was used, with the maximum value of the I-spline curve representing the total amount of variation in the compositional dissimilarity between sites explained by a particular driver (Ferrier et al., 2007; Fitzpatrick et al., 2021, 2013; Jewitt et al., 2016). Variable importance was assessed with 1000 permutations in the full model (at each spatial grain) in order to select the best subset of variables for the large, intermediate and small grain GDMs.

The percentage deviance change when a variable was included in the fitted model versus when the variable was excluded was used to assess variable importance. In turn, each variable was permuted 1000 times, while the other variables were kept constant. Variable importance and significance were assessed with each permutation (Ferrier et al., 2007; Fitzpatrick et al., 2021). Backward selection allowed the model to drop the least important variable at each permutation, until the three most important significant variables remained. This process was repeated for each spatial grain, in order to construct three GDMs, one for each spatial grain. GDM analyses were subsequently conducted on the three most important predictor variables (as outlined by the model selection processes of each spatial grain) to assess if these drivers had a significant effect on beta-diversity at small, intermediate and large spatial grains and how much deviance was explained by each driver.

Diversity data analyses at different spatial scales was done with the use of R (R Core Team, 2018), using the following packages: the "psych" (Revelle, 2021a), "psychTools" (Revelle, 2021b), "BiodiversityR" (Kindt and Coe, 2005), "dplyr" (Wickham et al., 2021), "vegan" (Oksanen et al., 2019), the "ggplot2" package (Wickham, 2016), the "permute" package (Simpson, 2019), "lattice" (Sarkar, 2008), "leaps" (Lumley and Miller, 2020), "bestglm" (McLeod et al., 2020), "MASS" (Ripley et al., 2013), "stats" (R Core Team, 2021), "geosphere" (Hijmans, 2019), "gdm" (Fitzpatrick et al., 2021).

Results

Alpha-diversity

The estimated gamma-diversity of the grassland forbs sampled across the study area was considerably higher than the observed species richness (S), at large (486), intermediate (284) and small spatial grains (136), by all four non-parametric estimators. The estimated richness by the four non-parametric estimators ranged between 605.15 and 1067.82 at a large spatial grain, between 352.81 and 559.68 at an intermediate spatial grain and between 170.96 and 280.40 at a small spatial grain (*Figure 8, Table S6*). The Bootstrap estimator gave the most conservative estimates of gamma-diversity at all spatial grains (*Figure 8, Table S6)*, while the Chao 2 estimator mostly provided the highest estimate (*Figure 8, Table S6*). Estimated gamma-diversity was highest at a large spatial grain and decreased as spatial grain decreased. Therefore, spatial grain had a pronounced influence on the estimation of gamma-diversity.

The rarefaction curves did not flatten off at any spatial grain, however the slope was the steepest at a large spatial grain, followed by the intermediate and small spatial grain curves (*Figure* 9 *A*). This indicated that, even at a large spatial grain, the 42 sampled sites did not approach the true number of species in the sampled grasslands, as confirmed by the non-parametric estimators. Marked differences were observed between all spatial grains in terms of the rate of the number of species added (*Figure 9 B*). Initially, the large spatial grain graph showed the largest change in the rate of species added, as new sites were sampled. Compared to the large and intermediate spatial grain graph, the small spatial grain graph showed little change in the rate of new species being added (*Figure 9 B*).

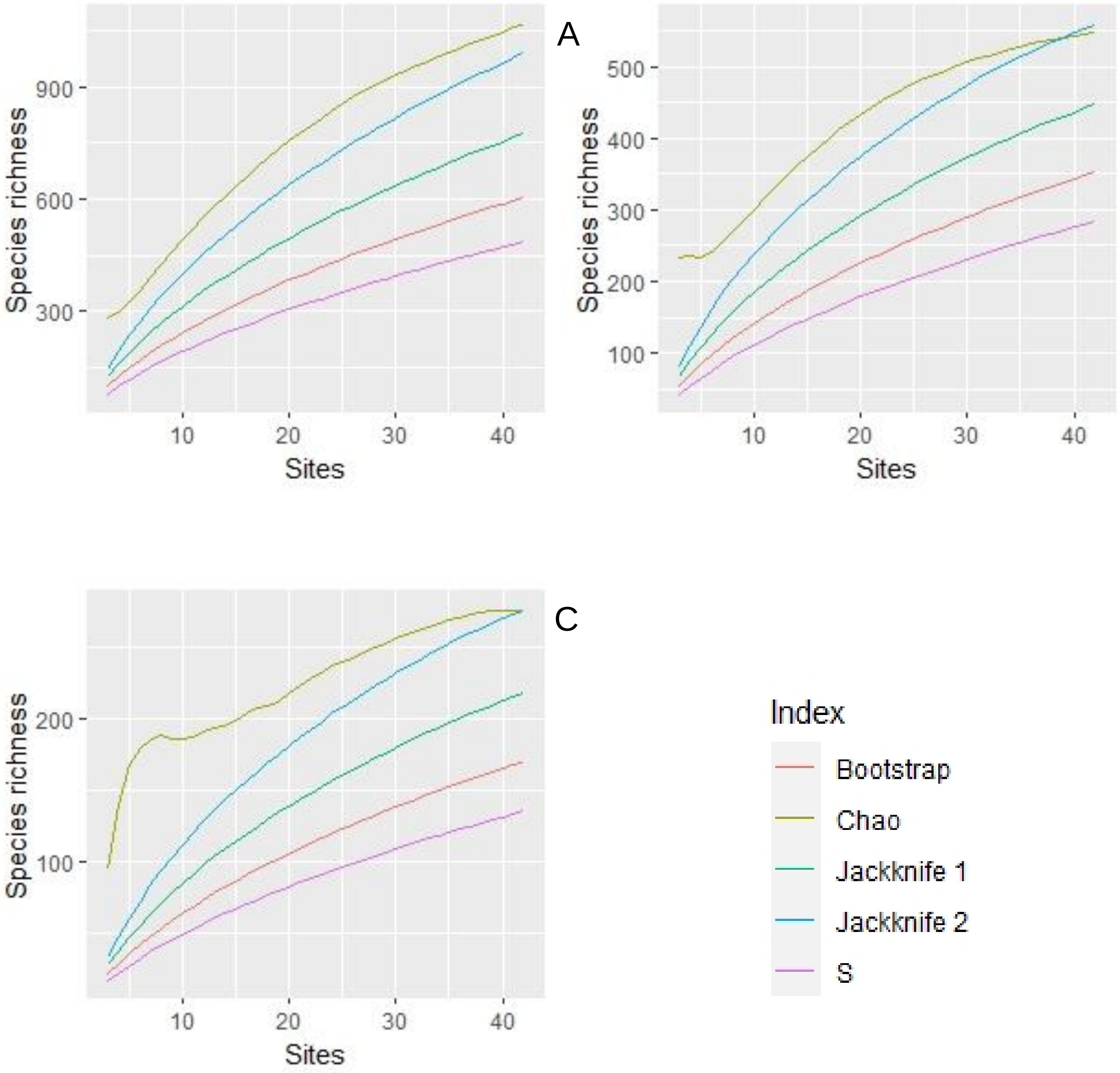

Figure 8: The estimated total forb species richness and accumulation of 42 grassland sites at a large (A), intermediate (B) and small (C) spatial grain estimated using four non-parametric estimators, namely Bootstrap, Chao 2, Jacknife 1 and Jacknife 2. The total observed species richness is also shown (S).

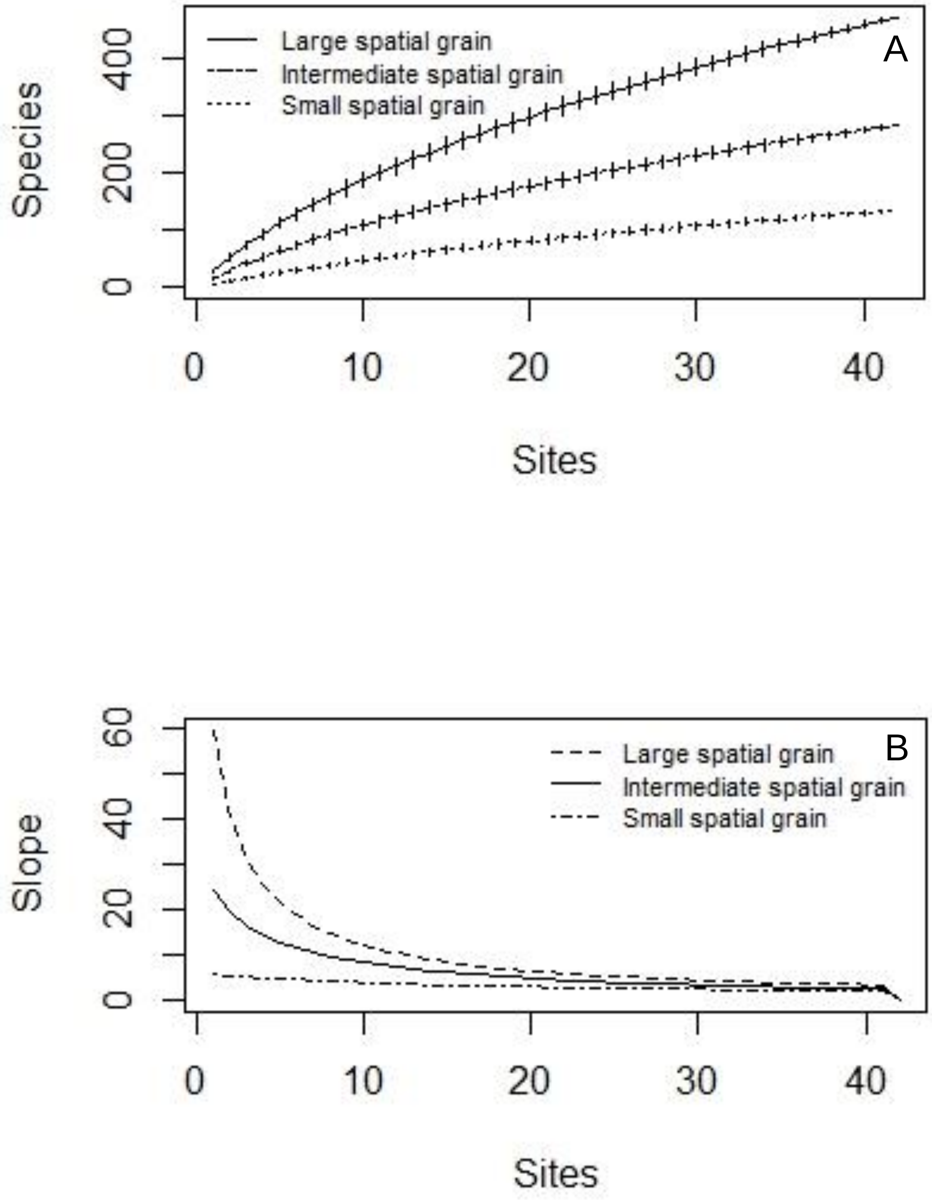

Figure 9: Three sample-based rarefaction curves, constructed with 1000 permutations, of 42 grassland sites at large, intermediate and small spatial grains (A). The change in the rate of the addition of new forb species across 42 grassland sites at large, intermediate and small spatial grains are depicted in (B).

The results from the GLMs assessing the effect of the different climate, landscape and disturbance drivers on alpha-diversity at different spatial grains, indicated that the drivers of alpha-diversity differed between spatial grains. At a large spatial grain, soil type, slope, geology and climate PC-3 had a significant effect on forb alpha-diversity ($p < 0.05$) (*Table 4, Table S2*), with a pseudo Nagelkerke R^2 value of 0.83. Northness was most strongly and inversely related to climate PC-3 (*Figure S2*). Alpha-diversity increased with slope and decreased with northness. The acrisol soil type and igneous geology were associated with the highest species richness. The results from the intermediate spatial grain GLM indicated that soil type and soil texture had significant effects on alpha-diversity ($p < 0.05$) ((*Table 4*), with a pseudo Nagelkerke R^2 value of 0.66. Sandy soils had lower alpha-diversity than clayey soils. At a small spatial grain only disturbance PC-1 had a significant effect on alpha-diversity ($p < 0.05$) (*Table 4, Table S3*), with a pseudo Nagelkerke R^2 value of 0.32. The disturbance drivers that were most strongly related to the first principal component of the disturbance PCA were grazing pressure and fire return interval (*Figure S3*). An increase in grazing pressure resulted in a decrease in alpha-diversity, while an increase in the fire return interval resulted in an increase in alpha-diversity at a small spatial grain.

Beta-diversity

The compositional dissimilarity at a regional extent was significantly higher than the compositional dissimilarity at local extent (*Figure 10 A*) ($t = 23.74$, $p < 0.05$, $df = 42.33$). The highest compositional dissimilarity was recorded at a small spatial grain, followed by the intermediate and large spatial grains (*Figure 10 B*) ($p < 0.05$). Neither turnover (*Figure 10 C*), nor nestedness (*Figure 10 D*) differed significantly between large, intermediate and small spatial grains ($p > 0.05$). However, it is important to note that most of the compositional dissimilarity between sites was due to turnover (the replacement of species between sites) and not nestedness (the loss of species between sites) (*Figure 10 C & D*).

Table 4: Best subset results from generalized linear models at large, intermediate and small spatial grains to assess the effect of climate, landscape and disturbance on alpha-diversity. Significant drivers are highlighted in bold. The Tukey 'honest significance difference' method was used to determine if significant differences exist between soil types (Table S7).

	Large spatial grain				**Intermediate spatial grain**				**Small spatial grain**			
Drivers:	**Estimate**	**χ^2**	**Df**	**p-value**	**Estimate**	**χ^2**	**Df**	**p-value**	**Estimate**	**χ^2**	**Df**	**p-value**
Soil type	-	18.55	8	**1.75×10^{-2}**	-	33.58	8	**4.84×10^{-5}**	-	-	-	-
Soil texture	-	-	-	-	-0.18	4.30	1	**3.82×10^{-2}**	-	-	-	-
Geology	-	9.73	3	**2.11×10^{-2}**	-	-	-	-	-	-	-	-
Slope	0.11	6.68	1	**9.75×10^{-3}**	-0.10	2.33	1	1.27×10^{-1}	-	-	-	-
Climate PC-1	-	-	-	-	0.18	3.42	1	6.44×10^{-2}	0.15	2.62	1	1.06×10^{-1}
Climate PC-3	-0.24	16.75	1	**4.27×10^{-5}**	-	-	-	-	-	-	-	-
Disturbance PC-1	-	-	-	-	-	-	-	-	-0.41	14.21	1	**1.64×10^{-4}**

Some drivers of beta-diversity were similar, and others differed when assessed for different spatial grains. At a large spatial grain, the best subset model retained geographic distance, northness and the vertisol soil type ($p < 0.05$). An increase in the geographic distance between sites, an increase in the difference in the degree of northness between sites and the presence of the vertisol soil type were associated with an increase in beta-diversity (*Table 5, Figure S5 A*). The percentage deviance explained by the large spatial grain GDM model was 28.95% (see Chapter 2). At an intermediate spatial grain, the best subset model retained geographic distance, northness and days since the last fire ($p < 0.05$); greater dissimilarity in these drivers between sites resulted in increased beta-diversity (*Table 5, Figure S5 B*). Less variation was explained by the intermediate spatial grain model with 16.12% deviance explained. At a small spatial grain, an increase in the distance between sites and the difference in the degree of northness between sites and the presence of the igneous geology type were associated with an increase in beta-diversity ($p < 0.05$). However, the small spatial grain model explained very little variation in compositional dissimilarity with 5.91% deviance explained *(Table 5, Figure S5 C*). Northness was an important driver of beta-diversity across all spatial grains.

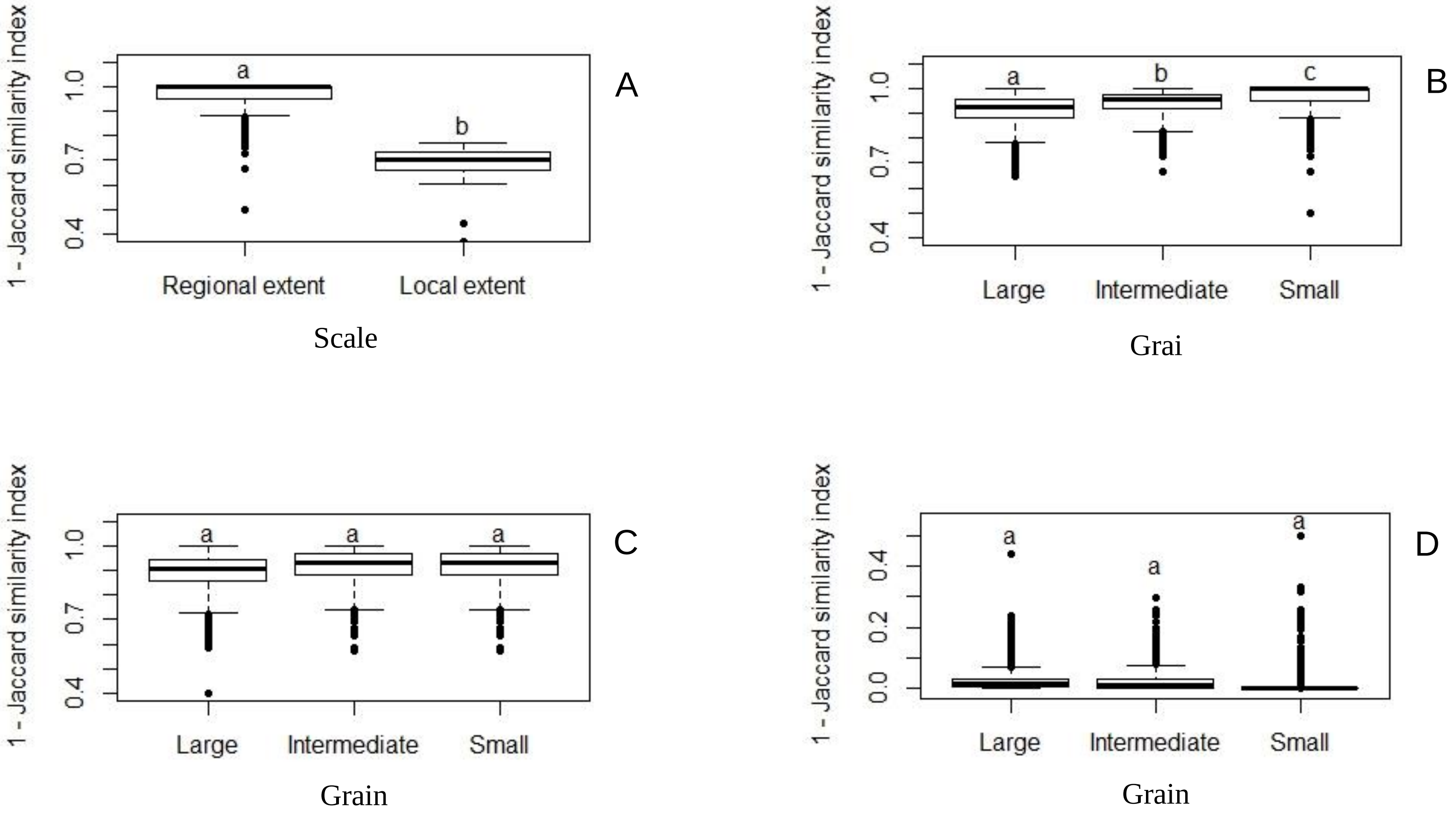

Figure 10: Boxplots depicting the difference in beta-diversity (Jaccard distance) between different spatial extents (A); the difference in beta-diversity measured at different grains, (B); and dissimilarity in the two components of beta diversity, namely turnover (C) and nestedness (D), measured at different grains.

Table 5: Results from the generalized dissimilarity models at large, intermediate and small spatial grains, constructed with 1000 permutations, to assess the effect of climate, landscape and disturbance on beta-diversity as estimated with the Jaccard distance index. The relative variable importance of each driver, calculated as the sum of the three I-spline coefficients is displayed. Significant drivers are highlighted in bold.

	Large spatial grain		**Intermediate spatial grain**		**Small spatial grain**	
Driver	**Variable importance**	**p-value**	**Variable importance**	**p-value**	**Variable importance**	**p-value**
Geographic distance	73.15	**$< 1.0 \times 10^{-3}$**	44.75	**$< 1 \times 10^{-3}$**	39.48	**$< 15 \times 10^{-3}$**
Northness	8.65	**2.5×10^{-2}**	20.24	**9×10^{-3}**	36.97	**2.3×10^{-2}**
Vertisol	9.82	**3.6×10^{-2}**	-	-	-	-
Days since the last fire	-	-	13.96	5.1×10^{-2}	-	-
Igneous	-	-	-	-	20.20	**2.4×10^{-2}**

Discussion

The results from this study confirm the notion that spatial scale influences the estimates of diversity patterns, as well as the associated importance of the drivers of alpha- and beta-diversity.

Different non-parametric estimators yielded varying estimations of gamma-diversity at different spatial grains. This highlights the importance of using the appropriate grain of assessment to improve estimates of diversity. Diversity data forms the basis of effective systematic conservation planning; thus, the effect of spatial grain should be considered when selecting areas to conserve (Manhães et al., 2016; Rondinini et al., 2006). The effect of spatial grain on the non-parametric estimates of species richness is further exemplified by the rarefaction curves. None of the rarefaction curves completely flattened off. Therefore, even at a large spatial grain, the sampling effort did not approach the true number of species in these systems, as was also indicated by the large grain non-parametric estimators. The initial change in the rate of the addition of new species at a large grain and to a lesser extent at an intermediate grain, were notable when compared to the small grain graph. This indicated that the rate of adding new species at a large spatial grain was comparatively higher than the rate at which new species were added at an intermediate or small spatial grain. The large grain change in slope decreased rapidly after a few sites were sampled, but never reached zero. This indicated that new species were still being added in later sites, but at a slower rate. This is due to the large number of localised species in the grassland biome of South Africa (Bond, 2016; Carbutt et al., 2011; Uys, 2006). The change in rate of new species being added at a small spatial grain was minimal when compared to the intermediate and large spatial grain graphs, which indicated that rate at which new species were added as new site was sampled was similar across all 42 sampled sites. This was due to high compositional turnover at small grains which resulted in almost as many new species being added after >40 sites had been added as when only a handful of sites had been sampled. This confirmed that, in grasslands, larger grains are more exhaustive and suitable to capture the compositional turnover across a landscape (Gotelli and Colwell, 2001; Moreno et al., 2018).

The drivers of alpha-diversity varied substantially with spatial grain. At a large spatial grain northness (a measure of aspect), slope, soil type and geology were significant drivers. This is consistent with other grassland studies that were conducted at spatial grains similar to the large and intermediate spatial grains in this study, where plant species richness was significantly affected by soil type and chemistry, as well as slope and heat load index (Dembicz et al., 2021b; Polyakova et al., 2016; Zuo et al., 2012). An increase in the degree of northness resulted in a decrease in the observed species richness, suggesting that the effect of microclimate (i.e., increased heat- and drought-stress due to the higher levels of solar radiation experienced by north-facing slopes) may be more pronounced than the effect of macroclimatic precipitation and temperature gradients in driving alpha-diversity (Coops et al., 2000; Mahmoudi et al., 2018; Polyakova et al., 2016; Zeng et al., 2005). This may be due to the limited precipitation and temperature range (250 mm and 4.32 °C, respectively) that was covered by the sampled sites. The precipitation range for the South African grassland biome for example, is between 400 mm and 2500 mm annually (Mucina and Rutherford, 2006), which is notably higher than the range that was covered by the sampled sites in this study. Therefore, climate may be an important driver of grassland diversity across larger climatic gradients. Higher forb alpha-diversity on steeper slopes could be driven by more variable microhabitats which may facilitate species co-existence (Stein et al., 2014). Climatic drivers, especially annual precipitation and temperature have been demonstrated elsewhere to have a strong positive effect on alpha-diversity at large spatial grains (Polyakova et al., 2016; Talebi et al., 2021). However, the results from this study indicated that effect of precipitation and temperature were not as important as the effect of other drivers in driving alpha-diversity across sampled sites.

Some similarities were found between the drivers of alpha-diversity at a large- and intermediate spatial grain, with soil type being a significant driver of species richness at both spatial grains. The acrisol soil type was associated with higher species richness. Soil texture also had a significant effect on alpha-diversity at an intermediate spatial grain, with loam soil being associated with higher species richness. The importance of soil type and texture in driving alpha-diversity may result from the differences in soil pH, nutrient availability and water availability that vary between different soil types and textures, which in turn affected forb species richness (Critchley et al., 2002; Pausas and Austin, 2001; Ratliff et al., 1983). The high water carrying capacity and bulk density of the acrisol soil type potentially leading to the high species richness (Cavalli et al., 2020; Paltineanu et al., 2020). The formation of different soil types and textures in turn are regulated by the underlying geology of a region (Rahbek et al., 2019; Simon et al., 2021). Therefore, it was not surprising that geology also had a significant effect on alpha-diversity at a large spatial grain, with the presence of the igneous geology type resulting in increased alpha-diversity.

At a small spatial grain, the effects slope, geology, soil type and texture, and aspect were not important in driving forb alpha-diversity when compared to large and intermediate spatial grains. Instead, disturbance factors emerge as the most important correlates of alpha-diversity. Fire return interval and grazing pressure were most strongly related to the first principal component of the disturbance PCA. Higher fire return intervals and lower grazing pressure increased forb alpha-diversity. Intermediate fire return intervals, which simulates lightning-driven fire regimes, that partly lead to the evolution of grasslands, have been linked to increased alpha-diversity (Bond and Parr, 2010; Gordijn et al., 2018; Hobbs and Huenneke, 1992). Decreased alpha-diversity has also been found to be correlated with high intensity grazing or moderately selective grazing (Little et al., 2015; Neke and Du Plessis, 2004; O'Connor et al., 2011; O'Connor and Kuyler, 2009; Venter et al., 2020). Disturbance drivers were not important at larger spatial grains, where the effect of aspect and landscape drivers were more important in regulating alpha-diversity (Harrison et al., 2003; Hobbs and Huenneke, 1992; Joubert et al., 2016). This suggests that the effects of disturbances on diversity are fairly localised in the grassland landscape, i.e., smaller than the 50 x 50 m area of the largest grain at which we sampled.

Beta-diversity was significantly higher at regional extents than at local extents. This is expected due to an increase in the landscape and habitat heterogeneity at larger spatial extents, which shapes unique species assemblages due to varying evolutionary histories (Mistral et al., 2000). Decreased spatial autocorrelation will also increase the probability of sampling increasingly different species assemblages over greater spatial extents (Mistral et al., 2000).

The effect of spatial grain on overall compositional dissimilarity (beta-diversity) across the study region was also significant. Beta-diversity significantly increased with a decrease in the spatial grain. This may result from the increased probability of sampling different species assemblages that occupy specific microhabitats at each site, as the sampled area is too small to stretch across multiple microhabitats (Barton et al., 2013; Steinbauer et al., 2012). Microhabitat heterogeneity and variable niche occupation may also be responsible for the relatively high beta-diversity (68. 74 ± 07.70%) at local extents, as unique suites of species are adapted to and occupy each microhabitat or niche (Barton et al., 2013; Steinbauer et al., 2012). However, even at a large spatial grain, high beta-diversity (89.52 ± 5.93%) was observed, which indicated that the forb species composition differed vastly even if a larger pool of species are sampled in the diverse grassland biome (Carbutt et al., 2011; Uys, 2006). No significant difference was detected in dissimilarity due to the turnover, nor to the nestedness components of beta-diversity at different spatial grains. It is, however, clear that, overall, beta-diversity at all spatial grains can be attributed primarily to forb turnover between sites as very little of dissimilarity could be ascribed to nestedness. Therefore, forb species replacement is the largest driver of beta-diversity at all spatial grains in the grassland biome of South Africa (Baselga, 2010). This is in contrast to some central European and North American grasslands, where high landscape-level diversity can mostly be ascribed to high alpha-diversity, with limited compositional dissimilarity between sites (Habel et al., 2013; Knapp et al., 2004; Michalcová et al., 2014). Large spatial grains are therefore necessary to capture the true diversity patterns in South African grasslands and to select appropriate areas for conservation (Barton et al., 2013; Kukkala and Moilanen, 2013).

Geographic distance between sites and differences in the degree of northness between sites were significant drivers of beta-diversity at all spatial grains. A decreasing amount of variation was explained by each GDM model as the spatial grain decreased, which indicated that unmeasured environmental drivers may be important in determining beta-diversity of forb species in these grassland systems at smaller spatial grains. Increased stochasticity of species occupation and extinction at small spatial grains may have led to the decrease in the amount of variation explained by the small spatial grain model, as the occupation or loss of species at small grains may not be driven by environmental or disturbance variables (Barton et al., 2013; Conradi et al., 2017; Segre et al., 2014).

An increase in the distance between sites is thought to increase beta-diversity as sites that are further apart are more likely to have distinct environmental features which will support unique suites of species due to niche-based community assembly processes, or simply due to stochastic community assembly (Barton et al., 2013; Conradi et al., 2017; Harrison et al., 2003; Stein et al., 2014). The role of aspect in regulating patterns of beta-diversity is well documented (Gallardo-Cruz et al., 2009; Yang et al., 2020). Similar trends were also observed for grassland diversity patterns in China, as aspect also had a significant influence on species composition (Yang et al., 2020). The results from this study indicated that the microclimatic effect of aspect was more important than macroclimatic precipitation and temperature gradients or soil texture in driving compositional dissimilarity across the sampled sites of this study. Also, the difference in the mean annual precipitation (250 mm) and mean annual temperature (4.32 °C) was not very varied across the sites, which may also explain why precipitation and temperature gradients were not important drivers of beta-diversity (*Table S1*).This may be due to increased habitat heterogeneity created by the varying levels of insolation on north- and south-facing slopes (Coops et al., 2000; Gallardo-Cruz et al., 2009; Yang et al., 2020). Higher insolation levels on north-facing slopes in the southern hemisphere may lead to increased heat and drought stress on north-facing slopes which will support distinct species assemblages that are adapted to these conditions. On the cooler southern-slopes, heat and drought intolerant species that form distinct species assemblages will survive (Coops et al., 2000; Gallardo-Cruz et al., 2009; Yang et al., 2020). Therefore, sampling grassland sites that are located on different slope aspects will increase beta-diversity. The presence of the vertisol soil type was also associated with unique species assemblages at a large spatial grain. This indicated that the specific structure and soil chemistry of the vertisol soil type is able to support very different species assemblages when compared to other soil types (Critchley et al., 2002; Pausas and Austin, 2001; Ratliff et al., 1983). It is important to note that soil types that were not sampled frequently may not have come out as significant drivers of beta-diversity. Differences between soil types that were sampled frequently are particularly strong and weaker for soil types that were sampled less frequently. At an intermediate spatial grain, the days since the last fire had a significant effect on beta-diversity. This aligns with the results from previous studies that also indicated that forb species composition was influenced by fire frequency and fire return interval in grasslands. High pyrodiversity and relatively short fire return intervals increased grassland forb beta-diversity (Gordijn et al., 2018; Gordijn and O’Connor, 2021).

Conclusion

The results of this study clearly illustrated that spatial scale, in terms of both extent and grain, impacts the overall estimation of diversity, as well as the drivers of the observed diversity. The influence of spatial scale on diversity assessments is thought to be more pronounced in highly diverse ecosystems, such as the grassland biome of South Africa (Barton et al., 2013; Carbutt et al., 2011; Chao and Chiu, 2016; Uys, 2006). The true number of species was not approached at the large spatial grain over 42 sampled sites in this study, which suggests that even at larger spatial grains many replicates of the sampled sites were required to estimate true diversity patterns. Based on the results from this study, it is recommended that the largest possible spatial grain and extent, with as many replicates possible, is used in highly diverse ecosystems in order to estimate true diversity patterns and drivers.

This relationship between the spatial scale of assessment and diversity estimates and drivers has important implications for both environmental impact assessments, as well as systematic conservation planning efforts. The danger of extrapolating the results from small spatial scale assessments to the large spatial scales at which conservation and anthropogenic transformation, such as mining and afforestation, takes place are highlighted by the results of this study. Vastly different overall species richness and beta-diversity estimates, as well as the drivers of alpha- and beta-diversity were obtained at different spatial grains, which indicated that small and even intermediate spatial scale results cannot be extrapolated to larger spatial scales. Therefore, the spatial scale of assessment should match the scale of the proposed transformation or conservation efforts (Kukkala and Moilanen, 2013; Manhães et al., 2016).

Chapter 4

General conclusion

Diversity is a core concept in describing and effectively preserving natural ecosystems (Jost, 2007; Whittaker, 1972; Whittaker et al., 2001). The Anthropocene has brought about unprecedented anthropogenically driven biodiversity loss (Jonsson, 2012; Rands et al., 2010). Therefore, now more than ever, the accurate quantification of diversity patterns and drivers is not merely of interest for the community ecologist, but is essential for sustaining ecosystem functioning and subsequently, human health and existence (Kukkala and Moilanen, 2013; Rands et al., 2010; Vitousek et al., 1997). Describing diversity patterns and drivers in under-studied natural ecosystems is critical in order to develop a unified global conservation framework. The results from this dissertation are therefore important to aid in the construction of an effective conservation framework for grassland ecosystems.

Open ecosystems, such as grasslands and savannas, have been historically under-estimated in terms of biodiversity value. Consequently, few studies have assessed grassland diversity patterns and drivers over the large spatial scales that conservation is conducted and anthropogenic disturbances takes place. A pronounced underappreciation of grasslands exist on a global scale with little information on large scale diversity patterns and drivers. This presented the opportunity for this dissertation to quantify the diversity patterns and drivers of forbs in the under-sampled and threatened grassland regions of South Africa in Chapter 2. The results from this assessment were used as a backdrop to evaluate the impact of the spatial scale on diversity patterns and drivers in terms of both spatial extent and grain in Chapter 3. This knowledge can aid in effective systematic conservation planning and accurate environmental impact assessments to minimize future diversity losses in these systems (Glasson et al., 2019; Kukkala and Moilanen, 2013; Margules and Pressey, 2000; Sonter et al., 2018).

The results from this study clearly illustrated the diversity, in terms of alpha- diversity, but especially beta-diversity, of forb species in the sampled natural grassland regions that vary with the spatial scale of assessment. The importance of considering the grain and extent of sampling was emphasized, as this had a notable effect on the interpolation of species richness. The high beta-diversity that was observed, even across small spatial extents, indicates that the species assemblages in different grassland areas are unique. Therefore, the transformation of even a small grassland patch can potentially lead to the loss of unique forb species assemblages and the associated ecosystem functioning (Carbutt et al., 2011; Costanza et al., 1997; Egoh et al., 2011). Therefore, sampling large grains across the greatest possible extents is required to obtain a comprehensive understanding of diversity patterns and drivers in highly diverse ecosystems, such as the grassland biome of South Africa. This study illustrated that the use of appropriate spatial grains and extents has to match the suspected diversity and biology of the species in question, as the use of small spatial grain and extent assessments cannot be extrapolated to the large spatial scales at which conservation is conducted (Barton et al., 2013; Kukkala and Moilanen, 2013; Turner et al., 1989). Therefore, this research can be used to inform environmental impact assessments and systematic conservation planning efforts as to the spatial grain and extent of sampling that is required to prevent the species loss associated with the ongoing anthropogenic transformation, such as mining, agriculture and afforestation by conducting accurate environmental impact assessments (Carbutt et al., 2017, 2011; Egoh et al., 2011; Olivier, 2020).

Forb alpha- and beta-diversity was also found to vary with aspect, and along certain landscape (at large spatial grains) and disturbance gradients (at small spatial grains). The effect of these gradients on species richness and compositional dissimilarity should also be considered when selecting regions for conservation (Dembicz et al., 2021b; Talebi et al., 2021). However, conservation is conducted at large spatial scales, therefore the effect of aspect and landscape gradients in regulating diversity patterns is more important than disturbance regimes, as the effect of burning patterns and grazing pressure was not important at large spatial grains. Grassland sites located on relatively steep slopes with southern aspects where acrisol soils are present will maximize local alpha-diversity. However, including other large, interconnected and geographically distant grassland conservation areas that are located on different aspects and stretch across the vertisol soil type, will maximize beta-diversity and thereby increase overall gamma-diversity. The effect of certain environmental and disturbance gradients on diversity patterns may also potentially be used as proxies for forb diversity in these systems, which will greatly decrease the labour intensity of grassland sampling efforts. The results from this study can consequently be used to guide both environmental impact assessments and systematic conservation efforts to prioritize certain grassland sites according to the aspect, and landscape gradients that are present in an area and the associated diversity patterns. However, the pronounced effect of the spatial extent and grain on patterns and drivers of diversity in a highly diverse system, such as the grassland biome observed in this study, warrants further investigation. Future research should focus on building a unified framework for diversity assessments in very diverse systems.

The forb sampling conducted in this study only represents a snapshot of the forb diversity at the time of sampling. Different grassland forb species are known to flower at different times of the year; however, a poor understanding still exists with regards to forb flowering phenology (Fidelis and Blanco, 2014; Lamont and Downes, 2011; Pillips, 1920). The proportion of flowering species during each season and at different time periods after a burning event has not been thoroughly explored in grasslands. Even though the days since the last fire was accounted for in this study, flowering phenology may still have an effect on forb diversity pattern estimates. A potential solution to this problem and a challenge for future studies may be to resample grassland sites at different times of the year, which will result in a more comprehensive understanding of grassland diversity patterns. Furthermore, exploring the relationship between spatial scale and diversity estimates and drivers further should be a priority in future ecological research, as this greatly influences the results of diversity assessments. The sampling effort of this study did not approach the true number of species in the grassland biome even at the largest spatial grain of sampling, therefore assessing the effect of using even larger spatial grains and extents of sampling across more sites than what was sampled in this study should be considered.

This study confirmed that small spatial scale diversity patterns and drivers cannot be extrapolated to the large spatial scales at which conservation efforts are conducted and at which anthropogenic transformation takes place, especially in highly diverse ecosystems, such as the grassland biome of South Africa (Harrison et al., 2003; Kukkala and Moilanen, 2013). The importance of considering the spatial scale at which ecosystem diversity assessments are conducted, as well as having recent, accurate richness and community composition data of the systems in question are of essence to describe and effectively conserve highly diverse ecosystems. This warrants further investigation to determine if similar patterns emerge in other highly diverse ecosystems. The world is witnessing the anthropogenically driven sixth mass extinction, therefore, having accurate, species-level diversity data is essential for goal-driven conservation in order to sustain the functioning of sensitive systems, the ecosystem services that are linked to these systems and subsequently, the health of our planet (Balvanera et al., 2006; Costanza et al., 1997; Rands et al., 2010).

Chapter 5

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Chapter 6

Supplementary results S1

Table S1: A summary table of each site and the associated climatic, landscape and disturbance drivers. MAP (Mean annual precipitation), MAT (Mean annual temperature), FRP (Fire radiative power), FRI (Fire return interval), MTCM (Minimum temperature of the coldest month), TR (Temperature range), DSLF (Days since the last fire).

Site	**Northness**	**MAP (mm)**	**MAT (^{o}C)**	**MTCM (^{o}C)**	**TR (^{o}C)**	**Grazing pressure**	**FRP (MW)**	**FRI (years)**	**DSLF**	**Slope**	**Soil type**	**Soil texture**	**Geology**
1	1	781.00	13.85	0.40	23.50	3	235.61	1.91	27	3	Lixisols	sandy	Sedimentary
2	1	811.00	14.60	1.10	22.80	3	292.47	1.56	53	1	Acrisols	sandy loam	Sedimentary
3	1	723.00	14.42	0.20	24.00	2	274.22	1.52	87	1	Acrisols	clay loam	Sedimentary
4	-1.83 x 10^{-16}	686.00	14.37	-0.60	25.20	2	329.27	1.52	20	3	Vertisols	clay	Igneous
5	-1	793.00	13.29	0.10	22.90	1	235.61	1.91	106	2	Regosols	loam	Sedimentary
6	1	781.00	17.20	2.70	25.10	1	235.61	1.91	107	3	Nitosols	clay	Igneous
7	6.13 x 10^{-17}	774.00	15.61	1.70	24.00	1	235.61	1.91	94	3	Ferrasols	clay	Sedimentary
8	-1	786.00	14.29	0.90	23.20	2	235.61	1.91	81	1	Regosols	loam	Sedimentary
9	1	794.00	14.00	0.90	22.80	1	235.61	1.91	98	2	Leptosols	loam	Sedimentary
10	-1.83 x 10^{-16}	779.00	15.85	1.90	24.10	2	235.61	1.91	99	1	Ferrasols	loam	Sedimentary
11	-1	777.00	17.42	2.80	25.30	1	235.61	1.91	130	2	Nitosols	clay	Igneous
12	6.13 x 10^{-17}	823.00	15.68	2.00	23.80	2	168.09	1.07	20	2	Regosols	sandy loam	Sedimentary
13	1	817.00	16.63	2.70	24.30	3	168.09	1.07	71	1	Regosols	loam clay	Sedimentary
14	-1.83 x 10^{-16}	816.00	16.63	2.70	24.30	3	168.09	1.07	71	1	Regosols	loam clay	Sedimentary
15	1	798.00	17.10	2.60	24.90	2	168.09	1.07	56	1	Acrisols	clay	Sedimentary
16	1	800.00	16.46	2.40	24.30	2	168.09	1.07	38	2	Lixisols	loam clay	Sedimentary
17	-1	825.00	16.61	2.80	23.90	2	120.89	1.32	73	1	Luvisols	loam	Igneous
18	-1	815.00	17.32	2.60	25.40	3	162.70	1.48	21	1	Vertisols	clay loam	Sedimentary
19	6.13 x 10^{-17}	813.00	17.08	3.00	24.40	2	162.84	1.32	22	1	Luvisols	clay loam	Sedimentary
20	1	813.00	17.03	2.90	24.50	1	162.84	1.32	22	1	Luvisols	sandy loam	Sedimentary
21	-1.83 x 10^{-16}	884.00	16.48	6.60	17.80	1	161.90	2.15	168	2	Regosols	loam sand	Igneous

22	1	884.00	16.48	6.60	17.80	1	161.90	2.15	168	1	Regosols	loam	Igneous
23	6.13 x 10^{-17}	836.00	13.13	0.60	21.70	1	268.54	1.48	54	3	Acrisols	sandy loam	Sedimentary
24	1	881.00	16.17	3.20	22.30	3	186.80	1.48	87	2	Acrisols	clay	Sedimentary
25	1	874.00	15.73	2.60	22.40	2	188.60	1.48	29	3	Acrisols	clay loam	Igneous
26	-1.83 x 10^{-16}	879.00	17.29	3.50	23.20	2	188.60	1.48	36	2	Acrisols	loam	Igneous-Metamorphic
27	-1.83 x 10^{-16}	877.00	16.35	3.10	22.60	1	188.60	1.48	91	2	Acrisols	loam	Igneous
28	-1.83 x 10^{-16}	898.00	16.28	3.40	22.10	2	188.60	1.48	58	1	Ferrasols	loam clay	Igneous
29	-1.83 x 10^{-16}	793.00	14.63	1.20	23.00	2	268.54	1.48	58	2	Leptosols	clay loam	Sedimentary
30	1	872.00	16.77	3.40	22.80	2	188.60	1.48	52	3	Acrisols	loam	Igneous
31	-1	687.00	14.40	-0.50	25.10	2	329.27	1.52	122	1	Vertisols	loam clay	Sedimentary
32	1	691.00	14.25	-0.10	24.10	1	274.22	1.52	146	1	Phaeozems	loam	Igneous
33	-1	707.00	14.25	-0.10	24.10	3	274.22	1.52	252	1	Phaeozems	loam	Igneous
34	-1	695.00	14.37	0.00	24.20	2	274.22	1.52	147	2	Phaeozems	loam	Sedimentary
35	-1.83 x 10^{-16}	823.00	15.00	2.20	22.00	2	248.26	1.56	132	2	Acrisols	sandy loam	Metamorphic-Sedimentary
36	1	861.00	14.33	1.80	21.60	1	248.26	1.56	132	2	Acrisols	loam	Metamorphic-Sedimentary
37	1	815.00	14.80	1.80	22.20	1	248.26	1.56	164	1	Acrisols	loam	Metamorphic-Sedimentary
38	-1.83 x 10^{-16}	656.00	17.00	2.80	23.70	3	136.08	1.26	183	1	Leptosols	loam sand	Sedimentary
39	-1.83 x 10^{-16}	648.00	17.80	3.50	24.00	3	136.08	1.26	257	1	Leptosols	loam sand	Sedimentary
40	-1	703.00	15.28	-0.10	25.50	1	190.96	1.44	176	1	Vertisols	clay	Igneous
41	-1.83 x 10^{-16}	691.00	15.21	-0.10	25.30	2	190.96	1.44	243	1	Vertisols	loam	Igneous
42	1	703.00	17.45	2.60	24.90	3	134.02	1.12	278	1	Leptosols	loam	Sedimentary

Table S2: A table displaying the eigenvalue, proportion explained and cumulative proportion of each PC axis of the climate variables.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	2.51	1.27	0.85	0.36	0.01
Proportion Explained	0.50	0.25	0.17	0.07	0.00
Cumulative Proportion	0.50	0.76	0.93	0.99	1.00

Table S3: A table displaying the eigenvalue, proportion explained and cumulative proportion of the disturbance variables.

	PC1	PC2	PC3	PC4
Eigenvalue	1.76	1.02	0.76	0.46
Proportion Explained	0.44	0.26	0.19	0.11
Cumulative Proportion	0.44	0.70	0.89	1.00

Table S4: A table displaying the eigenvalue, proportion explained and cumulative proportion of each PC axis of the landscape variables.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
Eigenvalue	2.42	1.99	1.50	1.35	1.17	1.11	1.09	1.08	1.04	0.78	0.64	0.41	0.36	0.07
Proportion Explained	0.16	0.13	0.10	0.09	0.07	0.07	0.073	0.07	0.07	0.05	0.04	0.03	0.02	0.01
Cumulative Proportion	0.16	0.29	0.40	0.48	0.56	0.64	0.71	0.80	0.85	0.90	0.94	0.97	1.00	1

Table S5: A correlation matrix of all variables. A Pearson's correlation coefficient of r > 0.6 cut-off value was used to eliminate highly correlated variables.

	Slope	**Temperature range**	**Min temp of coldest month**	**Northness**	**Mean annual temperature**	**Mean annual precipitation**	**Grazing pressure**	**Days since last fire**	**Fire return interval**	**Fire radiative power**	**Soil texture**
Slope	1.00	-0.15	-0.05	0.17	-0.22	0.26	-0.35	-0.36	0.30	0.27	-0.06
Temperature range	-0.15	1.00	-0.57	-0.25	0.13	-0.61	0.15	-0.05	-0.47	0.08	-0.39
Min temp of coldest month	-0.05	-0.57	1.00	0.25	0.73	0.60	0.13	-0.02	0.09	-0.69	0.08
Northness	0.17	-0.25	0.25	1.00	0.09	0.27	0.11	-0.15	-0.10	-0.11	0.08
Mean annual temperature	-0.22	0.13	0.73	0.09	1.00	0.17	0.27	0.02	-0.31	-0.77	-0.21
Mean annual precipitation	0.26	-0.61	0.60	0.27	0.17	1.00	0.00	-0.52	0.16	-0.26	0.02
Herbivory	-0.35	0.15	0.13	0.11	0.27	0.00	1.00	-0.20	-0.31	-0.24	0.06
Days since last fire	-0.36	-0.05	-0.02	-0.15	0.02	-0.52	-0.20	1.00	0.10	-0.07	0.16
Fire return interval	0.30	-0.47	0.09	-0.10	-0.31	0.16	-0.31	0.10	1.00	0.36	0.07
Fire radiative power	0.27	0.08	-0.69	-0.11	-0.77	-0.26	-0.24	-0.07	0.36	1.00	-0.10
Soil texture	-0.06	-0.39	0.08	0.08	-0.21	0.02	0.06	0.16	0.07	-0.10	1.00

Table S6: Estimated gamma-diversity of the sampled grassland area as estimated by four non-parametric estimators at a large, intermediate and small spatial grain. The standard errors of all estimators except the Jacknife 2 are displayed, as the package 'vegan' is not yet able to calculate the Jacknife 2 standard error.

	Total number of observed species	**Chao 2**	**Jacknife1**	**Jacknife2**	**Bootstrap**
Large spatial grain	486	1067.82 ± 98.57	775.93 ± 50.77	990.03	605.15 ± 21.73
Intermediate spatial grain	284	548.92 ± 57.32	448.00 ± 9.41	559.68	352.81 ± 13.13
Small spatial grain	136	280.40 ± 44. 14	219.95 ± 16.47	278.6283	170.96 ± 7.49

Table S7: A table showing the results from the Tukey 'honest significance difference' method to detect significant differences between all pairs of soil types, based on the range of the sample means, with a 95% confidence interval. Significant differences between soil types are indicated in bold.

Soil type comparison	Difference	Lower point	Upper point	p-value
Ferrasol-Acrisol	-4.08	-22.66	14.49	0.99
Leptosol-Acrisol	2.58	-12.74	17.90	0.99
Lixisol-Acrisol	2.58	-19.40	24.56	0.99
Luvisol-Acrisol	15.9	-2.66	34.49	0.14
Nitosol-Acrisol	-10.4	-32.40	11.56	0.81
Phaeozem-Acrisol	-5.08	-23.66	13.49	0.99
Regosol-Acrisol	-5.56	-19.25	8.13	0.91
Vertisol-Acrisol	-2.62	-17.94	12.70	0.99
Leptosol-Ferrasol	6.67	-14.35	27.68	0.98
Lixisol-Ferrasol	6.67	-19.61	32.94	0.99
Luvisol-Ferrasol	20.00	-3.50	43.50	0.15
Nitosol-Ferrasol	-6.33	-32.61	19.94	0.99
Phaeozem-Ferrasol	-1.00	-24.50	22.50	1
Regosol-Ferrasol	-1.48	-21.34	18.38	0.99
Vertisol-Ferrasol	1.47	-19.55	22.48	0.99
Lixisol-Leptosol	3.55×10^{-14}	-24.078	24.08	1
Luvisol-Leptosol	13.30	-7.68	34.35	0.49
Nitosol-Leptosol	-13.00	-37.08	11.08	0.69
Phaeozem-Leptosol	-7.67	-28.69	13.35	0.95
Regosol-Leptosol	-8.14	-24.99	8.71	0.80
Vertisol-Leptosol	-5.20	-23.40	13.00	0.99
Luvisol-Lixisol	13.30	-12.94	39.61	0.75
Nitosol-Lixisol	-13.30	-41.78	15.78	0.85
Phaeozem-Lixisol	-7.67	-33.94	18.61	0.99
Regosol-Lixisol	-8.14	-31.22	14.93	0.96

Vertisol-Lixisol	-5.20	-29.28	18.89	0.99
Nitosol-Luvisol	-26.30	-52.61	-0.06	**0.05**
Phaeozem-Luvisol	-21.00	-44.50	2.50	0.11
Regosol-Luvisol	-21.50	-41.34	-1.62	**0.03**
Vertisol-Luvisol	-18.50	-39.55	2.48	0.12
Phaeozem-Nitosol	5.33	-20.94	31.61	0.99
Regosol-Nitosol	4.86	-18.22	27.93	0.99
Vertisol-Nitosol	7.80	-16.28	31.88	0.97
Regosol-Phaeozem	-0.48	-20.34	19.38	1
Vertisol-Phaeozem	2.47	-18.55	23.48	0.99
Vertisol-Regosol	2.94	-13.91	19.79	0.99

Table S8: A list of all forbs that were recorded in this study. Two-hundred-and-eighty-eight forbs were identified to species level, 98 forbs to genus level and 22 to family-level, while 69 forbs were only identified as morphospecies. Exotic species are highlighted with an asterisk.

Species	**Family**
Acalypha glandulifolia	Euphorbiaceae
Acalypha punctata	Euphorbiaceae
Acalypha schinzii	Euphorbiaceae
Aeschynomene indica	Fabaceae
Aeschynomene rehmanii	Fabaceae
Afroaster hispidus	Asteraceae
Ajuga ophrydis	Lamiaceae
Albuca setosa	Hyacinthaceae
Alepidea natalensis	Apiaceae
Aloe davyana	Asphodelaceae
Aloe maculata	Asphodelaceae
Alysicarpus rugosus	Fabaceae
Anthospermum herbaceum	Rubiaceae
Anthospermum hispidulum	Rubiaceae
Anthospermum rigidum	Rubiaceae
Argyrolobium sericosemium	Fabaceae
Argyrolobium tomentosum	Fabaceae
Argyrolobium tuberosum	Fabaceae
Argyrolobium velutinum	Fabaceae
Aristea woodii	Iridaceae
Asclepias cucullata	Apocynaceae
Asclepias depressa	Apocynaceae
Asclepias eminens	Apocynaceae
Asclepias gibba	Apocynaceae
Asclepias stellifera	Apocynaceae
Aster bakerianus	Asteraceae
Athrixia angustissima	Asteraceae
Athrixia elata	Asteraceae
Berkheya echinacea	Asteraceae

Berkheya insignis	Asteraceae
Berkheya macrocephala	Asteraceae
Berkheya pinnatifida	Asteraceae
Berkheya radula	Asteraceae
Berkheya schinzii	Asteraceae
Berkheya setifera	Asteraceae
Berkheya speciosa	Asteraceae
Bidens pilosa	Asteraceae
Blepharis subvolubilis	Acanthaceae
Callilepis laureola	Asteraceae
Centella asiatica	Apiaceae
Chascanum latifolium	Verbenaceae
Chlorophytum cooperi	Agavaceae
Cleome maculata	Cleomaceae
Clutia hirsuta	Peraceae
Clutia monticola	Peraceae
Coleus heroensis	Lamiaceae
Commelina africana	Commelinaceae
Commelina benghalensis	Commelinaceae
Conostomium natalense	Rubiaceae
Convolvulus sagittatus	Convolvulaceae
Corchorus confusus	Malvaceae
*Cosmos bipinnatus**	Asteraceae
Crabbea acaulis	Acanthaceae
Crassula acinaciformis	Crassulaceae
Crassula lanceolata subsp. transvaalensis	Crassulaceae
Crassula pellucida	Crassulaceae
Craterocapsa tarsodes	Campanulaceae
*Crepis hypochoeridea**	Asteraceae
Crotalaria capensis	Fabaceae
Crotalaria natalensis	Fabaceae
Cucumis zeyheri	Cucurbitaceae
Cyanotis speciosa	Commelinaceae
Cynoglossum hispidum	Boranginaceae

Cyrtanthus tuckii	Amaryllidaceae
Delosperma caespitosum	Aizoaceae
Delosperma sutherlandii	Aizoaceae
Diclis reptans	Scrophulariaceae
Diclis rotundifolia	Scrophulariaceae
Dicoma anomala	Asteraceae
Dipcadi marlothii	Hyacinthaceae
Dipogon lignosus	Fabaceae
Drimia delagoensis	Hyacinthaceae
Dumasia villosa	Fabaceae
Dyschoriste burchellii	Acanthaceae
Dyschoriste costata	Acanthaceae
Dyschoriste setigera	Acanthaceae
Empodium monophyllum	Hypoxidaceae
Eriosema burkei	Fabaceae
Eriosema cordatum	Fabaceae
Eriosema kraussianum	Fabaceae
Eriosema salignum	Fabaceae
Euphorbia clavarioides	Euphorbiaceae
*Euphorbia indica**	Euphorbiaceae
Euphorbia striata	Euphorbiaceae
Felicia hirsuta	Asteraceae
Felicia linearis	Asteraceae
Felicia mossamedensis	Asteraceae
Felicia muricata subsp. muricata	Asteraceae
Gazania krebsiana subsp. serrulata	Asteraceae
Gerbera ambigua	Asteraceae
Gerbera aurantiaca	Asteraceae
Gerbera piloselloides	Asteraceae
Gerbera viridifolia	Asteraceae
Gnidia baurii	Thymelaeaceae
Gnidia gymnostachya	Thymelaeaceae
Graderia scabra	Orobanchaceae
Haplocarpha scaposa	Asteraceae

Helichrysum allioides	Asteraceae
Helichrysum aureonitens	Asteraceae
Helichrysum aureum	Asteraceae
Helichrysum auriceps	Asteraceae
Helichrysum caespititium	Asteraceae
Helichrysum cephaloideum	Asteraceae
Helichrysum chionosphaerum	Asteraceae
Helichrysum coriaceum	Asteraceae
Helichrysum cymosum	Asteraceae
Helichrysum grandibracteatum	Asteraceae
Helichrysum nudifolium	Asteraceae
Helichrysum oreophilum	Asteraceae
Helichrysum pallidum	Asteraceae
Helichrysum piloselum	Asteraceae
Helichrysum rugulosum	Asteraceae
Helichrysum thapsus	Asteraceae
Hermannia coccocarpa	Malvaceae
Hermannia cristata	Malvaceae
Hermannia depressa	Malvaceae
Hermannia transvaalensis	Malvaceae
Hermannia woodii	Malvaceae
Hibiscus aethiopicus	Malvaceae
Hibiscus engleri	Malvaceae
Hibiscus microcarpus	Malvaceae
Hilliardiella aristata	Asteraceae
Hilliardiella capensis	Asteraceae
Hilliardiella elaeagnoides	Asteraceae
Hilliardiella hirsuta	Asteraceae
Hilliardiella natalensis	Asteraceae
Hilliardiella oligocephala	Asteraceae
Hypericum aethiopicum subsp. aethiopicum	Hypericaceae
Hypericum aethiopicum subsp. sonderi	Hypericaceae
Hypericum lalandii	Hypericaceae
*Hypochaeris radicata**	Asteraceae

Hypoxis interjecta	Hypoxidaceae
Hypoxis acuminata	Hypoxidaceae
Hypoxis angustifolia	Hypoxidaceae
Hypoxis costata	Hypoxidaceae
Hypoxis filiformis	Hypoxidaceae
Hypoxis interjecta	Hypoxidaceae
Hypoxis ludwigii	Hypoxidaceae
Hypoxis multiceps	Hypoxidaceae
Hypoxis obtusa	Hypoxidaceae
Hypoxis rigidula	Hypoxidaceae
Indigastrum fastigiatum	Fabaceae
Indigofera comosa	Fabaceae
Indigofera dimidiata	Fabaceae
Indigofera evansiana	Fabaceae
Indigofera hedyantha	Fabaceae
Indigofera hilaris	Fabaceae
Indigofera oxytropis	Fabaceae
Indigofera purple margins	Fabaceae
Indigofera sanguinea	Fabaceae
Indigofera swaziensis	Fabaceae
*Inulanthera calva**	Asteraceae
Ipomoea crassipes	Convolvulaceae
Ipomoea oblongata	Convolvulaceae
*Ipomoea oblongifolia**	Convolvulaceae
Ipomoea sinensis	Convolvulaceae
Jamesbrittenia aurantiaca	Scrophulariaceae
Justicia anagalloides	Acanthaceae
Kalanchoe paniculata	Crassulaceae
Kohautia amatymbica	Rubiaceae
*Lactuca saligna**	Asteraceae
Lactuca tysonii	Asteraceae
Lasiosiphon caffer	Thymelaeaceae
Lasiosiphon capitatus	Thymelaeaceae
Lasiosiphon kraussianus	Thymelaeaceae

Launaea rarifolia	Asteraceae
Ledebouria apertiflora	Hyacinthaceae
Ledebouria asperifolia	Hyacinthaceae
Ledebouria cooperi	Hyacinthaceae
Ledebouria long leaf	Hyacinthaceae
Ledebouria luteola	Hyacinthaceae
Ledebouria ovatifolia	Hyacinthaceae
Leobordea divaricata	Fabaceae
Leobordea eriantha	Fabaceae
Leobordea foliosa	Fabaceae
Leobordea pulchra	Fabaceae
Linum thunbergii	Linaceae
Lobelia flaccida	Lobeliaceae
Lobelia sonderiana	Lobeliaceae
Lotononis amajubica	Fabaceae
Lotononis eriantha	Fabaceae
Lotononis amajubica	Fabaceae
Monopsis decipiens	Lobeliaceae
Monsonia burkeana	Geraniaceae
Monsonia angustifolia	Geraniaceae
Monsonia attenuata	Geraniaceae
Moraea stricta	Iridaceae
Nemesia fructicans	Scrophulariaceae
Nidorella anomala	Asteraceae
Nidorella undulata	Asteraceae
Nolletia rarifolia	Asteraceae
Ocimum obovatum	Lamiaceae
*Oenothera indecora**	Onagraceae
*Oenothera tetraptera**	Onagraceae
Oldenlandia herbacea	Rubiaceae
Ophrestia oblongifolia	Fabaceae
*Oxalis corniculata**	Oxalidaceae
Oxalis obliquifolia	Oxalidaceae
Pearsonia obovata	Fabaceae

Pearsonia sessilifolia	Fabaceae
Pelargonium alchemilloides	Geraniaceae
Pelargonium luridum	Geraniaceae
Pellaea calomelanos	Pteridaceae
Pentanisia angustifolia	Rubiaceae
Pentanisia prunelloides subsp. prunelloides	Rubiaceae
Phyllanthus maderaspatensis	Phyllanthaceae
Phyllanthus parvulus	Phyllanthaceae
Plantago longissima	Plantaginaceae
*Plantago major**	Plantaginaceae
*Plantago virginica**	Plantaginaceae
Polygala amatymbica	Polygalaceae
Polygala macowaniana	Polygalaceae
Polygala rehmannii	Polygalaceae
Polygala rhinostigma	Polygalaceae
Polygala transvaalensis	Polygalaceae
Psammotropha myriantha	Molluginaceae
Pygmaeothamnus chamaedendrum	Rubiaceae
Ranunculus meyeri	Ranunculaceae
Ranunculus multifidus	Ranunculaceae
Raphionacme hirsuta	Apocynaceae
Rhodohypoxis baurii	Hypoxidaceae
Rhynchosia angulosa	Fabaceae
Rhynchosia cooperi	Fabaceae
Rhynchosia minima	Fabaceae
Rhynchosia monophylla	Fabaceae
Rhynchosia totta	Fabaceae
*Richardia brasiliensis**	Rubiaceae
Rotheca hirsuta	Lamiaceae
*Rumex acetosella**	Polygonaceae
Rumex saggitatus	Polygonaceae
Scabiosa columbaria	Dipsacaceae
Schistostephium crataegifolium	Asteraceae
*Schkuhria pinnata**	Asteraceae

Sebaea grandis	Gentianaceae
Selago densiflora	Scrophulariaceae
Senecio coronatus	Asteraceae
Senecio discodregeanus	Asteraceae
Senecio glaberrimus	Asteraceae
Senecio inornatus	Asteraceae
Senecio isatidioides	Asteraceae
Senecio madagascariensis	Asteraceae
Senecio scitus	Asteraceae
Senecio speciosus	Asteraceae
Senecio subcoriaceus	Asteraceae
Senecio venosus	Asteraceae
*Solanum elaeagnifolium**	Solanaceae
*Solanum mauritianum**	Solanaceae
Solanum panduriforme	Solanaceae
*Solanum sisymbriifolium**	Solanaceae
Sonchus dregeanus	Asteraceae
Sonchus nanus	Asteraceae
Sonchus wilmsii	Asteraceae
Spermacoce natalensis	Rubiaceae
Stachys natalensis	Lamiaceae
Stoebe vulgaris	Asteraceae
Syncolostemon pretoriae	Lamiaceae
Syncolostemon thorncroftii	Lamiaceae
*Tagetes minuta**	Asteraceae
*Taraxacum officinale**	Asteraceae
Tephrosia bachmannii	Fabaceae
Tephrosia capensis	Fabaceae
Tephrosia elongata	Fabaceae
Tephrosia lupinifolia	Fabaceae
Tephrosia macropoda	Fabaceae
Tephrosia polystachya	Fabaceae
Tephrosia purpurea	Fabaceae
Tephrosia rhodesica	Fabaceae

Thesium costatum var. costatum	Santalaceae
Thesium pallidum	Santalaceae
Thunbergia atriplicifolia	Acanthaceae
Thunbergia dregeana	Acanthaceae
Thunbergia venosa	Acanthaceae
Trachyandra asperata	Asphodelaceae
Tragia wahlbergiana	Euphorbiaceae
*Tragopogon dubius**	Asteraceae
Trifolium africanum	Fabaceae
Ursinia tenuiloba	Asteraceae
*Verbena bonariensis**	Verbenaceae
Vigna unguiculata	Fabaceae
Wahlenbergia appressifolia	Campanulaceae
Wahlenbergia grandiflora	Campanulaceae
Wahlenbergia krebsii	Campanulaceae
Wahlenbergia virgata	Campanulaceae
Watsonia watsonioides	Iridaceae
Xerophyta retinervis	Velloziaceae
Xysmalobium undulatum	Apocynaceae
Zornia linearis	Fabaceae
Zornia milneana	Fabaceae
Acalypha spp. 3	Euphorbiaceae
Afroaster spp.2	Asteraceae
Albuca spp. 2 (small)	Hyacinthaceae
Albuca-like	Hyacinthaceae
Alepidea spp. 1	Apiaceae
Alepidea-like	Apiaceae
Anthospermum spp. 1	Rubiaceae
Anthospermum-like	Rubiaceae
Anthospermum long stem	Rubiaceae
Aristea spp. 1	Iridaceae
Asclepias basal	Apocynaceae
Asclepias spp. 2	Apocynaceae
Asclepias spp. 3	Apocynaceae

Asclepias spp.5	Apocynaceae
Asclepias thin leaf	Apocynaceae
Asclepias tiny	Apocynaceae
Athrixia spp. 1	Asteraceae
Athrixia spp. 4	Asteraceae
Barleria spp.	Acanthaceae
Berkheya-like (Aromatic)	Asteraceae
Brachystelma spp. 1	Apocynaceae
Brachystelma spp. 2	Apocynaceae
Brunsvigia spp.	Amaryllidaceae
Bulbine spp. 1	Asphodelaceae
Bulbine-like	Asphodelaceae
Bulbine-like 2	Asphodelaceae
Bulbine-like purple underside leaf	Asphodelaceae
Bulbine-like spp.	Asphodelaceae
Cleome trifoliate	Cleomaceae
Clerodendrum broad leaf	Lamiaceae
Commelina spp. 3	Commelinaceae
Conyza	Asteraceae
Conyza look alike	Asteraceae
Conyza spp.	Asteraceae
Conyza spp.2	Asteraceae
Crassula (baby)	Crassulaceae
Crassula tiny	Crassulaceae
Croscosmia spp. 1	Iridaceae
Cucumis-like	Cucurbitaceae
Cyphostemma	Vitaceae
Cyrtanthus spp. 2	Amaryllidaceae
Cyrtanthus spp. 4 only leaves	Amaryllidaceae
Dipcadi spp. 1	Hyacinthaceae
Dyschoriste spp. 2	Acanthaceae
Dyschoriste spp. 3	Acanthaceae
Eriosema (large red stipules)	Fabaceae
Euryops	Asteraceae

Euryops spp. 1	Asteraceae
Felicia-like	Asteraceae
Gladiolus spp.	Iridaceae
Gladiolus spp. 3	Iridaceae
Gladiolus spp. 7	Iridaceae
Gnidia spp. 3	Thymelaeaceae
Hairy *Indigofera*	Fabaceae
Helichrysum spp. 5	Asteraceae
Helichrysum tiny	Asteraceae
Helichrysum unknown light green	Asteraceae
Helichrysum yellow	Asteraceae
Hermannia -like	Malvaceae
Hybanthus spp. 1	Violaceae
Hypericum-like	Hypericaceae
Hypericum-like (lamiacea)	Hypericaceae
Indigastrum spp. 3	Fabaceae
Indigofera	Fabaceae
Indigofera-like	Fabaceae
Ipomoea spp. 2	Convolvulaceae
Kohautia spp. 2	Rubiaceae
Ledebouria purple underside	Hyacinthaceae
Ledebouria spp. 3	Hyacinthaceae
Ledebouria spp. 7	Hyacinthaceae
Ledebouria spp. Narrow leaves	Hyacinthaceae
Ledebouria spp1.	Hyacinthaceae
Monsonia narrow leaf	Geraniaceae
Nidorella like not sticky	Asteraceae
Nidorella-like	Asteraceae
Oxalis purple margins	Oxalidaceae
Pelargonium spp.	Geraniaceae
Rhynchosia spp. 5	Fabaceae
Rotheca spp. 2	Lamiaceae
Scabiosa-like	Dipsacaceae
Schistostephium	Asteraceae

Senecio (Richardia)	Asteraceae
Senecio clasping leaf	Asteraceae
Senecio hairy stem	Asteraceae
Senecio narrow leaf	Asteraceae
Senecio silky	Asteraceae
Senecio-like (sticky leaves) sp3	Asteraceae
Senecio-like light green leaf (aromatic) (nudifolium)	Asteraceae
Solanum spp2	Solanaceae
Tephrosia hairy	Fabaceae
Tephrosia pink	Fabaceae
Thesium long leaf	Santalaceae
Thesium spp. 2	Santalaceae
Thesium spp. 3	Santalaceae
Thesium spp. 4	Santalaceae
Ursinia spp. 1	Asteraceae
Wahlenbergia spp.1	Campanulaceae
Watsonia spp. 2	Iridaceae
Watsonia spp. 3	Iridaceae
Alliaceae spp. 1	Alliaceae
Aponaceae 1	Apocynaceae
Asclepid too small	Apocynaceae
Baby asteraceae	Asteraceae
Boranginaceae	Boranginaceae
Cotula-like	Asteraceae
Crabbea like (no hairs)	Acanthaceae
Galled fabaceae	Fabaceae
Iridaceae	Iridaceae
Lamiaceae	Lamiaceae
Malvaceae prostrate	Malvaceae
Rubiaceae	Rubiaceae
Orchid	Orchidaceae
Orchid (big)	Orchidaceae
Orchid 4	Orchidaceae
Orchid big	Orchidaceae

Orchid spp. 2	Orchidaceae
Orchid spp. 3	Orchidaceae
Orchid spp. 7	Orchidaceae
Orchid spp. 8	Orchidaceae
Orchid spp. 9	Orchidaceae
Orchid spp5	Orchidaceae

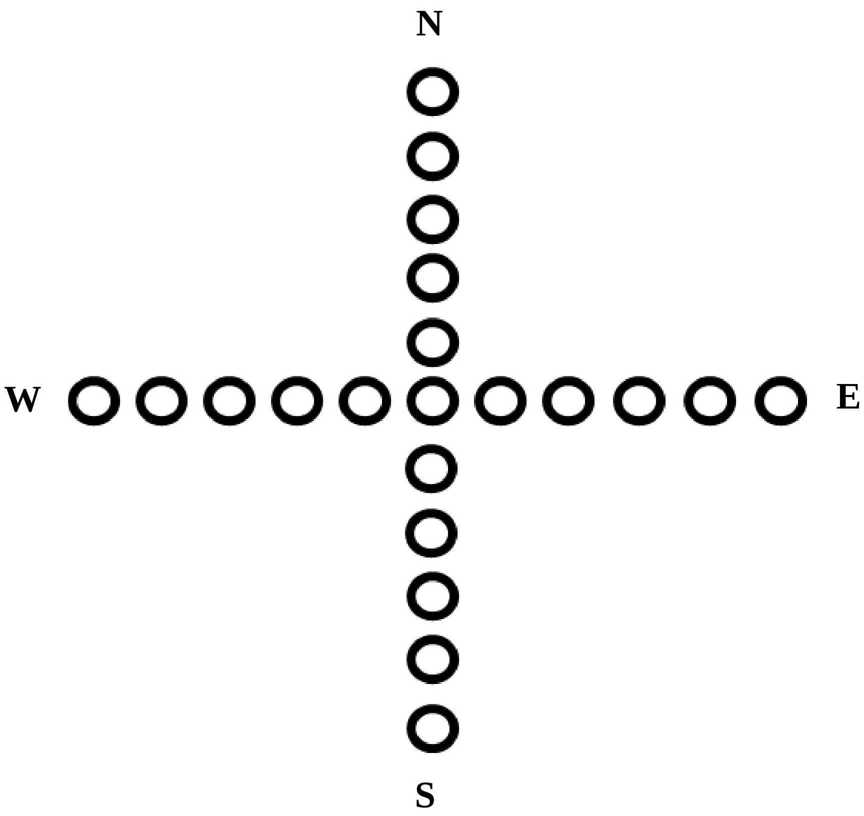

Figure S1: A diagram of the site sampling design: one site was made up of 21 one circular plots (individual plot area: 0.79 m^2) laid out in a cross-shaped pattern, orientated along cardinal directions as north-south and east-west. Each plot had a diameter of 1 m. The plots were spaced five metres apart, with each transect (north-south and east-west) covering a total distance of 50 meters. The total area of each site (as the sum of all plots) was 16.59 m^2.

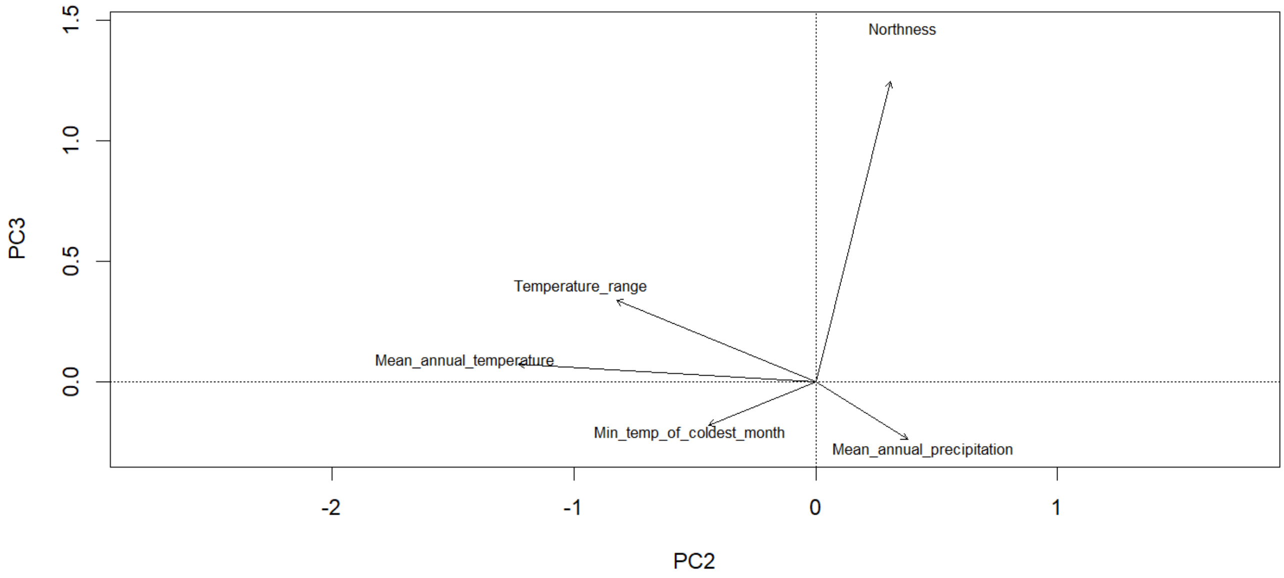

Figure S2: A PCA ordination biplot depicting how strongly climate variables collected across 42 grassland sites relate to the second and third principal components.

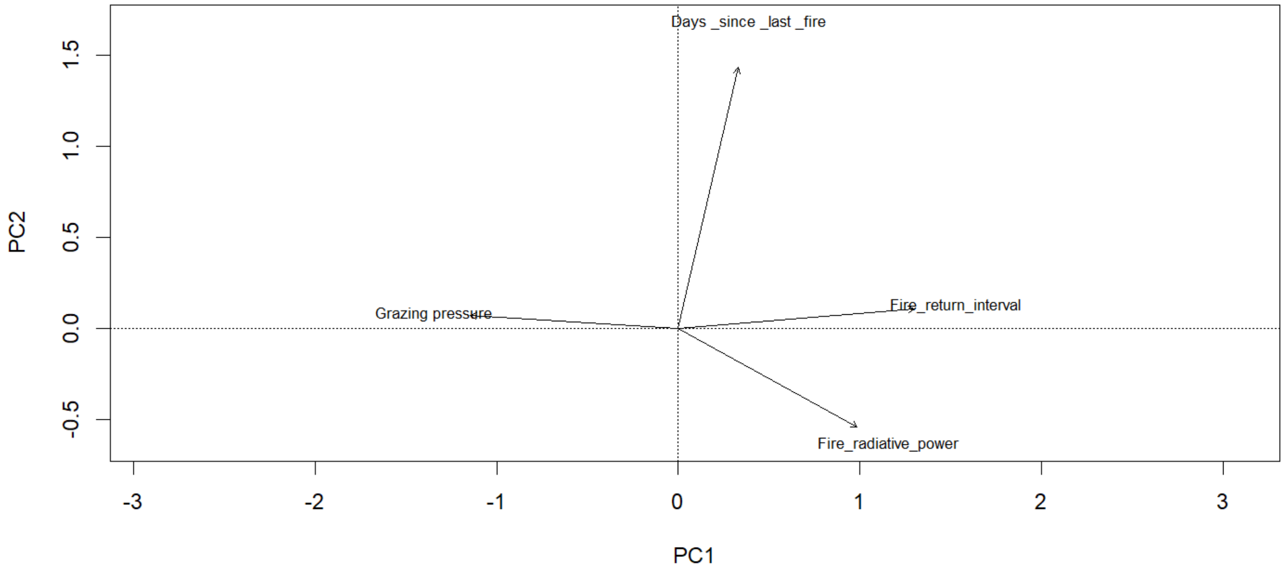

Figure S3: A PCA ordination biplot depicting how strongly each of the disturbance variables collected across 42 grassland sites relate to the first and second principal components.

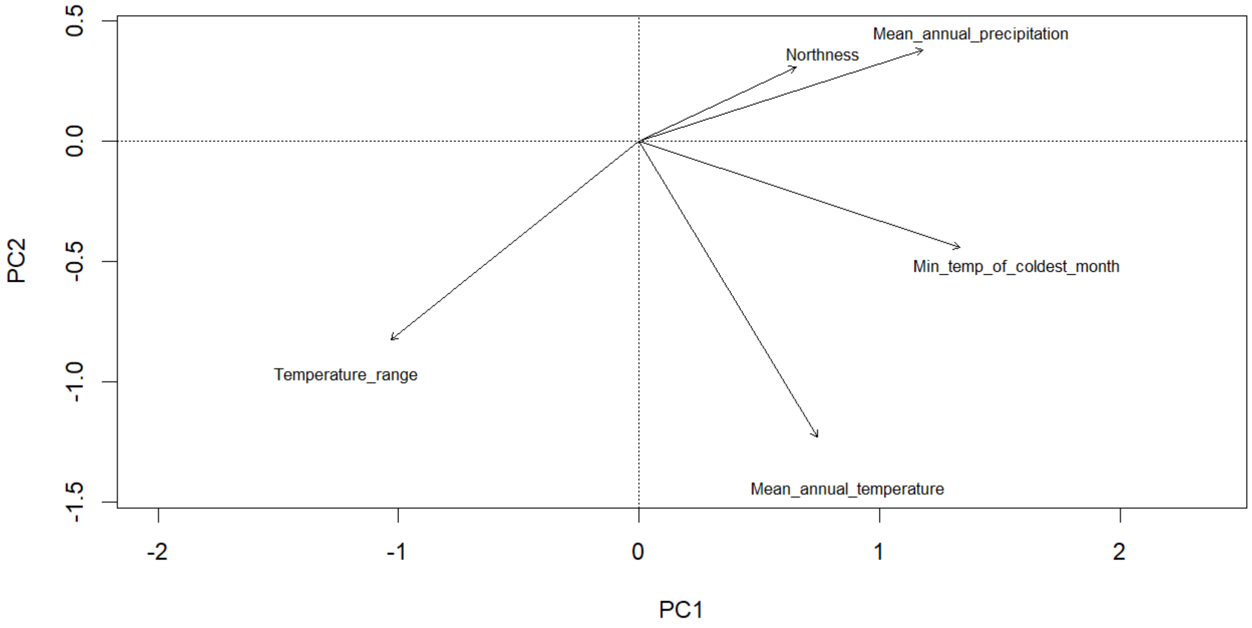

Figure S4: A PCA ordination biplot depicting how strongly each of the climatic variables collected across 42 grassland sites relate to the first and second principal components.

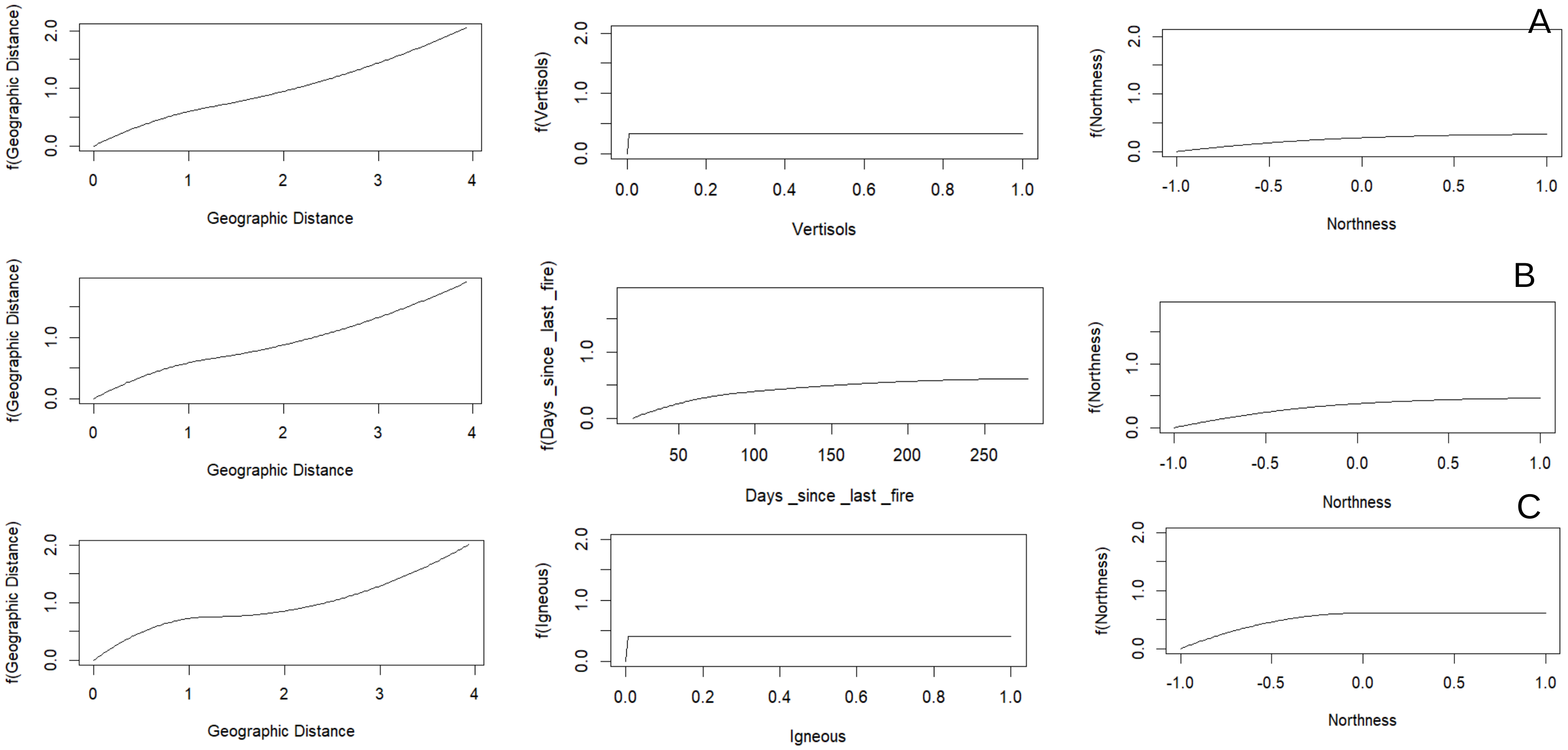

Figure S5: Fitted function for the generalised dissimilarity modelling of the relationship between each predictor variable and beta-diversity between sites at a large (A), intermediate (B) and small spatial grain (C).

Supplementary methods S1

The process of estimating species richness is complex, especially in ecosystems with exceptionally high species richness, where a multitude of rare species will not be sampled even with extensive sampling efforts (Chao and Chiu, 2016). Therefore, observed species richness often falls short from even approaching the true number of species in a very diverse system (Brose, 2002). One of the widely recognized approaches to interpolate species richness is the asymptotic approach (Brose, 2002; Chao and Chiu, 2016; Colwell et al., 2012; Gotelli and Colwell, 2011). In this approach, species accumulation curves are constructed, with the asymptote of these curves being used as a proxy for true species richness (Chao and Chiu, 2016).

Two methods exist to fit the species accumulation curve, namely parametric and non-parametric approaches (Brose, 2002; Chao and Chiu, 2016; Kunin et al., 2018). The parametric approach fits a parametric curve to predict species richness through the asymptote (Chao and Chiu, 2016). One of the greatest limitations of parametric approaches is that frequency information regarding rare species (singletons and doubletons), as well as common species are often not included in the model (Chao and Chiu, 2016). Underlying assumptions of the species accumulation curves or species abundances are also required to apply parametric methods (Brose, 2002).

Non-parametric estimators, on the other hand, do not make any assumptions about the shape of the species abundance or incidence data and are consequently very versatile in their application (Brose, 2002; Chao and Chiu, 2016). Non-parametric estimators depend on the frequency occurrence of singletons and doubletons in the estimation of species richness. The assumption is made that abundant species are sampled frequently and suggest no information about the abundance of rare species, while the frequency of sampling singletons and doubletons will indirectly infer information about the abundance of rare species in the community and lead to an overall improved estimation of species richness (Brose, 2002; Chao and Chiu, 2016).

A variety of different non-parametric estimators exists that are useful to predict species richness without assuming any knowledge about the underlying distribution patterns of species richness. However, all non-parametric estimators tend to underestimate true species richness and have considerable variances (Gotelli and Colwell, 2011). Consequently, species richness estimation by these estimators should be handled as lower-bound estimate (Brose, 2002; Gotelli and Colwell, 2011). For incidence data, the following non-parametric estimators are widely used: Chao 2 index; the Jacknife 1 and Jacknife 2 estimators, as well as the Bootstrap estimator (Brose, 2002; Chao and Chiu, 2016; Gotelli and Colwell, 2011). It has been demonstrated repeatedly for incidence data that the Chao 2 index and two Jacknife estimators outperform the Bootstrap estimator and parametric estimators, especially for small data sets, with the bias towards underestimating true species richness being the lowest for the Chao 2 and Jacknife 2 estimator (Brose, 2002; Butler and Chazdon, 1998; Colwell and Coddington, 1994; Gotelli and Colwell, 2011).