

**The importance of renosterveld conservation based on insect
diversity sampled in West Coast Renosterveld in the Western Cape
of South Africa.**

by

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Declaration

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Abstract

Research in the Fynbos Biome mainly focussed on the exceptional high plant diversity, especially of the Cape Floristic Region. However, there remains a poor understanding of the insect life found in this region and throughout the Fynbos Biome. The purpose of this thesis was to reduce this knowledge gap, and to add conservation value to the West Coast Renosterveld based on the insect life. This thesis is also the first to document insect diversity at community-level in a highly threatened and fragmented vegetation type in South Africa. West Coast Renosterveld, along with the other renosterveld types, is threatened by agricultural development due to the nutrient richer soils compared to the other vegetation types of the Fynbos Biome. From a conservation point of view, West Coast Renosterveld is in a critical state, with only 3-4% remaining. I used various sampling methods over four seasons to document insect species found in four West Coast Renosterveld patches in the Cape Winelands district, Western Cape. I analyse the effects of sampling methods, seasons and location on the community composition data, and calculate both alpha and beta diversity measures for the four renosterveld patches.

The first part of the thesis investigated the effects of sampling method and season on the documented insect species richness and abundance of the insect communities. D-vac, sweep net, pitfall traps, and pan traps were the sampling methods used for this study. Sampling was done over all four seasons in the first year. The combined sampling methods and seasons delivered a total of 851 morphospecies, consisting of 17 orders and 155 families. The sampling methods used in the West Coast Renosterveld did not have an effect on insect species richness or abundance, but did determine how many species and what number were sampled. In addition, seasons are an important driving factor of insect communities in the remaining renosterveld areas. The sampling effort applied in this study thus suggests that renosterveld is rich in insect fauna. Furthermore, a feasible and logistical sampling strategy is proposed which can be used for future insect sampling in renosterveld and other Fynbos Biome vegetation types.

The second part of the thesis compared the insect species richness and abundance of four renosterveld areas and investigated what the possible variables are affecting the variation between these areas. J.N Briers-Louw had the highest insect diversity compared to Papegaaiberg, Kooppmanskloof and Spier. Beta diversity was the smallest between any combination with J.N.Briers-Louw and yielded greater diversity when representing the whole region, compared to any other two renosterveld areas. Based on the results, it can be assumed that habitat characteristics have the biggest effect on insect diversity in renosterveld, which is altered by factors including disturbance and topography. Considering these

contributing factors, this study found that insect diversity differs greatly between West Coast Renosterveld remnants with only about 20% of the species shared between patches. This emphasises the importance of conserving all remaining renosterveld patches. Also, with this study's documentation and explanation for the insect diversity found in West Coast Renosterveld, future research can now focus on specific taxa and plant-insect relationships to better understand the ecological functionality of insects in renosterveld.

Opsomming

Navorsing in die Fynbos Bioom het grotendeels op die besondere hoë plant diversiteit gefokus, veral in die Kaap Floristiese Ryk. As 'n gevolg is daar nogsteeds 'n gebrek aan kennis en begrip van die inseklike in hierdie streek en die Fynbos Bioom. Die doel van die tesis was om hierdie gaping in die studieveld kleiner te maak, asook om waarde tot Weskus Renosterveld bewaring toe te voeg. Hierdie tesis is ook die eerste om insekdiversiteit op 'n gemeenskapsvlak in 'n hoogs bedreigde en gefragmenteerde plantegroei tipe in Suid-Afrika te dokumenteer. Weskus Renosterveld, tesame met die ander renosterveld tipes, word bedreig deur landbou ontwikkeling as gevolg van die geskikte grond wat hoër in voedingswaarde is, teenoor die ander plantegroei tipes van die Fynbos Bioom. Uit 'n bewarings oogpunt is Weskus Renosterveld in 'n kritieke toestand waar daar slegs 'n oorblywende 3-4% van hierdie plantegroei-tipe oor is. Ek het verskeie versamelingsmetodes gebruik oor vier seisoene om die verskillende insekspesies te dokumenteer wat in die vier afsonderlike Weskus Renosterveld areas in die Kaapse Wynland distrik voorkom. Ek analiseer die effekte van versamelingsmetodes, seisoene en area op spesies voorkoms en getalle, en bereken beide alpha en beta diversiteitwaardes vir die vier renosterveld areas.

Die eerste deel van die tesis het die effekte van versamelingsmetodes en seisoene op die gedokumenteerde insekspesies se spesiesrykheid en getalle van die insekbevolkings ondersoek. D-vac, swaainet, pitvalle, en gekleurde pan-lokvalle was die gekose versamelingsmetodes vir hierdie studie. Veldwerk was oor vier seisoene in die eerste jaar uitgevoer. Die gekombineerde versamelingsmetodes en seisoene het 'n totaal van 851 morfospesies, wat uit 17 orders en 155 families bestaan, opgelewer. Die versamelingsmetodes het geen effek op die spesiesrykheid en getalle van die insekte gehad nie, maar het wel die hoeveelheid spesies en hul ooreenstemmende getalle bepaal. Verder is seisoen 'n belangrike drywende faktor van insekbevolkings in die oorblywende renosterveld areas. Die geïmplimenteerde versameling van hierdie studie stel voor dat die renosterveld ryk is in insek fauna. Daar is ook 'n gepaste en logiese versamelingsstrategie voorgestel vir verdere insekversameling in renosterveld en ander plantegroei tipes van die Fynbos Bioom.

Die tweede deel van die tesis het die insekte se spesiesrykheid en getalle van die vier renosterveld areas vergelyk en het ondersoek wat die moontlike veranderlikes kan wees wat die variasie tussen hierdie areas beïnvloed. J.N. Briers-Louw het die grootste insekdiversiteit in vergelyking met Papegaaiberg, Koopmanskloof en Spier. Beta diversiteit was die kleinste tussen enige areas met J.N. Briers-Louw as kombinasie en het die grootste diversiteit wat die hele gebied verteenwoordig opgelewer, in vergelyking met enige ander twee renosterveld areas. Op grond van die resultate kan mens aanneem dat habitat karaktereienskappe die

grootste effek op die insekdiversiteit van die renosterveld het, wat bepaal word deur faktore soos graad van versteuring en topografie. Deur hierdie bydraende faktore in ag te neem, het die studie bevind dat die insekdiversiteit grotendeels verskil tussen Weskus Renosterveld areas met slegs 20% van die spesies wat tussen areas gedeel word. Dit beklemtoon die belangrikheid om al die oorblywende renosterveld areas te bewaar. Met hierdie studie se dokumentasie en verduideliking van die insekdiversiteit wat in Weskus Renosterveld gevind word, kan toekomstige navorsing nou op spesifieke taksa en plant-insek verhoudings fokus om 'n beter begrip van die ekologiese funksionaliteit van insekte in renosterveld te kry.

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CHAPTER 1

General introduction

The question of how many species there are on the planet has inspired naturalists for centuries. As insects represent about half of all extant species on Earth, answering this question about their diversity seems natural. The number of studies that document insect diversity are however very limited, and even estimates on the number of species yet to be discovered are highly variable (Ehrlich & Wilson 1988). Many published sources indicate that about 1.4 million species of living organisms have been described comprising of approximately 750 000 insects, 41 000 vertebrates, 250 000 plants (vascular and bryophytes) and the remainder consisting of invertebrates, microorganisms, fungi and algae (Ehrlich & Wilson 1988; Willers 1991). The poor understanding of insect diversity necessitates that such surveys should be linked to other patterns of diversity. For example, studies examining the high plant diversity in the tropics has preceded studies on documenting gross insect species richness (for examples see Godfray *et al.* 1999 and Novotny *et al.* 2006). In this study, I focus on insect diversity in West Coast Renosterveld in the context of the Fynbos Biome with its well documented, rich plant diversity.

Renosterveld as part of the Fynbos Biome and Cape Floristic Region

The Greater Cape Floristic Region (GCFR) includes the whole winter-rainfall region (arid and mesic climates) of South Africa and has a high level of endemism, containing two biodiversity hotspots namely fynbos and succulent Karoo (Born *et al.* 2007) (Figure 1). This region includes the following vegetation types: fynbos, renosterveld, subtropical thicket; succulent Karoo; southern afro-montane forest; and southern coastal forest (Allsopp *et al.* 2014).

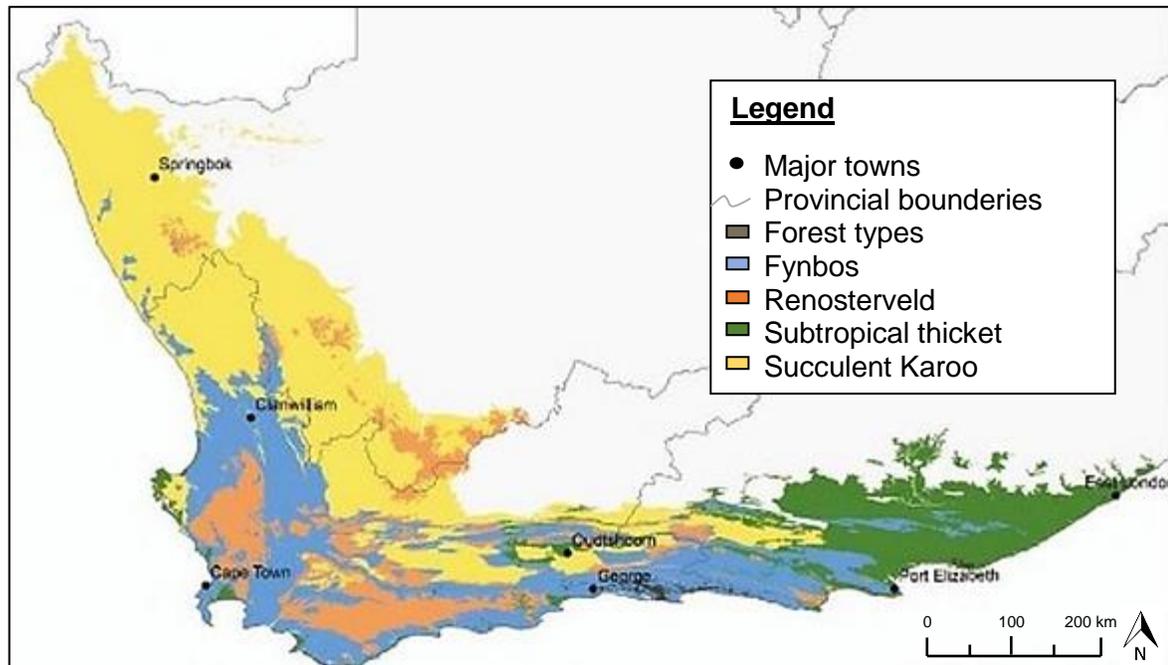


Figure 1 - The Greater Cape Floristic Region comprising five vegetation types namely, forest, fynbos, renosterveld, subtropical thicket, and succulent Karoo. The two forest types are the same colour because the southern coastal forest patches are too small to be visualized at this scale (modified from Allsopp *et al.* 2014).

The Cape Floristic Region (CFR) which falls solely within the Fynbos Biome, is known as one of the world's biodiversity hotspots for plants and invertebrates (Peron & Altwegg 2015; Pressey *et al.* 2003), containing over 9000 plant species of which 70% are endemic to this region (Rouget *et al.* 2003a). Currently 30% of the CFR is transformed, comprising cultivated land including forestry plantations (25.9%), urban areas (1.6%) and dense stands of alien invasive trees (1.6%) (Frazee *et al.* 2003; Rouget *et al.* 2003a). The majority of biodiversity research has been done on plant diversity, while the ecology of insects in this region is poorly understood (Matenaar *et al.* 2014), as is the relationship between plant and insect diversity (Augustyn *et al.* 2013).

The Fynbos Biome is unique due to its high floral diversity relative to its small surface area within a single country, compared to other floral kingdoms across the globe which may span across different countries and much larger areas (Littlejohn & De Kock 1997; Matlhako *et al.* 2010; Taylor *et al.* 2001;). The distribution of the Fynbos Biome stretches from north of Clanwilliam in the west, to Port Elizabeth in the east (Manning 2007).

Fynbos is one of the distinctive vegetation types in this biome, with a Mediterranean climate (hot, dry summers and cool, wet winters) (Cowling 1994; Davis *et al.* 1996; Naveh & Whittaker 1980). Four distinct growth forms are found in the fynbos: tall protea shrubs with large leaves (proteoids); heath-like shrubs (ericoids); reed-like plants (restoids); and bulbous herbs (geophytes). Depending on the landscape, the abundances of these growth forms may vary. Despite possible variations in growth forms, restoids are always present and is therefore the unique distinguishing feature of fynbos (Cowling 1995).

Renosterveld is the most threatened vegetation type of the Fynbos Biome due to large-scale transformation and fragmentation (Bergh *et al.* 2014). Renosterveld is a fire-prone shrubland which typically consists of renosterbos (*Elytropappus rhinocerotis*), a variety of geophytes and C3 grasses (Curtis *et al.* 2013; Kraaij 2010; Newton & Knight 2004; Parker & Lomba 2009; Rebelo 1992; Shiponeni & Milton 2006). Older veld can be richer in a variety of legumes and succulents (Curtis *et al.* 2013). The term 'renosterbos' was applied to the vegetation in the Olifants valley by Simon van der Stel in 1685, because black rhinoceros were frequently found in it (Boucher 1980; Parker & Lomba 2009). Therefore, it is believed that 'renosterveld' came in use either by the black rhinos roaming in it, or due to the similarity of these shrubs to the colour of rhino hide (Parker & Lomba 2009).

Renosterveld is restricted to the semi-arid and semi-humid coastal forelands of the southern and south-eastern Cape. (Von Hase *et al.* 2003). The most common shrubs in renosterveld are of the daisy family (Asteraceae), pea family (Fabaceae) and the cocoa family (Malvaceae). Almost a third of the plant species are endemic to renosterveld, and it is possible that in one square meter about 1 000 bulbs can be found (Parker & Lomba 2009). Most of the bulbs are of the iris (Iridaceae) and orchid (Orchidaceae) families (Cowling 1984; Cowling *et al.* 1986; Parker & Lomba 2009). Flora of renosterveld is transitional and thus has a lower level of endemism than Cape Fynbos (Cowling 1984). One of the characteristic species, *Elytropappus rhinocerotis* (Renosterbos), is an evergreen shrub with small, green-grey, leathery leaves (Cowling 1984; Cowling *et al.* 1986). Renosterveld is defined often as non-fynbos, because of the lack of distinctive fynbos plants such as Proteaceae, Ericaceae and Restionaceae. However, some of these fynbos elements may be found intermittently in renosterveld. This usually occurs when annual rainfall exceeds 800mm or when fynbos and renosterveld are transitional and renosterveld occurs on sandier soils associated with fynbos (Parker & Lomba 2009; Rebelo 1995; Von Hase

et al. 2003). Renosterveld requires an annual rainfall of 350mm to 600mm (Parker & Lomba 2009; Von Hase *et al.* 2003).

The geology of the fynbos and renosterveld differs even more markedly than the plant taxonomic composition, and it is a useful way to distinguish between these two vegetation types. Fynbos soils are acidic and nutrient poor, whereas soils of renosterveld are relatively nutrient rich (Cowling *et al.* 1986; Parker & Lomba 2009; Shiponeni & Milton 2006). Renosterveld occurs on shale-, granite, and silcrete-derived soils (Kemper *et al.* 2000; Kraaij 2010; Von Hase *et al.* 2003) which are mainly converted to wheat lands and vineyards (Bigalke 1979; Moll & Bossi 1984; Parker & Lomba 2009; Shiponeni & Milton 2006). Its conservation status is Critically Endangered due to the fragmentation of this veld type for agricultural purposes. Fragmentation of renosterveld has occurred since the early settlers required these richer soils for cultivation (Groenewald 2014; Parker & Lomba 2009).

In addition to habitat fragmentation, fire management of these patches is also a reason for conservation concern. Whereas the fire ecology of mountainous fynbos is well researched, this is not the case for lowland fynbos and renosterveld (Bond *et al.* 2004; Kraaij 2010). Fire management in renosterveld is also not simple, because of the surrounding cultivated lands, which are also at risk during controlled fires (Parker & Lomba 2009). Incorrect fire management could thus further compound the effects of fragmentation on preserving the remaining renosterveld biodiversity and maintaining ecosystem functioning.

The effect of grazing on renosterveld 'ecosystem integrity' is also a concern. Remnant patches on livestock farms are usually used for grazing (Groenewald 2014). The ecology of renosterveld (before agricultural activities) was historically tied to herbivory and fire. Natural herbivores (grazers and browsers) were found in renosterveld. The diversity of herbivores were greatly reduced when many of the large herbivores were exterminated and replaced by selective-feeders in the form of livestock (sheep and cattle). The livestock tend to forage on the most palatable grasses in a very selective manner. They are sedentary, whereas game would have been more nomadic. Therefore, the theory exists that the grazing pressure of livestock resulted in an increase in less palatable species (including renosterbos and other Asteraceae species) at the expense of the more palatable species (including Themeda and other palatable C3/C4 grasses). Consequently, the heavy grazing pressure of livestock on the grasses results in the transformation of renosterveld to a even greater shrubland as there are nothing feeding on the woody shrubs

(Newton & Knight 2004). Natural grazers fed on palatable grasses, preventing the transformation of renosterveld to grassland. These grazers have gone extinct as they were hunted by the earlier settlers (Groenewald 2014; Raitt 2005). When overgrazing occurs, it can lead to transformation of renosterveld to a very dense shrubland (Raitt 2005). Therefore, livestock grazing on renosterveld patches should not be excluded by all means, but rather managed properly to keep the vegetation structure and composition intact.

Today, there are ca. 18 000 fragmented patches of renosterveld, most of which are smaller than one hectare in size (Parker & Lomba 2009). It is thus important to understand the effects of the size of remnant patches in terms of maintaining biodiversity. For example, avian diversity of renosterveld is affected by the size of the patch with some bird species requiring large tracts of renosterveld. In smaller patches they will be absent, as well as their contribution to the pollination of plants. Some plants, such as the wax creeper *Microlooma tenuifolium* rely on sunbirds to pollinate them. Therefore, with the absence of certain important pollinators, plant diversity can decrease. The total area of cultivated lands surrounding renosterveld also influence avian diversity. Gamebirds and seed-eating species can roam in cultivated lands, while fruit and insect eating birds are less able to adapt to croplands (Fox & Hockey 2007; Parker & Lomba 2009).

Although patch size influences avian diversity, this is not necessarily the case for insect diversity. Pollinator diversity has been researched in renosterveld, and it was found that the insect pollinators were more sensitive to habitat characteristics including percentage vegetation cover, percentage grass, and rockiness, rather than patch size (Donaldson *et al.* 2002). In addition the authors found that even patches smaller than one hectare contained a high diversity of insect pollinators. Furthermore, they postulate that small fragments may contain important populations of rare, threatened plant species, and therefore it is important to ensure that larger fragments in the vicinity are retained as potential sources of pollinators (Donaldson *et al.* 2002).

To add to the importance of renosterveld conservation, this vegetation type also has its own endemic fauna and flora. Fauna examples include the well-known Geometric tortoise *Psammobates geometricus* which is found only in the southwestern portion of the Western Cape, including the Southwestern Coastal Lowlands, Worcester-Tulbagh Valley and the Ceres Valley. The populations of these three areas are isolated from one another by mountain ranges (Baard & Mouton 1993; Boycott & Bourquin 2000). In their distribution range, they occur in West Coast Renosterveld and are listed as Critically Endangered (CR) in the Red Data Book (less than 5 000

individuals remaining) (Baard 1995; Hofmeyr *et al.* 2012; Parker & Lomba 2009; SANBI 2010). Another species, the Agulhas Long-billed lark *Certhilauda brevisrostris* is the only known endemic bird species confined to the Agulhas Plain renosterveld area in the Overberg (Parker & Lomba 2009).

Renosterveld vegetation type was thought to be transitional (Cowling 1984), where more recent research suggest that renosterveld should be an independent vegetation type, rather than a subtype of fynbos, succulent karoo or thicket (Allsopp *et al.* 2014). Renosterveld has lower levels of endemism compared to fynbos (Cowling 1984). In contrast, Groenewald (2014) found 403 plant species, comprising 226 genera from 76 families in an area of 28 ha Rûens Silcrete Renosterveld, a subtype of the South Coast Renosterveld near Swellendam, Western Cape. Asteraceae and Iridaceae were the most species rich families, followed by Fabaceae and Poaceae. This study by Groenewald (2014) shows the remarkable plant diversity which can be found in renosterveld. In addition, Curtis (2013) also demonstrates that renosterveld is not species-poor.

Different renosterveld types and their characteristics

Four major renosterveld types are found in South Africa, which slightly differ in floristic, edaphic and ecological characteristics (Cowling *et al.* 1986; McDowell & Moll 1992; Moll *et al.* 1984; Rebelo 1995; also see Figure 2). These include the West Coast Renosterveld which is comprised of mid-dense to closed (50-90% canopy cover) cupressoid and small-leafed, mid-high evergreen shrubs. Furthermore it has sparse perennial grass cover, and a high diversity of geophytes, and annuals, and has 'kapokbos' *Eriocephalus africanus*, 'geelblommetjies' *Leysera gnaphalodes*, and 'renosterbos' as major elements (Moll *et al.* 1984; Parker 2009). The plant communities of South West Coast Renosterveld are essentially mid-dense (50-75% canopy cover) cupressoid and small-leafed, mid-high evergreen shrubs, with rare broad-leaf shrubs. The understory is also herbaceous with occasional perennial grasses (Moll *et al.* 1984). Distinctive 'gombos' *Relhania*, 'sewejaartjie' *Helichrysum* and 'poprosie' *Hermannia* are found in this renosterveld type. The Mountain Renosterveld is more open to mid-dense (25-60% canopy cover) cupressoid and small-leafed, low to mid-high shrubs. Emergents of *Rhus*, *Acacia karoo*, *Euclea undulata* and *Aloe ferox* are scattered in this renosterveld type. The perennial grass component is usually absent due to veld deterioration (Moll *et al.* 1984). South Coast Renosterveld typically consists of open to mid-dense (25 – 60% canopy cover) cupressoid and small-leafed, low to mid-high shrubs. Scattered herbaceous shrubs are found in the understory and perennial grasses only on well-managed sites (Moll *et al.* 1984).

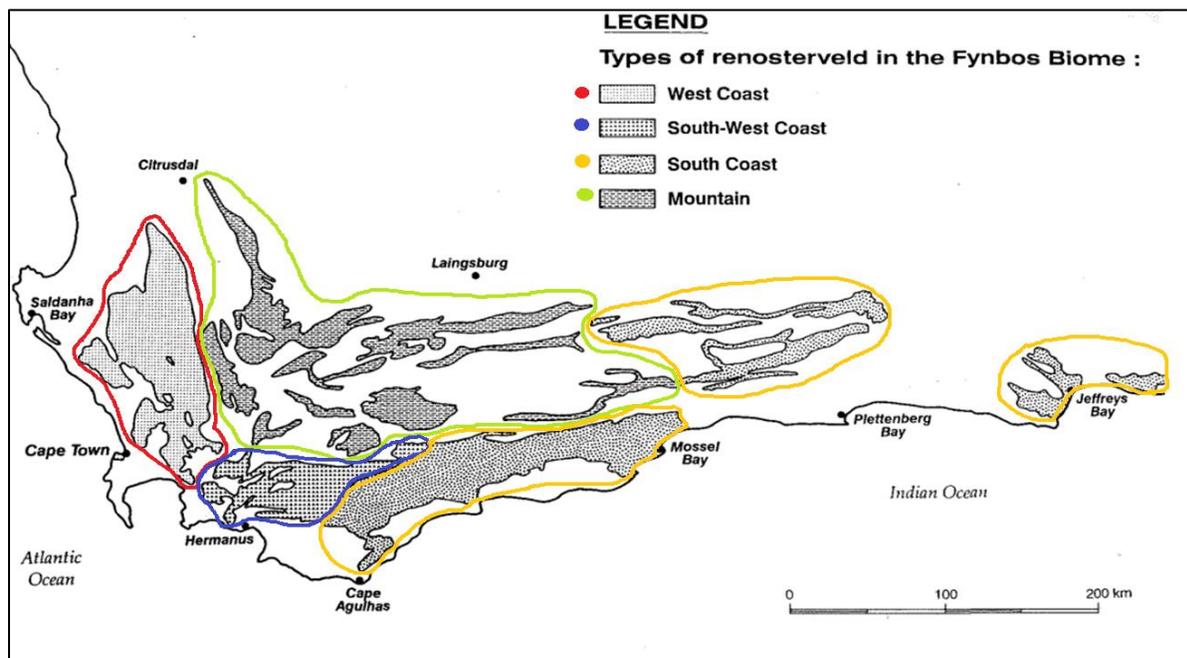


Figure 2 - The distribution and original extent of the four major renosterveld types in the Fynbos Biome (modified from Moll & Bossi 1984; Rebelo 1995)

Both South-West Coast Renosterveld and West Coast Renosterveld are considered as the most fragmented and transformed vegetation types in the CFR, due to their similarity in agricultural potential, reliable winter rainfall and the flat to gently rolling topography (Horn *et al.* 2011; Kemper *et al.* 2000). West Coast Renosterveld soils are derived from Malmesbury shales and Cape granites (McDowell & Moll 1992). Degradation of the West Coast Renosterveld has resulted in its conservation being in a critical state, with only 3-4% remaining undisturbed (Cowling *et al.* 1986; Parker & Lomba 2009) (see Table 1). Boucher (1983) and Von Hase *et al.* (2003) support this finding stating that less than 6% of West Coast Renosterveld remains. McDowell and Moll (1992) identified 55 renosterveld remnants, and calculated the proportional area of these remnants and suggested that the remaining West Coast Renosterveld is slightly over 3%. Hall and Veldhuis (1985) estimate the original area of West Coast Renosterveld to be 7280 km². Consequently, with a total of only 3-4% of remaining renosterveld (\pm 295 km²), the conservation status of West Coast Renosterveld is exceptionally poor (Heelemann *et al.* 2012; McDowell & Moll 1992). Despite the heavy transformation of renosterveld to agricultural lands, some marginal land was abandoned during the 1980s (Krug & Krug 2007), constraining these old fields to slow succession and

recovery rates of indigenous plant species due to soil degradation, alien grass competition and depleting the indigenous soil seed bank (Heelemann *et al.* 2012). The remaining percentage of renosterveld types are guesstimates within the literature (Table 1). The total area of Coastal renosterveld (including the three types: West Coast, South West Coast and South Coast) (McDowell & Moll 1992) remaining is an estimated 2256 km² (85% natural vegetation lost) and the remaining Mountain renosterveld is 3448 km² (27% natural vegetation lost) (Moll & Bossi 1984).

Table 1 - The remaining percentage of the four renosterveld types (from Curtis 2013; McDowell & Moll 1992; Moll & Bossi 1984; Rebelo 1995)

Renosterveld type	% remaining	Area remaining (km ²)
West Coast	3-4	295
South West Coast	5-8	N/A
South Coast	4-6	N/A
Mountain	73	3448

There are 230 Red Data plant species in West Coast Renosterveld, and it has the highest concentration of threatened plants, with an estimated average of about one threatened species per square kilometer of remaining vegetation (McDowell & Moll 1992). Farmers privately own as much as 80% of the remaining renosterveld remnants. Therefore, collaboration with farmers (for example the CapeNature Stewardship Program) (Milne & Niesten 2009) is crucial for conservation, as their attitudes and behaviour towards maintaining this scarce vegetation type need to be positive (Curtis 2013; McDowell & Moll 1992; Rouget *et al.* 2003b). Future conservation of threatened ecosystems, including renosterveld, lies in the hands of private landowners and farmers (Winter *et al.* 2007).

Current knowledge on the insect diversity of the Fynbos Biome

While the high plant diversity of the region has received much interest, the region's insect diversity is poorly known (Braschler *et al.* 2012; Johnson 1992). Furthermore, it is proposed that the CFR does not reflect a strong association between high plant species richness and high insect species richness (Giliomee 2003). However, the small guild of endophagous insects could be an exception. Based on the results from Giliomee (2003), it is assumed that the CFR vegetation is a

poor source of nutrition for herbivores, considering the sclerophyllous nature of the plants and chemical defenses against herbivory (Giliomee 2003; Johnson 1992). Giliomee (2003) also emphasizes the need to determine levels of biodiversity and endemism for invertebrates in the CFR given that this is already fairly documented for both plants and vertebrates. Species richness of certain taxa and the endophagous guild of specific areas in the CFR are fairly studied. He also highlights the challenges of obtaining a complete sample of invertebrate alpha diversity with specific reference to the large number of plant species found in the region, as well as the taxonomic input which will be required to identify sampled insect species. Taking this into account with incomplete distribution records makes it impossible to establish diversity of invertebrates of the region. However, Picker and Samways (1996) intensively sampled a small area and showed more invertebrate endemics present than plant endemics. The study area included palaeogenic zones such as upper-reach forest streams, riverine forests and caves. These zones differ in structure and conditions which could explain the high endemism and a high diversity of insects. Furthermore, Proches and Cowling (2007) assessed biome-specificity of insect assemblages using only sweep netting in the fynbos, grassland, subtropical thicket and Nama-Karoo biomes co-occurring in the Baviaanskloof area. Species richness was the highest in the subtropical thicket (158), followed by grassland (123), fynbos (97), and Karoo (55). Using multivariate analyses the authors found that insect samples from the same biome clustered together, although variation between sites within the same biome exists. The results showed that each biome is rich and has its unique insect fauna. More recently, Pauw and Stanway (2015) studied plant-pollinator interactions in the CFR. The study site, Sevilla which is close to the Pakhuis Pass near the Cederberg, included 62 plant species which are linked to 217 pollinator species. The authors concluded that the above findings regarding pollination networks is in contradiction to expectations derived from earlier literature. Long-term climatic stability might have allowed phenotypic and functional specialization to manifest and persist (Pauw & Stanway 2015).

Documentation on some insect taxa has been done within the Fynbos Biome as highlighted by Giliomee (2003), but this is at best only a random selection of insect taxa:

Orthoptera

Schlettwein and Giliomee (1987) sampled 19 species of grasshoppers with sweep nets in two broad firebreaks over a six month period in the Jonkershoek Valley. This cannot be compared to the 60 species found in the Nylsvlei Nature Reserve which is a savanna ecosystem (Gandar 1983), because the sampling times and sampling areas differ remarkably. However, 3 out of the

19 species are considered to be endemic to the CFR as these species are found to be associated with the restioids of the vegetation (Giliomee 2003).

Hemiptera

Schlettwein and Giliomee (1987) sampled 26 leafhopper species (Cicadellidae) over a period of 10 months in 3- and 5-year-old vegetation in Jonkershoek with a vacuum sampler. In contrast, Theron (1987) found 59 and 62 species of leafhoppers, respectively, on two small Bankenveld grassland sites (1.5 ha and 0.65 ha) nearby Pretoria, using sweep net for two days on two occasions.

Coleoptera

According to Donnelly and Giliomee (1988) fynbos has a high number of epigaeic beetle species (165 species) compared to other global Mediterranean-types, such as the Chilean matorral (136 species) and the Californian chaparral (118 species). In addition, Botes *et al.* (2007) assessed epigaeic beetle assemblages at different altitudes and diversity between different vegetation types, including Mountain Fynbos, Strandveld and succulent Karoo. A total of 49 epigaeic beetle species was sampled during two sampling occasions. Tenebrionidae contained 33 species and 16 species of Carabidae. The authors found that several beetle species showed habitat specificity and fidelity and clear differences existed between the three vegetation types. Also, a larger proportion of variation in tenebrionid species density compared to carabids was attributed to environmental variables and spatial factors.

Diptera

It is assumed that Diptera (true flies) in the CFR are species rich, specifically the Tabanidae family, where a total of 40 and 42 species are listed for the Western Cape and Southern Cape, respectively (Usher 1972).

Lepidoptera

Information on moth species in the CFR remains inconclusive, but according to Cottrell (1978) the CFR consists of 200 to 230 species of butterflies. Table Mountain itself has about 53 butterfly species (Claassens & Dickson 1980).

Hymenoptera

Many of the insect research in the Fynbos Biome focused on ant species and their functionality in this ecosystem. Bond and Slingsby (1983) estimated that approximately 20% of the plants in

the CFR produce seeds with elaiosomes (which depend on ant dispersal). However, the ant assemblages are not predominantly rich in species. Donnelly and Giliomee (1985) found a total of 45 ant species in the Jonkershoek Valley which is also comparable to the Mediterranean region of Australia. Braschler *et al.* (2012) also add that the local richness of ants in the Fynbos Biome and the succulent Karoo is not exceptional by global standards, given other similar habitats across the globe with greater ant species richness. Botes *et al.* (2006) studied ant assemblages in the northern CFR along an altitudinal gradient, ranging from sea level at Lamberts bay, over Sneekop in the Cederberg, to Wuppertal. Pitfall traps were used to sample 85 ant species representing 24 genera. Thirteen species were only found during October sampling while seven species were collected only during March, suggesting that seasonal variation affects the ant assemblages. Ericoid Fynbos had the greatest species density, while Proteid Fynbos had the highest abundance. The highest species richness was found at an altitude of 900 m. The authors concluded that spatial variables contributed to the variation in abundance while environmental variables, including area and temperature, contributed to the variation in species density. Ant species richness and abundance are found to vary according to temperature variation. Furthermore, abundance and richness increase with improvement in habitat quality, which is often associated with temperature, and both decline with more environmentally stressed habitats (Botes *et al.* 2006). Community structure of epigaeic ants was investigated in the fynbos in the Jonkershoek Valley (Donnelly & Giliomee 1985). The authors used pitfall traps to sample 45 species of ants. Species diversity was slightly lower in areas where fire has been excluded for 37 years than in firebreak sites where fire occurred regularly and species composition did not differ markedly between the two treatments. The authors also found low species diversity at one site where the alien invasive Argentine ant (*Iridomyrmex humilis*) was present (Donnelly & Giliomee 1985). This shows that both fire occurrence and alien invasive species can have effects on native insect diversity. Gaigher *et al.* (2015) assessed hymenopteran parasitoid diversity in fynbos remnants within agricultural landscapes. A total of 738 parasitoid individuals were sampled from 188 species and 21 families.

Gall insects

Of all insect assemblages, it appears that only gall insect species richness is high, seeing that there is a positive correlation with the plant species richness of the CFR (Wright & Samways 1998). According to Proches and Cowling (2006), gall insects are over-represented in the Fynbos Biome. A study done by Wright & Samways (1998) in the CFR, sampled in various vegetation types found in the CFR, including fynbos, renosterveld, afro-montane forest, etc. The authors

found that there is a positive correlation between gall insect species richness (GSR) and plant species richness in the CFR. A possible reason for this positive association between GSR and plant species richness, is that the gall insects can avoid nutritional and/or toxic restrictions of the sclerophyllous shrubs (Cornell 1989; Giliomee 2003). Therefore, the high plant species richness in the CFR made it possible for gall insect radiation, whereas other variables such as elevation and aspect had a negligible effect (Wright & Samways 1998). As gall insects are very host-specific (Ananthakrishnan 1984), it is suggested that the CFR will have a high endemism of gall forming insect species with respect to the high plant species richness of this region (Wright & Samways 1998).

Final remarks on expected insect diversity of the Fynbos Biome

The assumption has been made that species diversity generally has an inverse relationship with increasing latitude (Price 1997). Giliomee's (2003) assessment of the fynbos seems to support this as the herbivorous insect fauna is not exceptionally species-rich compared with the high floral diversity (with specific reference to the previous section). This is contrary to the statement by Rebelo (1992) that the invertebrate fauna is probably as diverse as the flora. Giliomee (2003) assumes that the environmental factors which contributed to the speciation of the flora (soil diversity, dissected landscape, moisture gradients and fire-created niches) did not promote equivalent speciation of the herbivorous insects. Possibly the only exception to this is the high numbers of endophagous insects found in the sclerophyllous vegetation (Price *et al.* 1998). The leafhoppers associated with Restionaceae could possibly be species rich, but host specificity is still not known (Giliomee 2003). The inverse relationship between plant diversity and insect diversity could possibly be explained by the sclerophyllous nature of the many plants, while certain plants contain a relatively high level of toxic compounds, such as phenolics, in the leaves (Johnson 1992). This means that many plant species found in the CFR also present these constraints which could have a negative impact on the insect diversity. As mentioned, renosterveld forms part of the CFR, and also consists of sclerophyllous plant species (Donaldson *et al.* 2002). Therefore, considering these constraints, this statement should be revisited after thorough sampling and documentation of the insect diversity in renosterveld has been conducted. Some research studies discussed above also reflect that more insect species can be sampled with an increase in sampling effort and time. Therefore, for a comprehensive diversity study, an array of sampling techniques should be used as they each target certain taxa. Seasonal differences in species richness and abundance is expected, and therefore seasonal sampling within an area is necessary. Comparisons to other biomes of South Africa based on the poor

knowledge of insects within the Fynbos Biome are not feasible, because there is insufficient insect documentation data which will lead to inconclusive results and assumptions.

In summary, insect species richness, abundance, and diversity have been documented for only certain insect assemblages found in the Fynbos Biome. A few studies have assessed insect diversity in fynbos remnants, but these were focusing on specific functional groups. Consequently, despite the integral ecological role and importance of insects in most ecosystems, general insect diversity has never been determined for any part of the Fynbos Biome. Given the very low percentage of renosterveld vegetation type remaining, it is important to know what the typical associated insect diversity is of remaining renosterveld patches. Therefore, the main aim of this thesis is to document the insect diversity found in the remaining large patches of West Coast Renosterveld which can also contribute to the conservation value of these remnants. Considering that renosterveld has a high diversity of plants, I hypothesize that renosterveld is rich in insect species despite the size of the renosterveld patch. To address this hypothesis, I will use an array of sampling methods over a course of four seasons. I will use statistical analyzes to calculate significant differences in species richness and abundance between the sampling methods and between sampling seasons. I will also calculate alpha diversity, using Shannon Wiener's Index, for each of the four West Coast Renosterveld remnants. This will reveal insect diversities for different sizes of renosterveld remnants which will indicate that every renosterveld patch, despite its size, is important to be conserved.

Thesis structure and outline

In Chapter 2, I investigated the effects of sampling method and season on the observed species richness and abundance of the insect communities found in four West Coast Renosterveld remnants. Two sampling strategies were compared with each other in terms of community composition.

In Chapter 3, I compared the insect species richness and abundance of four renosterveld remnants and investigated potential variables affecting the variation between these areas. Furthermore, alpha diversity was calculated for each renosterveld area to reflect local species diversity of each renosterveld patch using species richness and evenness indices. Beta diversity is still relatively new to the ecological literature. Numerous β -measures have been proposed, but there remains a lack of beta diversity studies for insect communities in fragmented landscapes. Therefore, a new β -measure specifically for abundance data was used to calculate beta diversity

between the different renosterveld areas to determine which of the four renosterveld remnants are most representative of the whole region's insect diversity.

This study is unique as it is the first to document (using various sampling methods) the diversity of an insect community found in a highly threatened vegetation type in South Africa. This research will thus contribute to promoting local biodiversity conservation with a new emphasis on insect diversity in threatened vegetation types, and provide a reference point for future research on the insect diversity of South Africa's biomes.

Chapters 2 and 3 were written as individual research papers. The same study area was used for both chapters. Both these chapters have introduction, materials and methods, results, discussion, and conclusion sections. Chapter 4 is a general conclusion which provides a summary of the main findings of the two data chapters, and discusses the conservation and management implications for West Coast Renosterveld in terms of insect diversity.

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CHAPTER 2

Determining the best strategy to measure insect diversity in a highly threatened and fragmented vegetation type, the West Coast Renosterveld: a comparison of sampling techniques and seasons.

Introduction

Although the total number of extant species remains unknown (Stork *et al.* 2015), roughly 1.5 million species of all organisms have been named and described (Costello *et al.* 2012). Insects are the most diverse group of animals in the world (Grimaldi & Engel 2005) representing ~57% of the total predicted eukaryotic species in the kingdom Animalia (Mora *et al.* 2011). This incredible biodiversity, however, provides a challenge when it comes to comprehensively documenting the insect diversity of the earth's habitats. Most studies concentrate on a functional group or assemblage, although this will only represent a small portion of the insect species found in a given area.

Over the last decades, the methodology for the sampling of insect populations has developed substantially (Kuno 1991), due to the importance of their functionality in ecosystems being recognized (Oliver & Beattie 1996a). However, there is no specified sampling method which will capture all the insect diversity of a particular area (Ward *et al.* 2001). It has been shown that an array of sampling methods will sample a wider range of species per site (Standen 2000; Hyvarinen *et al.* 2006). For example, flightless insects are understandably underrepresented in sweep net samples (Proches *et al.* 2009), and sample bias will occur if this is the sole method used for diversity studies per se. Consequently, it is important to select the adequate sampling methods to meet the study objectives (Doxon *et al.* 2011).

After Hertz (1927) and Barber (1931) first reported the use of empty tin cans as traps for insects, the use of pitfall traps has become the most widely used method for sampling litter-dwelling arthropods, especially beetles, ants and spiders (i.e. Coleoptera, Hymenoptera and Arachnida) (Spence & Niemelä 1994; Mommertz *et al.* 1996; Agosti *et al.* 2000; Neville & Yen 2007). It is an inexpensive, convenient and labour-efficient method (Luff 1975) which allows the collection of large numbers of arthropods for rigorous statistical analyses (Spence & Niemelä 1994). However, this method reflects arthropod densities and activity which is a disadvantage (Greenslade 1964).

Additionally, pitfall sampling is influenced by natural parameters such as temperature, moisture and surrounding vegetation. Excessive rain will cause the pitfalls to flood, which will prevent sampling of insects and loss of sampled individuals. Temperature controls insect activity and foraging capabilities. For example, heat-tolerant ant species can forage at high temperatures where they are close to their thermal limits. The opposite is found in heat-intolerant ant species which are sensitive to high temperatures and forage at lower temperatures (Cerdá *et al.* 1998). The more active the insects, the greater the probability that the insects will be sampled. The density of vegetation influences the total ground area exposed to sun radiation. Radiation will heat the ground which will control the insect activity (Luff 1975; Spence & Niemelä 1994; Sanders *et al.* 2003). The size, shape, preservatives, and the arrangement of the traps also influence the sampling efficiency (Luff 1968, 1975). A study undertaken by Work *et al.* (2002) compared pitfall traps of various sizes for collecting three taxa of litter-dwelling arthropods. They found in general that larger pitfalls collected more individuals, and more species of all three arthropod taxa. Based on their results, they suggest placing larger pitfall traps in the sampling regime which may aid with detecting rare species (Work *et al.* 2002). Small pitfall traps will be most efficient for the purpose of ecological monitoring, as they will collect the dominant epigaeic arthropod fauna. Another advantage of using small pitfall traps is that it lowers the non-target vertebrate by-catch and reduces the processing time (Pryke & Samways 2009; Work *et al.* 2002).

Another sampling method commonly used is coloured pan traps. Colour is one of the more important attractants for flower-visiting insects (Kevan 1972; Vrdoljak & Samways 2014). Flowering plants are also characterised by other traits including: rewards (pollen/nectar), fragrances, size and shape (Niesenbaum *et al.* 1998). Pan traps are useful to estimate insect abundance and diversity (Campbell & Hanula 2007), and are an efficient standardized method of bee sampling (Baum & Wallen 2011; Roulston *et al.* 2007; Wilson *et al.* 2008). The colour of pan traps influences the sampling efficiency. A study by Campbell and Hanula (2007) compared pan trap (blue, yellow, white and red) samples from three different forests. Overall, blue pan traps were most effective for sampling Hymenoptera and Lepidoptera species. The authors concluded that most of the pollinators in these forests preferred blue pan traps. Yellow traps have been used to catch phytophagous insects (Kirk 1984) and predatory insects (Leksono *et al.* 2005). Blue pan traps are used to catch various hymenopterans (Aguiar & Sharkov 1997). White or yellow pan traps catch various dipterans (Disney *et al.* 1982). According to Leong and Thorp (1999) quantitative bee sampling with pan traps can be highly sensitive to trap colour, and yellow pan traps are overall the most effective for bee sampling. Pan traps are also an inexpensive and simple sampling method. This makes it an effective way to determine the relative abundance and

species richness of flower-visiting insects (Campbell & Hanula 2007). Bees and other flower visiting insects thus respond to common floral colours which are associated with floral rewards, such as nectar and pollen (Kirk 1984; Leong & Thorp 1999). The combination of water and an additive, such as soap liquid, ensures to break the surface tension, preventing insects from escaping the pan trap (Baum & Wallen 2011; Leong & Thorp 1999). Pan traps are also used as a conservation monitoring tool to monitor insect populations in fragmented habitats (Aizen & Feinsinger 1994; Laubertie *et al.* 2006).

The Dietrick insect sampler, also known as the D-vac sampling method (Dietrick *et al.* 1960) was known for its considerable weight, bulkiness and noise. However, the original D-vac has been replaced by a petrol-driven suction apparatus called a 'Blow & Vac', making it simpler to handle due to the decrease in weight (Stewart & Wright 1995). This method samples insects inhabiting the interior areas of plants, as the nozzle of the blower passes through the plant canopy (Buffington & Redak 1998). These insects include members of the orders Diptera (especially weak fliers such as Tipulidae), Homoptera, Hymenoptera and Auchenorrhyncha which alternatively can be expected to dominate the vacuum samples (Brown *et al.* 1988; Buffington & Redak 1998; Doxon *et al.* 2011). Vacuum sampling is very effective to sample small (± 2.2 mm in length) insects (Doxon *et al.* 2011), especially parasitoids (Gaigher *et al.* 2015), but can give low population estimates of lepidopterans (Shepard *et al.* 1974).

The new 'Blow & Vac' apparatus was previously compared with the Dietrick sampler by sampling arthropods in grasslands (Stewart & Wright 1995). The authors found that the former caught more spider and beetle species, whereas the numbers of Auchenorrhyncha were almost equal. The unimpeded air velocity inside the suction tube which was approximately 4 times that of the original D-vac, can explain the greater sampling efficiency of the 'Blow & Vac' (Stewart & Wright 1995). Overall, this method is very popular for studying agricultural and grassland systems (Buffington & Redak 1998; Dietrick *et al.* 1960). Another advantage of this method is that it reduces damage done to the plant canopy (Pryke & Samways 2009; Stewart & Wright 1995).

The sweep net has become a standard item for entomological fieldwork (Buffington & Redak 1998). This method is very effective for sampling large (>5 cm), more active insects such as members of Lepidoptera and Orthoptera (Cooper *et al.* 2012; Cooper & Whitmore 1990; Doxon *et al.* 2011). The orders Homoptera, Orthoptera and Araneae can dominate the sweep net samples (Cooper *et al.* 2012; Doxon *et al.* 2011). Sweep-netting is very light-weight and simple to use (Buffington & Redak 1998; Doxon *et al.* 2011), and is ideal to sample insects in grasslands, but not those in sclerophyllous shrublands which prevent effective sweeps (Hyvarinen *et al.* 2006;

Pryke & Samways 2009). In addition, temperature and wind play significant roles in the success of sampling with a net (Hughes 1955; Saugstad *et al.* 1967; Sutherland 2006).

These four sampling techniques, as mentioned above, have been used for insect diversity studies by researchers in the Fynbos vegetation. These traditional sampling methods were used to sample insects at different sites and seasons, enabling species richness and abundance to be compared. Witt and Samways (2004) used pitfall traps and the D-vac suction sampler to determine arthropod diversity in a fynbos remnant over the summer months, collecting a total of 221 insect species. Magoba *et al.* (2015) compared surface-active arthropod species richness and abundance between different vegetation types (i.e. natural fynbos, alien cleared sites, vineyards, and alien infested sites) using pitfall traps during winter, spring, and summer months. Overall, the species richness and abundances were greater in natural fynbos compared to the other three areas. However, differences in species richness and abundance between the three sampling occasions were not tested. Proches and Cowling (2007) sampled insects with a sweep net in the fynbos (Kogelberg). Sampling took place only in spring and a total of 97 species were sampled. Vrdoljak and Samways (2014) assessed the diversity of flowering plants and their associated flower visiting insects (anthophiles) in sites representing a range of transformed land, remnants of fynbos, disturbed fynbos areas, and reserves. Sampling was done in spring and the authors used coloured pan traps. The authors found that many anthophile species were shared between the different areas, and that certain species are only found in specific mosaics of the agricultural landscape.

These studies show that each method samples certain functional groups or taxa. These studies also show that natural parameters, such as temperature, humidity, cloud cover, and wind velocity and direction, can alter the types of invertebrates collected even when using a specific sampling method (Cherry *et al.* 1977; Doxon *et al.* 2011; New 1998). Ramirez-Hernandez *et al.* (2014) studied changes in saproxylic beetle assemblages at the inter-annual scale in Mediterranean forests west of Spain by using window traps. The authors found that seasons act as a driver for saproxylic beetle assemblages, which make this type of sampling method a valuable tool for monitoring strategies. Gesse *et al.* (2014) studied true bug assemblages in four different habitats in the Garraf Natural Park (Barcelona, Spain), using sweep net, beating, and observations. They found that species richness, abundances, and diversity differed between the four sampled plant communities. Furthermore, they also found that the assemblages varied within the year, suggesting that seasons have an influence on true bug diversity.

Insect studies such as those described above generally provide seasonal variation in species richness, abundance, and diversity for only a few specific insect taxa. Consequently, there is a lack of research on the effect of seasons on species richness, abundance, and diversity of an entire insect community found in a specific vegetation type. Therefore, the purpose of this chapter was to test the effects of seasons and sampling methods on species richness, abundance, and composition of the insect communities found in the Mediterranean West Coast Renosterveld. Two different sampling strategies, varying sampling method and season, were compared in terms of diversity and complementarity for the sampling of insects in renosterveld. Based on the effects of seasons and methods on species richness and abundance documented by the two strategies, an effective sampling strategy is proposed for future documentation of insects in renosterveld and other vegetation types found in the Fynbos Biome.

Materials and methods

Study area

Sampling was carried out in and around the Cape Winelands district in the Western Cape, South Africa (Figure 3). Four renosterveld areas were selected, namely Papegaaiberg Nature Reserve, Koopmanskloof Nature Reserve; J.N. Briers-Louw Nature Reserve, and Slangkop at Spier (see Figures 4 & 5). I used four sampling sites per renosterveld area. All sites were approximately 100m apart from each other and placed where vegetation was not too dense to hinder efficient sampling. The vegetation of all the sites was of such a nature that it was possible to readily implement all four sampling methods. A suite of similar plant species were observed in all the sampling sites (see Table 2 for a brief summary of the 16 sampling sites).



Figure 3 - The four renosterveld patches around the Stellenbosch area. Furthest north lays J.N. Briers-Louw nature reserve; Spier lays south; Koopmanskloof nature reserve and Papegaaiberg in between and the latter is closest to Stellenbosch Town.



Figure 4 - a) Papegaaiberg Nature Reserve with *Acacia saligna* in the background. b) Koopmanskloof Nature Reserve with Simonsberg in the background.



Figure 5 - a) J.N. Briers-Louw Nature Reserve with many renosterbos. b) Spier with its dense vegetation.

Table 2 - Summary of sampling site details for four sampling areas.

Location	Site	GPS coordinates	Site information
Papegaaiberg Nature reserve	A	33°55'13.96" S 18°50'40.15" E	A & B: Similar in composition with tall <i>Elytropappus rhinocerotis</i> ("renosterbos"), but not dense. C: Very dense <i>Seriphium plumosum</i> ("slangbos"); only few <i>E. rhinocerotis</i> D: Dense <i>S. plumosum</i> ; only few <i>E. rhinocerotis</i> ; patches of restios All sites (especially C & D) disturbed by alien invasive plant species: <i>Acacia longifolia</i>
	B	33°56'02.72" S 18°50'28.59" E	
	C	33°56'09.63" S 18°50'30.45" E	
	D	33°56'13.29" S 18°50'37.81" E	
Koopmanskloof Nature reserve	E	33°54'13.29" S 18°46'14.02" E	E & F : Very dense <i>E. rhinocerotis</i> and <i>S. plumosum</i> G & H: More grassy with a few tall <i>E. rhinocerotis</i>
	F	33°54'03.96" S 18°46'15.68" E	
	G	33°53'57.96" S 18°46'16.25" E	
	H	33°53'55.55" S 18°46'11.26" E	
J.N. Briers- Louw Nature reserve	I	33°45'48.22" S 18°50'02.81" E	I – K: Similar in composition which is dense in <i>E. rhinocerotis</i> L: More grassy with restios and short <i>E. rhinocerotis</i> <i>E. rhinocerotis</i> tall in all sites
	J	33°45'51.32" S 18°49'56.71" E	
	K	33°45'54.15" S 18°49'55.34" E	
	L	33°45'57.08" S 18°49'51.96" E	
Spier	M	33°58'56.31" S 18°48'28.35" E	M: Very tall <i>E. rhinocerotis</i> and dense N: Very dense <i>S. plumosum</i> with tall <i>E. rhinocerotis</i> O: Vegetation short including <i>E. rhinocerotis</i> , <i>S. plumosum</i> and grasses P: Very dense <i>E. rhinocerotis</i> ; grassy; succulent groundcover
	N	33°58'47.25" S 18°48'32.40" E	
	O	33°58'51.13" S 18°48'55.17" E	
	P	33°58'56.91" S 18°48'39.27" E	

The biggest renosterveld patch, Papegaaiberg, lies in the heart of Stellenbosch and is 130 ha in total. It has a total area of 130 hectares. It has not been proclaimed a nature reserve yet. The most recent extensive fire was in 2010 when an intense fire burned almost the entire hill. As a result, the four chosen sampling sites have rehabilitated after a successional period of ± 4 years before the first sampling occasion. Currently, the hill is infested by alien invasive plants, especially various *Acacia spp.* The hill is surrounded by Stellenbosch town, Onderpapegaaiberg neighborhood, Middelvlei vineyards and Kayamandi and Enkanini settlements (Seeliger & Turok 2013) (see Appendix C).

Koopmanskloof nature reserve belongs to the Koopmanskloof winery and was proclaimed a nature reserve in about 1980. An area of 98 hectares of renosterveld is surrounded by vineyards and grazing fields for cattle (see Appendix C). Two soil types are found and divide this renosterveld into shale and granite renosterveld. The former burned in 2008, whereas the fire history of the latter is unknown. The reserve is surrounded by vineyards.

J.N. Briers-Louw nature reserve is situated on the Eenzaamheid farm with an area of 29 hectares, and was proclaimed a Provincial Nature Reserve in 1972 when a population of geometric tortoises (*Psammodromus geometricus*) was found on this remnant (Parker & Lomba 2009). These tortoises are endemic to renosterveld and are classified as Critically Endangered in the South African Red Data Book for endangered species (Hofmeyr *et al.* 2012; SANBI 2010). This is also the first tortoise reserve established in Africa. In 2001 & 2002 fires were recorded, but the extent of the area burnt is unknown. In 2011 the western side (± 10 ha) of the reserve was burnt, and mostly recently controlled fires were carried out during April 2015 in the southern side (± 7 ha) (Helene van der Westhuyzen: personal communication, 12 April 2016). The reserve is surrounded only by grazing fields for cattle (see Appendix C). A few alien invasive plants (*Acacia saligna*) were found on the reserve, but have been removed mechanically.

Slangkop is the biodiversity heritage site found on Spier's property. This heritage site has a total area of 100 hectares and is surrounded by Spier's vineyards and organic fields (see Appendix C). The last fire occurrence was about ten years ago, and the vegetation is conspicuously tall and dense with respect to the other three selected renosterveld areas.

Field sampling

Sampling was done over a course of four seasons. Sampling started in July 2014 and ended in April 2015 (see Table 3). Sampling methods were applied at each site according to a pre-

determined design in order to maintain consistency, which removed the possibility for variation in data produced by sampling efficiency (see Figure 6). Wooden dowels were used at the center and the corners of each site replication. The distance from each corner to the center was 25 meters. Danger tape was used to mark each wooden dowel and to find the boundaries of the sampling site.

Table 3 – Sampling schedule including seasons with corresponding methods applied.

Sampling occasions and applied sampling methods				
Season (Year)	Winter (2014)	Spring (2014)	Summer (2015)	Autumn (2015)
Month	July	October	January	April
Methods	D-vac & sweep net	D-vac; sweep net; pitfall traps; pan traps	D-vac & sweep net	D-vac; sweep net; pitfall traps; pan traps

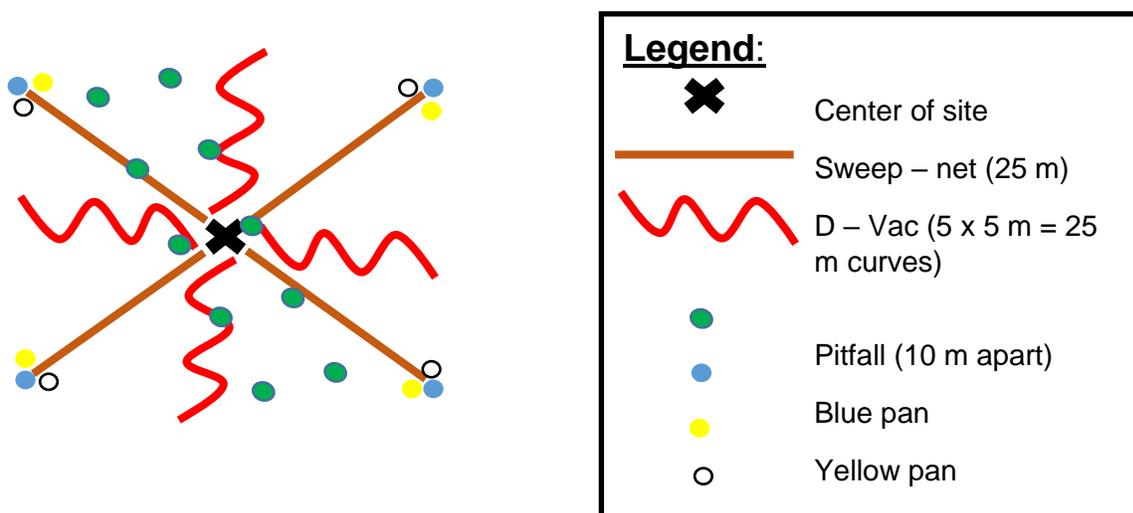


Figure 6 - Schematic display of the implemented sampling methods used at each sampling site.

Small pitfall traps (90 ml) were used for the already mentioned reasons: to sample the dominant epigaeic insects, and to lower non-target vertebrate by-catch, especially the geometric tortoise which is critically endangered (Hofmeyr *et al.* 2012). Pitfall traps were filled with a mixture of water

and glycerol glicerina (univAR®) in a ratio of 20:1 and approximately 50 ml of the mixture was added to each pitfall. The glycerol serves as a preservative, preventing bacteria and other microorganisms from growing on collected insects (Neville & Yen 2007). At each site, ten pitfall traps were marked individually and placed in a 2 x 5 design (see Figure 6; Neville & Yen 2007). Danger tape was attached to the bush nearest to each pitfall to facilitate location. Traps were set out in the field for 5 days.

White, yellow and blue coloured pan traps (20cm in diameter) were used to attract pollinating insects. They were placed on the ground surface at the corners of each sampling site (see Figure 6). Approximately 150ml of soapy water (Baum & Wallen 2011) was poured into each pan trap. Traps were also set out for 5 days. Insects were preserved in a 70% ethanol solution.

The two passive sampling methods, pitfall traps and pan traps, were implemented only during spring and autumn, and were left in the field for 5 days. When the pitfall traps were collected, the marked lids were placed onto the corresponding pitfall. When collecting pan traps, tweezers was used to extract all material from the pan traps into specimen jars filled with 70% alcohol.

The D-vac method was replicated four times in a wavy transect (see Figure 6). For this method, a Stihl SH-86 model leaf blower (Stihl, Germany) was used and a fine mesh bag was taped at the front of the nozzle, allowing insects to be vacuumed into this bag, instead of moving through the razor blades of the machine. The nozzle was inserted vertically with each step into the bushes and grasses to ensure a bigger catch than when done horizontally (Richmond & Graham 1969). At each insertion the throttle was on its highest level for 3 seconds to standardize catch intensity. After each transect was completed, a labeled Ziploc bag was held at the front of the nozzle, and all collected material was deposited into the bag.

For sweep net sampling, a sweep net with a diameter of 40 cm was used and replicated four times in a straight line transect (see Figure 6). With each step, two sweeps were made on both sides of the line transect, which gave a total of 100 sweeps per line transect. After each sampling occasion, the catch was placed into a Ziploc bag and labeled. All D-vac and sweep net samples were placed into a freezer at -18°C to kill collected insects.

Laboratory work and species classification

In total, 256 D-vac samples, 256 sweep net samples, 320 pitfall traps, and 384 pan traps were processed. After each sampling occasion, insect material was sorted in the laboratory. Species were sorted and identified to morphospecies level (see Derraik *et al.* 2002; Krell 2004; Neville &

Yen 2007; Oliver & Beattie 1996b). A reference collection was compiled of all species collected and is summarised in Appendix A. All the insects in the reference collection were pinned with insect pins (Ento Sphinx® No. 1-3). Smaller insects were pinned with micropins. If the insects were too small to be pinned at all, they were glued on a paper triangle with colourless nail polish. Insects that were not added to the reference collection was preserved in a 70% ethanol solution. The abundances of all morphospecies were notated for each sampling method and replication. After the completion of the reference collection, insects were classified to family level with the aid of Scholtz & Holm (1985), Unwin (1981 & 1984) and Prof Henk Geertsema's general knowledge.

Data analysis

Sampling effort was evaluated by estimating species richness using Chao2 and Jackknife2 estimators (Hortal *et al.* 2006), in the software EstimateS v.9.1.0 (Colwell 2013). These two non-parametric estimators were selected as overall both perform better for most data sets compared to other parametric estimators (Walther & Moore 2005). Species rarefaction curves were constructed to illustrate estimated species number given the sampling effort per method and for combined methods (Gotelli & Colwell 2001). I tested for significant differences in species richness and abundance (using Statistica 8.0 (Statsoft™)) between the four sampling methods using pooled spring and autumn data, and between seasons using the D-vac data respectively. Initially the data were tested for normality using the Shapiro-Wilk W test at α -value = 0.05. All non-parametric tests were performed with the Kruskal-Wallis test, while a One-way ANOVA was used where data were normally distributed.

Shared species between Strategy 1 (D-vac and sweep net implemented over four seasons) and Strategy 2 (all four methods implemented over spring and autumn seasons) were identified by EstimateS v.9.1.0 (Colwell 2013). Similarities in community composition between the four sampling methods and between the four different renosterveld sampling localities were tested by constructing a similarity dendrogram using Bray-Curtis similarity, with a stress value of 0.01 (Clarke 1993), after square root transformation of abundance data of pooled spring and autumn seasons, to reduce the effect of singletons (Anderson 2001). A permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences between sampling methods, sampling localities, and seasons based on Bray-Curtis similarity and 9999 permutations. This was done for both sampling strategies: when two methods were used over four seasons (Strategy 1); and four methods over two seasons (Strategy 2). Pairwise tests were performed for season comparisons of Strategy 1 and method comparisons of Strategy 2. These

analyses were carried out using the software program, PRIMER 6 (PRIMER-E 2008). A Canonical analysis of principal coordinates (CAP) based on Bray-Curtis similarity was performed to illustrate the PERMANOVA results for methods, seasons, and locations of both strategies.

Results

Reference collection

The combined sampling methods and seasons produced a total of 851 morphospecies, consisting of 17 orders and 155 families (see Appendix A and Figure 7). The D-vac sampling method collected the most species, 473 species. Sweep net, pan traps, and pitfall traps collected 312, 282, and 113 morphospecies respectively (Table 3). However, rarifying to the smallest sample size, D-vac sampling was closely followed by pan traps in sampled species richness, while sweep net and especially pitfall traps had a much lower species richness (Figure 8). D-vac sampled primarily Coleoptera, Hemiptera, Diptera, Hymenoptera and Orthoptera. The sweep net samples were generally dominated by Coleoptera, Diptera, and Hemiptera, but also collected many species of Lepidoptera and Orthoptera. Pan traps caught one more species of Lepidoptera and D-vac sampled twelve more species of Orthoptera. Coleoptera, Diptera and Hymenoptera dominated the pan trap samples. Pitfall traps were mainly dominated by Coleoptera and Hymenoptera (see Appendix B).

Coleoptera was the order with the most morphospecies with 234 different beetles (27.46% of all species), whereas Chrysomelidae contained the highest proportion (55 morphospecies). Other families which include most of the morphospecies are Curculionidae (28 morphospecies), Tenebrionidae (18 morphospecies), Coccinellidae (17 morphospecies), Scarabaeidae (15 morphospecies), Carabidae (14 morphospecies), and Buprestidae (14 morphospecies) (see Appendix A).

Diptera was the order with the most families, comprising a total of 35 families (164 morphospecies) of true flies. Tephritidae, Muscidae, Agromyzidae, Anthomyiidae, Chironomidae, and Empididae are the families containing most of the species, each having more than 10 morphospecies (see Appendix A).

Hemiptera (16.55%) and Hymenoptera (16.31%) were almost equal in number of morphospecies, however the former contains only 21 families of bugs (141 morphospecies), while the latter

contains 27 families (139 morphospecies) of bees, wasps and ants. The leafhopper family (Cicadellidae) had the highest number of morphospecies. Ants (Formicidae) were represented by 25 different species (see Appendix A).

Orthoptera (grasshoppers, locusts and crickets) was mainly represented by the Acrididae family with a total of 44 morphospecies. A total of 11 families and 97 morphospecies were sampled. Five of these families were represented by only one morphospecies (see Appendix A).

Three families of Blattodea (cockroaches) were sampled with a total of 12 morphospecies. Two families, with one morphospecies each, of Isoptera (termites) were sampled. Also two families of Mantodea (mantids), and one family of Phasmatodea (stick insects) were sampled (see Appendix A).

Lepidopterans (butterfly and moths) were sampled infrequently, but delivered 26 morphospecies comprising of 13 families. Tineidae was the family with the most morphospecies (four) (see Appendix A).

Rare orders (represented by one or a few morphospecies) which were sampled include: Mecoptera (hangingflies); Achaeanognatha (bristletails); Psocoptera (booklice); Odonata (dragonflies) and Ephemeroptera (mayflies) (see Appendix A).

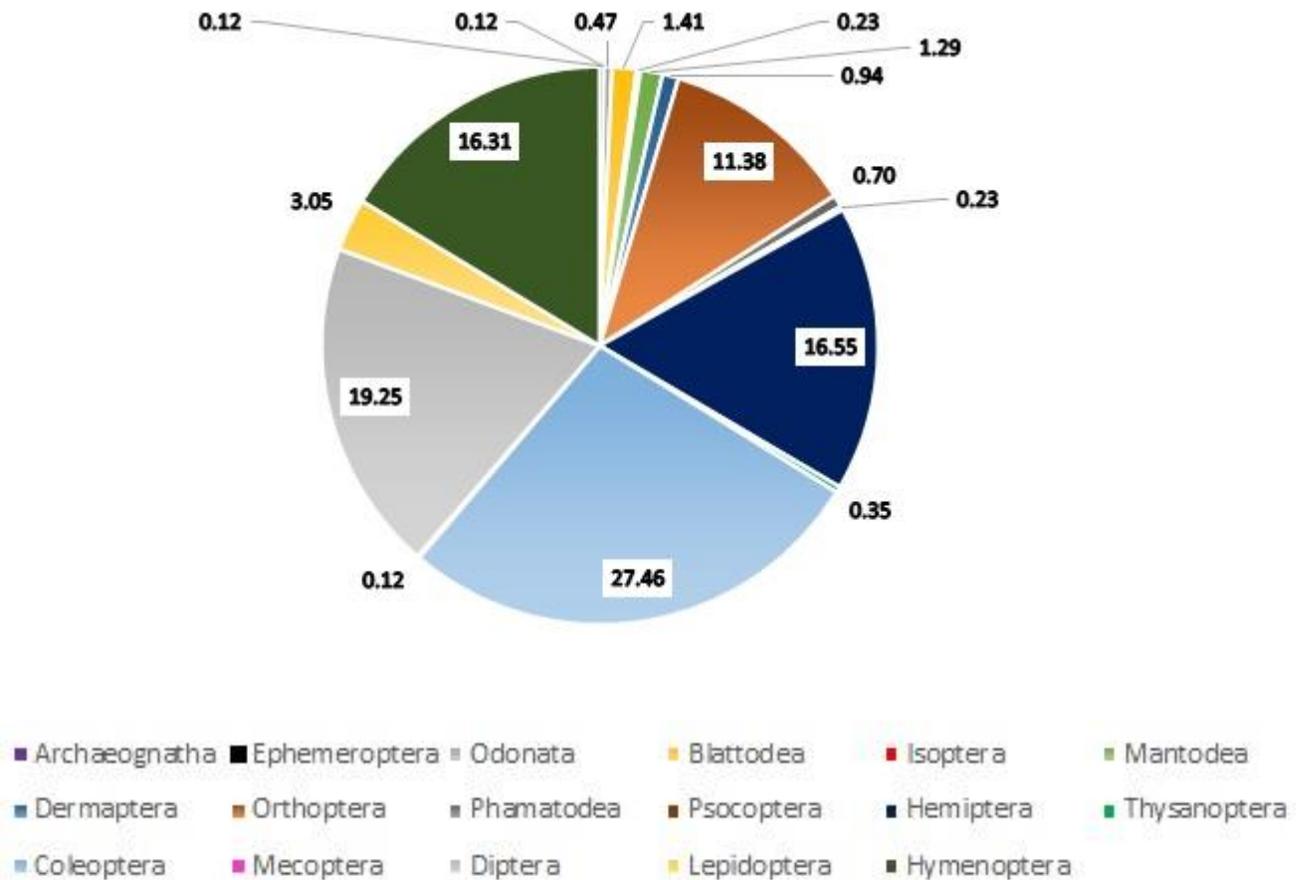


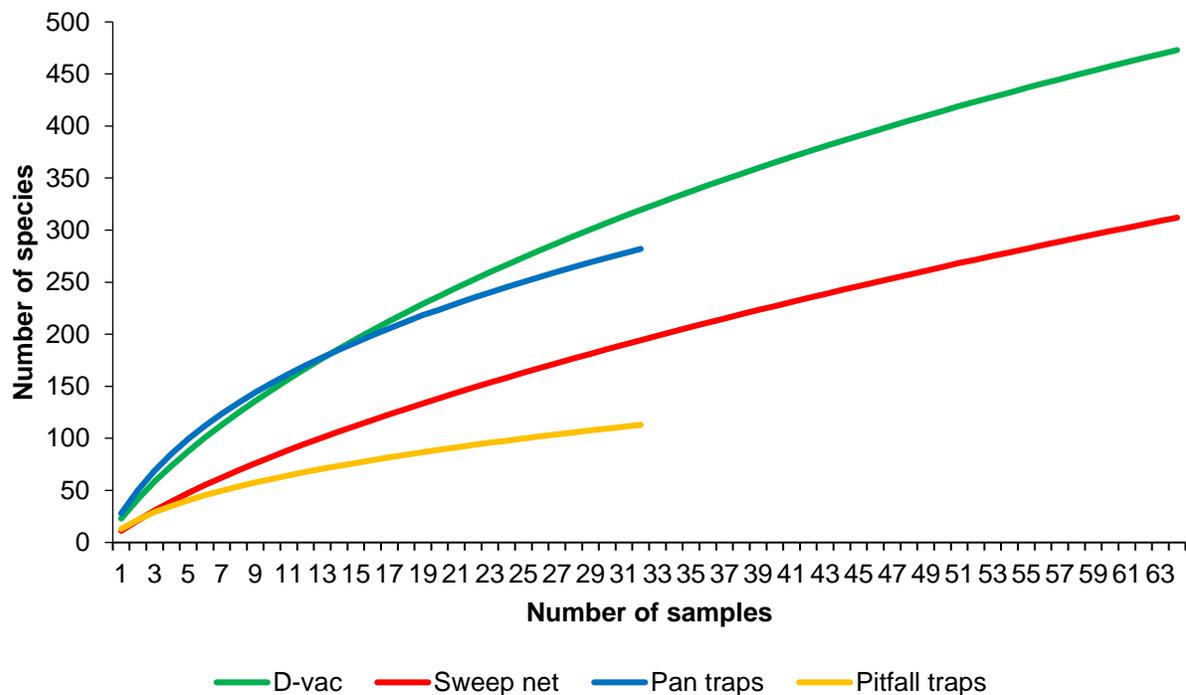
Figure 7 - Insect order composition of the project reference collection, given as percentage.

Sampling effort

The two estimators showed that the sampling effort was not optimal as many more species were expected to be sampled per sampling method, even when all methods were combined (Table 4). The species accumulation curves have steep slopes and do not reach an asymptote, illustrating that a greater sampling effort would be required to capture the entire community (Figures 8, 9 & 10). This is also the case when only two sampling methods are applied over a course of four seasons. The gap between the sampled species and the two estimators is the smallest for pitfall traps, showing that the epigaeic insect fauna is less than the other groups (Table 4).

Table 4 - Summary of total species sampled over total sampling period for each method with species estimators.

Sampling method	Sample size (n)	Species sampled	Chao2 (mean \pm SD)	Jackknife2 (mean)
D-Vac	64	473	795.88 \pm 55.97	866.76
Sweep net	64	312	747.13 \pm 89.76	668.58
Pan traps	32	282	469.82 \pm 44.54	496.7
Pitfall traps	32	113	197.39 \pm 31.44	203.42
All methods combined	192	851	1276.92 \pm 57.87	1445.68

**Figure 8** - Species rarefaction curves of all sampling methods over the total sampling period.

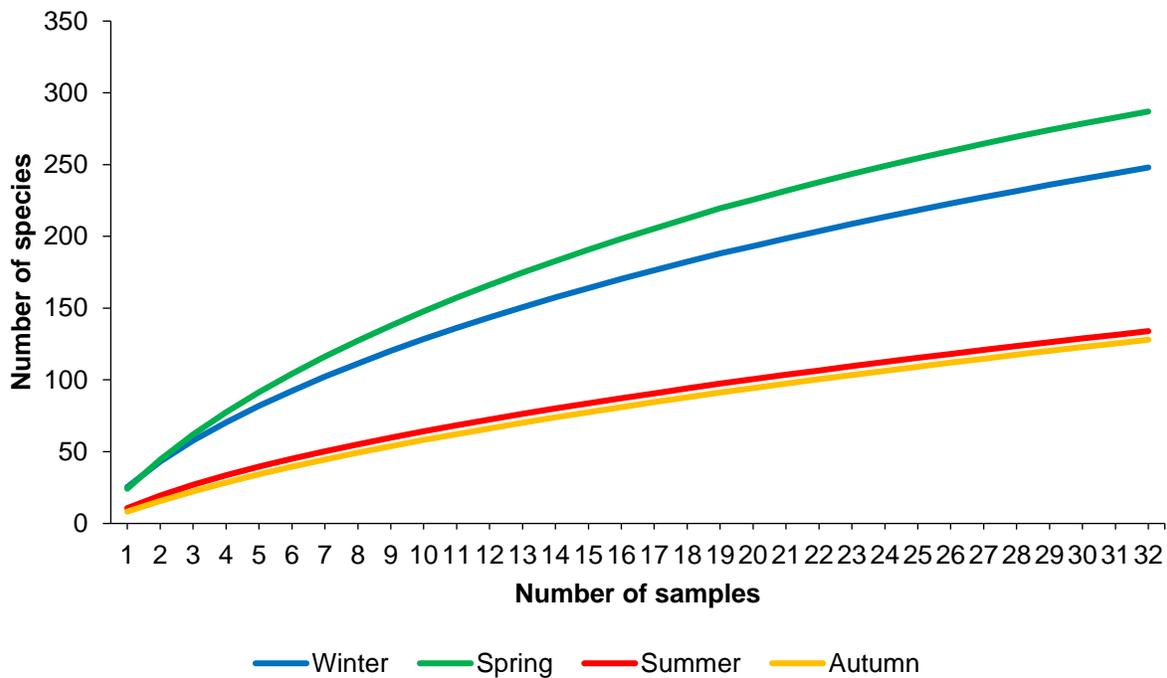


Figure 9 - Species rarefaction curves of combined D-vac and sweep net samples of all sampling seasons.

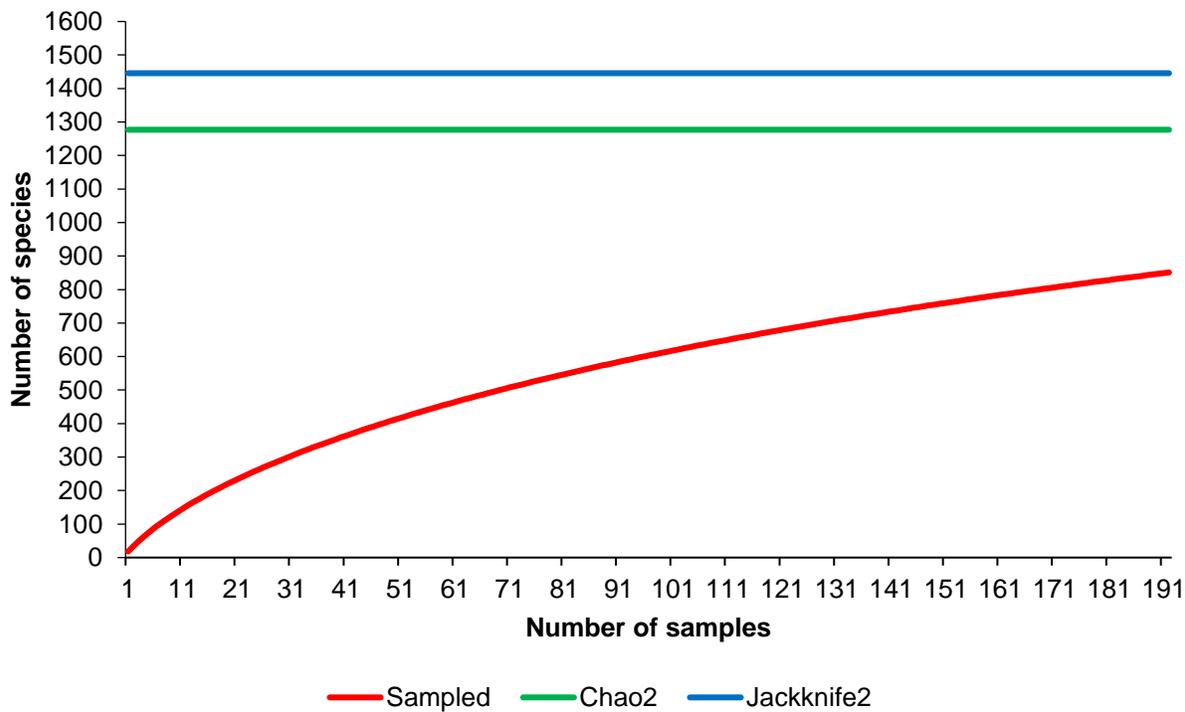


Figure 10 - Species accumulation curve for all sampling methods combined over total sampling period (n = 192). Chao2 and Jackknife2 estimators are also included.

Sampling methods effectiveness

Variation in species richness and abundances found between the four sampling methods, illustrate that an array of sampling techniques is required for insect diversity studies. Significant differences were found between the four sampling methods for both species richness ($H = 25.351$; $\chi^2_3 = 20.000$; $p < 0.007$) and abundance ($H = 22.891$; $\chi^2_3 = 18.000$; $p < 0.001$). There is interaction between seasons and methods, indicating that different methods are effective in different seasons (Figures 11 & 12).

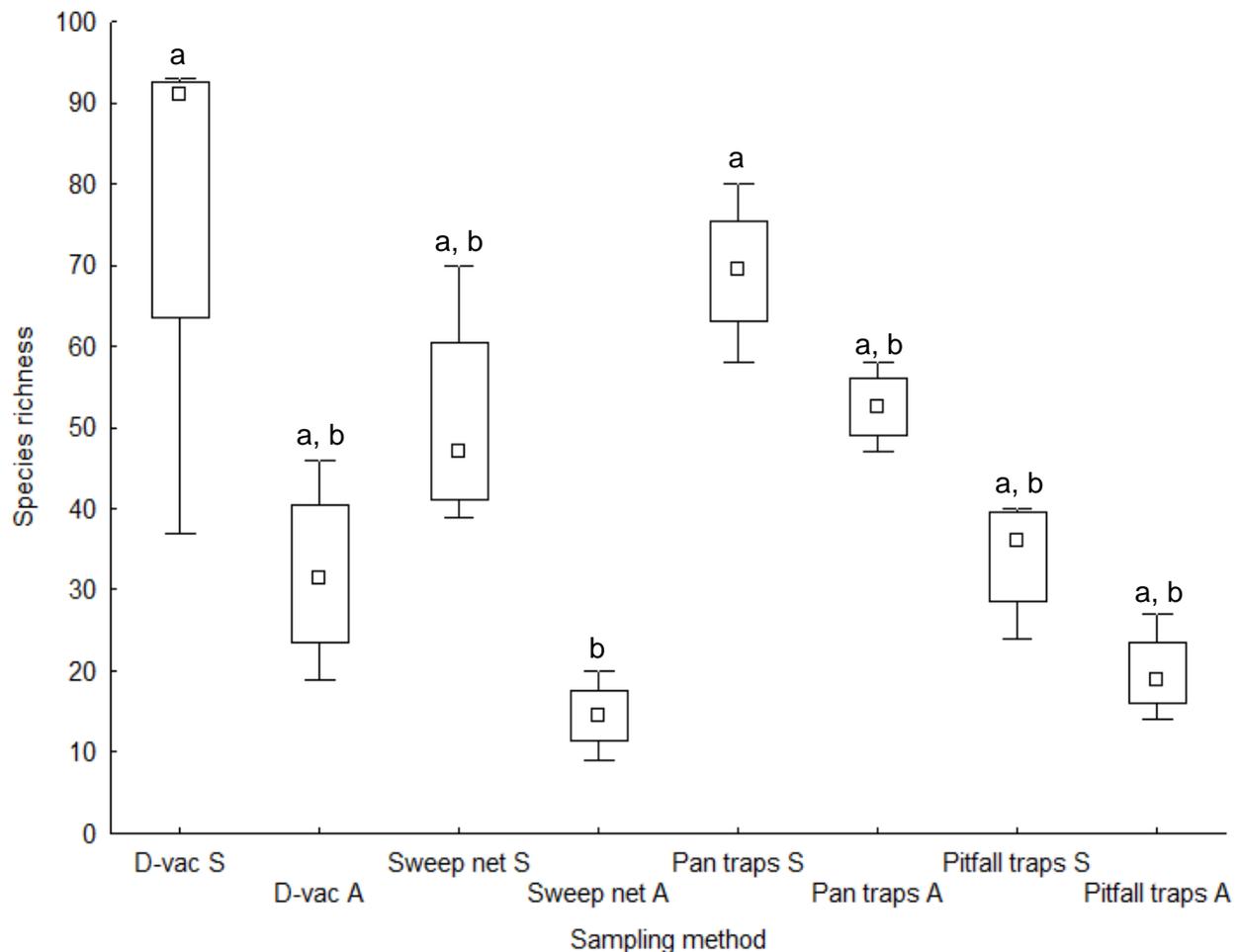


Figure 11 – Variation in insect species richness per site ($n = 16$) in spring (S) and autumn (A) for each sampling method. Kruskal-Wallis results based on method x season combination. The squares of the boxes denote the median; the boxes denote the 25% and 75% percentiles; the whiskers denote the min and max. Different letters indicate significant differences using multiple post-hoc comparisons.

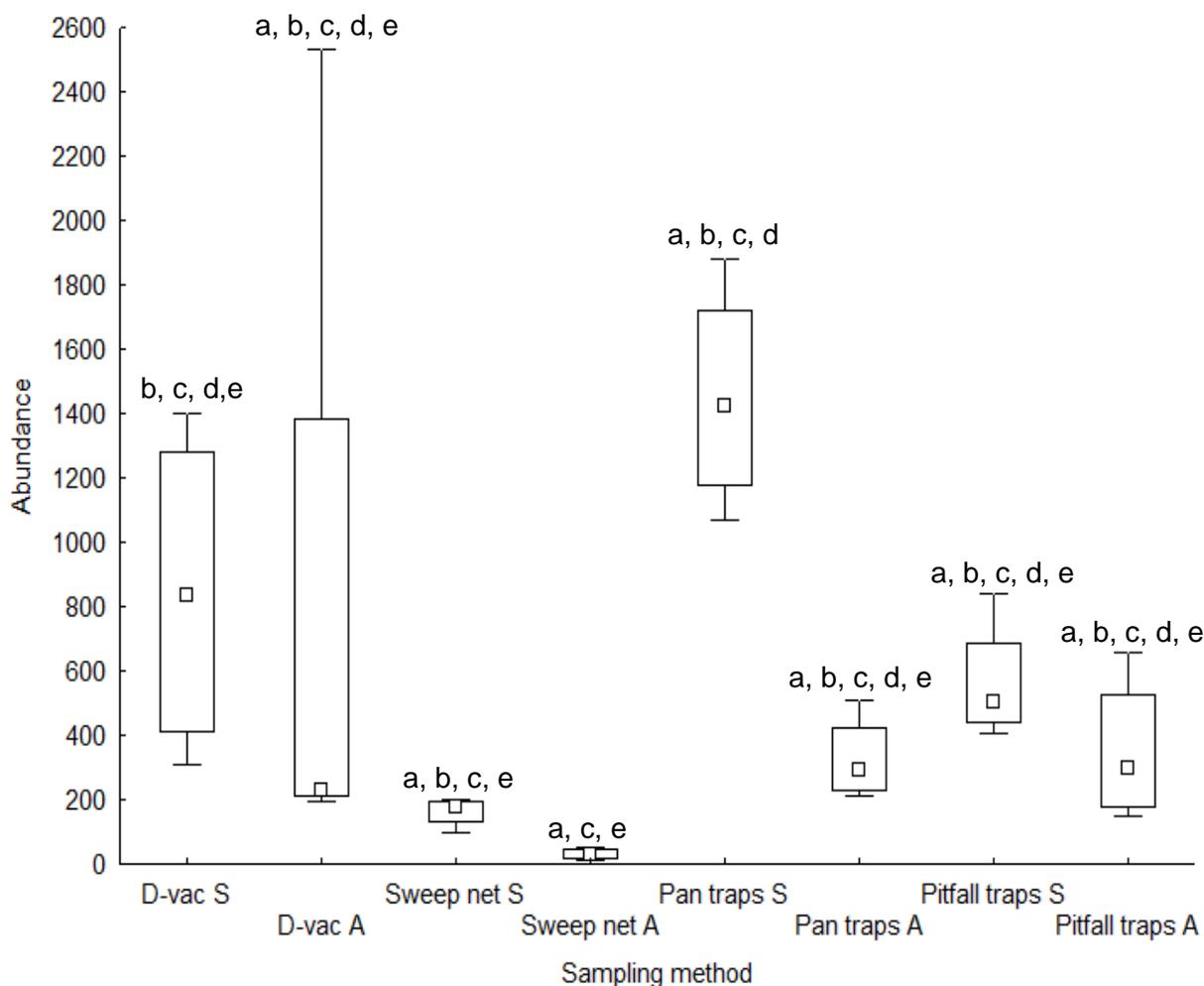


Figure 12 - Variation in insect abundance per site ($n = 16$) in spring (S) and autumn (A) for each sampling method. Kruskal-Wallis results based on method x season combination. The squares of the boxes denote the median; the boxes denote the 25% and 75% percentiles; the whiskers denote the min and max. Different letters indicate significant differences using multiple post-hoc comparisons.

When species richness and abundances are compared for a single sampling method, such as the D-vac (this method sampled the most species throughout the study and was included in all four sampling seasons), significant differences were found between the seasons in species richness ($H = 9.551$; $X^2_3 = 10.00$; $p < 0.05$) and abundance ($F = 68.30$; $p < 0.001$) (Figures 13 & 14), indicating that seasons had an effect on the sampling of species. Both winter and spring only differ significantly from autumn. Summer does not differ significantly from the other seasons, except autumn.

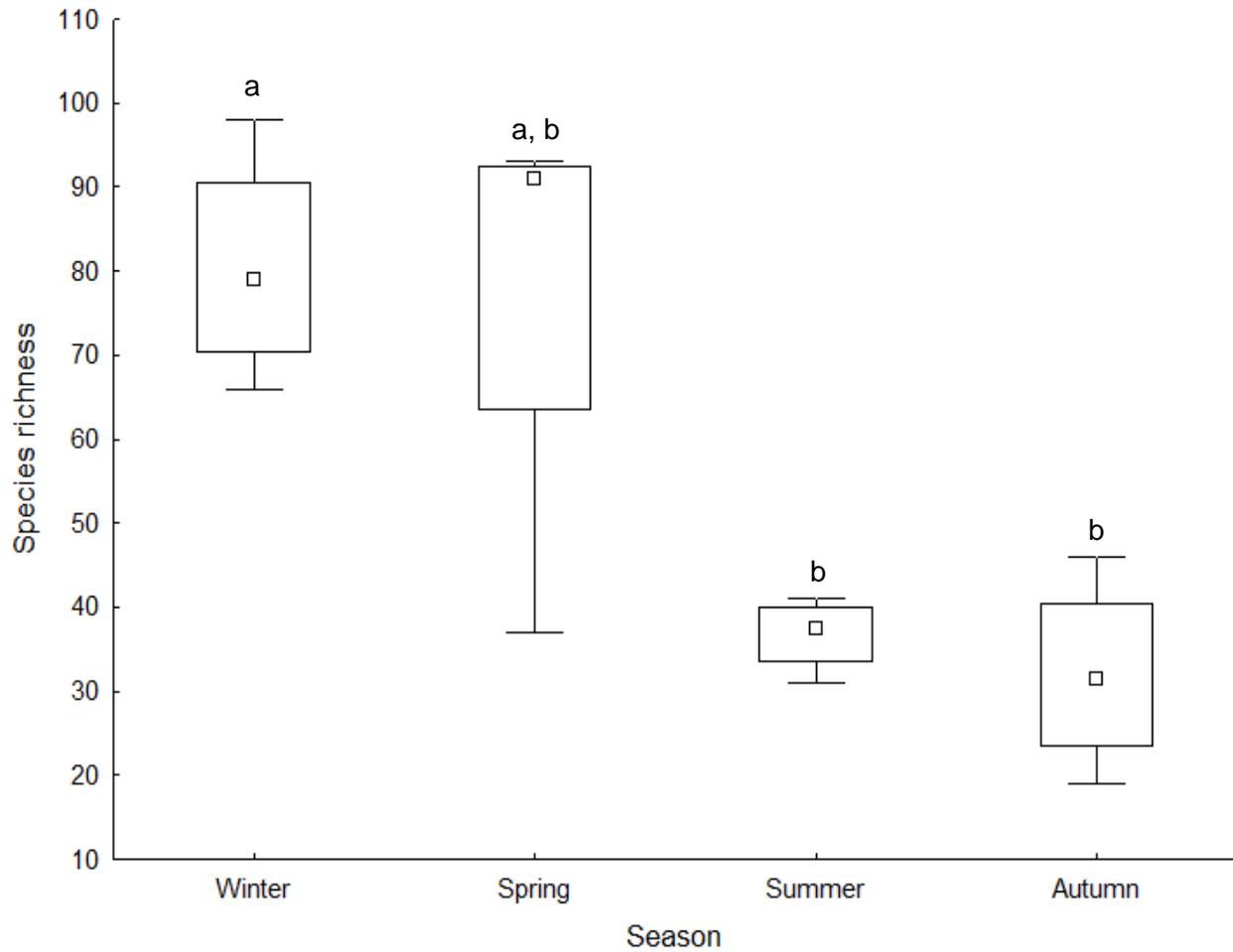


Figure 13 - Variation in insect species richness recorded per site ($n = 16$) using D-vac sampling across four sampling seasons. The squares of the boxes denote the median; the boxes denote the 25% and 75% percentiles; the whiskers denote the min and max. Different letters indicate significant differences computed by the multiple comparisons of mean ranks post hoc test.

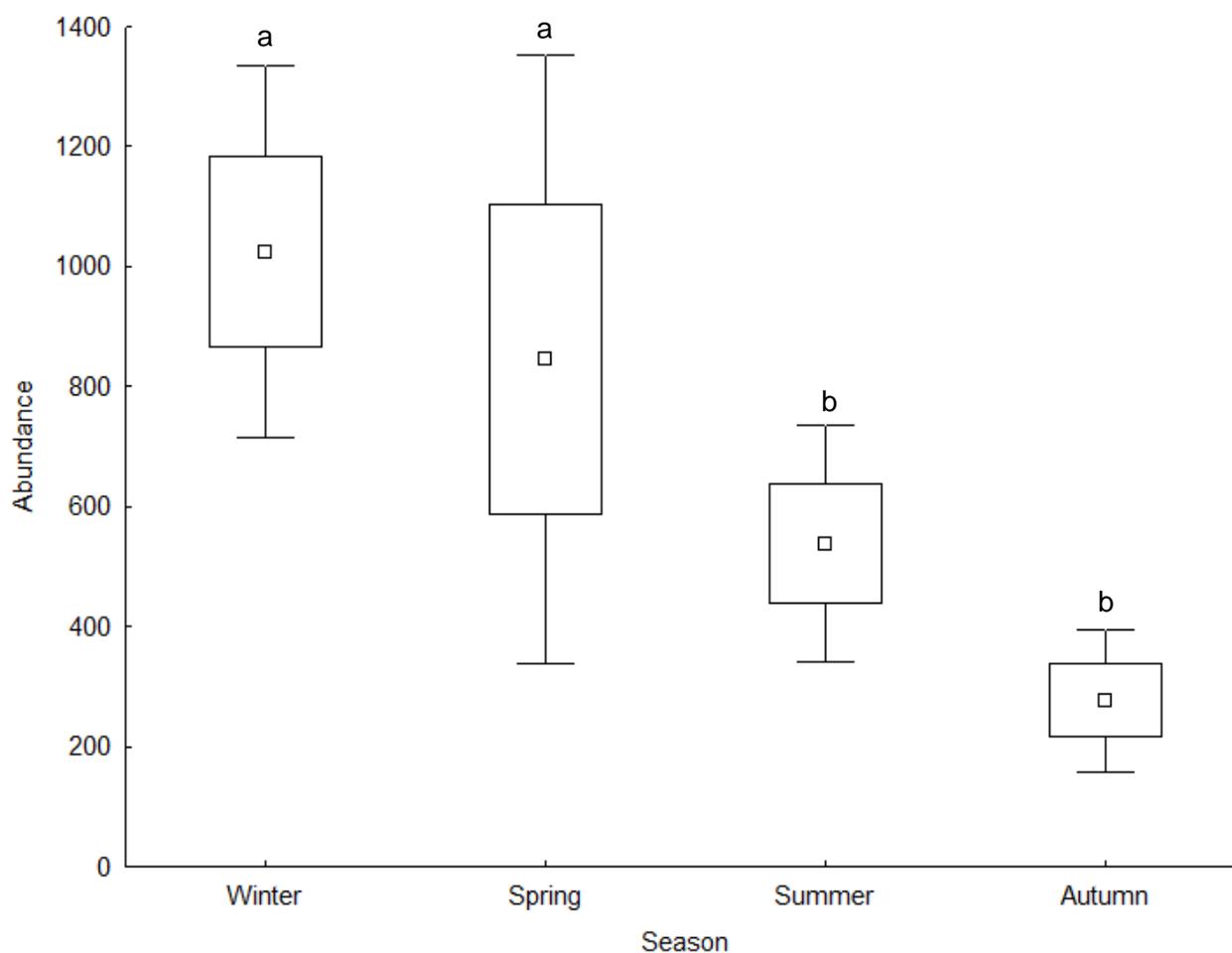


Figure 14 - Variation in insect abundance recorded per site ($n = 16$) using D-vac sampling across four sampling seasons. The squares of the boxes denote the mean; the boxes denote mean \pm SE; the whiskers denote mean $\pm 1.96 \times$ SE. Different letters indicate significant differences computed by the Fisher LSD post hoc test.

In terms of sampling method, it was clear that the combination of D-vac and sweep net delivered the most shared species compared to any of the other combinations (Table 5). D-vac and pan traps also seem to be an effective combination for sampling many different species, as this combination sampled the most species. In addition, the shared species between these methods are relatively low. The steep rarefaction curves could explain the low percentages of shared species (Figure 8). The combination of sweep net and pitfall traps sampled the least species and the proportion of shared species was the lowest (Table 5).

Table 5 - Number of insect species sampled and shared per sampling method combination for spring and autumn sampling combined (i.e. Strategy 1: four sampling methods used in both spring and autumn).

Combined sampling methods	Number of samples (n)	No. of species	No. of shared species	% species shared
D-vac and Sweep net	64	485	104	21.4
D-vac and Pan traps	64	575	59	10.3
D-vac and Pitfall traps	64	406	28	6.9
Sweep net and Pan traps	64	474	49	10.3
Sweep net and Pitfall traps	64	305	14	4.6
Pan traps and Pitfall traps	64	395	52	13.2

Winter and spring delivered the most species and also shared the most species (Table 6). Overall, shared species is low across the seasons. Given the two sampling methods used, it can be assumed that more insect species are found in the plant foliage and on the canopies during winter and spring compared to the other seasons.

Table 6 – Number of insect species sampled and shared per sampling season combination for D-vac and sweep net sampling combined (i.e. Strategy 2: four sampling seasons using D-vac and sweep net).

Combined sampling seasons	Number of samples (n)	No. of species	No. of shared species	% species shared
Winter and spring	64	535	74	13.8
Winter and summer	64	382	38	10.0
Winter and autumn	64	376	47	12.5
Spring and summer	64	421	33	7.8
Spring and autumn	64	415	34	8.2
Summer and autumn	64	262	31	11.8

The community composition data were clustered by the sampling method, rather than by sampling areas (Figure 15). This can be seen especially for pan traps and pitfall traps, as they strongly formed their separate clusters. There was less solely clustering in both the D-vac and sweep net sampling methods; however these two methods clustered together as a combination. However, this was definitely not due to sampling area differences, but could be expected due to the fact that these two methods both sample foliage insects.

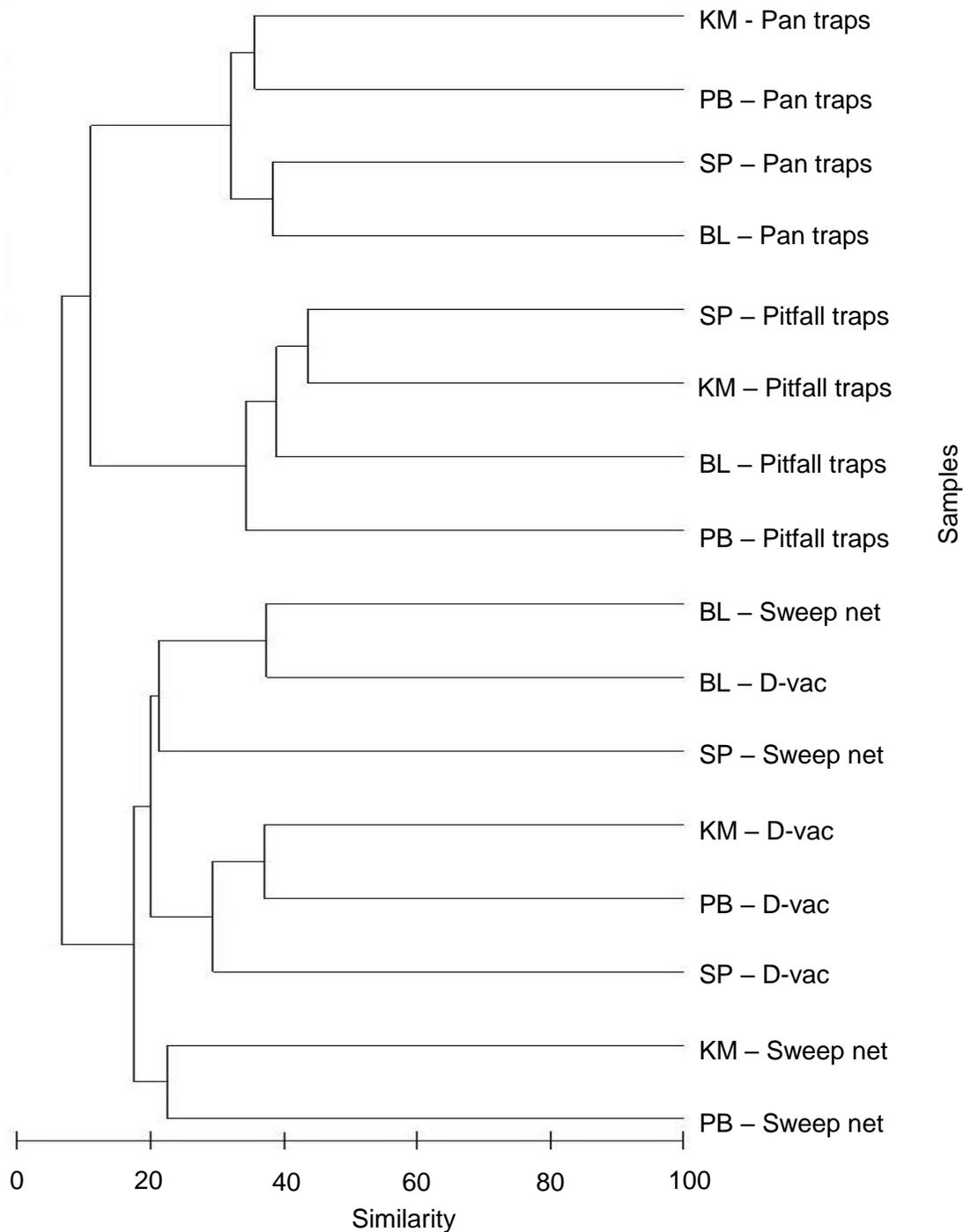


Figure 15 - Dendrogram based on Bray-Curtis similarity showing resemblance of insect communities between the different renosterveld areas and the four different sampling methods. The abundance data of both spring and autumn seasons was added together and then square-root transformed. PB = Papegaaiberg; KM = Koopmanskloof; BL = J.N. Briers-Louw; SP = Spier.

Sampling strategies

Evaluating sampling Strategy 1 (i.e. when D-vac and sweep net were implemented for all the seasons) also showed differences in seasons. Summer and autumn differed less from each other than any other pairwise comparison. Although the two sampling methods differ from each other, the four sampling areas differed more from each other, with Koopmanskloof, J.N. Briers-Louw differing from each other and the grouping of Spier and Papegaaiberg which differed less from each other (see Table 8 and Figure 16).

In turn, sampling Strategy 2 where all four sampling techniques were used, study area differences were relatively small, but strong seasonal difference still remained. Furthermore, the four sampling techniques differed considerably from each other. D-vac and sweep net differed less than any other pairwise sampling method comparisons (see Table 9 and Figure 17).

When two sampling methods were applied over four seasons (Strategy 1), 618 morphospecies comprised of 16 orders were sampled, while when four methods were used over two seasons (Strategy 2), 633 morphospecies comprised of 15 orders were sampled (Table 7). Although the first strategy sampled one more order, this is not significant as only one individual mayfly (Ephemeroptera) was additionally sampled. Also the total number of species does not differ considerably (only 15; see table 7). In contrast, 22 more insect families were documented when more sampling methods were used.

The PERMANOVA test confirmed that all the categorical variables (i.e. sampling locations, methods and seasons) differed significantly (Table 8). The test statistic for methods was the greatest and pairwise tests also confirmed significant differences for the seasons (Table 8). Although summer and autumn lies more closely to each other in the CAP plot, they still differed significantly (Figure 16). For Strategy 2 the PERMANOVA test also confirmed significant differences for all the categorical variables (Table 9). The test statistic for sampling methods was also the greatest. Although only comparing two seasons, these seasons accounted for more variation than sampling location which confirms that sampling during spring and autumn is important. The pairwise tests also indicated significant differences for the methods (Table 9). Although D-vac and sweep net lay closer to each other on the CAP plot (Figure 17), they still differed significantly from each other.

Table 7 - Order, family and species count per sampling strategy. Strategy 1 = D-vac and sweep net methods implemented over all four seasons. Strategy 2 = All four methods implemented over spring and autumn seasons.

Order	Strategy 1		Strategy 2	
	Family count per order	Species count per order	Family count per order	Species count per order
Archaeognatha	1	1	-	-
Blattodea	3	8	3	9
Coleoptera	22	162	27	182
Dermaptera	2	2	3	8
Diptera	26	110	32	127
Ephemeroptera	1	1	-	-
Hemiptera	20	130	19	92
Hymenoptera	21	83	24	112
Isoptera	1	1	2	2
Lepidoptera	8	15	13	23
Mantodea	1	9	2	5
Mecoptera	-	-	1	1
Odonata	2	4	2	2
Orthoptera	8	81	11	63
Phasmatodea	1	6	1	4
Psocoptera	1	2	1	1
Thysanoptera	2	3	1	2
Totals per strategy				
Orders	16		15	
Families	120		142	
Species	618		633	

The CAP analyses displayed the significant differences in the two strategies based on the PERMANOVA results. A higher degree of clustering means less variation within each individual categorical factor. For example, the clusters for sampling seasons and sampling methods are more concentrated, indicating less variation compared to location for both strategies (Figures 19 & 20). The seasons for Strategy 1 differed significantly from each other (Table 8 & Figure 19a). Summer and autumn seasons differed less significantly as they are closer to each other (Table 8 & Figure 19a). For Strategy 2, the four methods differed significantly from each other (Table 9 & Figure 20a). However D-vac and sweep net differed less significantly as they are clustered closer to each other (Table 9 & Figure 20a). In addition, the sampling areas differed significantly from each other in both strategies (Figures 19b & 20b). For Strategy 1 the sampling areas differed more or less equally from each other (Figure 19b), while for Strategy 2, Spier differed most from the other three sampling areas (Figure 20b).

Table 8 - PERMANOVA results illustrating the effects of the categorical variables (season, sampling area, method) on the differences between species identity and abundances for Strategy 1, (i.e. D-vac and sweep net methods over all four seasons; highlighting pairwise tests between seasons (df = degrees of freedom; SS = sum of squares; MS = variance; Pseudo-F = test statistic; P(Perm) = p-value generated by permutations; t = test statistic).

Source of variation	df	SS	MS	Pseudo-F	P(Perm)
Season	3	71251	23750	8.61	0.0001
Sampling areas	3	33063	11021	3.99	0.0001
Method	1	40163	40163	14.56	0.0001
Pairwise tests	t	P(perm)			
Winter, Spring	2.77	0.0001			
Winter, Summer	3.26	0.0001			
Winter, Autumn	3.11	0.0001			
Spring, Summer	2.66	0.0001			
Spring, Autumn	2.45	0.0001			
Summer, Autumn	1.79	0.0008			

Table 9 - PERMANOVA results illustrating the effects of the categorical variables (season, sampling area, method) on the differences between species identity and abundances for Strategy 2, (i.e. all four sampling methods over spring and autumn; highlighting pairwise tests between methods (df = degrees of freedom; SS = sum of squares; MS = variance; Pseudo-F = test statistic; P(Perm) = p-value generated by permutations; t = test statistic).

Source of variation	df	SS	MS	Pseudo-F	P(Perm)
Season	1	25960	25960	9.21	0.0001
Sampling areas	3	39659	13220	4.69	0.0001
Method	3	114520	38175	13.55	0.0001
Pairwise tests	t	P(perm)			
D-vac, Sweep net	2.50	0.0001			
D-vac, Pan traps	3.46	0.0001			
D-vac, Pitfalls	3.92	0.0001			
Sweep net, Pan traps	3.04	0.0001			
Sweep net, Pitfalls	3.76	0.0001			
Pan traps, Pitfalls	3.82	0.0001			

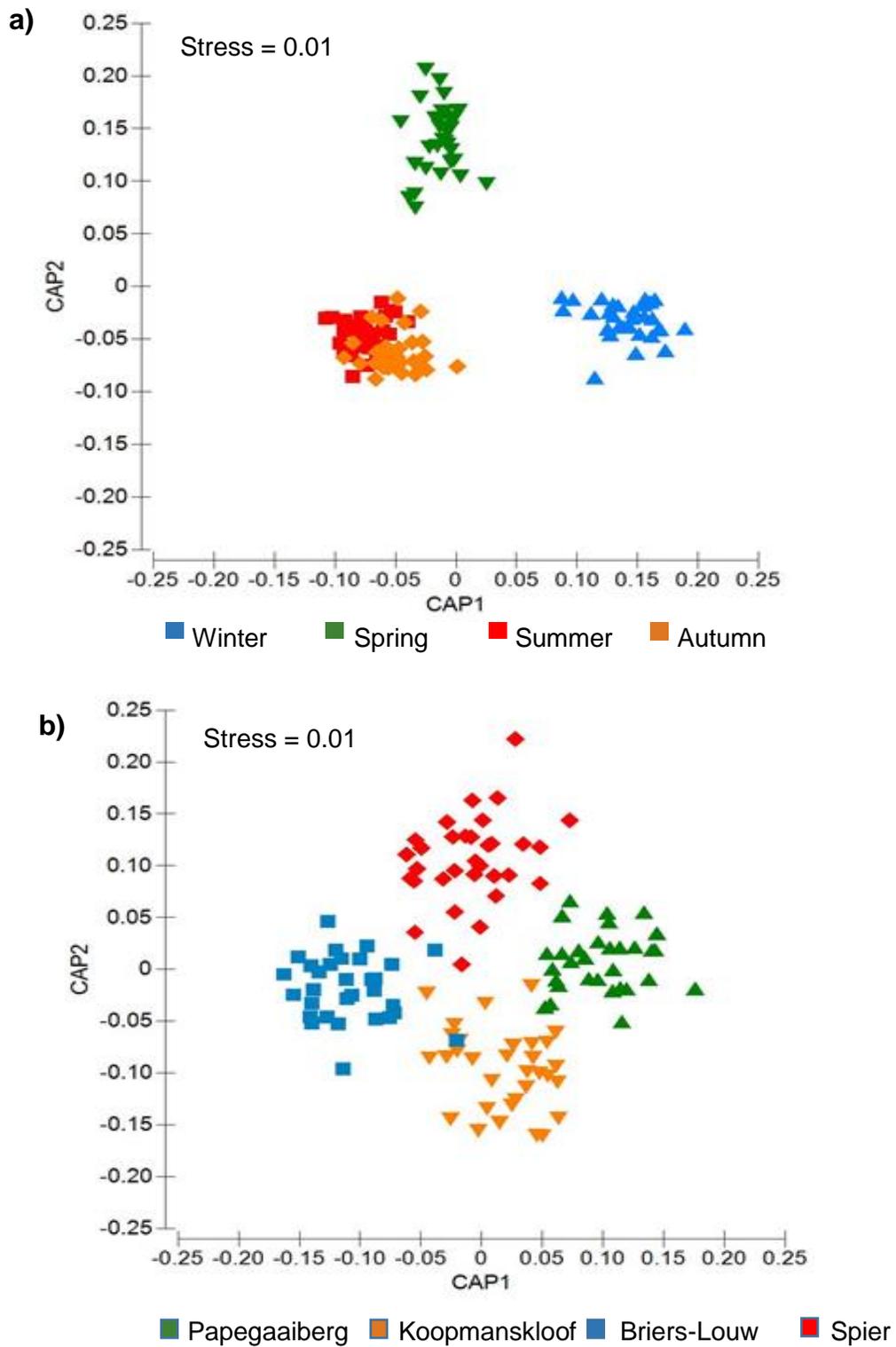


Figure 16 - Canonical analysis of principal coordinates (CAP) of Strategy 1: two methods (D-vac and sweep net) over four seasons based on Bray-Curtis similarity and PERMANOVA results: a) seasons; b) sampling areas. The stress value = 0.01.

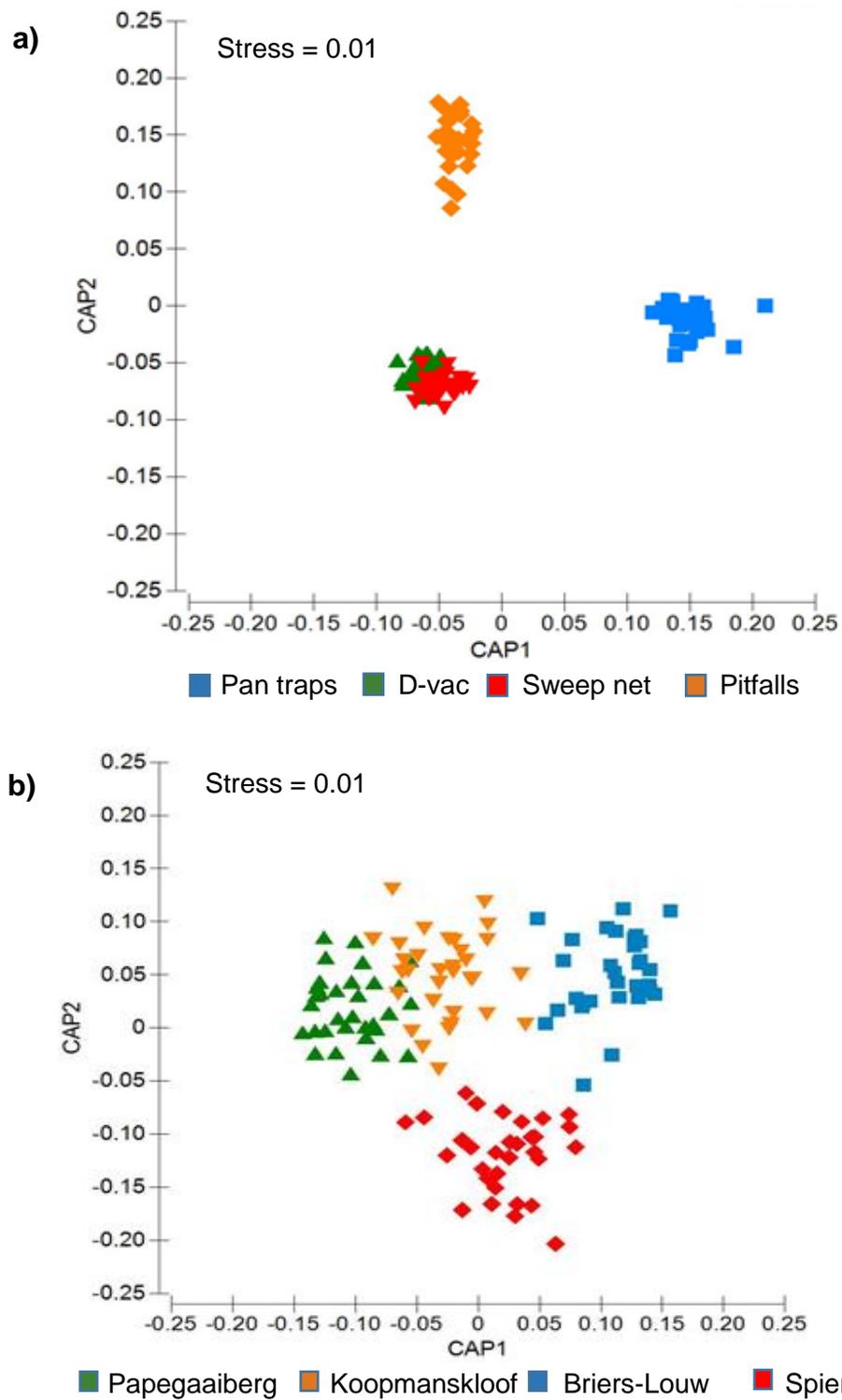


Figure 17 - Canonical analysis of principal coordinates (CAP) of Strategy 2: four methods over spring and autumn seasons based on Bray-Curtis similarity and PERMANOVA results: a) methods; b) sampling locations. The stress value = 0.01.

Discussion

Insect sampling in renosterveld

The four methods used in this study sampled the expected taxa as indicated by previous studies (Doxon *et al.* 2011; Shepard *et al.* 1974). The D-vac sampled primarily Coleoptera, Hemiptera, Diptera, Hymenoptera and Orthoptera (Brown *et al.* 1988; Buffington & Redak 1998; Doxon *et al.* 2011). Sweep net samples were generally dominated by Coleoptera, Diptera, and Hemiptera (Cooper & Whitmore 1990; Cooper *et al.* 2012; Doxon *et al.* 2011). Diptera and Hymenoptera dominated the pan trap samples (Aguar & Sharkov 1997; Disney *et al.* 1982), while pitfall trap specimens mostly consisted of Coleoptera and Hymenoptera (Agosti *et al.* 2000; Luff 1975; Mommertz *et al.* 1996; Spence & Niemelä 1994) (see Appendix B).

Most of the insect species found in renosterveld seem to be within the foliage as D-vac sampling caught most of the species, which cannot be reached by the sweep net (Cooper *et al.* 2012; Sutherland 2006). As many leafhopper species were sampled (see Appendix A), and have been shown to be host-specific in the CFR (Augustyn *et al.* 2013), a great proportion of these species is likely to be host-specific to renosterveld flora (Davies 1988a & Davies 1988b).

Sampling effort

It is common in the literature to find that species rarefaction curves do not reach asymptotes, despite intensive sampling (Gotelli & Colwell 2001). This is due to the relatively low abundance of rarer species sampled by the four sampling methods. The D-vac (which mainly samples the foliage) sampled the most species, therefore one can assume that the D-vac is most effective method to sample insects species in renosterveld plant foliage. In addition, many insect individuals can be sampled with the D-vac during a single season (Witt & Samways 2004), which also substantiates the assumption that many insects are found within the plant foliage. Pan traps delivered far more insect species in spring compared to autumn. This can be expected as the plants were flowering during spring and more anthophiles were attracted to the pan traps (Vrdoljak & Samways). In addition, with the high diversity of geophytes which mostly flower in spring (Kraaij 2010; Parker & Lomba 2009; Rebelo 1992; Shiponeni & Milton 2006), more pollinators can be expected when sampling in this season. Pitfall trap sampling came the closest to reaching an asymptote, which indicates that the sampling of ground-dwelling insect species was closest to being complete. It is possible to further reduce the gap between the number of species sampled

and the estimated species by using larger pitfall traps, which can sample the rarer ground-dwelling insects (Work *et al.* 2002).

Table 10 – Recent research of insect species richness found in South Africa’s biomes. Other studies found in the literature focusses only on a certain taxa or functional guild, whereas these studies include all insect species sampled.

Country	Vegetation type	Species richness	Sampling methods	Sampling area	Authors
South Africa	Fynbos	139	D-vac, Pitfall traps, Quadrat searches, Berlese-Tullgren funnel litter extractions, Aerial surveys, Window trapping	Table Mountain (eastern side)	Pryke & Samways (2009)
South Africa	Fynbos	97	Sweep net	Baviaanskloof (100m ²)	Proches & Cowling (2006)
South Africa	Grassland Biome	641	D-vac	1200 m ²	Botha <i>et al.</i> (2016)
South Africa	Savanna Biome	712	D-vac	1200 m ²	Botha <i>et al.</i> (2016)

Insect species richness are relatively high in the grassland and savanna biomes, seeing that only one sampling method, i.e. the D-vac, was used to sample the high number of species. This is much greater than the total number of species sampled with the D-vac for this study (see Table 4 & 10). However, the total number of species sampled with the sweep net for this study is far greater than what was sampled in fynbos (see Tables 4 & 10).

The sampling effort applied in this study suggests that renosterveld is rich in insect fauna, because even when combining the four sampling methods the rate of accumulating species did not reach an asymptote (barring pitfall sampling). Also, after each sampling occasion new species were found, even at the last locality sampled in autumn. If one extends the sampling period to two years, or intensifies sampling per area by selecting more sites within sampling areas, the increase in sampling effort could result in a tendency for the species rarefaction curves to reach asymptotes. However, simply doubling sampling effort is not logistically feasible or efficient (Gotelli & Colwell 2001). The total time spent on collecting samples, processing work in the laboratory, and the identification of morphospecies exceeded 500 hours. The literature also suggests that an array of sampling methods is necessary to sample many different insect species (Standen 2000; Hyvarinen *et al.* 2006). Differences in both species richness and abundances were found between the sampling methods, indicating that the application of only one sampling method will not be sufficient for insect diversity studies.

Variation in species richness, abundance, and composition

Variation in species richness and abundance between sampling methods and seasons indicate that the insect composition in renosterveld is not uniform throughout the year. Environmental and temporal factors can explain the variation in species richness, abundance, and composition (Botes *et al.* 2007; Neves *et al.* 2015). On average, more species were sampled during spring, although abundances were greater during winter. Based on these findings, different insect species and numbers can be expected to be sampled during different seasons. Also, insects undergo metamorphosis between developmental stages (Cheong *et al.* 2015) and can control their life cycles through actively regulating their development. This can be done by using various environmental cues to different degrees to either accelerate or retard the development in one or more life stages (Cheong *et al.* 2015). Therefore the insect species responses to the environment are very complex (Danks 2006), and in turn will alter the insects sampled in specific seasons.

It is expected that species richness and abundance of generalist insect species will increase with increasing plant diversity (Jonsen & Fahrig 1997). However, this does not mean that there will be a definite, strong, positive correlation between insect species diversity and plant diversity, due to the uncertainty of correlated diversification of insects to plant assemblages in fynbos (Proches & Cowling 2006). Consequently, in a renosterveld patch, more insect species can be expected in a more heterogeneous environment. Additionally, during certain times of the year, more plants are flowering which may result in more pollinator species being present. Flowering geophytes were

mostly observed during winter and spring sampling (Personal observation). Controversially, it has been postulated that the fynbos is not rich in insect fauna (Johnson 1992; Giliomee 2003), although others argue that the fynbos is not poor in insect fauna (Proches & Cowling 2006). This study lends support to the latter hypothesis.

In addition, insects have to take shelter from hot and dry conditions, as desiccation is one of the greatest threats posed to them (Lill & Marquis 2007; Doxon *et al.* 2011). This phenomenon can be expected especially in the hot, drier months of the year (January and February are considered as the hottest months in the Mediterranean Fynbos biome) (Mustart & Cowling 1993), as the insects will favor the available moister microhabitats (Akiko 2001). These microhabitats include: woody plant debris, soil, and different plant species morphology and structure (Altieri 1999; Thies *et al.* 2003; Bouget & Duelli 2004). When insects were sampled during summer and autumn, fewer species were caught and abundances were significantly lower than during winter and spring, indicating that these conditions influence the presence and abundance of insects. In addition, when sweep netting was applied only a few insects were sampled per line transect, supporting the notion that insects shelter from the heat by maintaining their body water content by means of feeding on plants, and taking shelter deeper in the foliage (Gray & Bradley 2005).

Environmental and seasonal conditions are thus driving factors which will determine insect activity and behaviour, which in turn affect insect diversity sampled at a specific time. Many new species were sampled within each sampling season, even at the last sampling site during autumn. However, intensification of the sampling effort is not necessarily logistically feasible or efficient, because the probability for sampling new species gets smaller in relation to the extended sampling period and therefore it can take a lot of effort to add only a few new species, while other species which have already been sampled are repeatedly sampled. Nevertheless, it is crucial to plan which sampling methods during which sampling seasons will meet the study objectives, which in the case of this study was to comprehensibly document insect diversity.

Sampling strategy for insect fauna of renosterveld

Based on the PERMANOVA results, it is clear that sampling methods certainly had an effect on variation in community composition for both sampling strategies. This highlights the importance of choosing the appropriate sampling methods for the desired objectives (Doxon *et al.* 2011). An important aspect to consider is that different types of data are needed for different purposes (i.e. high numbers and consistent catches for monitoring purposes, a wide range of taxa for inventory, and effective targeting of specific taxa for more focused ecological studies) (Lawton *et al.* 1998;

Magurran *et al.* 2010). In addition the two CAP plots for both strategies also indicated that each method samples different numbers of species and that the species sampled differed between the sampling methods, as generally found in previous studies (see Hyvarinen *et al.* 2006; Standen 2000).). In general, it is difficult to compare different sampling methods directly, because each method operates differently (i.e. they are in the field for different time periods, the size of the areas which they cover differ, different insects are susceptible to being trapped by different methods) (Meats & Edgerton 2008). D-vac and sweep net also differ significantly even though they cluster close to each other in the CAP plot, indicating these two methods are more similar in terms of the results they produce. Community composition differs significantly for all the seasons, even for summer and autumn which are more closely clustered in the CAP plot. This grouping of summer and autumn in the ordination plot can be explained by the hot and dry conditions which prevailed during summer and autumn. Unfavourable conditions during these two seasons will force the insects to move to microhabitats which are more favourable (Akiko 2001; Lill & Marquis 2007), which will reduce the number of individuals caught and produce greater similarity in species sampled between these two seasons. Another factor to consider is that population levels could be low during these periods.

For both sampling strategies, renosterveld sampling areas differed significantly from each other. Although weather conditions can cause fluctuations in the abundances of insects (Doxon *et al.* 2011), the effect of the weather variables in this study can be eliminated as they remained similar for all sampling areas during respective sampling occasions and seasons. Consequently, the remaining possible variables that might explain the significant differences could be vegetation structure and composition, and topography (Haddad *et al.* 2001; Hodkinson 2005). Vegetation structure and composition could be the factor causing Spier to differ most significantly to the other sampling areas for Strategy 2. As mentioned, the vegetation was very dense and tall, which causes more homogeneity in vegetation composition (Vilà & Sardans 1999), which in turn can influence the insect species found as well as their relative abundance.

Therefore, the ideal sampling strategy recommended for sampling insects in renosterveld is one where three complimentary methods are applied to three markedly different seasons. The method for this proposed strategy would include a combination of pan traps, pitfall traps and either D-vac or sweep netting. D-vac is preferable, because this method caught more species overall and was more suitable for sampling small insects (Gaigher *et al.* 2015), given that most of the insect species sampled were smaller than one centimeter. When choosing the D-vac over sweep net, one however has to consider that the processing time would be more time-consuming as the D-

vac samples contain considerably more plant debris and insects than sweep net. Despite this disadvantage, more species will be sampled, especially members of Coleoptera and Hemiptera (see Appendix B). Sweep net sampling in turn will sample more strong flying insects such as hymenopterans, but many could also be sampled with pan traps. These combined three methods should then be implemented in a sampling period including three seasons: winter, spring and anytime between the summer and autumn months (i.e. December – May), but preferably when maximum temperatures start to decline, i.e. the start of autumn. For this study, summer and autumn temperatures and rainfall were similar being hot and dry throughout this period. However if the sampling is done when temperatures start to decline, it would be equally representative of summer and autumn.

Using additional sampling methods (such as wing-type sticky traps, Malaise traps (Campbell & Hanula 2007; Hagstrum 2000; Tedeschi *et al.* 2003) would not necessarily imply sampling more insect orders, but based on the results it could be expected to sample more insect families (see Table 6). In addition, because sampling methods were only used throughout the day, sampling at night using a light trap could be considered to include nocturnal species (Epsky *et al.* 2008). Pitfall traps and pan traps were in the field for five consecutive days during each sampling occasion, which could have sampled some nocturnal species. However, although a specific night trapping method could possibly have sampled more insect species, night trapping was not within the scope of this study.

Conclusion

The sampling methods used in the West Coast Renosterveld did not have much of an effect on overall insect species richness or abundance. In addition, seasons are an important driving factor of insect communities in the remaining renosterveld areas. Seasons enhance variation in temperature, rainfall and insect phenology, and seasonal variation influences vegetation structure and composition and subsequently affects insect communities. Therefore, the variation in species richness and abundance sampled can be explained by the effects of seasons on insect communities. Due to controversy regarding the richness of insect fauna of the fynbos and Fynbos biome (Gilliomée 2003; Johnson 1992), more studies on insect diversity are required. This study is the first to document the insect diversity of renosterveld and link it to sampling method and season, indicating there are many different species representing various insect families found in renosterveld. Given that a small percentage of this vegetation type is remaining and that it is also highly fragmented, it is important to understand the insect diversity and ecology of this severely

threatened ecosystem. Ongoing documentation of the insect assemblages within renosterveld, with the application of adequate sampling strategies, is thus important. Despite the fact that renosterveld occurs mostly as small fragments, this vegetation appears to be rich in insect fauna. Most of the insects found in it, are very small and hidden within the foliage and are therefore unlikely to be casually observed. In an agricultural landscape, it is expected that overall insect diversity will be less in more isolated patches than patches with a low degree of disturbance (Fahrig & Jonsen 1998; Tschardtke *et al.* 2002). Systematic sampling over different seasons using complimentary methods will be required to quantify renosterveld diversity adequately. In addition, it is recommended that future insect diversity research should also include plant diversity, to examine insect functionality in renosterveld. Renosterveld and fynbos insect diversity studies should be compared to identify possible endemic insect species of renosterveld.

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CHAPTER 3

Insect diversity in West Coast Renosterveld: assessing species richness and evenness, and variation in community structure by calculating diversity metrics.

Introduction

Patterns in alpha diversity have been a major theme in the ecological literature, whereas beta diversity has received less systematic study (Blackburn & Gaston 1996; Harrison *et al.* 1992; Soininen *et al.* 2007). However, the start of the 21st century has witnessed an exceptional increase in studies under the theme of beta diversity (Anderson *et al.* 2011). This topic has become a well-known concept in community ecology, mainly focusing on what drives assemblages of species more or less equal to one another at different places and times (Vellend 2010). Beta diversity is generally defined as the variation in the presence of species among sites, and provides a direct link between biodiversity at local scales (α diversity) and the broader regional species pool (gamma diversity) (Anderson *et al.* 2006; Jost 2007; Legendre *et al.* 2005; Whittaker 1956). Beta diversity was initially introduced and defined by Whittaker (1960) as ‘the extent of change in community composition’ among sites (Baselga 2010; Legendre *et al.* 2005; Tuomisto 2010a), or “degree of community differentiation, in relation to a complex-gradient of environment, or a pattern of environments” (Tuomisto 2010a).

Ecological interest in beta diversity increased ever since the recognition of its essential role in identifying and understanding processes which drive diversity patterns at multiple scales (regional vs. local control of diversity), and underpins the meta-community concept (Heino 2013; Leibold *et al.* 2004; Ricklefs 1987). Wilson & Shmida (1984) emphasize the importance of beta diversity in indicating the extent to which habitats are utilized by species. Species-turnover can be expected to vary among different taxa. Sedentariness and habitat specificity have been referred to as the biological correlates of high beta diversity. To date, most studies have only focused on turnover of a single taxon across one or more gradients. The best outcome for beta diversity would be to compare the responses of different taxa to the same gradient of distance or environmental change (Harrison *et al.* 1992).

With this increase in interest of beta diversity in the ecological literature, many different measures of beta diversity have been proposed (see Jost 2007; Jurasinski *et al.* 2009; Koleff *et al.* 2003a; Tuomisto 2010a,b; Vellend 2001), but there is no consensus as to which ones are most adequate

for addressing particular ecological questions (Anderson *et al.* 2011). Consequently, the use of different measures and analytical approaches on a single data set can naturally result in different outcomes and interpretations (Smith & Lundholm 2010). In comparison, alpha diversity is frequently measured by species richness and evenness, using Shannon Wiener's index of diversity and E_{var} as an evenness index (Jost 2007; Legendre *et al.* 2005; Smith & Wilson 1996).

However, the multiplicative definition of beta diversity became entrenched in ecology and remains so today (Brown & Lomolino 1998, Gaston & Blackburn 2000; Haydon *et al.* 1993; Schluter & Ricklefs 1993).

For presence-absence data, Wilson & Shmida (1984) identified two β -measures: β_T (introduced by the authors) and β_W (Whittaker's (1960) original measure) (Vellend 2001). The authors concluded that the latter is best to use when samples cannot be arranged in a single gradient (Wilson & Shmida 1984). Tuomisto (2010a) also supports the assumption that this measurement is one of the most popular definitions of 'beta-diversity' in ecology when applied to presence-absence data (Harrison *et al.* 1992; Koleff *et al.* 2003a; Wiersma & Urban 2005; Anderson *et al.* 2006).

Jost (2007) has defined a new measure of beta diversity that also includes relative abundance data: $\beta_{Shannon} = H_V/H_\alpha$. This measure shares the property with β_W of being multiplicative (Anderson *et al.* 2011). As β_W is adequate for focussing on species alone (rather than individuals) which are often the units of interest in conservation and biodiversity studies, $\beta_{Shannon}$ (abundance information) is an important aspect of community structure (Anderson *et al.* 2011). To indicate a change in community structure, using relative abundance information, from one locality to another, β would be measured by a dissimilarity measure, including: Jaccard, Bray-Curtis, Gower, and chi squared (Anderson *et al.* 2006; Anderson *et al.* 2011; Chao *et al.* 2005;; Clarke *et al.* 2006). Bray-Curtis dissimilarities are one of the most popular abundance-based metrics (Clarke *et al.* 2006; Ellingsen 2001).

Beta diversity has direct application to questions of reserve design, fragmentation over the landscape, and the estimation of global diversity (Harrison *et al.* 1992; Hewitt *et al.* 2005; Wilson & Shmida 1984). Most beta diversity research has been done on large organisms and terrestrial ecosystems (Condit *et al.* 2002; Koleff *et al.* 2003b; Lennon *et al.* 2001; Witman *et al.* 2004). An increase in environmental heterogeneity will result in an increase in beta diversity, as the latter is positively associated with more fragmented and complex landscapes which show higher spatial variability in their biota (Nekola & White 1999). In contrast, while high dispersal ability increases

the species' capability to maintain viable populations in fragmented landscapes (Clobert *et al.* 2001), at the same time it reduces variation between sites, and therefore it can have a negative relationship with beta diversity (Sioninen *et al.* 2007).

West Coast Renosterveld is highly fragmented and exists as numerous patches within the landscape (see Chapter 1), thus alpha and beta diversities can reflect both local species diversity (i.e. diversity of a single patch) and determine which renosterveld areas best reflect the total diversity of the whole study area (Anderson *et al.* 2006; Jost 2007; Legendre *et al.* 2005). With all the different β -measures in the literature, redundancy exists with regard to the selection of the adequate measure for the specific outcome (Barwell *et al.* 2015). Given that beta diversity is still new to the ecological literature and a lack of research of beta diversity exists for insect communities, the aims of this chapter were to 1) determine species diversity of each area by calculating species richness and evenness indices; 2) test a β -measure specifically linked to abundance data, to determine whether this measure is suitable for insect communities; and 3) calculate the well-known Whittaker's β (β_w), to test the effect of focusing on species identity, when describing differences between renosterveld areas.

Materials and methods

Study site

The same sampling areas that were used to test sampling methods in Chapter 2 (see Chapter 2; Appendix B), were selected for this study (Table 11 & Figure 18) to calculate the regional diversity (γ), local diversity (α), and the change in insect community structure (β diversity).

Table 11 - Renosterveld areas with their properties.

Renosterveld area	GPS coordinates	Surface area (ha)	Altitude (m.a.s.l.)
Papegaaiberg	33°55'13.96" S 18°50'40.15" E	130	224
Koopmanskloof	33°54'13.29" S 18°46'14.02" E	98	366
J.N. Briers-Louw	33°45'48.22" S 18°50'02.81" E	29	140
Spier	33°58'56.31" S 18°48'28.35" E	100	99

Fieldwork and laboratory work

Please refer to Chapter 2 materials and methods section for a detailed description of how field sampling was conducted during which times, as well as the processing work of morphospecies and the classification thereof (see pp. 28-37).

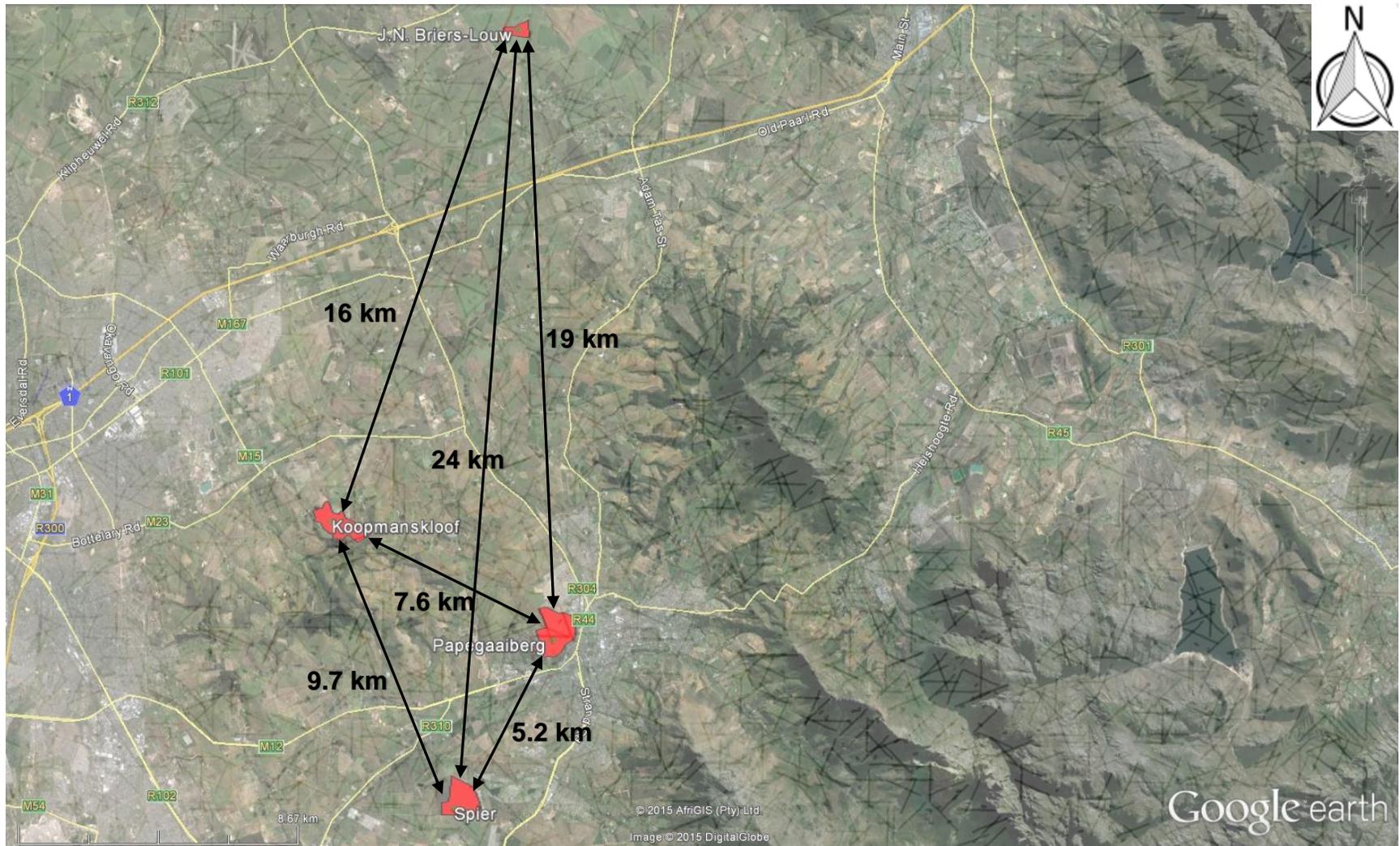


Figure 18 - The four renosterveld areas with the distances between them.

Data analyses

Sampling effort of the four areas was tested by comparing the total species to the two non-parametric, abundance-based estimators, Chao1 and ACE, using the software program, EstimateS v.9.1.0 (Colwell 2013; Samways *et al.* 2010). Species rarefaction curves for the four areas were constructed to illustrate the sampling effort (Gotelli & Colwell 2001). Shared species between the areas were also calculated with EstimateS.

Species diversity of a given area is determined by two components, namely species richness and evenness (Samways *et al.* 2010; Southwood & Henderson 2000). Species richness can be calculated by using species richness indices, such as the Shannon-Wiener index, which considers the total species with the abundances per species (Legendre *et al.* 2005; Veech *et al.* 2002). Species richness of the four renosterveld areas was calculated using this index, using the following formula:

$$\text{Shannon } H' = -\sum_{i=1}^S \sum p_i \ln(p_i)$$

where p_i is the proportion of individuals in the i th species (or n_i/N) (Jost 2007; Samways *et al.* 2010).

Evenness measures the abundance of each species in a community relative to the other species (i.e. the degree of equal abundances between species in a community) (Chao *et al.* 2006; Samways *et al.* 2010). Many evenness indices have been proposed and thus the choice of a suitable index became problematic (Smith & Wilson 1996). However, E_{var} is recommended as the best evenness index for general use as it is independent of species richness (Smith & Wilson 1996). The following formula was used to calculate evenness:

$$E_{var} = 1 - \frac{2}{\pi} \arctan \left\{ \frac{\sum_{s=1}^S \left(\ln(x_s) - \sum_{t=1}^S \ln(x_t)/S \right)^2}{S} \right\}$$

where X_s and X_t are the abundances of the species s and t , respectively; S is the total number of species in each area; arctan provides an angle in radians (Weiher & Keddy 1999; Laird *et al.* 2003).

Species richness and evenness were illustrated using rank-abundance curves for each area to assess which area is most diverse. The abundance of each species was log-transformed as it delivers better graphs as only the proportions are used (Southwood & Henderson 2000).

As beta diversity is the ratio of the total species richness in a region (regional species richness, RSR) to species richness of a locality (local species richness, LSR) (Soininen *et al.* 2007; Anderson *et al.* 2011), gamma diversity was determined first using the proposed measure by

Jost (2007) and Anderson *et al.* (2011): $\beta_{\text{Shannon}} = H_{\gamma}/H_{\alpha}$ where $H_{\gamma} = \exp(H'_{\text{pooled}})$ is an exponentiated Shannon-Wiener's index (i.e. effective diversity) for the γ -level sample unit, obtained by pooling the abundances for each species across all α -level sample units. $H_{\alpha} = \exp(\sum_{i=1}^N H'_i/N)$ is the exponential of the average of the indices calculated for each α -level sample unit (Anderson *et al.* 2011). This measure of beta diversity describes how many more species are present in the whole area rather than at an average site, and uses this value as the measure of beta diversity (Legendre *et al.* 2005).

Whittaker's multiplicative β -measure (β_w) was also calculated on species identities. The β -values refer to the times at which species richness in a region is greater than the average richness in the smaller-scale units (Anderson *et al.* 2011). Gamma (γ) is the total number of species found in the region (i.e. for all four renosterveld areas), α_i is the number of species per renosterveld area, $\bar{\alpha} = \sum_{i=1}^N \alpha_i/N$ is the average number of species obtained from N number of renosterveld areas (Anderson *et al.* 2011). A cluster analysis on presence/absence data was performed to compare insect communities of these renosterveld patches, and a dendrogram was used to illustrate similarities between the communities (Magurran 2004).

Analyses of species richness and abundance data were performed with the statistical software program Statistica Release 7 (Statsoft™). Species richness and abundance data were tested separately for normality using the Shapiro-Wilks W test at a 5% significance level. The Kruskal-Wallis test was used for the data which were not normally distributed.

The PERMANOVA is a multivariate extension of the traditional ANOVA, and tests for significant differences between two or more groups, based on any distance measure (Anderson 2001, McArdle & Anderson 2001). The PERMANOVA calculates an F-value by dividing the variances of all distances between observations that do not occur in the same group by the variances of all distances between observations that occur in the same group. The rows and columns are shuffled (permutations) of the corresponding dissimilarity matrix and a P-value, $P(\text{perm})$, is generated (Ricotta & Burrascano 2008). The Bray-Curtis index is adequate for equal-sampling situations (Chao *et al.* 2006). As equal amounts of sampling were done in all four areas, this dissimilarity index is suitable for the analyses of the data. The only disadvantage of this index is that it is unavoidably strongly affected by a few dominant species (i.e. species with highest abundances) and therefore ignores the effect of rare species (Chao *et al.* 2006).

Thus, the PERMANOVA was used to test significant differences between the sampling areas in terms of community composition. A Principal Coordinates Analysis (PCoA) plot was constructed to illustrate the differences between the sampling areas. Differences within sampling areas (i.e. sampling sites) were tested using the PERMANOVA test with additional

pairwise tests. A Canonical analysis of principal coordinates (CAP) based on Bray-Curtis similarity was performed to illustrate the PERMANOVA results for site differences.

Results

Community composition

Coleoptera, Diptera and Hymenoptera represented the most families and species in all four sampling areas (Table 12). There were no considerable differences in terms of orders and families between the four sampling areas, except when comparing Briers-Louw with Spier in terms of families. Spier also had the least species compared to the other areas. Some orders were only represented by one or a few families and species, which indicate that these orders are not very abundant, and that species which represent these orders are sampled occasionally (Table 12).

Many species rich families were shared between renosterveld areas (Figure 19), which indicate that these families represent species typically found in the West Coast Renosterveld. Chrysomelidae was the most species rich family in all the areas, except for Briers-Louw. However, the total species in Chrysomelidae was similar for Briers-Louw and Spier. Cicadellidae and Formicidae were also species rich families in all four areas. The ten most species rich families of Papegaaiberg, Koopmanskloof, Briers-Louw, and Spier comprised 33%, 30%, 35%, and 36% of the total species found per area, respectively. Therefore, these families contain a great fraction of the total species found in renosterveld, suggesting that these families will almost certainly be present when sampling in West Coast Renosterveld (Figure 19).

Table 12 – Total orders, families and species count for all four sampling areas. Fam. and Spp. denotes number of families and species respectively.

Order	<u>Renosterveld sampling areas</u>							
	Papegaaiberg		Koopmanskloof		J.N. Briers-Louw		Spier	
	Fam.	Spp.	Fam.	Spp.	Fam.	Spp.	Fam.	Spp.
Archaeognatha	-	-	1	1	1	1	-	-
Blattodea	3	9	2	4	1	4	2	4
Coleoptera	21	107	17	70	24	103	19	78
Dermaptera	2	4	1	2	2	5	2	3
Diptera	26	77	26	70	21	61	21	71
Ephemeroptera	1	1	-	-	-	-	-	-
Hemiptera	13	68	14	56	12	56	13	40
Hymenoptera	19	62	19	57	22	68	21	62
Isoptera	1	1	2	2	1	1	1	1
Lepidoptera	5	8	6	8	7	12	3	3
Mantodea	2	2	1	2	2	7	1	1
Mecoptera	-	-	-	-	1	1	-	-
Odonata	1	1	-	-	-	-	1	3
Orthoptera	5	24	7	31	9	46	5	18
Phasmatodea	1	3	-	-	1	2	1	1
Psocoptera	-	-	1	2	-	-	1	1
Thysanoptera	1	1	2	2	2	2	-	-
Total orders	14		13		14		13	
Total families	101		99		106		91	
Total species	368		307		369		286	

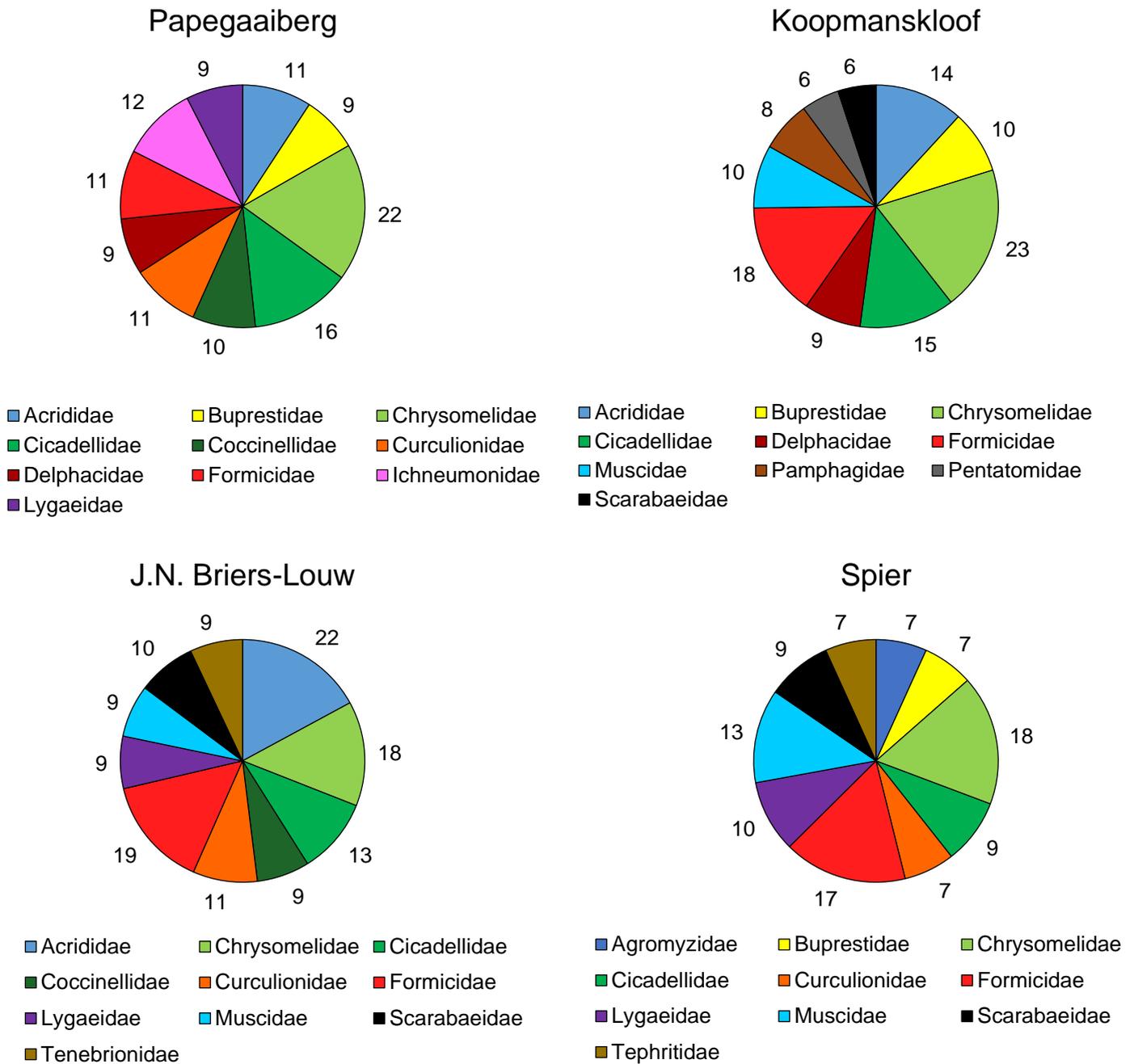


Figure 19 – Pie charts of the 10 most species rich families found in each renosterveld area. The numbers indicate the total species per family.

Sampling effort and shared species

According to the estimators (Chao1 and ACE), the sampling effort was not optimal as many more species are expected at each of the four renosterveld areas (Table 13). The species rarefaction curves also show that none of the four sampling areas reached an asymptote over a sample period including four seasons (Figure 20).

Table 13 – The total number of species sampled per renosterveld area compared to two different non-parametric, abundance-based estimators (Chao1 and ACE).

Renosterveld area	No. species sampled	Chao1 (mean±SD)	ACE (mean)
Papegaaiberg	368	736.7 ± 81.8	671.8
Koopmanskloof	307	476.8 ± 41.4	481.6
J.N. Briers-Louw	369	495.0 ± 29.7	511.8
Spier	286	457.1 ± 43.6	444.3

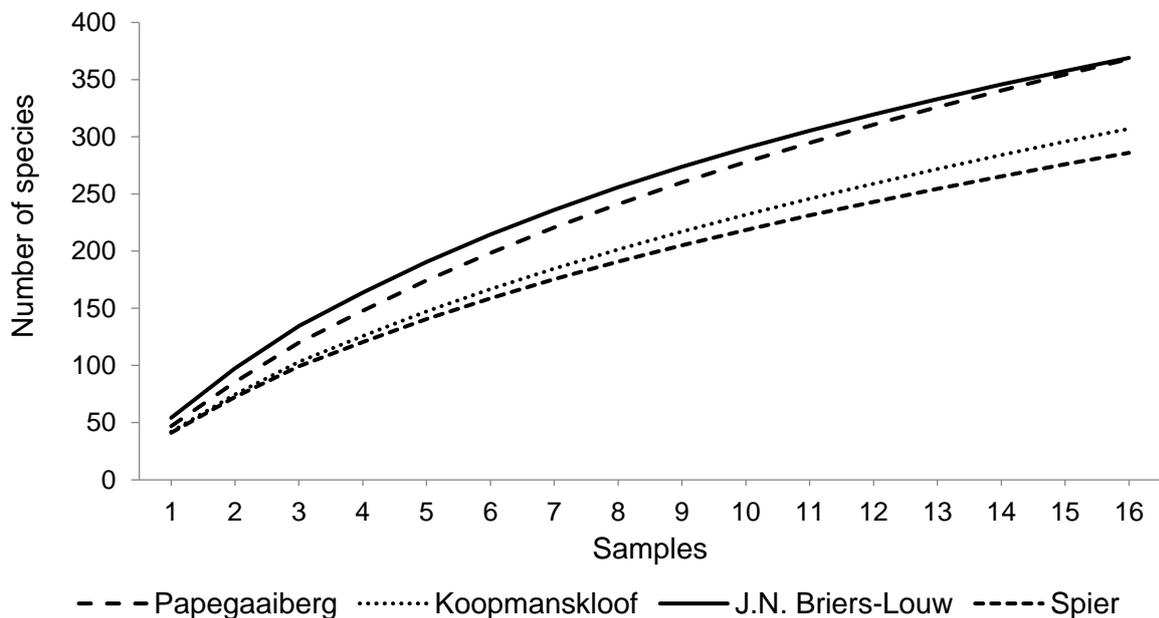


Figure 20 – Species rarefaction curves of the four renosterveld areas. The samples refer to four sampling sites per area over a course of four seasons (N = 16).

Only about a fifth of species between any two renosterveld areas was shared (Table 14), which indicates that each renosteveld area has its own unique suite of insect species. Seasonal variation was also found when looking at the shared species between the seasons (see Appendix D).

Table 14 – The total number of species sampled for each pairwise combination of sampled areas with percentage of shared species between areas

Renosterveld area	No. of species	No. of shared species	% species shared
Papegaaiberg and Koopmanskloof	675	134	19.85
Papegaaiberg and J.N. Briers-Louw	737	121	16.42
Papegaaiberg and Spier	654	112	17.13
Koopmanskloof and J.N. Briers-Louw	676	116	17.16
Koopmanskloof and Spier	593	107	18.04
J.N. Briers-Louw and Spier	655	127	19.39

Calculated indices

Briers-Louw had the highest insect diversity and had the lowest evenness value (Table 15 & Figure 21). Spier had the lowest insect diversity, followed by Koopmanskloof and Papegaaiberg. Papegaaiberg, Koopmanskloof and Spier were very similar in terms of the Shannon-Wiener's index. Koopmanskloof and Spier had similar evenness values. Spier also had the lowest diversity as the slope of the rank-abundance curve is steeper than the other curves (Figure 21).

Table 15 – Species richness and evenness indices calculated for each renosterveld area.

Renosterveld area	Shannon-Wiener's Index (H')	Evenness (E_{var})
Papegaaiberg	3.85	0.294
Koopmanskloof	3.80	0.281
J.N. Briers-Louw	4.28	0.277
Spier	3.72	0.281

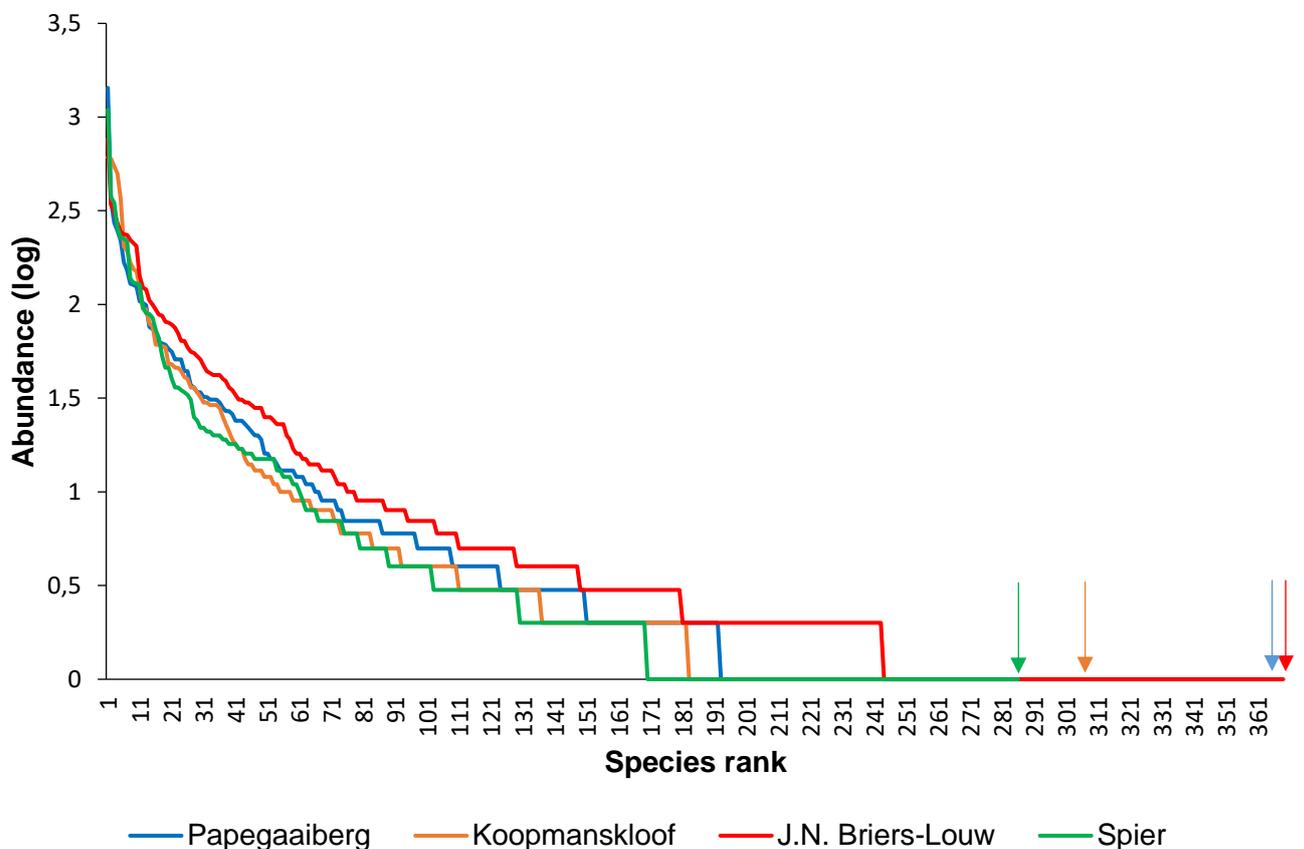


Figure 21 – Rank-abundance curves for the four areas, illustrating species richness and evenness. Abundance per species is log-transformed. As the areas overlap on the zero line (Y axis), the arrows indicate where each area ends on the zero line.

Variations in community composition

Overall, no significant differences were found for species richness between the sampling areas (Kruskal-Wallis: $H = 4.150$; $X_3^2 = 2.190$; $p = 0.246$) (Figure 22). Also, no significant differences were found for abundances between the four sampling areas (Kruskal-Wallis: $H = 1.932$; $X_3^2 = 2.000$; $p = 0.587$) (Figure 23), indicating that all areas are more or less equal in insect numbers, and that the E_{var} values are not statistically significant.

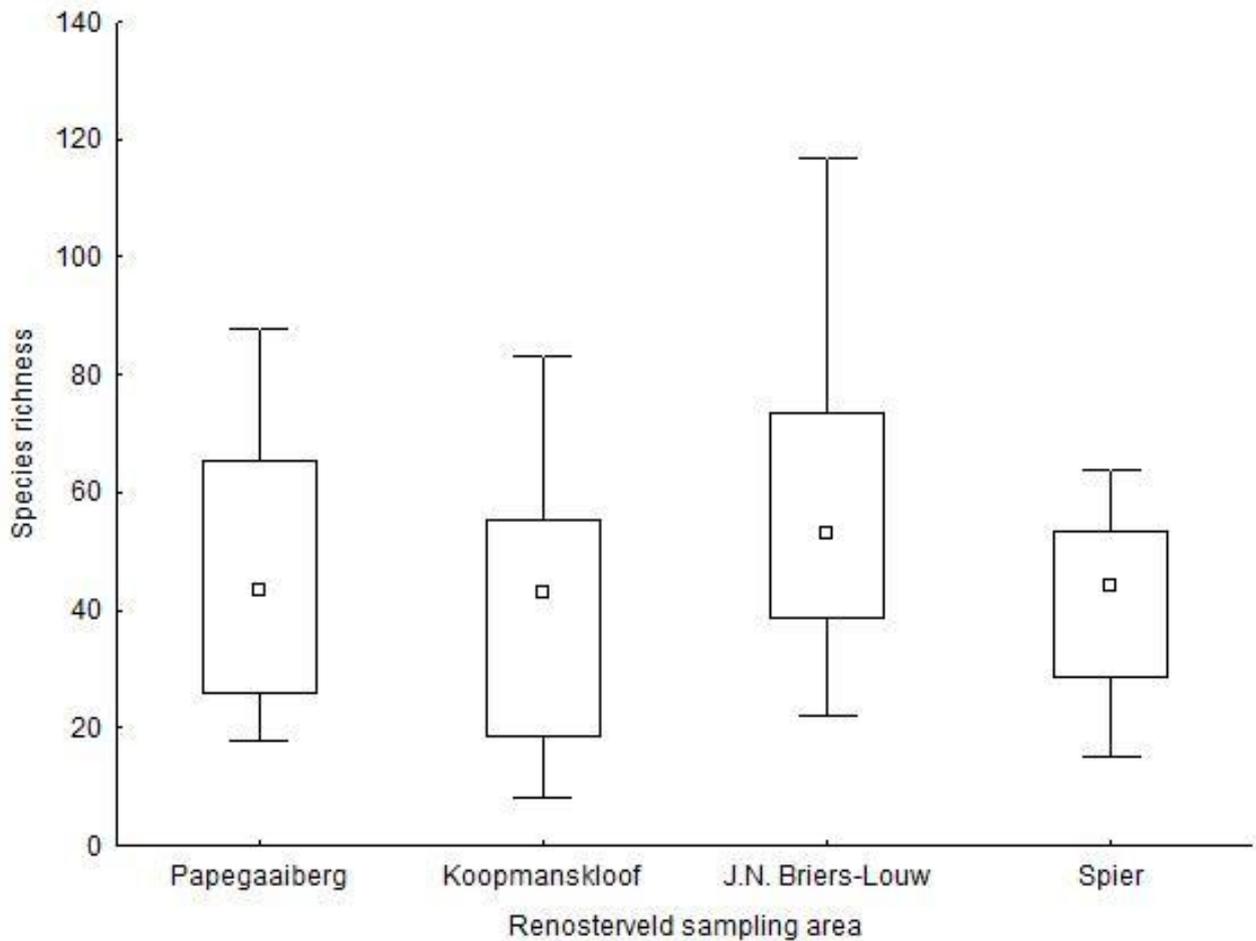


Figure 22 - Variation in species richness between sampling areas. The squares of the boxes denote the median; the boxes denote the 25% and 75% percentiles; the whiskers denote the min and max.

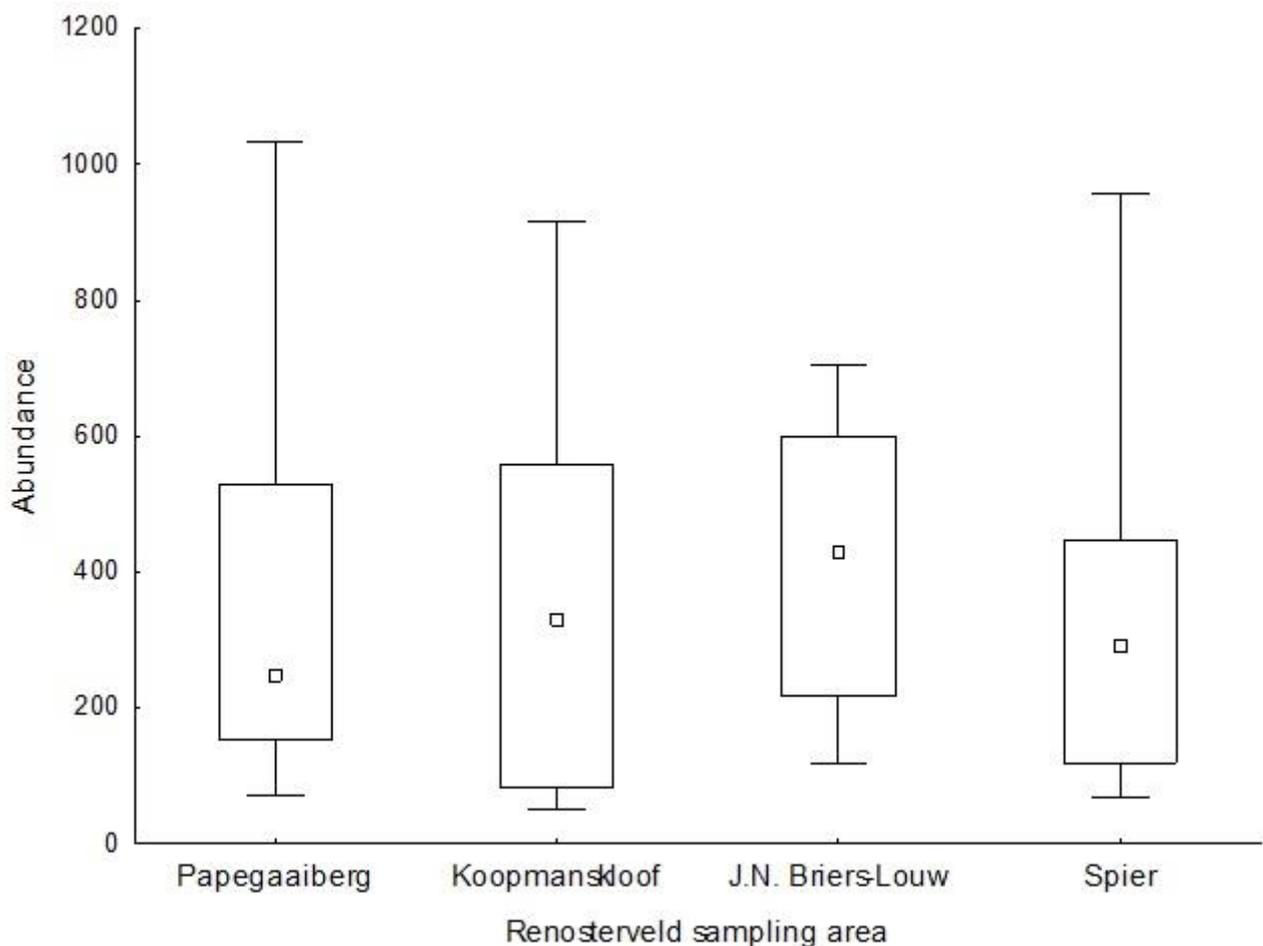


Figure 23 - Variation in species abundance between sampling areas. The squares of the boxes denote the median; the boxes denote the 25% and 75% percentiles; the whiskers denote the min and max.

Calculated diversity metrics

Both β -measures showed that the smallest three β -values were found between Briers-Louw and the other areas, meaning that any combination with Briers-Louw yields a greater diversity representing the whole region, compared to any other β -diversity between any two of the study areas. Thus, the combined Briers-Louw and Papegaaiberg areas were most species rich, showing the smallest β -values for both indices. However, there were differences between the β_{Shannon} and β_{W} values (Tables 16 & 17).

Table 16 – Calculated Shannon-Wiener's β -measure (H_β) between renosterveld areas, as well as the pooled Shannon-Wiener (H') obtained from all the areas combined. Gamma diversity was calculated by the exponential of the pooled H' . Averages between sampling areas (H/N), where N indicates the number of sample areas, were calculated in order to get the exponential of the average of the indices calculated for each α -level sample unit (H_α) which is the exponential of H/N . Beta diversities (H_β) between areas were then calculated by H_γ/H_α . PB = Papegaaiberg, KM = Koopmanskloof, BL = J.N. Briers-Louw, SP = Spier.

Sampling areas	Shannon-Wiener (H')	Between areas	Average of the indices (H/N)	$H_\alpha = \exp(H/N)$	$H_\beta = H_\gamma/H_\alpha$
Papegaaiberg	3.865	PB & KM	3.831	46.10	2.01
Koopmanskloof	3.797	PB & BL	4.074	58.80	1.58
J.N. Briers-Louw	4.284	PB & SP	3.793	44.40	2.09
Spier	3.722	KM & BL	4.040	56.84	1.63
H' pooled	4.530	KM & SP	3.759	42.92	2.16
$H_{\gamma \exp}(H' \text{ pooled})$	92.780	BL & SP	4.003	54.75	1.69

Table 17 – Calculated Whittaker's β -measure (β_w) on presence/absence data (species identities) for the four renosterveld areas. PB = Papegaaiberg, KM = Koopmanskloof, BL = J.N. Briers-Louw, SP = Spier.

Sampling areas	Number of species (α_i)	Between areas	Average number of species between areas ($\bar{\alpha}$)	Whittaker β measure ($\beta_w = \gamma/\bar{\alpha}$)
Papegaaiberg	368	PB & KM	337.5	2.52
Koopmanskloof	307	PB & BL	368.5	2.31
J.N. Briers-Louw	369	PB & SP	327.0	2.61
Spier	286	KM & BL	338.0	2.52
Total number of species in the region (γ)	852	KM & SP	296.5	2.87
		BL & SP	327.5	2.61

The PERMANOVA showed significant difference in community composition between the four renosterveld areas. According to the PERMANOVA pairwise tests, Papegaaiberg and Koopmanskloof seemed to differ less significantly from each other as the t-value is the smallest (Table 18). The PCoA plot also illustrated this as two sampling sites from Papegaaiberg (i.e. sites A & B) are closely clustered with the sampling sites of Koopmanskloof (Figure 24). Overall differences in community composition are found within all four sampling areas (Table 19).

Table 18 – PERMANOVA results showing significant differences of combined species richness and abundance data between the sampling areas. Pairwise tests between sampling areas also show significant differences.

Source of variation	df	SS	MS	Pseudo-F	P(Perm)
Sampling area	3	17618	5872.6	4.647	0.0001
Res	12	15166	1263.8		
Total	15	32783			

Pairwise tests	t	P(perm)
Papegaaiberg, Koopmanskloof	1.882	0.03
Papegaaiberg, J.N. Briers-Louw	2.318	0.03
Papegaaiberg, Spier	2.055	0.03
Koopmanskloof, J.N. Briers-Louw	2.325	0.03
Koopmanskloof, Spier	2.107	0.03
J.N. Briers-Louw, Spier	2.299	0.03

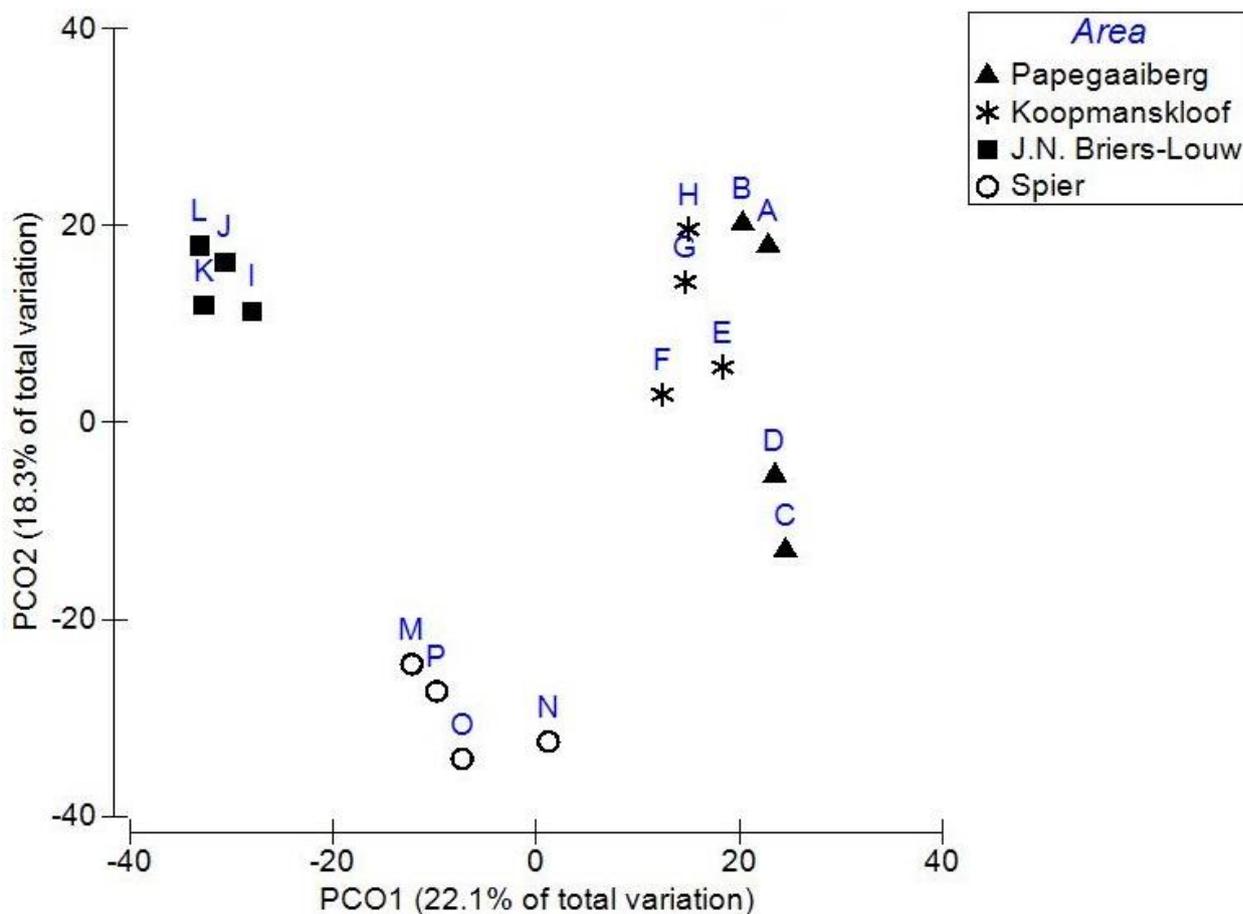


Figure 24 – Principal coordinates analysis (PcoA) ordination plot using the Bray-Curtis similarity matrix for the community composition data of the sampling areas. All the sampling sites are denoted by A-P, of the different sampling areas. Numbers in brackets are the percentage of variance associated with each principal coordinate axis.

Table 19 – PERMANOVA results showing significant differences in community composition data within sampling areas (i.e. differences between sampling sites).

Source of variation	df	SS	MS	Pseudo-F	P(Perm)
Sampling sites	15	59820	3988.0	1.1632	0.0456
Res	48	164559	3428.4		
Total	63	32783			

Furthermore, the dendrogram also illustrates that the community composition differs between all four sampling areas, with all sites from the same area clustering together (Figure 25). Briers-Louw was the only area with no significant differences in community composition between any sites, and Spier had only two sites significantly different from each other. In contrast most sites were significantly different from one another for both Papegaaiberg and Koopmanskloof (Table 20). The CAP analysis also illustrates that Briers-Louw is the only area which clustered best, showing no significant differences between sampling sites. For the other remaining areas, sites are more scattered in the ordination plot (Figure 26).

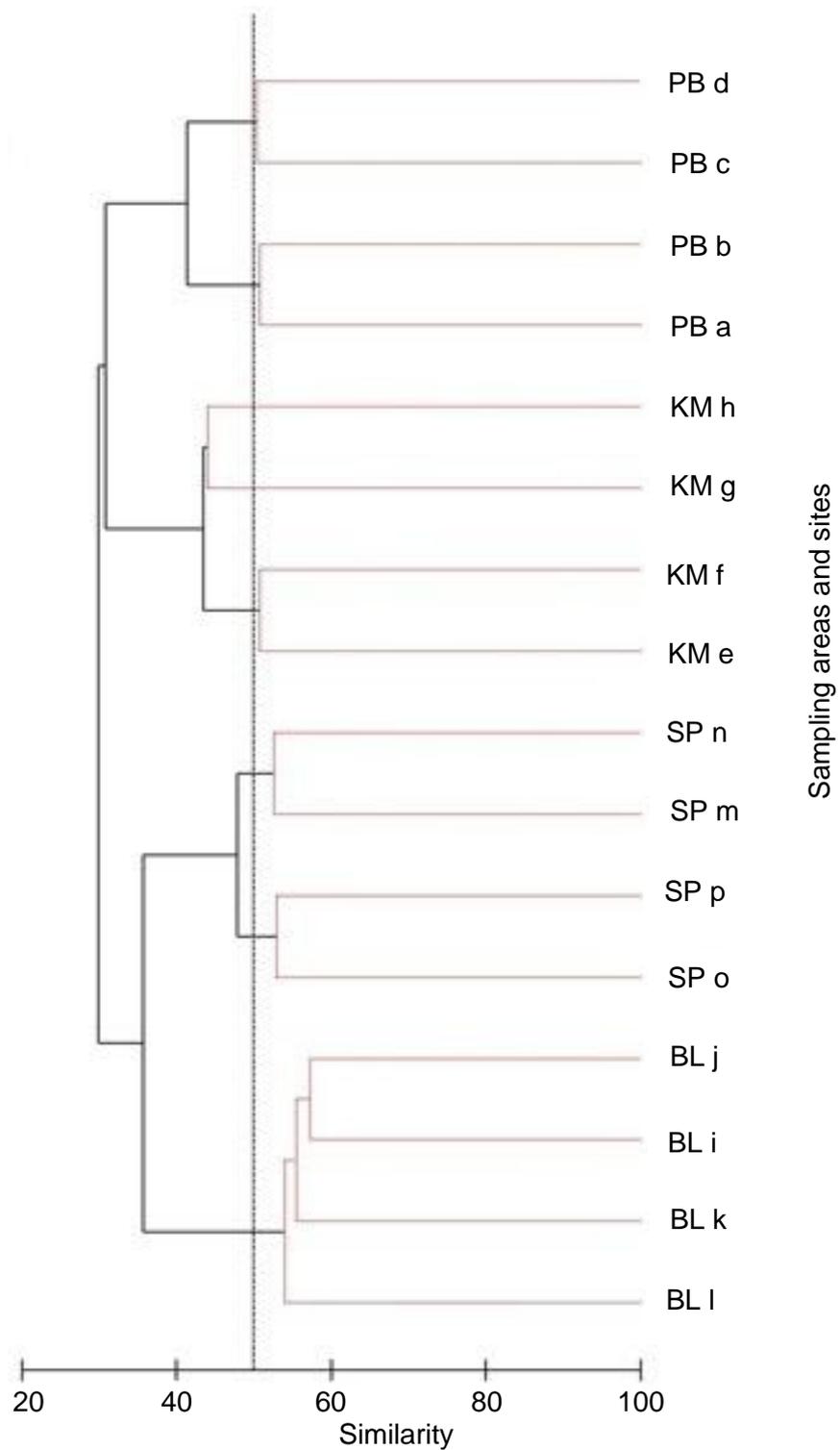


Figure 25 – Dendrogram based on Bray-Curtis similarity of presence/absence data showing similarities and dissimilarities between sampling sites (a - p) of the four renosterveld areas. The vertical dot line denotes 50% similarity. PB = Papegaaiberg, KM = Koopmansklouf, BL = J.N. Briers-Louw, SP = Spier.

Table 20 – Pairwise T-tests showing differences in community composition data between sampling sites of each renosterveld area. The asterisk (*) symbols indicate the statistically significant differences between sites.

Pairwise tests	t	P(perm)
Papegaaiberg sites		
A, B	1.1054	0.3149
A, C	1.5374	0.0013*
A, D	1.3771	0.0390*
B, C	1.7709	0.0008*
B, D	1.5630	0.0019*
C, D	1.3956	0.0630
Koopmanskloof sites		
E, F	1.3006	0.1136
E, G	1.2929	0.0450*
E, H	1.6738	0.0011*
F, G	1.3204	0.0631
F, H	1.7272	0.0009*
G, H	1.4293	0.0377*
J.N. Briers-Louw sites		
I, J	1.1091	0.3610
I, K	1.0311	0.4971
I, L	1.0135	0.5487
J, K	1.0512	0.4350
J, L	0.9650	0.6680
K, L	0.8587	0.7664
Spier sites		
M, N	1.3515	0.0520
M, O	1.2899	0.1100
M, P	0.9356	0.6389
N, O	1.5378	0.0337*
N, P	1.3078	0.0842
O, P	1.2091	0.1784

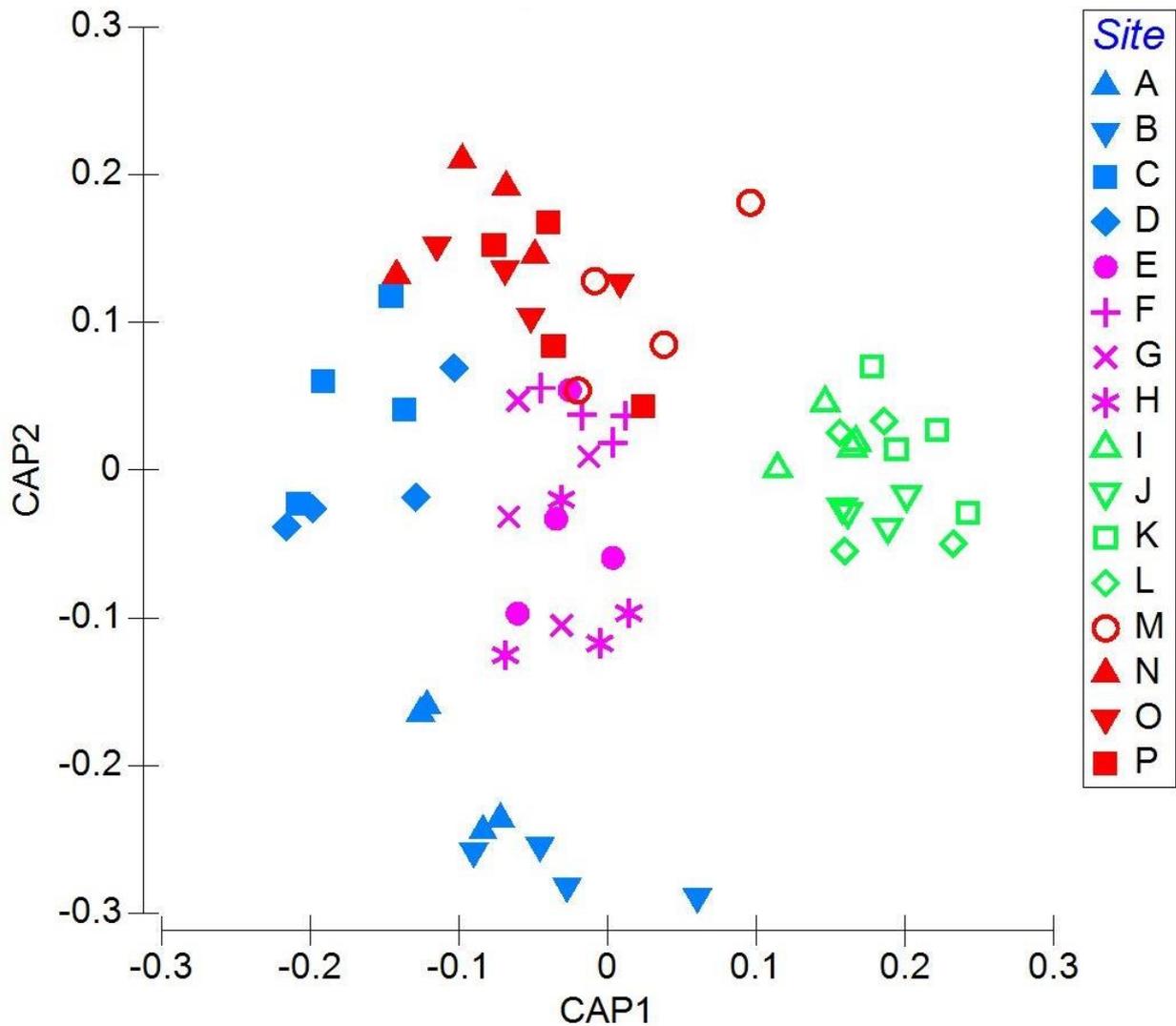


Figure 26 – Canonical Analysis of Principal coordinates (CAP) ordination plot illustrating the differences in community composition data between sampling sites of the four renosterveld areas over the total sampling period. Papegaaiberg (A – D), Koopmanskloof (E – H), J.N. Briers-Louw (I – L), and Spier (M – P) at two dimensions, CAP1 and CAP2.

Discussion

Sampling effort

It is important to note that the species accumulation curves of the four sampled renosterveld areas did not reach asymptotes. Consequently this can have an effect on the variation in communities between the four areas (Samways *et al.* 2010). However, it is commonly found in the literature that these curves do not reach asymptotes, despite the sampling intensity (Gotelli & Colwell 2001). Briers-Louw and Papegaaiberg sampled the most species, Koopmanskloof was intermediate and Spier had the least species. Also, Papegaaiberg combined with Briers-Louw delivered the most species relative to the total sampled species (86.5%), which exceeds all other combinations of renosterveld areas. A great proportion of the total species sampled are rare species. Considering rare and common species sampled, possible explanations for differences in species numbers between renosterveld areas could be that Briers-Louw is in a more natural state, considering the structure and composition of the vegetation (Personal observation). Papegaaiberg's high species richness could possibly relate to the surrounding gardens of the Onderpapegaaiberg neighbourhood where insects move from the gardens into renosterveld. Koopmanskloof's intermediate insect species richness could be a result of isolation from nearby gardens and the natural state of the vegetation structure and composition. Conversely, the low insect species richness of Spier could be explained by the homogenous state of the vegetation composition and structure, where *Elytropappus rhinocerotis* (renosterbos) and *Seriphium plumosum* (slangbos) are very dense and tall (see Chapter 2: Table 2), and therefore suppress ground cover growth forms. The significant differences in species richness between Briers-Louw and both Koopmanskloof and Spier could thus indicate that the former area's vegetation is in a more heterogenous state. All four renosterveld areas had more or less the same numbers of insect individuals, but the difference in species identity could be the result of Briers-Louw containing rare insect species not found in disturbed renosterveld (i.e. Papegaaiberg has suburban insects, Koopmanskloof is relatively isolated and Spier was overgrown). Nevertheless, the results indicate that Papegaaiberg and Koopmanskloof are relatively similar in insect diversity, which might be explained by various environmental and spatial factors.

The direct influence of environmental variables

Environmental variables and spatial factors (Botes *et al.* 2007; Neves *et al.* 2015) are most probably the reasons for these similarities in insect diversity. Donaldson *et al.* (2002) also concluded that pollinator diversity is more sensitive to habitat characteristics, rather than patch size, as pollinator diversity remained high within patches smaller than one hectare. Fahrig &

Jonsen (1998) investigated the effects of patch size, patch isolation, and level of disturbance on herbivorous insect diversity. They found that the more isolated and least disturbed patches contained a higher diversity of insects, but that patch size did not have a significant effect on the diversity. Donaldson *et al.* (2002) reached similar conclusions in their study. Therefore, the assumption can be made that due to less disturbances, and a more natural vegetation structure, Briers-Louw nature reserve has the highest insect diversity.

The vegetation composition of Spier is considerably more homogenous as dense stands of *Elytropappus rhinocerotis* (renosterbos) and *Seriphium plumosum* (slangbos) are abundant, which could explain the lower insect diversity relative to the other renosterveld areas. During site visits, more flowering plants were observed during spring at Briers-Louw than the other renosterveld areas, which suggest that this area will attract more pollinators and other flower-visiting insects. Overall this area also appears more heterogeneous (Personal observation). In addition, the 'habitat heterogeneity hypothesis' assumes that structurally more complex habitats provide more niches and enhances diversity (Martin & Ferrer 2015; Tews *et al.* 2004). This reserve is also more isolated as it is surrounded by only grazing fields. Therefore, the dispersal ability of many insect assemblages, especially ground-dwelling insects, will be limited.

The other three renosterveld areas are surrounded by vineyards and organic fields, which can provide shelter and food for insects, and in turn enhance movement out of the natural areas. Controversially, when vineyards and farmlands are sprayed with insecticides, they prevent some insects from moving through these lands, forcing them to the field edges and non-crop habitats (Bianchi *et al.* 2006). Therefore, when the surrounded farmlands of the three renosterveld areas are sprayed with insecticides, some insects might persist in the natural veld. Sioninen *et al.* (2007) postulate that an increase in dispersal abilities will result in a decrease of variability between areas. Therefore, it could be expected that Papegaaiberg and Koopmanskloof would be more similar. Two sites of Papegaaiberg are more or less similar to the sites of Koopmanskloof (see Figure 24). The other two sites were more infested with alien invasive plants, which could explain the variation in the PCoA graph. Several studies also substantiate the negative influence of alien invasive plants on insect communities (Bezemer *et al.* 2014; Elleriis *et al.* 2015; Fenesi *et al.* 2015; Fickenscher *et al.* 2014). Alien invasive plants can alter the vegetation composition and structure where most of the native plant community can be replaced (Fickenscher *et al.* 2014). Thus the invasive plants can disrupt trophic interactions in insect communities and affect the performance of herbivorous insects and their natural enemies such as parasitoids and predators. Furthermore, the spatial and temporal dynamics of native insect communities and metapopulations can also be impacted by alien vegetation leading to alterations at the landscape level (Bezemer *et al.* 2014).

The attempts to understand the variation in species richness across the study sites have merit, but one can only postulate on these given the small number of study areas in this study. Only four sampling areas were sampled, where each one is different in veld age, invasion by alien vegetation, patch size, aspect, and slope. The successional adaptations evolved by plants have received extensive studying. However, little is known about the response of insects after fire occurrences (Kaynas & Gurkan 2005). In addition, veld age (i.e. time-since-last-burn) will have an impact on the diversity of insect, as renosterveld is a fire-prone vegetation type (i.e. renosterveld needs to burn in order to rejuvenate the plant diversity) (Rouget *et al.* 2001). Therefore, it could be expected that differences in veld age of the renosterveld areas should affect insect diversity.

The indirect influence of spatial factors

Possible spatial factors that explain the similarity between Papegaaiberg and Koopmanskloof the fact that both these areas are hills. These hills comprise of slopes which can differ from each other in terms of plant composition, where differences in insect diversity can be expected compared to flat terrains (Binz *et al.* 2014). Spier and Briers-Louw are flat areas in the landscape.

Various studies postulate that topography creates complex mosaics of exposure to wind and radiation. In addition, sheltered locations may be buffered from regional climate change (Ashcroft 2010; Ashcroft *et al.* 2009; Hampe & Petit 2005; Williams *et al.* 2008). Sampling was done on West-facing slopes of Papegaaiberg and Koopmanskloof (see Appendix C), and thus the moisture levels and microclimates would likely be similar for both areas.

Assessing the β -measures

The above sections explaining the potential factors influencing the diversity of each sampled area could explain the variation in the calculated β -measures. The values calculated by these two β -measures did not overlapped entirely (compare β values of Tables 16 & 17), which can be a typical result when using multiple β -measures on a single data set (Smith & Lundholm). Considering that both β_{Shannon} and β_{W} have their shortcomings (e.g. both are multiplicative meaning that beta diversity is dependent on alpha diversity) which are explained in the literature (Jost 2007; Wilson & Shmida 1984), it appears that neither of these two β -measures are suitable for this study. Although the multiplicative definition of beta diversity became entrenched in ecology (Gaston & Blackburn 2000; Haydon *et al.* 1993) and that it is still used for β -diversity studies to date, a new β -measure is needed to assess variation in insect communities between areas. It is expected to find an increase in beta diversity with an increase in environmental heterogeneity in fragmented landscapes (Nekola & White 1999).

Therefore, an ideal β -measure for insect communities in fragmented landscapes (including renosterveld) should: 1) be independent of α -diversity; 2) use abundance data (including both common and rare species); 3) considering spatial and temporal variation between areas (i.e. patch size, aspect, veld age, etc). With a more suitable β -measure, questions such as the effect of fragmentation on renosterveld insect communities, and designing renosterveld reserves can be properly assessed when β -diversity can be properly calculated.

Conclusion

It can be assumed that habitat characteristics have an effect on insect diversity in renosterveld, which is altered by factors including disturbance, altitude and topography. This study found that insect diversity differs between West Coast Renosterveld remnants with only about 20% of the species shared between patches. Therefore, it is important to consider the conservation value of each remnant, regardless of the size, such as 30ha of renosterveld patch. Newton and Knight (2010) made two conclusions on the plant diversity of West Coast Renosterveld: it is home to many wide-spread micro-habitat endemics, rather than local endemics. Also, that small fragments are not subpopulations of larger patches, but may contain unique species. Kemper *et al.* (1999) also emphasizes the importance of small renosterveld patches retaining similar community structure as larger patches. This study and Donaldson *et al.* 2002 suggest the same for insect communities (i.e. patch size does not influence species richness). More research needs to be carried out on insect diversity in renosterveld to monitor changes in insect community composition, and identify which factors contribute most significantly towards the variation in insect diversity between patches. Martin and Ferrer (2015) found that habitat spatial heterogeneity increased α -diversity, but did not significantly affect β -diversity among sites. Therefore, disturbed landscapes may show higher spatial variation in habitat and thus a higher α -diversity due to the contribution of highly generalist species that are widely distributed and do not differ in composition (β -diversity) among the different areas within the landscape (Martin & Ferrer 2015). This infers that disturbed renosterveld areas could potentially be monitored by examining β -diversity between the different areas in a specific region. Therefore, in order to monitor the state of renosterveld in a specific region, it is recommended and necessary for a β -measure which is suitable for insect communities in fragmented landscapes. Differences between different patches (α -diversity) within the landscape can then be monitored more thoroughly.

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CHAPTER 4:

Diverse insect fauna of a highly threatened vegetation type: conservation and management implications for the West Coast Renosterveld

General conclusion:

The outcomes of the thesis

There remains an outstanding gap in the literature regarding insect diversity within the Fynbos Biome (Braschler *et al.* 2012; Johnson 1992). Research on insect-based studies within the Fynbos Biome has mostly focused on specific insect taxa or assemblages, as opposed to examining all insect taxa within a specific vegetation type (see Chapter 1). Previous studies on insect diversity within the Fynbos Biome assumed that the Fynbos Biome is poor in insect fauna (Giliomee 2003; Proches & Cowling 2007). Therefore the main objective of this thesis was to fill the gap by providing more information on insect diversity found in a threatened vegetation type within the Fynbos Biome.

The first part of the thesis proposed an adequate sampling strategy, based on the shortcomings of the two compared strategies, for insect sampling in renosterveld. Sampling effort was also determined for each sampling method. Species rarefaction curves remained steep and did not reach asymptotes, illustrating that more insect species are expected to be sampled for each sampling technique with increased sampling effort. The proposed sampling strategy, after comparing two strategies, can be used for future insect documentation work in renosterveld, and more broadly the entire Fynbos Biome. This sampling technique consists of three sampling methods: D-vac, pan traps, and pitfall traps. These methods should be applied over three seasons: winter, spring, in early autumn. The three methods will target different insect taxa and will thus be complementary to one another for insect diversity documentation. The recommended protocol will be more time-efficient, considering there will be only three sampling occasions. This will mean that more time can be spent on processing samples in the laboratory. However, the sampling strategy can be adjusted to address specific research questions or to target certain insect taxa (see Chapter 2). Thus this proposed sampling strategy can be used as a baseline when considering limitations such as time and labour-intensity. Future studies which examine insect diversity should incorporate plant diversity assessments in order to assess correlations between insect and plant communities.

It is also important to assess the influence of other management-related variables such as fire (time-since-last-burn), grazing and alien plant infestations. Larger studies would need to take other factors into account, such as slope, aspects, presence of a watercourse, etc., or at least control for these variables. There is a large suite of variables which can influence both plant and insect diversity in fragmented habitats especially.

The second part of the thesis assess correlations between insect and plant communities plus the influence of veld age and other management-related influences, based on species richness and abundance, in West Coast Renosterveld (considering the methods applied to the corresponding sampling occasions), and compared diversity between four renosterveld patches. The species rarefaction curves for all study areas did not reach asymptotes, indicating that more insect species are expected to be found at each renosterveld patch, with increased sampling effort. Various biotic and abiotic characteristics could explain the differences in insect diversity between the four study areas (see Chapter 3). The purpose of this chapter is to highlight the importance of managing and conserving the West Coast Renosterveld, based on the findings of this thesis; and how this thesis contributes to the conservation of South African insects, especially in threatened vegetation types.

Conservation and management of renosterveld

The Mediterranean-type ecosystems (including the Mediterranean regions of Australia, California, Chile, Mediterranean Basin and South Africa) constitute one of the rarest biomes and they are so extraordinary diverse. These regions have favourable climatic and coastal conditions which make them highly desirable human habitats. Consequently, the rapid population growth will result in continuous shrinking of the Mediterranean biome. Only 43% of this biome falls within protected areas specifically designed for biodiversity protection (IUCN classes I – IV), which is less than half of the accepted global protection goal for ecological systems. Existing protected areas are disproportionately concentrated in “left-over land” (not suitable for economical uses, such as areas of high elevation and steep slopes in the Cape Region of South Africa. South Africa’s Mediterranean Fynbos Biome comprises an area of 96 000+ km² of which ~19% of the area is IUCN (classes I – VI) protected. Another 56% of the area has conservation potential. However, conservation competes poorly with privately-held agriculture and urban areas, such as South Africa’s renosterveld (Cox & Underwood 2011).

Approximately 80% of Coastal Renosterveld has been transformed to agricultural lands due to its nutrient-rich soils (Rouget *et al.* 2003; Shiponeni & Milton 2006). Less than 1% of Coastal Renosterveld is formally conserved in statutory reserves (Winter *et al.* 2007). Therefore, Coastal Renosterveld is one of the most poorly protected veld types in the CFR and the most

transformed habitat type in South Africa (Bergh *et al.* 2014; Winter *et al.* 2007). The major threats to renosterveld conservation include further agricultural development, alien plant invasions, overgrazing, frequent fires, chemical drift from crop spraying and climate change (Bergh *et al.* 2014; Rouget *et al.* 2003; Winter *et al.* 2007). Renosterveld now exists in the form of thousands of fragmented patches in the landscape spread across the lowlands of the Fynbos Biome (Horn *et al.* 2011; Jenkins *et al.* 2013; Kemper *et al.* 2000; Newton & Knight 2010). Given the current condition and the Critically Endangered status of renosterveld (Heelemann *et al.* 2012; Radloff *et al.* 2014), the results of this thesis which highlight insect diversity should also be incorporated in the conservation and management of renosterveld biodiversity.

Most of these fragmented patches are smaller than one hectare in size (Parker & Lomba 2009). Despite the small sizes of these patches, the flora of renosterveld remains extremely diverse and unique. The diversity of geophytes is exceptionally high and almost a third of the plant species are endemic (Cowling 1984; Cowling *et al.* 1986). Even the smallest patches of renosterveld can contain populations of rare and threatened plant species (Donaldson *et al.* 2002), which highlights the importance of conserving renosterveld and proper management thereof.

Two important management factors contributing to the condition of renosterveld includes livestock grazing and fire frequency (Radloff *et al.* 2014). These two components can alter the composition and structure of renosterveld (Kemper *et al.* 1999; Radloff *et al.* 2014). For example, tend to graze mostly on grasses allows the shrub elements to become more dominant. Cattle dung was only found on Briers-Louw reserve, indicating that the livestock may feed occasionally on the grasses within the reserve. Fire on the other hand will prevent the shrubs from dominating over the grasses and geophytes (Curtis 2013; Groenewald 2014; Radloff *et al.* 2014). However, when these two components are mismanaged, it can lead to severe veld deterioration (Curtis 2013; Radloff *et al.* 2014). For example if all livestock gather in a small post-fire area, the result will be a shrubland with no grass. This will lead to intense veld deterioration as shrubs and resprouters will struggle to re-establish in grazing lawns (Curtis *et al.* 2013; Listopad *et al.* 2015; Radloff *et al.* 2014). Curtis (2013) also advises farmers to avoid grazing over winter and spring months, but preferably between late November and late March. Also, controlled burns should be implemented during late summer and early autumn months. A non-grazing period of minimum 18-24 months after fire is also recommended. These three strategies are based on related fynbos management, considering a lack of data specific to renosterveld (Bond *et al.* 2004; Curtis 2013; Kraaij 2010). The strategies are believed to favour palatable grasses and geophytes which usually emerge and flower during the winter and spring months (Curtis 2013).

Conservation authorities have set out strategic management plans for renosterveld conservation (Winter *et al.* 2007). The Cape Action for People and the Environment (C.A.P.E.) program in South Africa has coordinated a suite of complementary conservation interventions since 2001. The main aim of this program is to conserve terrestrial and marine plants, animals, and ecosystems of the CFR while delivering economic benefits. Furthermore, two conservation programs focusing on privately-owned land operate in the Cape Lowlands region: the Conservation Stewardship Program (CapeNature) since 2003, and the Overberg Lowlands Conservation Trust (OLCT). The conclusions of this study supports the findings of previous studies that 'patch size does not matter' and is a reminder to conservation planners that in a system as fragmented and complex as renosterveld, we need to consider the importance of every remnant that remains within this transformed landscape. Antwood *et al.* 2008 also state that promoting arthropod diversity in native vegetation, surrounded by agricultural lands (such as renosterveld), retention of the native vegetation may well be the most effective method of conserving arthropod diversity. Therefore, each renosterveld patch matters in order to conserve the insect diversity. This study also shows that each renosterveld patch is unique in terms of species richness. Only 20% of species is shared between any two sampled areas, indicating that insect communities can strongly vary among patches found in the landscape. Considering the uniqueness and irreplaceability of each renosterveld patch, conserving the remaining remnants is crucial.

The cooperation of farmers and private landowners is crucial for fulfilling biodiversity conservation of renosterveld (Winter *et al.* 2007). Winter *et al.* (2007) point out three factors which will contribute to the success of the private-conservation programs. These include the attitudes of the landowners towards the specific habitat or species requiring conservation; the conservation agency responsible for the area; and the willingness of landowners to participate in such a conservation program. Landowners' attitudes towards the conservation value of their renosterveld are affected by the land use (livestock or crops), climate and topography. These factors all contribute to the economic success the landowners can obtain by optimizing their agricultural activities. However, landowners are requested not to plough through renosterveld, because once it is ploughed, resprouters and geophytes are removed which results in monospecific stands of renosterbos (Midoko-Iponga 2004). Certain renosterveld patches could also act as sources for insect dispersal to nearby patches (Diekötter *et al.* 2008). Thus, if a farmer ploughs up renosterveld, the connectivity to nearby patches can be suppressed, which will limit the dispersal abilities of insects between patches.

Landowners have identified both advantages and disadvantages regarding their renosterveld. Advantages include: source of natural grazing, valuable especially during drought and winter conditions; grazing provides a form of natural medicine for livestock which improves resistance

to diseases; intact veld protects steep slopes from water run-off and thus controls soil erosion; ecosystem services such as soil carbon sequestration which maintain soil fertility; certain plants have human medicinal value; source of cultivated garden plants especially the bulbs; habitat of many animals and birds which allows activities such as bird-watching. Perceived disadvantages of renosterveld on a farmer's land include: renosterbos is costly to keep under control and decreases the grazing value of pasture; unploughed land leads to no generated income; from a farmer's perspective, unploughed natural vegetation is seen as untidy; the wool of sheep is damaged by renosterveld shrubs; caracal live and breed in renosterveld and cause livestock losses and conflict with farmers (Winter *et al.* 2007). Based on these advantages and disadvantages, it is understandable why some farmers are uncertain what to do with their renosterveld piece. Certain farmers are also unsure whether they should burn their piece of renosterveld or not. While fire management can suppress dense stands of renosterbos, the restrictions of burning permits and seasons, the risk of escaping fires, and the complexity of constructing fire breaks to protect fences and agricultural land all need to be considered by the farmers (Curtis 2013). One major advantage for the farmers is that if their patches are put into stewardship with conservation agencies, and proclaimed as nature reserves, the farmers can get tax rebates (Antoinette Veldtman, personal communication, July 5, 2016).

The importance of insect conservation

In general, insects are essential to terrestrial ecosystems as they provide many ecosystem functions. These functions include nutrient cycling (via decomposing leaf-litter, dispersal of fungi, disposal of carrion and dung, and soil turnover), plant propagation (pollination and seed dispersal), maintaining plant community composition and structure (herbivorous insects), food for insectivores (birds, reptiles, mammals, amphibians, and fish), and maintaining animal community structure (transmission of diseases to large animals, and predation and parasitism of smaller animals). Furthermore, many insect species are keystone species, meaning with the loss of these species (e.g. a predator of an insect herbivore), a population explosion of certain insect species (e.g. insect herbivores) could result in a decrease in overall insect diversity (Gullan & Cranston 2004). These numerous functions indicate the critical role of insects in our ecosystems. Future research on ecosystem processes is important such as pollination webs (especially at community level), which is barely known for fynbos systems (Stanway 2009). Pauw (2007) found that the oil-collecting bee *Rediviva peringueyi* was absent in small conservation areas in an urban matrix within the CFR. The absence of this bee species resulted in failure of seed set in six specialist plant species that are pollinated only by *R. peringueyi*. Generalist pollinators are essential to maintain the structure of pollination webs.

Furthermore, generalist pollinators are also important to maintain plant diversity (Pauw 2007; Stanway 2009). Renosterveld is already severely transformed – and fragmented habitats are subjected to a suite of influences that affect intact habitats to a far lesser extent – e.g. chemical drift from surrounding agriculture, edge effects, extinction debts, etc. Therefore, the maintenance of particularly pollinators is crucial for the continuation of the functioning of these habitats – if we lose ecosystem processes, we lose the entire system.

Differences in insect diversity (considering species richness and abundance of species) between different renosterveld patches, further highlights the need for renosterveld conservation. The IUCN status of the sampled insects are not currently determined. Furthermore, plant diversity was not studied and therefore no correlations between the plants and insects were possible. The necessity for renosterveld conservation with regards to insects is merely based on the total different insect species sampled, and also the amount of species expected to be sampled with increased effort. The assumption can be made that renosterveld has an exceptional insect fauna, which emphasises the importance of conserving renosterveld. Proper identification of the sampled insects to genus and species level will result in the determination of rare/common/endemic/threatened species which will add significance to renosterveld conservation. Only about a fifth of the insect species found were shared between the four sampled renosterveld areas. This small fraction indicates that each renosterveld area, irrespective of size, is unique in terms of insect species composition. West Coast Renosterveld patches are also not homogeneous in terms of plant diversity, and certain plant species are unique to certain patches (Newton & Knight 2010). Considering the important role that vegetation plays in insect communities (i.e. many insects can be host-specific to certain plant assemblages), heterogeneity in plant diversity can possibly explain these observed differences in insect communities. For example, a renosterveld patch with a high geophyte diversity could be expected to have more flower-visiting insects (pollinators) during spring than a patch with low geophyte diversity. Maintaining heterogeneity of patches for conserving plant diversity would thus also benefit the maintenance of insect diversity.

Given South Africa's diverse biomes, many more insect documentation and interaction studies are still required before their functionality and thus their importance in our biomes can be better understood. More specifically, insect communities need to be documented in threatened landscapes to enhance their conservation importance. Diversity studies should not only include a specific order or functional group, but rather the whole spectrum of orders. Such diversity studies would reveal keystone species, specialist species, and generalist species which function in our ecosystems and keeping our ecosystems intact. In addition, identifying endemic and threatened insect species will allow the conservation value of a piece of land not to only be determined by the plant community and structure, but also the insects found in these

plant communities (Irl *et al.* 2015). Certain patches could act as sources of insect species which can disperse to nearby patches by linked corridors between patches (Diekotter *et al.* 2008).

Recommendations and future research

- Future collaboration between conservation bodies (OLCT) and private landowners should be enhanced to protect the small percentage of remaining renosterveld.
- Farmers and landowners need to be persuaded by the advantages they will gain by leaving renosterveld patch intact.
- When farmers want to plough their renosterveld, conservation authorities need to work with the farmers to investigate any possible alternatives to keep renosterveld intact and enforce the law when they plough illegally (CARA and NEMA).
- Farmers need to know that renosterveld is Critically Endangered and that the existence of renosterveld does not lie in the hands of conservationists, but mostly in their own hands.
- More insect work could also be beneficial to highlight the importance of a renosterveld patch.
- With a clearer documentation of the insects found in West Coast Renosterveld, more research can be conducted on certain taxa and plant-insect relationships for a greater understanding of the functionality of insects in renosterveld.
- Pollination studies can showcase the natural pollinators by which farmers can benefit.
- Plant-insect interactions can be investigated to determine which insect species are of major concern for renosterveld's flora pollination.
- Successional studies after fire occasions can also be investigated to determine which insects establish first and what their roles are in the restoration of renosterveld.
- Insect documentation and diversity studies should be carried out throughout the CFR in order to identify species endemic to renosterveld and the other vegetation types.
- Night sampling of insects can also reveal more nocturnal species which are found in renosterveld.
- Research on the connectivity between nearby renosterveld patches can be conducted to determine dispersal abilities of insect species moving between patches.
- Future research on basic pollination webs for renosterveld communities is necessary.

These are only a few suggestions, but they will all contribute to the conservation and management of threatened vegetation types, especially to the Critically Endangered renosterveld. Renosterveld should not only be conserved and managed based on the unique

flora, but also due to the insect life found in this extraordinary, yet threatened vegetation type. Ecosystem functioning is important and insects may be the best indicators of how well these systems are still functioning.

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Appendix A – Reference collection family composition

Order & Family	# of morphospecies/family
ARCHAEOGNATHA	
Meinertellidae	1
1 family of bristletail	1
EPHEMEROPTERA	
Unknown family	1
1 family of mayfly	1
ODONATA	
Aeshnidae	1
Libellulidae	3
2 families of dragonflies	4
BLATTODEA	
Blaberidae	2
Blatellidae	3
Blattidae	7
3 families of cockroaches	12
ISOPTERA	
Hodotermitidae	1
Termitidae	1
2 families of termites	2
MANTODEA	
Empusidae	1
Mantidae	10
2 families of mantids	11
DERMAPTERA	
Forficulidae	1
Labiduridae	6
Lepismatidae	1
3 families of earwigs	8
ORTHOPTERA	
Acrididae	44
Bradyporidae	1
Gryllidae	13
Lentulidae	10
Mogoplistidae	1
Pamphagidae	14
Pneumoridae	1
Shizodactylidae	1
Stenopelmatidae	1
Tetrigidae	2
Tettigionidae	9
11 families of grasshoppers, locusts and crickets	97

PHASMATODEA	
Phasmatidae	6
1 family of stick insects	6
PSOCOPTERA	
Hemipsocidae	2
1 family of booklice	2
HEMIPTERA	
Alydidae	2
Anthocoridae	3
Aphididae	2
Aphrophoridae	1
Cicadellidae	29
Cercopidae	1
Cydnidae	1
Coreidae	3
Delphacidae	16
Fulgoridae	2
Issidae	1
Lygaeidae	21
Margodidae	2
Miridae	8
Nabidae	1
Pentatomidae	17
Plataspidae	1
Psyllidae	5
Reduviidae	15
Scutelleridae	1
Tingidae	9
21 families of bugs	141
THYSANOPTERA	
Phlaeothripidae	2
Thripidae	1
2 families of thrips	3
COLEOPTERA	
Anthicidae	6
Apionidae	4
Biphyllidae	1
Bostrichidae	1
Bruchidae	2
Buprestidae	14
Byrrhidae	2
Carabidae	15
Cerambycidae	3
Chrysomelidae	55
Cicindelidae	1
Cleridae	11
Coccinellidae	17
Colydidae	1
Corylophidae	4

Curculionidae	28
Dermistidae	7
Discolomidae	1
Elateridae	2
Endomychidae	1
Meloidae	4
Melyridae	9
Mordellidae	2
Nitidulidae	5
Pselaphidae	3
Scarabaeidae	15
Silphidae	1
Staphylinidae	1
Tenebrionidae	18
29 families of beetles	234
MECOPTERA	
Bittacidae	1
1 family of hanging fly	1
DIPTERA	
Agromyzidae	14
Anthericeridae	1
Anthomyiidae	13
Asilidae	10
Bibionidae	8
Bombyliidae	1
Calliphoridae	1
Canacidae	1
Cecidomyiidae	2
Ceratopogonidae	1
Chironomidae	12
Cryptochetidae	1
Culicidae	1
Curtomidae	1
Drosophilidae	8
Empididae	12
Fanniidae	2
Heliozelidae	1
Milichiidae	2
Muscidae	19
Mycetophilidae	3
Mydidae	1
Nemestrinidae	1
Pupinculidae	4
Rhagionidae	2
Sarcophagidae	6
Scatophagidae	2
Sepsidae	2
Simuliidae	3
Syrphidae	8
Tabanidae	2
Tachinidae	6

Tephritidae	11
Therevidae	1
Xylomyidae	1
35 families of true flies	164
LEPIDOPTERA	
Adellidae	1
Arctiidae	3
Brachypodidae	1
Crambidae	2
Geometridae	1
Hepialidae	1
Lycaenidae	3
Noctuidae	3
Nymphalidae	1
Pyralidae	3
Satyridae	2
Tineidae	4
Tortricidae	1
13 families of butterflies and moths	26
HYMENOPTERA	
Anthophoridae	12
Apidae	4
Braconidae	9
Chalcididae	6
Chrysididae	3
Colletidae	4
Elasmidae	2
Encyrtidae	3
Eulophidae	4
Eumenidae	1
Eupelmidae	10
Eurytomidae	1
Formicidae	25
Halictidae	6
Ichneumonidae	14
Masaridae	1
Megachilidae	5
Megalyridae	1
Mutillidae	2
Perilampidae	1
Pompilidae	6
Pteromalidae	5
Sclerogibbidae	1
Scoliidae	4
Sphecidae	3
Tiphiidae	3
Vespidae	3
27 families of bees, wasps and ants	139
Total of 17 orders & 155 families of insects	852 morphospecies

Appendix B – Total number of species per order sampled for all sampling methods

Order	D-vac	Sweep net	Pan traps	Pitfall traps
Archaeognatha	1	1	-	-
Blattodea	8	-	4	5
Coleoptera	138	77	66	47
Dermaptera	2	-	3	5
Diptera	72	75	81	6
Ephemeroptera	1	-	-	-
Hemiptera	104	64	23	6
Hymenoptera	63	37	74	27
Isoptera	1	-	1	2
Lepidoptera	7	10	11	2
Mantodea	7	3	1	1
Mecoptera	-	-	1	-
Odonata	-	4	-	-
Orthoptera	59	37	17	12
Phasmatodea	5	2	-	-
Psocoptera	2	1	-	-
Thysanura	3	1	-	-
Total orders/method:	15	12	11	10
Total species/method	473	312	282	113

Appendix C – Study sites with their surroundings



Papegaaiberg study area with four (A-D) sampling replications. On the Western side of the hill lies Onderpapegaaiberg neighborhood; South and East lies Stellenbosch town; North is Middelvllei and Enkanini/Kayamandi townships.



Koopmanskloof study area with four (E-H) sampling replications. The reserve is completely surrounded by a mosaic of agricultural land.



J.N. Briers-Louw Nature reserve with four (I-L) sampling replications. The reserve is surrounded by the Eenzaamheid farm's grazing fields.



Spier study site with four (M-P) sampling replications. The heritage site is surrounded by the property's organic crop fields and vineyards.

Appendix D - Shared species between the four renosterveld areas at different sampling occasions

Renosterveld area	No. of species	No. of shared species	% species shared
Winter (July 2014)			
Papegaaiberg and Koopmanskloof	168	35	20.83
Papegaaiberg and J.N. Briers-Louw	188	29	15.43
Papegaaiberg and Spier	177	32	18.08
Koopmanskloof and J.N. Briers-Louw	208	32	15.38
Koopmanskloof and Spier	197	38	19.29
J.N. Briers-Louw and Spier	217	44	20.28
Spring (October 2014)			
Papegaaiberg and Koopmanskloof	392	82	20.92
Papegaaiberg and J.N. Briers-Louw	400	62	15.50
Papegaaiberg and Spier	344	51	14.83
Koopmanskloof and J.N. Briers-Louw	368	63	17.12
Koopmanskloof and Spier	312	55	17.63
J.N. Briers-Louw and Spier	320	58	18.13
Summer (January 2015)			
Papegaaiberg and Koopmanskloof	101	15	14.85
Papegaaiberg and J.N. Briers-Louw	104	14	13.46
Papegaaiberg and Spier	96	13	13.54
Koopmanskloof and J.N. Briers-Louw	95	18	18.95
Koopmanskloof and Spier	87	15	17.24
J.N. Briers-Louw and Spier	90	15	16.67

Renosterveld area	No. of species	No. of shared species	% species shared
Autumn (April 2015)			
Papegaaiberg and Koopmanskloof	192	28	14.58
Papegaaiberg and J.N. Briers-Louw	227	32	14.10
Papegaaiberg and Spier	207	37	17.87
Koopmanskloof and J.N. Briers-Louw	187	34	18.18
Koopmanskloof and Spier	167	27	16.17
J.N. Briers-Louw and Spier	202	34	16.83
