Persistence of insect biodiversity within a timber production landscape mosaic

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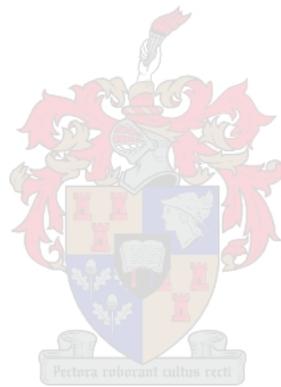
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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights, and that I have not previously in its entirety, or in part, submitted it for obtaining any qualification.

General summary

Habitat transformation, through the expansion of agricultural activities, leads to the formation of novel landscapes, which drives global declines in insect diversity. This leads to high contrast between the structural characteristics of neighbouring biotopes, changing the local distribution of species across the landscape. Contrast between natural and anthropogenic biotopes determines the degree to which species are able to spillover between adjacent biotopes. Agroecosystems are complex landscapes, where natural and transformed biotopes are adjacent. Across the South African timber production landscape, ecological networks (ENs) of conservation corridors are employed to mitigate the adverse effects of plantation forestry. Boundaries between the natural and planted areas are sharp, and provide the opportunity to investigate how contrast between multiple biotopes drives insect assemblages. The aim here is to determine how ecological contrast in a forestry landscape affects local distribution of insect diversity, and to investigate its significance for insect conservation.

I used various sampling methods, specifically pitfall traps, active searches, sweep netting, and vacuum sampling to assess how multiple epigaeic and foliage-dwelling insect taxa and their assemblages respond to changing contrast. I also use a newly-developed ecoacoustics approach, where male bush cricket calls were recorded to determine their response to landscape contrast.

Across all sampled assemblages (epigaeic, foliage-dwelling, and calling bush crickets), contrast played an important role in driving the distribution of insect diversity. Epigaeic and foliage-dwelling assemblages were highly distinct in grasslands due to high contrast between these and all other biotopes. Contrast was lowest between *Eucalyptus* plantations of different ages, resulting in insect assemblages that were indistinguishable from each other. Plantations maintain their own unique insect assemblage, with some spillover from indigenous forest into mature plantations, which is indicative of lower contrast at the plantation-forest interface.

Bush crickets and their soundscape, responded to changing contrasts across this landscape. Their diversity and activity were highest at the natural forest-grassland ecotones. For all ecotone types, there was spillover. Bush cricket species are highly adapted to their specific acoustic environments, and there is acoustic niche partitioning between them. *Thoracistus thyraeus*, a threatened species, was recorded in both natural and transformed biotopes, representative of local bush cricket diversity, as well as promoting insect conservation and the high value of ENs in this transformed landscape.

Natural biotopes, such as forest, grasslands and ecotone between them, are essential for maintenance of all insect species in this transformed landscape, and require active management. Owing to low contrast between plantation age classes, transformed plantations maintain their own unique insect assemblage no matter plantation age. Forest species spillover into plantations, supporting the value of natural forests in this production landscape. Furthermore, low contrast promotes higher insect diversity and is

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beneficial at the plantation edges. Acoustic sampling provides a rapid and easy sampling technique showing the underlying spatial patterning of biodiversity, with very low sampling effort. Overall, the South African forestry landscape conserves insect biodiversity, provided patches of grasslands, natural forests, and their edges are maintained in their natural state.

Opsomming

Habitattransformasie, veroorsaak deur die uitbreiding van landboubedrywighede, lei tot die vorming van nuwe landskappe, wat die wêreldwye afname in insekdiversiteit dryf. Dit lei tot 'n hoë kontras tussen die strukturele eienskappe van naburige biotope, wat 'n verandering veroorsaak in die plaaslike verspreiding van spesies oor die landskap. Die kontras tussen natuurlike en antropogeniese biotope bepaal die mate waartoe spesies tussen aangrensende biotope kan versprei. Agro-ekosisteme is komplekse landskappe waar natuurlike en getransformeerde biotope aanliggend is. Regoor die Suid-Afrikaanse houtproduksie landskap word ekologiese netwerke (EN's) van bewaringskorridors gebruik om die nadelige gevolge van plantasiebosbou te verlig. Grense tussen natuurlike en aangeplante gebiede is skerp, en bied die geleentheid om te ondersoek hoe kontras tussen verskeie biotope insek gemeenskappe dryf. Die doel hier is om te bepaal hoe ekologiese kontras in 'n bosboulandskap die plaaslike verspreiding van insekdiversiteit beïnvloed, en om die belangrikheid daarvan vir insekbewaring te ondersoek.

Ek het verskillende steekproefmetodes gebruik, spesifiek pitval strikke, aktiewe soektogte, vee-net en lugsuier monsterneming om te bepaal hoe verskeie grond en plant-wonende insektaksa en hul gemeenskappe reageer op veranderende kontras. Ek gebruik ook 'n nuut ontwikkelde eko-akoestiese benadering, waar manlike boskriek roepgeluide opgeneem is om hul reaksie op landskap kontras te bepaal.

Kontras het 'n belangrike rol gespeel as dryfkrag in die verspreiding van insek-diversiteit oor alle gemeenskappe (grond, plant-wonende insekte en roepende boskrieke). Grond en plant-wonende insek gemeenskappe was hoogs onderskeidend in grasvelde vanweë die hoë kontras tussen hierdie en alle ander biotope. Die kontras was die laagste tussen *Eucalyptus* plantasies van verskillende ouderdomme, wat gelei het tot insek gemeenskappe wat nie van mekaar onderskei kon word nie. Plantasies handhaaf hul eie unieke insek gemeenskappe, met 'n bietjie verspreiding van inheemse woud na volwasse plantasies, wat 'n aanduiding is van 'n laer kontras by die plantasie-woud-ekotoon.

Boskrieke en hul klankbeeld het gereageer op veranderende kontraste oor die landskap. Hul diversiteit en aktiwiteit was die hoogste by natuurlike woud-grasveld ekotone. Daar was verspreiding tussen alle ekotoontipes. Boskriekspesies is hoogs aangepas tot hul spesifieke akoestiese omgewings, en daar is akoestiese nisverdeling tussen hulle. *Thoracistus thyraeus*, 'n bedreigde spesie, is opgeneem in natuurlike en getransformeerde biotope. Die spesie is verteenwoordigend van die plaaslike boskriek diversiteit, en as 'n vlagskip spesie bevorder dit die bewaring van insekte en die hoë waarde van EN's in hierdie getransformeerde landskap.

Natuurlike biotope, soos woud, grasvelde en die ekotoon tussen hulle, is noodsaaklik vir die instandhouding van alle insek spesies in hierdie getransformeerde landskap en benodig aktiewe bestuur.

Vanweë die lae kontras tussen plantasie-ouderdomsklasse, handhaaf getransformeerde plantasies hul eie unieke insek gemeenskap, ongeag planttyd. Woud spesies versprei na plantasies, wat die waarde van natuurlike woude in hierdie produksie-landskap ondersteun. Verder bevorder lae kontras groter insek diversiteit en is dit voordelig vir plantasie grense. Akoestiese monsterneming bied 'n vinnige en maklike tegniek wat die onderliggende ruimtelike patroon van biodiversiteit toon, met 'n baie lae steekproefneming inspanning. In die algemeen bewaar die Suid-Afrikaanse bosboulandskap insekte se biodiversiteit, mits kolle grasveld, natuurlike woude en hul grense in hul natuurlike toestand behou word

Graphical abstract

Sampled Biotopes

Low contrast: some spillover

Increased bush cricket activity

Low contrast: resilience within plantations

High contrast: distinct assemblages

Open canopy planation

Closed canopy planation

Indigenous forest

Forest – grassland ecotone

Grassland
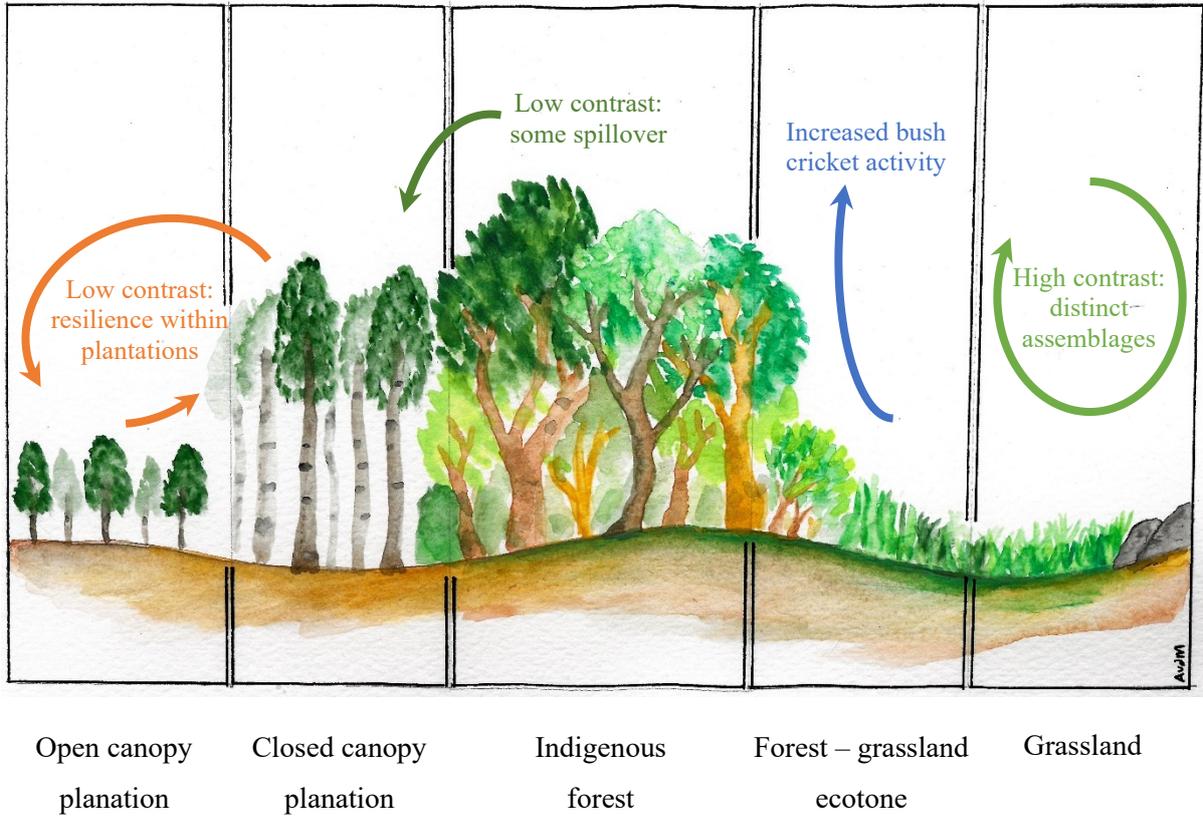
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Although as a geographer, my mom's ability to walk a straight transect while swinging a net is remarkedly questionable, I would like to thank my parents, Sue and Rob Thompson for their constant support, both in life and in my academic career. They have never questioned my need to listen to insects, or at least not to my face, but rather have put on their fieldwork pants, grabbed various nets and clipboards and headed out into the field with me. For this unrelinquishing support, I thank them.

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Tea, please?

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Chapter 1:

General introduction

> "Giddy Grasshopper,
>
> take care… do not leap and crush
>
> these pearls of dewdrop."
>
> -Issa

1.1 The global biodiversity crisis

Anthropogenic disturbance is directly linked to the loss of biodiversity (Pimm & Raven 2000; Thomas et al. 2004). There are 36 identified global biodiversity hotspots (Conservation International 2019), promoted as tools to aid in the successful conservation of biodiversity (Myers et al. 2000; Zachos & Habel 2011) and facing inordinate threat due to habitat loss. These regions cover an estimated 2.4% of the earths land surface. Yet these 34 hotspots are home to an estimated 50% of all vascular plant species, and 33% of all vertebrate species (Myers et al. 2000; Zachos & Habel 2011). Global biodiversity hotspots are therefore defined as regions of exceptional richness and endemism that are faced with anthropogenic threat (Myers et al. 2000; Mittermeier et al. 2004).

Insect declines are of interest given that insects are essential for the maintenance of ecosystems as well as the provisioning of many essential ecosystem services, such as pollination (Allsopp et al. 2008), without which many systems, natural and anthropogenic, would collapse (Daily 1997; Losey et al. 2006). Recently, a paper investigated the drivers of global insect declines. In it, it is suggested that worldwide, 41% of all insects are in decline, largely as a result of habitat transformation and loss due to agricultural practices, pollution, pathogens and introduced species as well as climate change (Sánchez-Bayo & Wyckhuys 2019). Highly criticized as being biased in its methods, and creating sensationalism within the public domain, this paper nevertheless highlights the fact that long-term monitoring and gathering of scrupulous data is of paramount importance to accurately determine the trajectories of global insect diversity (Thomas et al. 2019).

1.2 Habitat transformation and the creation of novel landscapes

The rate at which habitat fragmentation and loss is occurring is alarming and is a leading cause of the global biodiversity crisis (Fahrig 2003, 2017; Krauss et al. 2010). Through habitat transformation, naturally occurring tracts of native vegetation become divided into smaller more isolated parcels of varying sizes. Habitat transformation can lead to habitat loss (the shear loss of land to anthropogenic transformation) and fragmentation (the spatial isolation and compartmentalization) of remnant vegetation (Fahrig 2017). Habitat transformation results in the formation of novel landscapes, where

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patches of natural habitat become isolated and surrounded by tracts of transformed habitat (Pickett & Cadenasso 1995; Fahrig 2003) (Figure 1.1). These transformed areas are characterized by changes to vegetation structure (Fahrig 2003) and species composition (Schneider & Fry 2001; Reino et al. 2009; Tscharntke et al. 2012), changes in the microclimates along edges (Didham et al. 1996), as well as in the number and distribution of resources available for species to utilize.

Figure 1.1: A novel landscape situated in Baynesfield KwaZulu-Natal, South Africa. The original extent of the grassland has been reduced and fragmented through the expansion of timber production. Further pressure is exerted on the grassland by inappropriate cattle grazing regimes.

Species responses to transformation vary in accordance to the specialization of individuals and their propensity to adapt and persist in an altered environment (Laurance et al. 2001; Ricketts 2004; Rand et al. 2006; Evans et al. 2016; Gray et al. 2016). Specialist species, those with narrow ecological niches, and those species that require specific resources for the completion of their lifecycles, are not expected to persist in altered landscapes if their basic ecological needs are not met, and as a consequence are more susceptible to habitat transformation (Didham et al. 1996; Reino et al. 2009). Generalist species, those whose ecological requirements can be satisfied by a range of resources, and who neither depend on specific host species or climatic conditions are able to derive benefit from the transformed regions

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of a landscape (Tscharntke et al. 2012). Environmental filtering prevents highly adapted species from persisting in transformed areas (Dröse et al. 2019; Santoandré et al. 2019).

The impact of habitat fragmentation on biodiversity can vary depending on the characteristics of the system, and species, experiencing habitat fragmentation (Fahrig 2017). Although responses to fragmentation are not static, isolation, size, and shape of these remnant fragments are important factors in determining how biodiversity is able to respond to fragmentation (Fahrig 2003, 2017). Fragments may be closely clumped, whereas other fragments can be widely dispersed across the landscape and surrounded by tracts of transformed habitats (Fahrig 2003). This in turn affects how isolated patches are from one another and how species are able to move between patches. More mobile (Brudvig et al. 2009; Evans et al. 2016), and tolerant species will be able to disperse further afield than less mobile (Evans et al. 2016) and sensitive species (Rand et al. 2006). This in turn will impact the degree of reproductive isolation in each remnant patch. Size and shape of fragments, in turn, determine the edge ratio of patches, the higher the amount of edge habitat, the less pristine habitat remains in a patch (Ewers & Didham 2008; Tscharntke et al. 2012). Edges are characterized by altered microclimates, resource distribution, as well as novel species assemblages, due to mixing of species from adjacent biotopes occurring (Ewers & Didham 2008; Boetzl 2016; Dröse et al. 2019). Remnant patches of habitat in a landscape are not without value, in fact they are essential in maintaining landscape, and habitat heterogeneity. Many specialist species would be lost from transformed landscapes should the remnants disappear (Duelli & Obrist 2003; Phalan et al. 2011).

However, it is not just habitat alterations that structure insect communities in transformed landscapes, but also novel insect assemblages that can result in novel species interactions (Boetzl 2016) which, in turn, can lead to ecosystem level changes in the landscape (Frost et al. 2015). These novel species interactions can have unpredictable effects on both the abiotic and biotic features found within these transfromed landscapes (Rand et al. 2006; Ewers & Didham 2008). Little is known about the species level interactions and how these interactions function in the structuring of insect communities found within transformed landscapes (Andersson et al. 2013).

1.3 Edge contrast and species spillover

Contrast refers to the degree of dissimilarity in structural characteristics, as well as the abiotic and biotic components between two neighboring biotopes, or landscape features (Hunter 2005; Biswas & Wagner 2012). Contrast occurs between both natural habitat features, as well as between anthropogenically transformed patches. As habitat fragmentation, loss and transformation occur in a landscape, the degree of contrast between remnant habitat patches and transformed habitat also changes (Ries et al. 2004). This, in turn, impacts the distribution of native species across the landscape (Figure 1.2).

Species movement across boundaries can be facilitated, or hindered, by edge contrast (Tscharntke & Brandl 2004), and by the species' in question own characteristics (Tscharntke & Brandl 2004). Where

the surrounding transformed habitat is "softer", or ecological conditions such as microclimates, vegetation structure, and niche availability are similar to the natal habitat of species, these transformed regions may in fact facilitate the spillover of species into these transformed regions (Purtauf et al. 2005; Laurance et al. 2011; Tscharntke et al. 2012). Care must be taken, however, in interpreting the distribution of species within these transformed areas, as presence of a species does not imply that the species is a permanent resident of a transformed area (Tscharntke et al. 2012; Madeira et al. 2016). In fact, there are many examples of species utilizing transformed regions depending on the seasonal availability of resources (Tscharntke et al. 2005). Carabid beetle numbers increase in meadows surrounding wheat field post-harvest as they follow the distribution of prey species in response to disturbance (Rand et al. 2006). Presence of a species in transformed regions can also be attributed to the life stage of the individual insect. The bush cricket, *Platycleis affinis*, moves from surrounding natural vegetation into barley fields as they mature into adults (Samways 1977).

Figure 1.2: Lower edge contrast between an indigenous forest and *Eucalytus* plantation (light orange arrow), and higher contrast between an indigenous forest and grassland (light green arrow).

As not all species perceive edges, or respond to edges in the same way, some edges may appear as "hard", when ecological conditions are perceived as highly contrasting. Specialist predators are more

at risk to fragmentation effects, than generalist prey species. In many instances, species belonging to higher trophic levels are disadvantaged when encountering edges (Rand et al. 2006). For example, grassland-forest edges often present as hard contrasting edges to species, as the thermal conditions of these two biotopes are vastly different, forests are shaded and thermally buffered, while grasslands are not (Dröse et al. 2019; Santoandré et al. 2019). Grassland ant species that are shade intolerant, are therefore not found within the forests due to the contrasting conditions (Dröse et al. 2019). Not all edges are created equal and while many anthropogenic edges are hard, other edges, such as those between forest and plantations, are perceived as softer by a range of species due to the similarities in physical structure and climatic conditions between the two biotope types (Peyras et al. 2013). Furthermore, mixing of forest plant species at the interface between indigenous forest and pine plantations has been found to facilitate spillover of forest species into the plantations (Yekwayo et al. 2016a, 2017).

Ecotones are zones of transition between two adjacent biotopes and are characterize by a mixture of ecological conditions (Dröse et al. 2019). As gradual changes occur at these ecotones, complementary species assemblages are often observed (Ries et al. 2004; De Smedt et al. 2019). Due to the novel assemblages occurring at the ecotones, spillover of ecosystem services and not just species results (Ricketts 2004), although the direction and nature of resultant interactions is difficult to determine (Tscharntke et al. 2005). Conditions that allow for the positive spillover of one species at an ecotone, may in turn facilitate conditions so that a negative response occurs with a second species, such as conditions that allow pest species to thrive (Tscharntke et al. 2005).

Distribution of resources at edges drive the spillover response of many species. Increasing individual numbers at an edge, with a gradual decrease in numbers as distance into the non-natal biotope increases, indicates complementary resources use, where resources from both adjacent biotopes are present at the edge (Rand et al. 2006; De Smedt et al. 2019). Productivity differences between adjacent biotopes results in spillover from a highly productive biotope into a less productive biotope. Therefore, no increase in species numbers at the edge is observed and a gradual decrease in numbers occurs as distance into the non-natal biotope increases. While, finally, a sharp drop in numbers at the boundary of adjacent biotopes indicates that the species in question is a habitat specialist, and that the non-natal biotope does not meet its specific resource requirements (Tscharntke et al. 2005; Rand et al. 2006; Loreau et al. 2013).

1.4 Agroecosystems

Agroecosystem comprise of natural, seminatural and transformed production lands in proximity (Duelli & Obrist 2003). The diversity of invertebrates within agroecosystems is influenced by the various landscape features present (Purtauf et al. 2005; Tscharntke et al. 2005). Some insect species present with the ability to move between agricultural lands and surrounding natural vegetation in response to resource availability and habitat requirements (Tscharntke et al. 2005, 2012). Although resources are

available in transformed habitats, natural remnant patches are essential for the maintenance of comprehensive species assemblages in agroecosystems (Duelli & Obrist 2003; Fahrig 2017). In a vineyard production landscape, parasitoid wasps found in remnant fynbos patches show limited spillover, and are unable to survive in the surrounding vineyards, effectively isolating specialist fynbos species in these remnant patches. These remnants are therefore essential in the conservation of indigenous parasitoid diversity across this production landscape (Gaigher et al. 2015).

Species distribution patterns in agroecosystems are driven by the individual species' characteristics which in turn influence a species fidelity to biotopes within the landscape. As such, fidelity refers to a species preferred biotope, or combinations thereof (Duelli & Obrist 2003). In the above example, parasitoid wasps perceive the vineyards as unsuitable likely due to pesticide use, low resource availability and the lack of suitable host species. These factors likely combine and hamper the dispersal abilities of the species across the landscape (Gaigher et al. 2015). Although challenging to determine the individual dispersal capabilities of species, species fidelity to landscape features is a useful way in which to determine which landscape features species are dependent on, and therefore which landscape features are of conservation significance (Duelli & Obrist 2003). By identifying which landscape features within agroecosystems are pertinent to regional biodiversity, conservation measures can be designed to best benefit, manage and conserve overall biodiversity in production landscapes (Gaigher et al. 2015; Samways & Pryke 2016).

1.5 Ecological networks

Ecological networks (ENs) are interconnected areas of natural habitat that are surrounded by transformed production land (Jongman 1995). Within the South African timber production landscapes, there exists an extensive network of grasslands, indigenous forests and riparian zones (Figure 1.3). These regions were either set aside, or were previously afforested and have been subsequently cleared in an effort to restore ecosystem services lost due to inappropriate silviculture regimes (Samways & Pryke 2016). Ecological networks are essential to the maintenance of landscape wide habitat quality and heterogeneity (Crous et al. 2014a; Kietzka et al. 2015; van Schalkwyk et al. 2017), as well as provisioning ecosystem services (Bazelet & Samways 2011a, 2012; Yekwayo et al. 2016b) within the timber production landscape. In addition, ENs effectively act as extensions of local protected areas, as they support complementary arthropod assemblages (Samways & Pryke 2016).

By managing ENs, landscape wide connectivity between habitat patches is improved (Pryke & Samways 2001, 2012, 2015; Samways & Pryke 2016). The width and size of these ecological corridors is also an important consideration (van Schalkwyk et al. 2017), larger corridors ensure that more intact core habitat is conserved (Pryke & Samways 2001; Fahrig 2017; van Schalkwyk et al. 2017). With more intact habitats available, more complete, and permanent, assemblages of arthropods are conserved in the ENs. Narrow corridors are not without value, and while they may not provide intact habitat to

arthropods, they do facilitate the movement of species across a production landscape (Pryke & Samways 2001; Samways & Pryke 2016). Grassland butterflies traverse narrow corridors 13 times faster than they do wider ENs, providing evidence that these ENs are utilized for more than resource provision, such as movement between patches(Pryke & Samways 2001).

Figure 1.3: A timber production landscape characterized by a mix of timber stands, indigenous grasslands and forests

Active management and conservation of these ENs ensures that ecosystems services remain present in the landscape (Duelli 1997; Pryke & Samways 2015; Fahrig 2017). Resources are available for a more complete cohort of species, than if ENs did not exist in these timber production landscapes, also resources availability will prevent one species becoming dominant at the expanse of another (Fahrig 2017). By conserving a range of habitat types, ENs promote the maintenance of habitat heterogeneity and increase the total number of species protected (Fahrig 2017). For example, by including remnant patches of indigenous forests with in an EN, it was found that the forest patches conserve additional arthropod assemblages not present in grasslands, thereby adding to the regional pool of species protected within the EN (Yekwayo et al. 2016b). Landscape heterogeneity does not only pertain to biotic resources, but also to abiotic landscape features within the ENs. Rocks, at the mesoscale, including rocky outcrops within grasslands, have been found toconserve a full cohort of plants, butterflies and grasshoppers within ENs (Crous et al. 2013, 2014a).

Ecological networks within the timber production regions of South Africa are a model system for studying anthropogenic affects, as natural biotopes, such as grasslands and forests can be found near transformed timber stands. The importance of the ENs is well understood in the region. Previous work has investigated design features and the best practice to maintain diversity in these systems (Bazelet & Samways 2011b; van Schalkwyk et al. 2017), as well as the complementarity of various features and landscape components to landscape wide diversity (Crous et al. 2013; Yekwayo et al. 2016b). Although, much of what is known about these systems has been determined from the natural biotopes viewpoint, and the roles that they play in landscape wide biodiversity conservation, little has been done on the role that the timber stands play in the maintenance, or lack thereof, of regional biodiversity.

1.6 Biodiversity monitoring in across a timber production landscape

1.6.1 Traditional methods

As different insect species exhibit different traits and rely on a variety of different resources to complete their lifecycles, methods employed to sample and monitor insect biodiversity vary vastly in accordance (Sawmways et al. 2010). In addition to this, various insect groups are often used as indicators of habitat quality. In South Africa, dragonflies are used as indicators of freshwater and riparian habitat quality (Samways & Simaika 2016), while grassland dwelling grasshoppers are indicative of grassland quality (Bazelet & Samways 2011a; Matenaar et al. 2015). Dung beetles (van Schalkwyk et al. 2017), and ants are also known indicators of habitat quality (Ekschmitt et al. 2003). With this is mind, multi-taxon sampling enables a wide range of insects to be sampled, allowing for all possible responses of insects to perturbation to be determined. These methods have enabled the responses of many invertebrate assemblages to ENs to be determined (Bazelet & Samways 2011b; Crous et al. 2014b; Samways & Pryke 2016; van Schalkwyk et al. 2017; Yekwayo et al. 2017). These sampling techniques require the individual insects to be caught, killed and stored in order to be identified. High numbers of individuals caught, not to mention by-catch of non-target taxa, such as small vertebrates falling into pitfall traps. These sampling techniques are often time-consuming and not always appropriate when time is a limiting factor.

1.6.2 Ecoacoustics - a complementary and novel technique

Ecoacoustics is emerging as a novel, rapid and non-invasive method to characterize landscapes and to complement traditional biodiversity assessment and sampling techniques (Figure 1.4). Sound is inherent in all natural systems, and until recently (Laiolo 2010), often overlooked as feature of the natural world (Farina & Pieretti 2014). Across landscapes, sound is emitted from a diverse array of sources (Villanueva-Rivera et al. 2011). Sounds can originate from natural abiotic processes, such as weather phenomena like wind and rain, or from flowing water (Krause et al. 2011) and is termed geophony, while sounds originating from animal signals is termed biophony. While, noise originating from human sources, such as traffic and machinery, is termed anthrophony (Pijanowski et al. 2011). Ecoacoustics, the study of these soundscapes, is a rapidly growing field, with many novel indices aimed at

characterizing community level soundscapes being developed (Sueur et al. 2008; Sueur & Farina 2015; Bradfer-Lawrence et al. 2019). Yet the importance of species identity and ecological interactions of species cannot be overlooked (Sueur et al. 2014; Sueur & Farina 2015), as the differential responses of

Figure 1.4: Non-invasive acoustic sampling of an indigenous forest patch.

species in response to the availability of specific resources in a landscape, can help explain differences in the emerging soundscape (Rodriguez et al. 2014).

Many insect species produce sound, but most notable, are the sounds produced by Orthoptera (Bailey & Rentz 1990; Riede 1998; Nischk & Riede 2001).Within South Africa, ecoacoustics methods focusing on orthopteran communication, have been used to study sexual selection, and communication methods of the southern African endemic Bladder grasshoppers (Couldridge & van Staaden 2004; Couldridge & van Staaden 2006). The response of bush cricket assemblages to changing elevation and vegetation gradients has been determined (Grant 2014), as well as to determine the predator-prey dynamic between acoustically communicating bush crickets and a listening predator, the Bat-eared Fox, *Otocyon megalotis* (Grant & Samways 2015). Furthermore, ecoacoustics methods have successfully been used to determine the distribution of bush crickets in response to the landscape mosaic present in the highly diverse Cape Floristic Region (Thompson et al. 2019). Within a timber production mosaic landscape, *Eucalytus* plantations were found to be devoid of all but one acoustically communicating cricket species, while the neighboring ENs were highly diverse, with a soundscapes consisting of calls from many cricket and bush cricket species, as well as birds and other vertebrates (Grant & Samways 2016).

Acoustic monitoring of ENs is therefore a viable method to assess changes in acoustically communicating insect assemblages, as well as the soundscapes occurring across a wide range of biotope types, ecotones and changing contrasts. Bush crickets have been shown to be appropriate study organisms as they produce obvious calls and are easily sampled acoustically in addition to being sensitive to ecological condition (Grant & Samways 2015; Thompson et al. 2019). Acoustic assessments, producing large amount of data in a relatively short period of time, offer a complementary method in with which biodiversity in ENs can be monitored. In addition, acoustic sampling techniques add additional valuable insight into how species respond to landscape transformation that the traditional methods may overlook.

1.7 Overall aim

As ENs comprise many different biotope types (Samways & Pryke 2016), a wide range of biotope combinations are possible between naturally occurring and transformed timber stands. This thesis focuses on the role that both the timber stands, and natural biotopes play in conserving biodiversity within the landscape.

In addition, this thesis investigates the role that contrast between adjacent biotopes plays in hampering, or facilitating, the distribution of species across a timber production landscape mosaic. Structural differences between grasslands and timber plantations are known to prevent grassland specialists from occurring within the plantations (Pryke & Samways 2001), but research has shown that there is indeed evidence for spillover from indigenous forests into plantations (Yekwayo et al. 2016a, 2017), therefore by focusing on many contrasting biotope pairs it will be possible to determine how boundaries of differential contrast impact distributions of species.

Furthermore, this thesis also provides a contrast between traditional sampling methods and the novel approach of monitoring biodiversity acoustically. Traditional sampling methods can be time consuming, intensive and laborious, while acoustic sampling provides straightforward method whereby animal diversity can be recorded without interfering with the individual in question. The streamlining of biodiversity monitoring could potentially improve conservation management of these ENs, benefiting both biodiversity and sustainable timber production.

In addition, ecoacoustic methods can provide additional ecological information that cannot easily be obtained by traditional sampling methods. For example, it is possible to assess acoustically how different species partition their calls to prevent masking, resulting in niche partitioning of the acoustic space, which in turn leads to co-occurrence, or not, of acoustically communicating species (Krause 1993). It is not just co-occurrence of species that determine soundscape diversity, but also the adaptation of the individual species to the acoustic environment in which they occur (Morton 1975). As a result, this additional information gained through soundscape assessments allows for further understanding of species distributions and assemblage dynamics.

1.8 Thesis outline and chapter aims

This thesis is comprised of six chapters, chapters 2 to 5 present the findings of the research and are presented as individual research papers. In chapter 6 the results are discussed, and recommendations made.

Chapter 2: Patch contrast in a fragmented landscape: the persistence of epigaeic insect biodiversity within a timber production landscape.

Here, I aim to determine how contrast between four distinct biotope types affect the maintenance of epigaeic insect diversity across the landscape. By applying a multi-taxon approach, responses from a wide range of species are investigated. To determine this the following research questions and objectives were addressed:

1. How does epigaeic species diversity and assemblage structures change across the edges of different biotope combinations?
2. How do the different age classes of *Eucalyptus* trees impact biodiversity within the plantations?
3. Assess the potential of natural biotopes to function as source populations for epigaeic insects to recolonise young *Eucalyptus* plantations.
4. Determine the affect different age classes of *Eucalyptus* trees have on the insect assemblages within adjacent remnant habitat patches.
5. Identify the environmental factors driving the observed patterns and how these can be accommodated into management practices to further improve the conservation of biodiversity across this production landscape.

Chapter 3: Role of contrast and species biotope fidelity in determining spillover of foliage-dwelling insect assemblages between natural and timber production patches.

In this chapter, foliage-dwelling arthropod species displaying fidelity to the various biotopes are used to determine how biotope fidelity impacts the spillover of species between biotopes. Once again a multi-taxon approach is used here. To do this I address the following aims and objectives:

1. Determine the environmental drivers of species richness and assemblage structure between the different biotopes.
2. Ascertain how spillover between biotopes is impacted by contrast.
3. Determine how spillover is impacted by species biotope fidelity and how species richness of these species change in biotopes where fidelity is not observed.

Chapter 4: Soundscapes among bush crickets (Tettigoniidae) vary in accordance to vegetation structural diversity and across the ecotones of a complex timber production landscape.

In this chapter, in addition to the four previously sampled biotopes, the ecotones occurring between the previously sampled forests, grasslands and plantation age classes were also sampled. In doing so, I

determine how changing contrast at the ecotones between biotopes affects the diversity and singing activity of bush cricket assemblages. The following aims and objectives are answered in this chapter:

1. Determine how the complexity of bush cricket soundscape changes across the landscape and between sampled biotopes.

2. Ascertain if there are compositional changes in the bush cricket assemblages across the different biotopes and ecotones.

3. Identify key species and frequency bands associated to each biotope and determine if there is evidence of acoustic adaptation of the bush cricket species to the sampled biotopes.

Chapter 5: Acoustic response of a threatened bush cricket species (Tettigoniidae: Thoracistus thyraeus) to a complex production landscape mosaic.

An endangered and charismatic bush cricket, *Thoracistus thyraeus*, was identified acoustically. In this chapter I aim to determine where in the landscape it is found, and how habitat transformation and interspecific competition impacts this species' distribution. To do this the following aims were identified:

1. Identify landscape elements that *Thoracistus thyraeus* occupies, as little is known about this species' distribution.

2. Determine the environmental variables that drive the distribution of *Thoracistus thyraeus* in this landscape.

3. Investigate whether inter-specific acoustic competition occurs between *Thoracistus thyraeus* and other co-occurring bush cricket species.

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Chapter 2:

Patch contrast in a fragmented landscape: the persistence of epigaeic insect biodiversity within a timber production landscape

"Earthworm pops up.

how quick

the ants."

-Issa

Abstract

Habitat fragmentation leads to the formation of novel, mosaic landscapes comprising natural fragments surrounded by transformed habitat. These remnant patches help maintain natural biodiversity across these landscape mosaics. Yet, the response of species depends on the contrast between these natural remnant patches and the surrounding transformed habitat. Ecological networks (ENs), large-scale conservation corridors, are vital for conserving a variety of habitats and species, as well as ecosystem processes and habitat heterogeneity in the timber production landscapes in South Africa. However, little is known about the response of species to contrast within these ENs. A multi-taxon approach was used to investigate the role of contrast in these timber production landscapes as well as the impact that eucalypt tree age has on the epigaeic insect species in the EN grasslands, indigenous forests and plantation. Transects spanning different combinations of adjacent biotopes were sampled with pitfall traps and active searches. Here, the response of species richness, and species assemblages, to the contrast inherent in these timber production landscapes is assessed. Contrast between biotope types results in species richness in grasslands and indigenous forests being higher than in open or closed canopy plantations. There was no difference in the species richness, or assemblage composition between the plantation age classes, indicating low contrast between the two biotopes. The species assemblages of the grasslands and forests were also highly distinct. There is some evidence for similar assemblages of species at the interface of the indigenous forests and closed canopy plantations, indicating lower contrast between these structurally similar biotopes. Due to the low contrast between the two plantation age classes, the species assemblages within the plantations are resilient to changes in plantation age, as the same species are present in both the open and closed canopy plantations. Across this landscape, it is contrast between elements, that drives species responses. Natural areas within this production landscape are invaluable for the maintenance of species.

2.1 Introduction

Fragmentation of natural habitats is one of the leading causes of the global biodiversity crisis (Fahrig 2003; Hoekstra et al. 2005; Krauss et al. 2010). Attrition of remaining patches, results in further habitat loss (Fahrig 2003, 2017), leading to the formation of novel landscapes. These altered landscapes can be viewed as a mosaic of remnant patches surrounded by an anthropogenically altered habitat (Pickett & Cadenasso 1995). Although remnant patches are more isolated than intact habitat, are not without value, as many native species are able to find refuge in them, enabling these species to persist in novel landscapes. However, species persistence often varies across mosaic landscapes in response to contrast between habitat features and landscape elements (Hunter 2002; Biswas & Wagner 2012; Yekwayo et.al 2017).

Contrast is an inherent landscape feature in fragmented landscapes, affecting species responses across a landscape mosaic. Contrast exists between all elements of a mosaic landscape, and can be defined as the relative dissimilarity between the abiotic and biotic elements of two adjacent biotopes (Hunter 2005; Biswas & Wagner 2012). In fragmented landscapes, there is often a high degree of contrast between remnant fragments and the surrounding transformed biotope (Tscharntke & Brandl 2004; Fahrig 2017). For example, there is a high level of contrast between grasslands and pine plantations (Yekwayo et al. 2016a, 2017). Grasslands are open grass-dominated systems, while mature pine plantations have closed canopies with little understorey vegetation. This high level of contrast could discourage movement of species between grasslands and plantations, as is the case with grassland butterflies. These butterflies are unable to penetrate into the plantation, and so turn around at the edge (Pryke & Samways 2001). However, contrast is not solely an artefact of fragmentation, but also occurs naturally across a landscape. Indeed, indigenous forests and grasslands have a high degree of contrast. In Brazil, forest-grassland ecotones affect the diversity of ant species adapted to typical grassland conditions of oscillating thermal conditions, low microhabitat complexity, as well as low vegetation complexity. Ants decreased in species richness, as well as phylogenetic diversity, when the woody layer of the grasslands increased with forest expansion (Dröse et al. 2019).

A landscape mosaic comprises a variety of land-use types and remnant fragments. Connectivity between patches, size, and shape of the fragments interact, and influence the degree of resilience to further disturbance (Bender et al. 1998; Fahrig 2003). Contrast plays between natural and non-natural biotopes plays an integral role in determining the responses of native species (Tscharntke & Brandl 2004). Although spillover across edges occurs, this does not necessarily indicate that these species are establishing in these non-natal biotopes (Madeira et al. 2016). However, some appropriate resources are present while the species are present in the non-natal biotopes.

Spillover from non-natural patches into small and isolated remnant habitat patches, can change the local assemblages by increasing the primary productivity of the patch, and increase the abundances of

common, generalist species (Boetzl 2016), further threatening native species in these sensitive patches. As distance to the edge decreases in natural fragments, species occurrences change. In birds, woodland specialists decrease towards the edge of woodland patches, and do not extend beyond it and into the surrounding grasslands, as their ecological requirements are not met. This is important for conservation as, in many cases, species that benefit from edge effects and persist, or even increase in abundances at these interfaces, may not be of conservation concern (Reino et al. 2009).

The spillover of species across edges is partially determined by the neighbouring biotope, which may allow, or prevent, movement of individuals (Tscharntke et al. 2012). Edge effects, such as microhabitat changes and altered plant assemblages, permeate habitat fragments to various degrees (Ewers & Didham 2008). Where contrast is high, edge effects from changes in abiotic and biotic conditions, are able to penetrate deeper into habitat fragments (Reino et al. 2009). Ground beetle assemblages in cultivated fields vary in accordance to the various surrounding natural habitats (Boivin & Hance 2003). Landscape heterogeneity drives coccinellid assemblages, e.g. in cornfields, assemblages are partially determined by the surrounding landscape mosaic and the resources that the species are able to glean from it (Elliott et al. 2002), while a variety of surrounding habitat types helps to conserve coccinellid diversity in citrus orchards (Magagula & Samways 2001). Composition of mobile species in farmlands, such as butterflies, is greatly affected by surrounding landscape complexity, whereas sessile species, such as plants, are not as impacted to the same extent (Weibull & Östman 2003).

Responses to contrast at the assemblage level depend on the contrast between resources of two biotopes (Fahrig 2003; Reino et al. 2009; Boetzl 2016), while species level responses depend on the specialisation of the individual species (Tscharntke & Brandl 2004). Forest-grassland ecotones function as hard edges, with ant assemblages changing at these ecotones (Dröse et al. 2019). Differences in microclimates and vegetation structure result in variable species responses, depending on their resource requirements, and distribution patterns of these resources (Lindenmayer et al. 2008; Peyras et al. 2013; Dröse et al. 2019). However, contrast between mature plantations of both eucalypt and pine species, and the indigenous Southern Atlantic forest in Argentina, is lower due to similarities in the microclimates and vegetation structure of the two biotopes (Peyras et al. 2013). Although responses vary between functional groups, dung beetles show stronger responses to the contrast between young and intermediate-aged plantations, than they do to mature plantations and surrounding indigenous forests due to differences in the thermal conditions of the two biotope types (Peyras et al. 2013).

Ecological networks (ENs) are interconnected patches of natural habitat surrounded by highly transformed production land (Samways & Pryke 2016). The forestry sector in South Africa maintains about 500 000 ha of remnant natural habitat patches, largely comprised of grasslands, indigenous forest, and riparian areas (Samways & Pryke 2016). ENs provide a wide range of benefits to native biodiversity, and by actively managing remnant natural habitats in these timber production landscapes,

ecosystem function, landscape heterogeneity, and connectivity are maintained, which further promote conservation of indigenous biodiversity (Bazelet & Samways 2012; Crous et al. 2013; van Schalkwyk et al. 2017). ENs effectively extend the range of formally protected areas into production and transformed landscapes (Samways & Pryke 2016). Connectivity across the network of exotic timber production is improved with ENs, ensuring that conduits, source habitats, and resources are maintained for indigenous biodiversity.

ENs have been used extensively for study of biodiversity response to fragmentation and transformation. Consequently, their vital role in maintaining biodiversity in these transformed landscapes is fairly well understood (Pryke & Samways 2001; Kietzka et al. 2015; Samways & Pryke 2016; van Schalkwyk et al. 2017). However, little work has focused on the role of contrast within these timber production landscapes. There is some evidence of structurally-similar pine plantations supporting native biodiversity at the interface between indigenous forests and plantations (Peyras et al. 2013; Yekwayo et al. 2016b, 2017). Low contrast between these two biotope types, as well as the plant assemblages and environmental conditions at the edge, enable persistence of species into the plantations (Yekwayo et al. 2016a, 2017).

This study determines how contrast between four distinct biotope types occurring in a timber production landscape, affect the recovery and maintenance of epigaeic biodiversity within the different biotopes. I do this by assessing species diversity and assemblage structure across the edges of different combinations of biotopes. Firstly, I hypothesise that those *Eucalyptus* plantations close to indigenous forest patches will share some species at the interface between the two biotopes, as a result of superficial structural similarity. Secondly, I hypothesize that *Eucalyptus* plantations of different age classes will host distinct epigaeic insect assemblages. To do this I study an extensive network of grasslands and indigenous forest surrounded by both mature and young *Eucalyptus* plantations.Iindirectly assess how age classes of the trees, impacts biodiversity within the plantations. To do this, I use a multi-taxon approach to compare epigaeic insect assemblages between young and mature plantations. I hypothesise that the differences in structure between open and closed canopy plantations will impact insect assemblages, resulting in two distinct assemblages. Thirdly, I hypothesize that remnant grasslands, forests and mature *Eucalyptus* plantations will serve as source habitats from which epigaeic insects can recolonise young eucalypt plantations. Forthly, I investigate the effect of varying tree age, resulting from clear-felling and reestablishment of plantations, on epigaeic insect assemblages within remnant habitat patches. I hypothesise that there will likely be a marked change in insect assemblages and species richness in natural areas close to sites that had been more recently disturbed such as the open canopy plantations. Finally, I identify the environmental factors driving the observed patterns, and suggest how these can be accommodated in management practices to further improve conservation of biodiversity across the entire timber production landscape, and not just in the remnant patches.

2.2 Methods

2.2.1 Study area and design

The study area covered Baynesfield, Richmond, and the Byrne Valley areas in KwaZulu-Natal, South Africa (29° 48' 24.14" S; 30° 14' 4.4" E). This area is dominated by forestry production surrounding remnant patches of Southern Mistbelt Forest and Midlands Mistbelt Grasslands (South African National Biodiversity Institue 2018). Sampling was conducted on three timber estates (Baynesfield, Enon and Highlands), as well as on a privately-run conservancy. A mix of land-use types, with the focal biotopes adjoining each other were present at the study area. Biotopes identified were: 1) closed canopy plantations (CC), consisting of mature *Eucalyptus* trees >7 years old which formed a closed canopy, 2) open canopy plantations (OC), where the trees (*Eucalyptus*) were 2-5 years old and the canopy still open, 3) natural remnant grasslands (G), that make up the majority of the natural areas and formed an extensive EN, and 4) small patches of indigenous forest (F) (Figure 2.1).

Transects were laid out across the interface of two adjacent biotopes. Where transects of the same type were alongside each other, a distance of 300 m was maintained to ensure relative independence of samples. Each transect was 120 m long and composed of two 60 m-lengths extending perpendicular to the edge, into each biotope. The transect types identified for sampling were CC - G (n = 7); OC – G (n = 8); CC – F (n = 8); CC – OC (n = 7) and F - G (n =6) (Figure 2.1). In total, 36 transects were sampled. Spatial distribution and availability of grasslands, indigenous forests, and *Eucalyptus* plantations limited the number of transects per transect type, as well as the distribution of transects across the study site. At each transect, six stations were established at increasing distances from biotope edge, at stations 15 m, 30 m, and 60 m into both biotopes.

2.2.2 Invertebrate sampling

Sampling was conducted during March and April 2017. Four pitfall traps, 70 mm in diameter, were deployed at each sampling station along each transect, resulting in 24 traps per transect. Traps were half filled with a 1:1 mixture of ethylene glycol and water, with a small amount of dishwashing soap added to help break the surface tension of the trap solution (Sawmways et al. 2010). Traps were positioned in a line parallel to the biotope interface and were spaced 1 m apart. To ensure adequate sampling, traps were left out for one week. After this, the traps were collected, contents washed, and samples placed in 75% ethanol until sorting. Traps were kept separate and sorted as such. For data analyses the data from the four individual traps was pooled so that each station was represented by one collective sample.

Active searching was conducted for 10 min at each station by two observers. This entailed searching the soil surface and disturbing the leaf litter of a randomly selected 1 m^2 quadrat, and then collecting any invertebrate individuals visible to the naked eye. Specimens were placed directly into 75% ethanol and stored until sorted.

Insects in the following groups were identified: Coleoptera, Diptera, Hemiptera, Orthoptera, Hymenoptera (excluding Formicidae), and Formicidae on their own. Due to time constraints and the large number of specimens collected, as well as a lack of taxonomic knowledge among all insect groups, individual samples were identified to morphospecies. The morphospecies were then identified to family level. Parasitic wasps were excluded, due to their diverse and complicated taxonomy, although easily recognisable groups, such as Ichneumonidae, Braconidae and Chalcididae were included. Only adult specimens were identified, to prevent duplicate records of the same species at different life stages.

2.2.3 Environmental variables

At each station, soil type was classified, according to appearance, into three groups: sandy, loamy, and clay soils. Five measures of soil compaction were taken (using a Dicky-John Penetrometer), and averaged to get a single value per station. The point intercept method was conducted parallel to the interface of the biotopes to measure plant related variables. A graduated pole, in increments of 10 cm, was used to record the height of the vegetation layer intercepted, in addition, the number of plant structural classes intercepted were also recorded. Structural classes identified were grasses, shrubs, forbs and dead vegetation matter. Alien vegetation was also recorded. From these measures the average height at a station was calculated, as well as the percentage ground cover (defined as the layer of dead plant material including leaf litter), percentage bare ground and percentage alien vegetation at a station were determined. In the indigenous forests only the understory was sampled. Canopy cover in the indigenous forests and *Eucalyptus* plantations were estimated. In addition, *Eucalyptus* trees left to coppice were recorded. Topology was recorded by measuring the slope and elevation at a station.

2.2.4 Statistical analyses

To measure the overall effectiveness of sampling, the species accumulation curve for all species samples along all stations was plotted, and the Chao estimator (Chao 1987) used to estimate species richness across all sites. These analyses were conducted in the package *Vegan* (Oksanen et al. 2018) in *R* (R Core Team 2018).

Moran's I in the package *Ape* (Paradis et al. 2004) and a mantel test in the package *Ade4* (Dray & Dufour 2007) were conducted in *R* to test for spatial autocorrelation. However, there was no evidence for spatial autocorrelation within the data (Moran's I = -0.004, p = 0.998; Mantle test = 0.039, p = 0.124). Nevertheless, a random spatial variable was included in all linear models to account for any influence from spatial distribution of sampling sites (Bolker et al. 2009). To investigate the effect of transect type and sampling station position along the transects, as well as the interaction between the two, overall species richness, as well as the Shannon evenness of each sampling station was calculated using the package *Vegan* in *R*. A linear mixed effects model (LMM), was conducted for species richness, which was normally distributed. The Shannon evenness values were then transformed by dividing the values by the maximum Shannon evenness score calculated, resulting in values that ranged

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between 0 and 1. A generalized linear mixed model (GLMM), with binomial distribution and Laplace approximation was used for the transformed Shannon values. In both cases, 'farm' (i.e. location) was used as the random variable. LMM's and GLMM's were conducted using the *lme4* package (Bates et al. 2015) in *R*. These models contained no other main effects. Tukey post hoc comparisons were conducted when significance existed for the main effects.

Correlation between environmental variables was first determined to ensure that variables included in the models did not correlate, correlation between environmental variables was determined by calculating Spearman's R-vales for each possible pair, and when rho > 0.5 and < - 0.05, only one variable in a pair would be included in the model. Variables showing covariance were: grassland and compaction (rho = 0.583), vegetation cover and grasslands (rho = 0.750), and ground cover and closed canopy plantations (rho = 0.516). Therefore compaction and vegetation cover were excluded from any models involving grasslands, and ground cover from models pertaining to closed canopy plantations. When environmental variables showed covariation only one variable was included in the models, with the selection based on ecological appropriateness of each variable.

In order to investigate the role of the various environmental variables, model selection for the best fitting environmental variables was performed using the *AICcmodavg* package (Mazerolle 2017). This was done for species richness of overall dataset, individual transects types, as well as for the species richness of the individual biotopes. Responses of species richness along the transects and biotopes were used to assess the relative impact of differences in plantation age. For the model selection procedure, individual models were created with all possible combinations of environmental variables. LMMs were used, as all datasets here were normally distributed. The best candidate model was the one with the lowest AICc value. To determine the effect of each environmental variable on species richness, selected variables were tested following the LMM procedure, as some variables were categorical, and model averaging could not be used. An entire model, containing the variables in the best candidate model, was created, and compared against successive models, where an alternating environmental variable was removed to determine those variables with significant effects on species richness. 'Farm' was used as the random variable for the overall model, while 'transect number' (the unique identification code for each sampled transect) was used as the random variable for the transect type and biotope models. For the biotope specific models, "neighbouring biotope" was included to determine if changes in neighbour impact species richness responses.

For the multivariate statistical analyses, permutational multivariate analysis of variance (PERMANOVA) (Clark & Warwick 2001) and Primer-6 (Clark & Gorley 2006) were used. Initially, a PERMANOVA considering transect type, position along the transects as well as their interaction, for the entire assemblage was conducted. To investigate the role of contrast between the different biotopes, pairwise PERMANOVAs were run on the species assemblages of the five different transect types. To

do this, data was square-root transformed to reduce the influence of common and abundant species, and then Bray-Curtis similarity measures calculated. A pairwise PERMANOVA was then run to establish the influence of position along transect, unrestricted permutation of the raw data was used as well as 9999 permutations. Distance based linear models (DistLM) where used to determine the environmental variables driving assemblage structure across the entire assemblage of samples. The Bray-Curtis similarity matrix was used in the DistLM procedure, following a step-wise selection procedure, with the selection of the models based on the AICc values. DistLMs were used to determine the most influential environmental variables along each transect type following the same selection procedure as before. Following this, the environmental variables driving the assemblage structures of the individual taxonomic groups was investigates by separating the data by taxonomic groups, and DistLMs following the above mentioned procedure were run.

Overall beta diversity between the individual biotopes was investigated using the beta function in the *R* package *BAT* (Cardosa et al. 2014), and using Sørensen measure of dissimilarity. This was done to determine which proportion of turnover between biotopes is due to species replacement vs. changes in number of species. Beta diversity was calculated by partitioning overall beta diversity into its constituent parts of species turnover (differences in species identity between sites, i.e. species replacement) and change in species richness, between biotopes.

2.3 Results

Overall, 20 182 specimens belonging to 491 morphospecies were sampled across all six target taxa. The species accumulation curve did not reach an asymptote (Appendix 2.1) and the Chao-estimated total species richness was 714.74 (±43.33) species. Ants were the most abundant group, contributing 50.68% of the total number of specimens collected yet they only represented 5.30% of all the morphospecies identified across all six taxa. Coleoptera were the most speciose group of insects, representing 37.27% of all sampled morphospecies, but only making up 13.27% of the total sampled number of individuals. Diptera were the second most abundant and speciose group (29.58% and 20.93% respectively), as well as being the most family rich group sampled, and represented by 28.72% of all families sampled. Hemiptera, Hymenoptera and Orthoptera were the least abundant and diverse groups.

2.3.1 Contrast between biotopes

Sampling station, and transect type, were both significant for observed species richness ($\chi^2 = 150.79$, p < 0.001, and $\chi^2 = 14.83$, p < 0.01 respectively) although show no interaction ($\chi^2 = 22.45$, p > 0.05). Shannon Evenness values show that position and transect type ($\chi^2 = 16.69$, p > 0.05, and $\chi^2 = 2.58$, p > 0.05 respectively), as well as interaction between the two, are non-significant ($\chi^2 = 9.33$, p > 0.05).

Assemblage composition results indicate that sampling station (Pseudo-F = 3.14, p < 0.001), and transect type (Pseudo-F = 5.82, p < 0.001), are significant assemblage composition. As with species richness, there was no significant interaction between transect type and position along the transect.

Species composition differed significantly between stations of both biotopes along all transect types, except for stations along the CC-OC transects (Appendix 2.2). These differences in overall species composition become more apparent when considering each insect order independently. Coleoptera, Diptera and Hymenoptera species compositions are significantly different between all biotopes. While Formicidae, Hemiptera and Orthoptera species assemblages are also all significantly different across all biotopes, except for the species in the closed and open canopy plantations (Appendix 2.3).

Distance based linear models showed that overall species composition of grasslands and forests differed from each other, as well as from open and closed canopy plantations, while species composition of open and closed canopy plantations did not differ significantly from one another (Figure 2.3). Elevation, slope, leaf litter, average vegetation height, ground and vegetation cover, as well as canopy cover and presence of loamy soils, were driving the observed patterns in species assemblages (Table 2.2). Various environmental variables were responsible for different patterns observed between different insect orders, with canopy cover and ground cover being important variables for all orders sampled (Table 2.2).

Species assemblages differed between the four biotope types. Grasslands and forests were the most distinct from one another, with beta diversity being largely driven by species replacement (Table 2.3). Between CC and OC plantations, beta diversity was solely driven by species replacement, and not by differences in species richness. Overall beta diversity values between OC and CC plantations was low, indicating less dissimilarity between the two biotopes (Table 2.3). Both CC and OC plantations were equally dissimilar to grassland sites (Table 2.3), yet grassland assemblages appeared to be closer to those of the plantations than to those of natural forests (Table 2.3).

2.3.2 Environmental factors and plantation ages

Overall, the best model selected average vegetation height and alien vegetation presence, yet only average vegetation height had a significant positive effect on species richness (Table 2.1). Species richness, when sampled along transects bisecting natural and plantation biotopes, was highest in the natural biotopes (Figures 2.2 A, C and E). Forests supported higher numbers of species than grasslands when F-G transects are considered (Figure 2.2 D), and in CC-OC transects, there were no significant differences in species richness (Figure 2.2 B). Along the OC-G transects, aspect, soil type, and ground cover were important variables, with all three having significant impacts. Notably, insect species richness decreased as ground cover increased. In the case of the OC-CC transects, aspect and vegetation cover were both selected for during model selection, with both having significant effects on species richness, i.e. as vegetation cover increases so too does insect species richness (Table 2.1). Differences between species richness of the OC-CC transects occurred between different aspects. As with the OC-CC transects, variables important for F-CC transects are aspect and vegetation cover, with the addition of soil type, although only vegetation cover had a significant and positive effect on insect species

richness. For the F-G transects, only aspect and soil type were selected, with soil type the only variable having a significant impact on species richness.

Species richness within the separate biotopes was influenced by a range of environmental factors (Table 2.1). In CC plantations, neighbouring biotope, soil type, average vegetation height, and vegetation cover, were selected, yet only vegetation cover and average height had significantly positive effects on insect species richness. In OC plantations, no environmental variables had any significant effect on species richness, even though neighbouring biotope, soil type, leaf litter depth, and vegetation cover were all selected. In forests, neighbouring biotope, soil type, and leaf litter were selected, but it was only leaf litter that significantly affected insect species richness, where it was a negative relationship. Insect species richness within the grasslands was only influenced by neighbouring biotopes, with species richness in grasslands adjacent to forests being lower than that of grasslands adjacent to CC or OC plantations.

2.4 Discussion

Indigenous forests and grasslands had higher species richness than either closed or open canopy plantations, highlighting the importance of natural habitats for maintaining epigaeic insect species within these production landscape mosaics. Presence of *Eucalytus* plantations, closed or open canopy, did not affect insect species richness of adjacent natural areas. In contrast, pine plantations adjacent to natural forests have been shown to affect species richness and abundance of arthropods in adjacent natural forests (Yekwayo et al. 2016a, 2017).

2.4.1 Contrast between biotopes

Mature *Eucalyptus* plantations are superficially similar to indigenous forests in terms of structure, leaf litter, stumps, and wood fragments which are available as resources to epigaeic insect species (Peyras et al. 2013), thereby reducing the contrast between these two biotope types. A more notable difference between indigenous forests and the mature *Eucalyptus* plantations is the lack of vegetation diversity in the plantations, being dominated by a single cultivated species. The resultant contrast can reduce the various niches available, resulting in a lack of specialist species, with only generalist or opportunist species remaining (Magoba & Samways 2012; Roets & Pryke 2013).

Indigenous forests are higher in species richness and have species assemblages distinct from those of the open canopy and closed canopy plantations. Although, similar to the pine-forest interface (Yekwayo et al. 2016b, 2017), richness here is higher at closed canopy sampling stations 15 m from the indigenous forests. This is likely due to the interface between the two biotopes containing a mix of forest plant species, so enabling the persistence of insect species under the plantation canopy (Geldenhuys 1997; Kotze & Samways 1999; Pryke & Samways 2012). Forest dung beetles in Brazil, have stronger responses to high contrast landscapes, open or younger plantations alongside indigenous forest, than they do to lower contrast landscapes, such as the mature plantation-forest interface (Peyras et al. 2013).

Here, contrast between *Eucalyptus* plantations and indigenous forests appears to be high, as only superficial spillover at the edge sites was recorded. No open canopy-forest transects were possible, and so it is not possible to determine whether the insect assemblages here respond to an interface of higher contrast, where temperature differences and structural differences, between the open canopy plantations and indigenous forests, would be greater (Peyras et al. 2013).

Afforestation of grasslands leads to shifts in species assemblages, as grassland species are replaced by forest species (Reino et al. 2009; Dröse et al. 2019). Ant diversity of grasslands decreases with an increase of herbaceous vegetation during natural forest expansion (Dröse et al. 2019). Similarly, afforestation of grasslands by non-native, and often invasive, hardwood trees leads to loss of specialist insect species, as well as a reduction in species richness and abundance (Armstrong & Hensbergen 1995; Brockerhoff et al. 2008; Bremer & Farley 2010; Nepstad et al. 2014; Rodriguez et al. 2014). Here, afforestation of grasslands by *Eucalyptus* plantations, leads to the localised loss of grassland species, these grassland species are not supported by plantations as they lack the necessary environmental conditions. Plantations are structurally similar to forests (Peyras et al. 2013)**,** and so provide a buffered, shaded, thermal environment. In contrast, grasslands species are adapted to oscillating thermal conditions, and are often shade-intolerant (Dröse et al. 2019), and therefore are not expected to persist in the plantation environment. Contrast between grasslands and plantations, both closed canopy and open, is high, and results in environmental filtering, where species adapted to specific habitat conditions are selected for (Dröse et al. 2019). Grasslands here, had higher species richness than both plantation age classes, as well as highly distinct assemblages compared to the other sampled biotopes.

Environmental factors responsible for the formation of these different assemblages are highly specific for each biotope, indicating that this is a landscape of high contrast. Species assemblages of the individual biotopes are not affected by the surrounding biotopes, be it grassland, forest, or plantation. Species richness of grassland did not differ in accordance with distance to edge, nor in response to different neighbouring biotopes. Although the species richness observed at 15 m in grasslands neighbouring forests is significantly lower than further into the grasslands, grassland assemblages next to indigenous forests were the same as grassland assemblages alongside both plantation age classes.

Spatial scale is an important aspect to take into account (Elliott et al. 2002; Steffan-Dewenter et al. 2002; Purtauf et al. 2005; Reino et al. 2009), perhaps, as Ewers et al (2008) argue for temperate beech forests in New Zealand, edge effects can penetrate >2 km, rather than the 30 m currently employed as a cut off value here for grasslands (Pryke & Samways 2012). This would result in an entirely different assemblages at the edges of the patches, devoid of habitat specialists, and lead to heterogeneous microhabitats and increased stochastic events at the edges (Ewers & Didham 2008). If this were the case in this study, it would effectively mask any effect due to the distance covered by the transects in

the indigenous forests (60 m). Yet, the relative lack of assemblage mixing at the ecotones here (15 m sampling stations), would imply that the edge effects here are smaller and contrast higher, resulting the near-edge assemblages being the same as those further away (De Smedt et al. 2019)**.**

When considering the three most abundant taxa individually, Coleoptera, Diptera and Formicidae, it is clear that the grassland assemblages are very distinct, as all three groups form separate assemblages in each biotope. Coleptera and Diptera in the forest also form assemblages separate from those in the plantations. Ant assemblages between the forests and plantation age classes overlap as a result of similar environmental conditions between the three biotopes. Coleoptera can show strong responses to habitat alteration and fragmentation, with loss of specialist species from the surrounding altered biotopes, and an increase of generalist species along the edge (Rainio & Niemela 2003; Soga et al. 2013; Yu et al. 2014). As known indicators of habitat quality (Rainio & Niemela 2003), Coleoptera respond to changes in resource distribution, niche environments, as well as changes in microclimate. Similarly, ant species assemblages respond in a similar way as Coleoptera, although here the ants showed a stronger association with the environmental conditions of plantations and indigenous forests, likely due to similarities in leaf litter load and nature of the soils between indigenous forests and plantations (Yekwayo et al. 2017). Above-ground habitat disturbance affects ant assemblages mainly indirectly, as most species nest below the ground, and activities directly affecting the soil tend to have more direct influences on ant assemblages (Andersen 2019). The effects of landscape moderation are slower below ground than above ground (Tscharntke et al. 2012). Ants, however, do respond to habitat disturbance and biotope type, but based on life cycle characteristics, the various species respond differently (Dröse et al. 2019).

2.4.2 Plantation age classes

Overall species assemblages within closed and open canopy plantations were indistinguishable from each other. Small beta diversity changes between the two canopy types were solely driven by species replacement, i.e. the turnover of species between the two plantation age classes. Beetle, fly, and wasp assemblages were the only three to show any significant differences between the two biotopes. This evidence for low beta diversity between the two plantation biotopes, as well as low distinction between assemblages, is perhaps due to environmental conditions on the plantation floor of the two plantation age classes being similar. Distance based linear models help to explain this finding. Grassland and forest assemblages are very distinct, with grassland assemblages responding to vegetation cover, while forest assemblages responded to elevation and canopy cover. Open and closed plantation assemblages responded to the same environmental variables, namely: ground cover, slope and loamy soils. Ground cover and leaf litter, provides considerable resources for ground dwelling insects (Yekwayo et al. 2017), and increases microhabitat complexity (Dröse et al. 2019), as was the case here in both plantation age classes. The species assemblages within both plantation age classes are not dependent on the adjacent biotopes in order to survive. With the high contrast across this landscape, very few species are able to

move into these plantations from the indigenous biotopes. So, the diversity observed within the plantations results largely, from within the plantation assemblages as they are able to maintain themselves.

Timber production has dominated this landscape for about 50 years, and it is likely that the generalist species in the plantations have had time to colonise and become resident in this changing production landscape. Although in disturbed environments, generalist species in both open canopy and closed canopy plantations are able to tolerate these conditions, and populations are able to maintain themselves. Resilience exists within in the plantation network, with the insect species that are present being able to recover and maintain levels of diversity irrespective of the age or canopy cover of the plantation. Source populations of insects exist within the plantation network, and post felling, these populations will enable the reestablishment of assemblages at recently felled sites as source populations can be found in other regions within the plantations. Therefore, maintaining connectivity between plantations of varying ages during felling is essential, as this will allow insect assemblages to re-establish within the felled areas.

Pryke and Samways (2012) argue that the different growth stages of pine plantations has little effect on the core of the adjacent grassland corridors. Here, there was no significant difference in response of insects to different aged *Eucalyptus* plantations. Grassland and forest assemblages, as well as species richness, were significantly different from those of plantations, irrespective of transect type, neighbouring biotope, and station position sampled. As various taxa respond to disturbance in a variety of ways, a 32 m edge zone is considered the approximate maximum distance over which plantation forestry impacts grasslands (Pryke & Samways 2012).

2.4.3 Environmental factors as drivers of biodiversity

No singular environmental factor was identified as a sole driver for patterns observed here, although average vegetation height and vegetation cover can be the most frequently occurring important variables, positively impacting species richness (Morris 2000; Boulton et al. 2005; Dröse et al. 2019). This is likely due to an increase in resource availability with both increasing height and cover, providing resources integral to the persistence and maintenance of species (Peyras et al. 2013; Dröse et al. 2019). Depending on the target taxon or assemblage associated with any particular biotope, an array of environmental factors was found be important for driving both richness and composition of assemblages here. Habitat heterogeneity is therefore an important management consideration across a landscape mosaic, as it ensures that a suite of ecological resources are present and able to fulfil the requirements of specialist and generalist species alike (Bazelet & Samways 2012; Crous et al. 2013; van Schalkwyk et al. 2017).

2.5 Conclusions

Natural areas within timber production landscapes mosaics are invaluable for maintenance of indigenous species. Both plantation age classes supported lower species richness, and very different

assemblage composition to the natural biotopes. Without these ecological networks within this timber production landscape, the unique assemblages of insects, characteristic of the grasslands and indigenous forests, would be lost. Ecological integrity of the system would also be compromised, as the ENs play an integral role for natural ecosystem function and provision of ecosystem services (Samways et al. 2010; Samways & Pryke 2016). As no one environmental factor is responsible for the patterns observed, maintenance of natural areas (Fischer et al. 2013), as well as habitat heterogeneity (Crous et al. 2013), is imperative for ensuring that all required conditions for maintenance of these insect assemblages are met. Furthermore, the indigenous forests here play a role, albeit small, in the maintenance of species richness and species assemblages within the plantation mosaic.

The rotational nature of timber production in these areas does not have an impact on insect diversity, in both the surrounding grasslands and indigenous forests, as well as within the plantations. Assemblages are homogenized between open and closed plantations, with recolonization after harvesting likely occurring from other plantation areas, leading to re-establishment of pre-felling insect assemblages after felling has occurred.

Management of these timber production landscapes should continue to focus on the preservation of natural areas in the form of conservation corridors making up ecological networks, as these are integral for maintenance of biodiversity across the landscape. Contrast between the grasslands and plantations will continue to exist, but at the interface of forests and plantations, contrast should be actively managed to be minimised by managing alien plant encroachment along the forest edges. This may assist in the conservation of indigenous species beyond the boundaries of the indigenous forests. Finally, plantations are not devoid of biodiversity, but maintain their own unique insect assemblages and, even though it appears that felling is not impacting these assemblages over the rotation cycle, care should be taken while planning where to fell to ensure that there is some form of connectivity between cleared and young growth areas, with the more established mature *Eucalyptus* plantations enabling recovery of insect assemblages within the felled areas.

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2.7 Tables and figures

Table 2.1: Results of model selection and models conducted on overall species richness of all transects sampled, on species richness of individual transects sampled, as well as on species richness of individual biotopes sampled. x Denotes environmental variables included in model selection procedure. Chi square (χ^2) values are shown (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). Abbreviations: CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland and F- Forest.

Model	Variables									Best model		
	Aspect	Average plant height (cm)	Alien vegetation	Soil type	Soil compaction	Vegetation cover (%)	Ground cover (%)	Neighbour biotope	Leaf litter depth (cm)	Variable	χ^2	df
Overall species richness	x	x	x	x						Average plant height	6.83**	1
										Alien vegetation	0.15	1
Transect types												
CC-G	x	x	x	x						Average plant height	44.06***	1
										Alien vegetation	1.03	1
CC-OC	x	x	x		x	x				Aspect	16.33**	4
										Vegetation cover	3.90*	1
F-CC	x	x		x	x	x				Aspect	8.67	5
										Soil type	1.09	2
										Vegetation cover	34.12***	1
F-G	x	x	x	x			x			Aspect	4.074	2
										Soil type	10.91***	2
OC-G	x	x	x	x			x			Aspect	12.10*	5
										Soil type	8.54*	2
										Ground cover	13.94***	1
Biotope types												
CC		x		x		x		x	x	Neighbouring biotope	4.19	2
										Soil type	0.09	1
										Vegetation cover	7.43**	1
										Average plant height	17.64***	1
OC		x		x		x		x	x	Neighbouring biotope	0.063	1
										Soil type	0.13	1
										Vegetation cover	2.94	1
										Leaf litter depth	1.71	1
F		x			x	x		x		Neighbouring biotope	3.67	1
										Soil type	3.13	2
										Leaf litter depth	6.08*	1
G		x			x	x		x		Neighbouring biotope	11.32**	2

Table 2.2: Results from DistLM following a step-wise selection procedure indicating the environmental variables, from the sequential model, significantly impacting the species assemblages sampled across each transect type, as well as the environmental variables responsible for shaping the assemblages of the six insect orders. Pseudo-F values are shown here (* $p < 0.001$). Abbreviations: CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland and F- Forest.

	Aspect	Average height	Canopy cover	Clay soil	Coppice	Distance to edge	Elevation	Ground cover	Leaf litter	Loam soil	Sandy soil	Slope	Vegetation cover	Total percentage explained variance
Transect types														
CC-G			5.81*				3.33*					3.52*		26.37
OC-G							3.04*					4.61*	7.77*	27.41
CC-OC	3.30*						5.01*							18.06
F-CC									3.68*				8.51*	21.98
F-G	3.13*		10.14*											29.65
Insect orders														
Coleoptera			10.95*	2.53*			4.03*	2.56*	3.33*				21.31*	18.43
Diptera	2.53*	2.94*	21.53*				4.67*	14.39*	5.08*	2.85*		2.31*	2.71*	23.7
Formicidae			23.81*				17.49*	11.55*	3.52*	3.14*		3.78*	4.44*	26.49
Hemiptera	2.39*		24.74*	3.60*				4.23*				2.08*		15.39
Hymenoptera			11.33*					20.02*	2.55*	2.89*				15.38
Orthoptera			28.50*	2.13*			3.30*	10.89*		4.82*		10.52*		23.79

Table 2.3: Beta diversity between biotope types, shown here are total beta diversity, replacement beta diversity and richness beta diversity. Abbreviations: CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland and F- Forest.

	CC	OC	G
Total beta diversity			
OC	0.41		
G	0.57	0.56	
F	0.51	0.56	0.66
Beta diversity due to replacement of species			
OC	0.41		
G	0.27	0.26	
F	0.44	0.50	0.41
Beta diversity due to differences in species richness			
OC	0.00		
G	0.31	0.31	
F	0.07	0.07	0.24

Figure 2.1: Map showing details of study sites, including the farms sampled and transect positions (symbols represent the central point of the transect). Green blocks indicate location of *Eucalyptus* plantations.
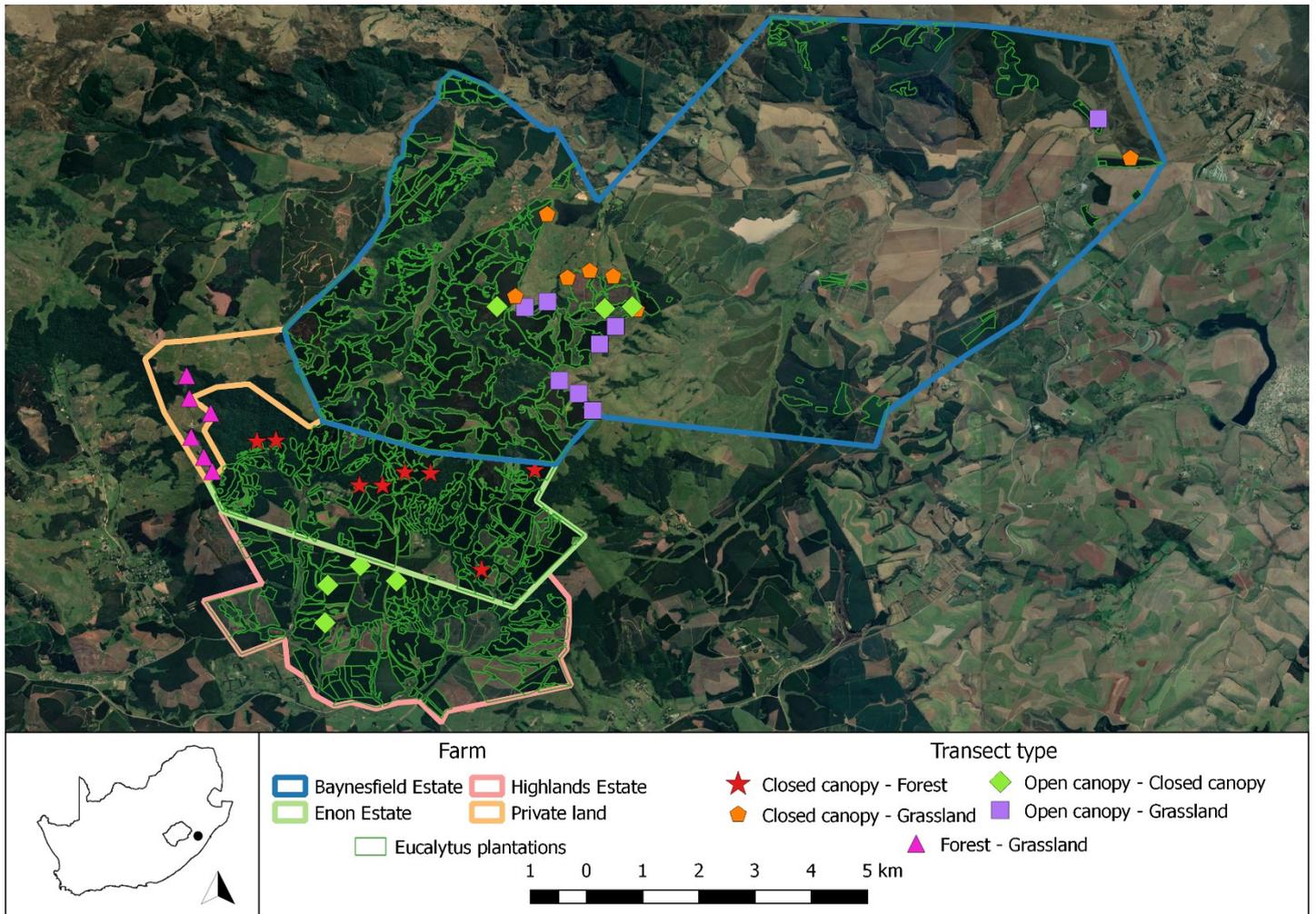
Satellite image from Google Earth (Image@2019 Maxar Technologies)

Figure 2.2: Species richness at each sampling station along the individual transect types sampled, A: Closed canopy plantation - Grassland; B: Closed canopy plantation – Open canopy plantation; C: Closed canopy plantation - Forest; D: Forest - Grassland and E: Grassland - Open canopy plantation.
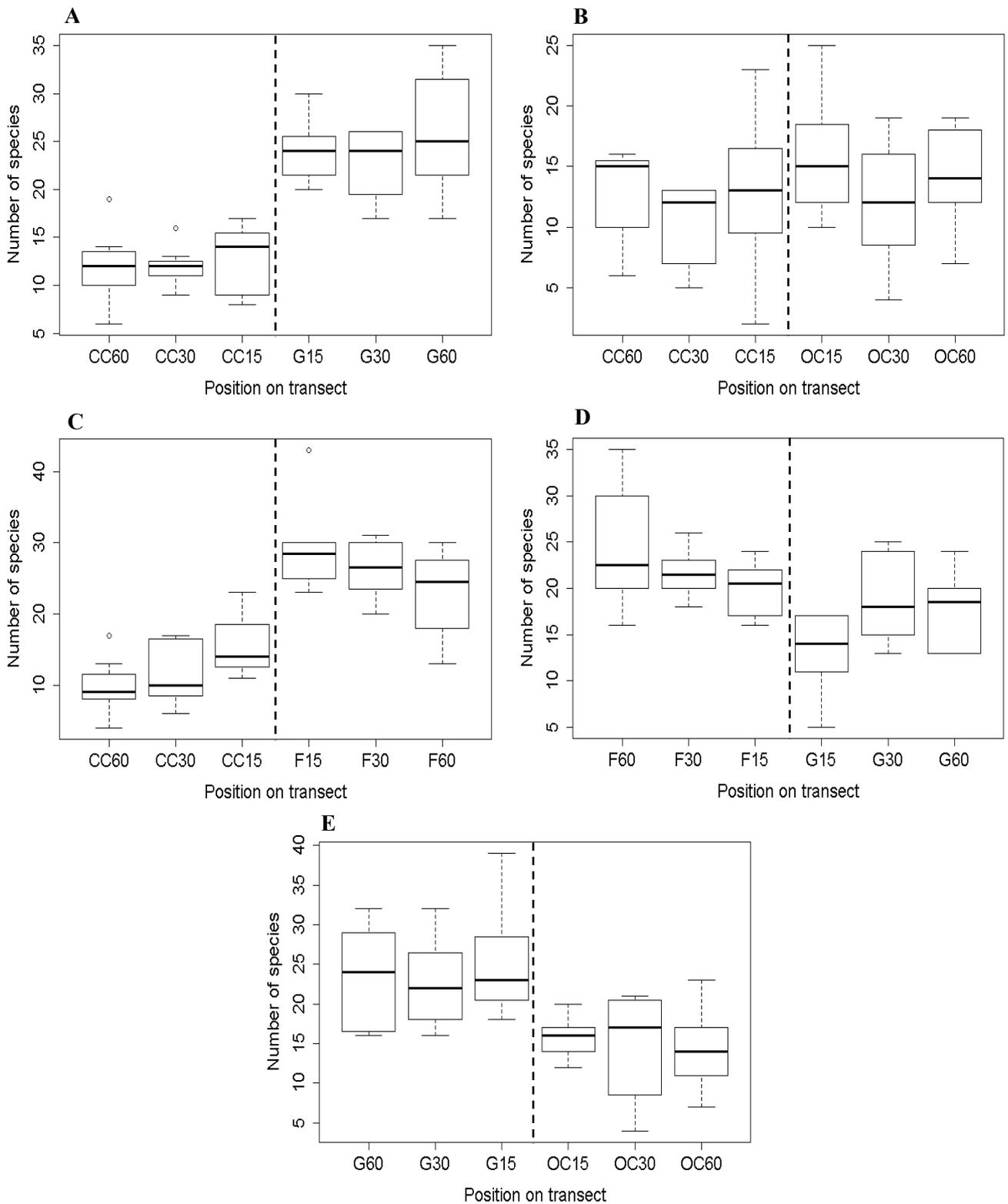
Figure 2.3: Similarity of overall species assemblages, categorized by biotope types: CC- Closed canopy plantation, G – Grassland, OC – Open canopy plantation and F- Forest. Environmental variables depicted are: GC – Ground cover, Loam type soil, Slope, Canopy cover, AveH – Average vegetation height, Elev – Elevation, and VC – Vegetation cover. Lit – Leaf litter depth.
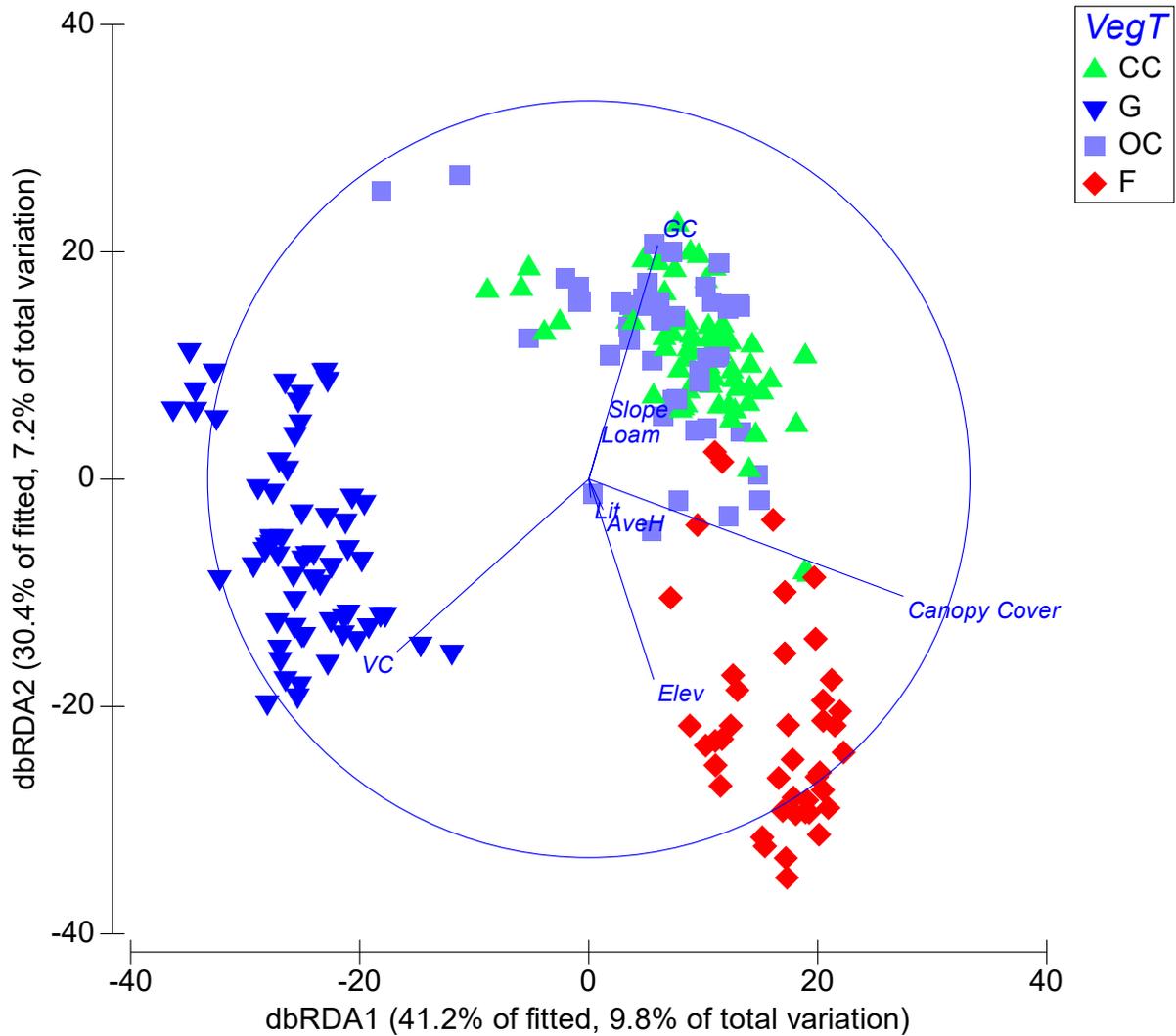
2.8 Appendices

Appendix 2.1: Species accumulation curve, and 95% confidence interval, for epigaeic species sampled in both pitfalls and active searches across all sampling stations (n=216) irrespective of transect or biotope type.
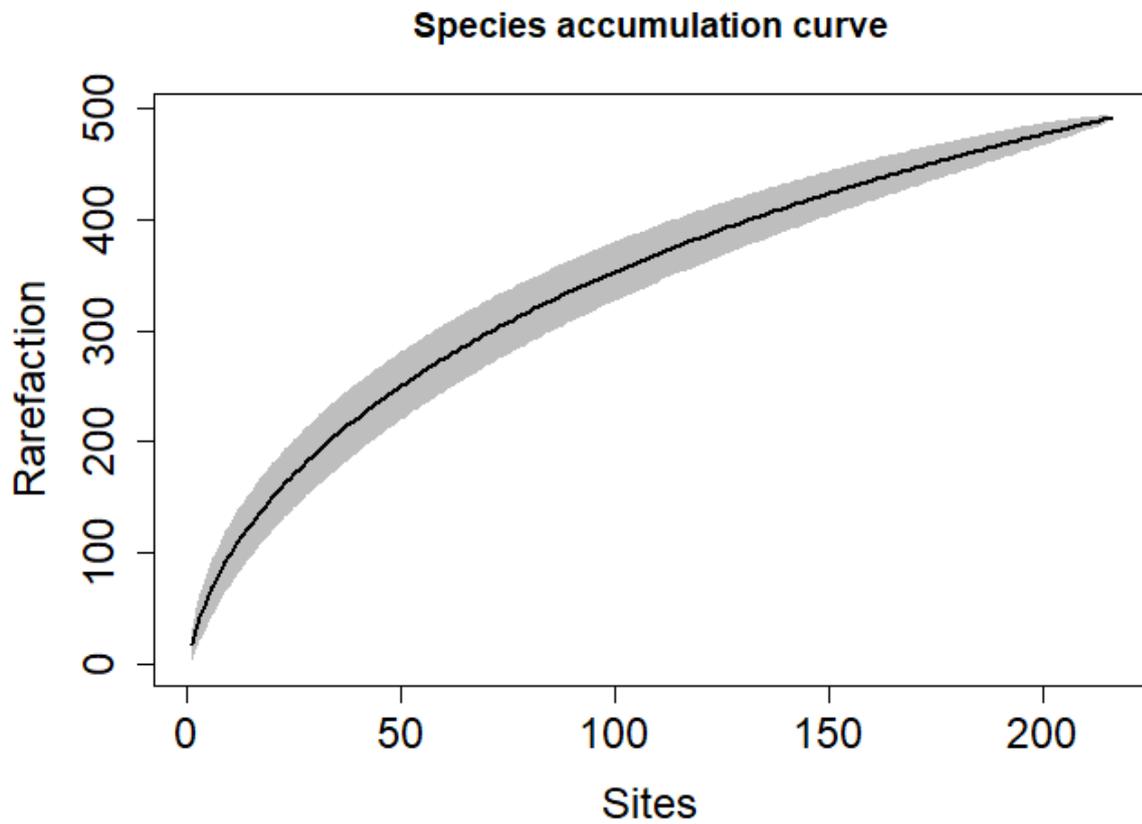
Appendix 2.2: Results from PERMANOVA posthoc tests showing t-values and the significant differences in insect assemblages at the various sampling stations of each transect type (* p < 0.05). Abbreviations: CC- Closed canopy plantation, OC- Open canopy plantation, G-Grassland and F- Forest. 60 – 60 m sampling station, 30 – 30 m sampling station, 15 – 15 m sampling station.

	CC15	CC30	CC60	G15	G30
Closed canopy – Grassland					
CC30	0.74	-			
CC60	0.91	0.66	-		
G15	1.52*	1.30*	1.50*	-	
G30	1.59*	1.38*	1.6*	0.67	-
G60	1.56*	1.35*	1.49*	0.68	1.68

	OC15	OC30	OC60	G15	G30
Open canopy – Grassland					
OC30	0.76	-			
OC60	0.72	0.56	-		
G15	2.06*	1.72*	1.87*	-	
G30	1.80*	1.80*	1.69*	0.63	-
G60	1.70*	1.48*	1.59*	0.80	0.70

	CC15	CC30	CC60	OC15	OC30
Closed canopy – Open canopy					
CC30	0.89	-			
CC60	1.07	0.97	-		
OC15	1.04	1.13	1.19	-	
OC30	1.49	1.08	1.48	1.21	-
OC60	1.13	1.07	1.07	0.86	1.10

	CC15	CC30	CC60	F15	F30
Forest – Closed canopy					
CC30	0.75	-			
CC60	0.83	0.80	-		
F15	1.96*	2.10*	2.32*	-	
F30	1.90*	1.97*	1.21*	0.80	-
F60	1.63*	1.99*	1.9*	1.05	0.74

	F15	F30	F60	G15	G30
Forest – Grassland					
F30	0.94	-			
F60	1.03	0.85	-		
G15	1.74*	1.8*	1.77*	-	
G30	1.97*	1.96*	1.92*	0.57	-
G60	2.03*	2.00*	1.98*	0.58	0.75

Appendix 2.3: Results from PERMANOVA posthoc tests showing t-values, and the significant differences between biotope types for the six sampled insect orders (* p < 0.05). Abbreviations: CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland and F- Forest.

	Coleoptera		
	CC	**OC**	**G**
OC	1.74*	-	
G	4.20*	2.91*	-
F	5.01*	3.86*	3.37*
	Diptera		
	CC	**OC**	**G**
OC	3.11*	-	
G	3.89*	4.58*	-
F	4.90*	3.82*	4.75*
	Formicidae		
	CC	**OC**	**G**
OC	1.36	-	
G	4.03*	3.60*	-
F	4.06*	4.95*	5.97*
	Hemiptera		
	CC	**OC**	**G**
OC	1.1408	-	
G	4.3595*	3.8146*	-
F	2.2063*	1.7351*	3.4362*
	Hymenoptera		
	CC	**OC**	**G**
OC	1.56*	-	
G	1.96*	1.79*	-
F	4.69*	4.32*	5.10*
	Orthoptera		
	CC	**OC**	**G**
OC	0.99	-	
G	4.55*	3.69*	-
F	2.57*	2.26*	2.79*

Chapter 3:

Role of contrast and species biotope fidelity in determining spillover of foliage-dwelling insect assemblages between natural and timber production patches

"Don't kill that fly!

Look- it's wringing its hands,

wringing its feet."

-Issa

Abstract

Boundaries between biotopes exist the world over. Depending on the degree of contrast between adjacent biotopes, these boundaries can either help or hinder the spillover of species between one biotope and another. Spillover is highly dynamic and depends on species specialisation, as well as biotope characteristics, making spillover and the resultant species composition difficult to predict. Differences in primary productivity of biotopes can play a role in species spillover. Agroecosystems comprise a landscape mosaic of croplands interspersed with semi-natural and natural biotopes. Across these mosaics, species dependencies on particular biotopes can help to determine how spillover impacts species distributions across these agroecosystems. By assessing species associations of foliage dwelling insects, I aim to determine which environmental factors impact the ability of species to persist in adjacent biotopes, how contrast impacts species spillover, and finally, how spillover in this mosaic landscape is influenced by species fidelity. Here, the grasslands and forest assemblages were very distinct, while the open and closed canopy plantation assemblages were not. Biodiversity within the plantations is self-sustaining, and more likely to spillover between open and closed plantation than between plantations and natural areas (or vice versa). Interestingly, cultural species from the open canopy plantations spillover into the grasslands, but not from the closed canopy plantations. There is also evidence for spillover of forest species into the closed canopy plantations. In both cases, lower contrast between adjacent biotopes facilitates this spillover, which was supported by the positive response of forest species to vegetation cover. The indigenous forests and grasslands are unique and diverse in their insect assemblages, and so are essential for maintenance of biodiversity overall, as these natural, and semi-natural biotopes enable a wide range of environmental variables to be maintained, which further enables species conservation in the contrasting landscape. Furthermore, biotopes with similar contrast levels to plantations are most likely to have greater spillover into the plantations. In sum, having a mix of natural biotopes with a variety of open and closed canopies would best promote biodiversity at landscape and patch levels across this production mosaic.

3.1 Introduction

Boundaries between biotopes, habitats or topographic features, exist the world over. These boundaries, or edges, can be natural or anthropogenic (Fahrig 2017), and are dynamic and ever changing. The degree of contrast, or structural dissimilarity, between biotopes impacts the degree to which biotic and abiotic factors of two neighbouring biotopes are able to mix (Didham & Lawton 1999; Fagan et al. 1999). Edges, depending on the structural contrast, can be classified along a gradient from hard to soft, and either facilitate or inhibit the spillover of species (Didham & Lawton 1999). Depending on species adaptations, life history traits, and degree of specialisation, the direction and magnitude of spillover between patches can change, as a result spillover between biotopes is highly dynamic (Loreau et al. 2013).

Spillover occurs when species from one biotope move, usually across a habitat boundary, into an adjacent, non-natal biotope. Flow of organisms and resources follow concentration gradients (Rand et al. 2006; Loreau et al. 2013). Areas of higher concentrations of individuals act as sources, and facilitate spillover into neighbouring biotopes (Tscharntke et al. 2005; Rand et al. 2006). Spillover of species between biotopes is also linked to changes in habitat quality, where resources are low, species will spillover when they respond to better habitat quality, be it climatic conditions, resources such as food, or refuges (Reis & Sisk 2004; Rand et al. 2006). Indicative of spillover occurring in a non-target biotope, is the decrease in the abundance of spillover species with an increase in distance from the edge and source biotope (Brudvig et al. 2009).

Driven by resource distribution around an edge, species responses to edges may be positive, negative, or neutral (Ries et al. 2004; Rand et al. 2006). Inferences regarding the patterns of species spillover can be made according to the spatial distribution of resources, as well as the type of edge. Low contrast edges, where complementary resources from both biotopes are present, would be expected to support higher numbers at the edge, and then decrease with increasing distance into the non-natal biotope (Rand et al. 2006). Where resources transition from a highly productive biotope into a less productive and lower quality biotope, no peak or complementary resource use at the edge is expected, while a typical decrease in spillover is expected deeper into the non-target biotope (Reis & Sisk 2004).

Specialist species typically exhibit a hard edge response, where the required resources, host species, or other specific resources are present up to the edge. However, beyond the edge, little to no spillover occurs. Specialist species at high contrast edges tend to aggregate at the edges and not spillover into the surrounding matrix (Rand et al. 2006). Such is the case in the prairie planthopper (*Prokelisia crocea*), where individuals aggregate at the edges of prairie patches when surrounded by bare open mudflats. This edge is highly impermeable, and the surrounding biotope unable to provide the resources the planthopper requires, and so spillover does not occur (Haynes & Cronin 2003).

Edge contrast between adjacent biotopes impacts the structure of the edge and degree to which various species are able to permeate the edge (Inclán & Marini 2015). Intermediate environmental conditions between adjacent biotopes occur at biotope edges (Ries et al. 2004). Depending on the species habitat requirements, one edge may be differentially permeable to a suite of species, as habitat specialists may be unable to permeate, yet the altered edge may not prove to be such a harsh barrier to generalist species. Species spillover is predicted to be low when contrast between biotopes is high, as the higher the contrast (Laurance et al. 2001), the more impermeable to movement the edge becomes (Inclán & Marini 2015). Forests are able to better support biodiversity in adjacent perennial crops such as apple orchards, while grasslands have a greater effect if adjacent agroecosystems dominated by annual crops (Inclán & Marini 2015). Permeability of edges, as well as contrast, is not a static feature, and can change over time. Amazonian forest edges, for example, when initially formed due to fragmentation, allow edge effects to penetrate up to 100 m, with wind damage occurring up to 400 m into the forest. As regrowth of pioneer species occurs at the edges, the microclimates are buffered, contrast is reduced, and species edge avoidance is reduced. This culminates in the constant change of edge permeability over time (Laurance et al. 2002).

Spillover of species from one habitat to another is able to alter both the species interactions, as well as the ecosystem services in the non-target biotopes (Tscharntke & Brandl 2004). Evidence for spillover of pollination services comes from Costa Rican coffee plantations benefitting from the enhanced native pollinator activity when situated 100 m or closer to tropical forest fragments. Native bee species reside in these fragments and increase the rate of pollination of nearby coffee trees in the plantations. Deeper into the coffee plantations, generalist, introduced bee species occur (Ricketts 2004). Species spillover impacts are not always positive. In tall grass prairie fragments, corn-root worm beetles (*Diabrotica barberi* and *D.* virgifera) invade from the neighbouring corn fields and cause significant damage to native sunflower species. Flowers within 30 m of the edge sustain significantly more damage through adult beetles feeding on flower heads, than those flowers situated further away from the edge. Spillover of these beetles into tallgrass prairie fragments, may pose a significant risk to prairie plant populations (Mckone et al. 2001), especially when fragments are small.

Agroecosystems comprise a landscape mosaic of croplands interspersed with semi-natural and natural habitats (Duelli & Obrist 2003; Fahrig 2003; Inclán & Marini 2015). The semi-natural, and natural habitat patches provide important and alternative resources for species, such as overwintering sites, alternative host species, and energy sources (Rand et al. 2006). Within these landscapes, there are stenotopic species, which depend solely on natural and semi-natural areas. These species would not occur in agricultural landscapes when semi-natural and natural areas are not present. Spillover of these species is minimal, as surrounding non-target biotopes would not be able to provide the necessary resources, and the edge contrast would be harsh. Cultural species are those which prefer croplands, and whose numbers are significantly lower in natural and semi-natural areas of the landscape. It would be

expected that these species would be able to spillover into adjacent natural biotopes more so than stenotopic species are able to. Finally, ubiquitous species are those whose numbers are equally well maintained in all habitats in the landscape, being able to persist in a range of habitats. To ensure ecological resilience of these agroecosystems, a broad range of the taxonomic spectrum must be maintained. For example in a valley constrained agroecosystem, 69% of all hoverflies identified, as well as 83% of all bee and wasp species were dependent on the semi-natural areas, without which they would not persist in the landscape (Duelli & Obrist 2003).

Ecological Networks (ENs) are formed by interconnected natural remnant habitat in the form of conservation corridors (Samways et al. 2010), with high habitat heterogeneity (Bazelet & Samways 2012; Crous et al. 2014a; van Schalkwyk et al. 2017) that are interspersed among timber plantations. They also effectively extend protected areas into the timber production areas (Samways & Pryke 2016; Yekwayo et al. 2016a), and conserve about 500 000 ha of remnant natural habitats within the South African timber production areas (Samways & Pryke 2016). Many current studies of spillover in agroecosystems focus solely on a single combination of biotopes. These ENs conserve a range of diverse habitats, all close to commercial tree stands, and so provide the opportunity for investigating spillover across a complex landscape, and between more than a single biotope pair.

This study aims to determine how a species biotope fidelity impact the spillover of species between different biotopes. To do this I address the following aims. 1) Determination of the environmental factors driving observed species richness and assemblages along the different transect types sampled. I hypothesise that ss a diverse array of biotopes is being investigated, important environmental variables will vary between biotope types which, in turn, may impact the degree to which species are able to persist in neighbouring biotopes. 2) Does contrast between biotope types impact the degree to which spillover occurs between the biotopes? High-contrast interfaces, such as those between grasslands and indigenous forests, present harsh, impermeable barriers to species, and therefore spillover is not expected to occur among many species. However, where contrast is low, between two tree dominated biotpes, much higher levels of spillover is expected. Finally, 3) determination of how spillover is affected by species displaying biotope fidelity by ascertaining whether there are any changes in species richness across the different pairs of biotopes and assemblages. Stenotopic species are expected to occur in significantly lower numbers in their non-target biotopes, as their resource requirements will not be met, while the ubiquitous species will occur in comparable numbers across all biotope types. Cultural species are expected to be present at higher numbers in the production areas of the landscape.

3.2 Methods

3.2.1 Study area and design

Baynesfield, Richmond and the Byrne Valley areas in KwaZulu-Natal (29° 48' 24.14" S; 30° 14' 4.4" E), South Africa, were chosen as the study area. Dominated by timber production, this transformed

landscape surrounds patches of Southern Mistblet Forest and Midlands Mistbelt Grasslands (South African National Biodiversity Institue 2018). Sites were located on a privately-run conservancy, as well as on three plantation estates (Baynesfield, Enon and Highlands). A mix of the four focal biotopes were present on all estates. Focal biotopes were identified as: 1) indigenous forest (F), 2) natural remnant grassland (G), 3) close canopy plantation (CC) of mature *Eucalyptus* forming a closed canopy with trees >7 years old, and 4) open canopy plantation (OC), where *Eucalyptus* trees were 2-5 years old, and the canopy not yet closed (Figure 3.1).

Biotope pairs were identified, and transects laid out perpendicularly across the interface. In total, 36 transects were identified: CC –G (n = 7); OC – G (n = 8); CC – F (n = 8); CC – OC (n = 7) and F-G (n = 6). The spatial distribution of the grasslands, indigenous forests and *Eucalyptus* plantations limited the number of transects that were possible per type. At least 300 m separated transects of the same type when situated alongside each other. This was done to maintain independence of the samples. Transects were 120 m long, extending 60 m either side of the boundary between a biotope pair. Along each transect, six sampling stations were established, with increasing distance from the edge, and at 15 m, 30 m, and 60 m into each biotope.

3.2.2 Invertebrate sampling

Six taxonomic groups were targeted: Coleoptera, Diptera, Hemiptera, Orthoptera, Hymenoptera (excluding Formicidae), and Formicidae. Samples were collected at each sampling station along a transect using a sweep net and a vacuum sampler. Samples of both methods were collected along a secondary transect that ran parallel to the biotope edge to ensure that the sampling distance was at 15 m, 30 m and 60 m respectively. All samples were collected from vegetation, in the grassland samples were collected from the grass layer, while in the forests and plantations only the understory was sampled. Sweep net (net was 50 cm wide) and suction samples (using a Stihl SH56 petrol powered leaf blower in reverse, and fitted with an oval nozzle 14 cm x 10 cm wide, combined with a fine grain mesh collection net) were collected in opposite directions to each other to prevent one method from interfering with the success of the other. Sampling consisted of 200 sweeps with the sweep net (at each step the net was passed through the vegetation in front of the collector, sweeps therefore alternated direction with each step), and 100 insertions of the suction sampler into the vegetation in front of the collector. Specimens were stored in 75% ethanol until identification at a later stage.

Time constraints, coupled with the sheer number of samples collected, as well as a lack of taxonomic knowledge of all the insect groups, meant that adult specimens could only be identified to morphospecies. These were then identified to family level. For Hymenoptera, only the easily recognisable groups of Ichneumonidae, Braconidae and Chlacididae were included.

3.2.3 Environmental variables

A graduated pole, in 10 cm increments, was used for measuring both plant height and percentage plant cover, at each sampling station. A hundred points were dropped while walking parallel to the biotope edge. Grass types or other vegetation touching the pole was recorded. The maximum height of the understory vegetation at each point was also noted. Alien plant species along the walks were also noted. Structural characteristics of the vegetation were also recorded, examples of these are forbs, shrubs, dead vegetation as well as the presence of bare ground and rocks. Average plant height, percentage plant and ground cover, as well as bare ground, was then calculated for each sampling point along the transect. Grass heterogeneity was then calculated by adding together the number of grass types. Topology was recorded by measuring the slope and elevation at a station, as well as determining the aspect.

3.2.4 Statistical analyses

The overall effectiveness of sampling was determined by plotting a species accumulation curve as well as calculating the Chao2 estimator (Chao 1987) to estimate the expected species richness across all sites. These analyses were conducted in *Vegan* (Oksanen et al. 2018) in *R* (R Core Team 2018).

To test for spatial autocorrelation in the data, a Mantel test in was performed in R using *Ade4* (Dray & Dufour 2007). The Mantel test indicated spatial autocorrelation within the data (Mantle test = 0.086, p = 0.014). To account for the influence of the spatial distribution of sampling sites (Bolker et al. 2009), random spatial variables were included in all linear models.

The effect of transect type, sampling station and the interaction between these two variables on species richness, and the Shannon evenness of all sampling sites calculated using *Vegan*, was investigated. To do this generalised linear mixed effects models (GLMM) were run with Laplace approximation and fitted with a Poisson distribution for species richness. Negative binomial distribution was used for the Shannon index as values were transformed by dividing all evenness values by the maximum Shannon evenness score, and ranged between 0 and 1. No other main effects were included in the models, and the random variable included in both was 'farm' (i.e. location of the transect). The package *lme4* (Bates et al. 2015) was used to conduct both models, and Tukey post hoc comparisons were run when significance existed between any of the main effects.

Using the *AICcmodavg* package (Mazerolle 2017), model selection was used to find the best fit model for the environmental variables on species richness in the overall dataset, as well as the for the species richness observed along the different transect types. Firstly, correlation between environmental variables was assessed to ensure that pairs of correlating variables were not included. To do this Spearman's R-values were calculated for each possible pairing. When rho > 0.5 or < -0.5, one variable of a pair would be excluded from the model. Correlating variables were: grass heterogeneity and forests (rho =-0.56), grass heterogeneity (ie. total number of grass species at a site) and grasslands (rho = 0.82) and finally, total plant heterogeneity (the diversity of plant structural types) and grasslands (rho = 0.56).

Therefore, grass heterogeneity was excluded from models involving forest data, and grass and total plant heterogeneity were excluded from models involving grasslands. To identify the best fitting model, individual models were created using all possible variable combinations. The best fitting model is that with the lowest AICc value. As some variables were categorical, models could not be averaged. Therefore, a complete model was created using all the variables in the best model. This was successively compared to models where alternating variables were removed, to determine the variables with significant effects on species richness. As all data were non-normal, GLMMs, described above, were used. For the overall model, 'farm' was used as the random variable, while 'transect number' was used for the transect specific models.

Primer-6 (Clark & Gorley 2006) and the PERMANOVA+ add-on (Clark & Warwick 2001) were used to investigate the sampled insect assemblages. Initially, data were square-root transformed, and a Bray-Curtis similarity matrix was calculated for the entire assemblage. Using this similarity matrix, a permutational multivariate analysis of variance (PERMANOVA) was run to investigate the effect of transect type, sampling station and their interaction. Unrestricted permutation of the raw data was used, as well as 9999 permutations. Secondly, using the same Bray-Curtis similarity matrix, the effect that position along each transect in each biotope was determined for both main and pairwise effects across the entire data set. This approach enabled the role of contrast in determining spillover between biotopes to be identified. Finally, the influential environmental variables in each biotope were identified using distance based linear models (DistLM). This used the Bray-Curtis matrix, and followed a step-wise selection procedure, with the selection of the models being based on AICc values.

To investigate spillover of species exhibiting biotope fidelity, these species were first identified using *indicspecies* (De Cáceres & Legendre 2009) in *R*. Multi-level pattern analysis was used, which determines any association between species patterns and various site groupings. Species associated with only CC, OC, G and F were identified, as well as those associated with all possible combinations of biotope types. Biotope associations were then inferred from these groupings, and the species grouped accordingly.Four groups were identified:stenotopic grasslands species were only associated with the grouping "G", and stenotopic forest species were only associated with 'F'. Cultural species were taken to be those species showing association only with plantation biotopes, these 'OC', 'CC' and 'OC+CC' groupings. The species in these two groups were added together to form the cultural species assemblages. Ubiquitous species were identified as those with associations across a range of biotope types, namely: 'F+G', 'G+OC', 'CC+F', 'CC+F+OC' and 'CC+G+OC'. These 5 assemblages were added together to form the ubiquitous species group. Appropriate transect types were then identified for each species grouping. A transect was considered appropriate if it incorporated a biotope to which a fidelity group was associated. Thus it would be possible to investigate the potential spillover of an associated group from a natal biotope into a non-natal biotope. For example, it was not appropriate to investigate the response of grassland stenotopic species along the CC-OC transects, as neither biotope

in question would be expected to host a population of grassland stenotopic species. Yet the CC-G transects would be appropriate for the grassland stenotopic species. This resulted in eighteen transect-species grouping combinations being identified. Species richness of each sampling station along selected transects was determined for all species fidelity groups. GLMMs, following the approach outlined for the overall richness, were used to model the response of species richness to sampling station along the various transect types.

The package *MuMIn* (Barton 2019) was used for the model averaging to determine which environmental variables were significant for species richness in the four species groups showing biotope fidelity. Data was combined across all samples for each individual species group. All possible model combination of four non-correlated environmental variables (average plant height, vegetation cover, grass heterogeneity and elevation) are automatically generated using the dredge function, and then their AICc values compared and the models ranked. The top model is that with the lowest AICc values, where the AICc between top models differed by < 2, these models were then averaged. The significant environmental variables were then identified from the averaged models by both p value, and confidence interval. If the confidence interval did not include 0, the variables influencing species richness, could be interpreted as significant. The variable 'farm', was used as the random factor in all models.

PERMANOVAs were calculated to investigate how the separate assemblages of the grassland and forest stenotopic species, as well as those of the cultural and ubiquitous species, differed across the four biotope types. Canonical analysis of principal coordinates (CAP) of the four biotope types was then constructed. Finally, following the previous method, distance based linear models (DistLM) were used to calculate the environmental variables driving the assemblage patterns of the four species groupings.

3.3 Results

Across all transects, 15 034 individuals, belonging to 883 morphospecies, were sampled. The species accumulation curve did not reach an asymptote (Appendix 3.1), while the Chao2 estimate indicated that 1 325.31 ($\pm$ 64.22) species should be present. Diptera was the most abundant group overall, representing 30.83% of all sampled specimens. The Diptera was also the taxonomic group with the highest overall species and family richness (24.46% and 35.09% respectively). Hemiptera was the second most abundant group, comprising 26.61% of all sampled specimens as well as second mose speciose group at 24.43%. The third most abundant and speciose group was the Coleoptera with 19.25% and 21.78% respectively. Hymenoptera and Orthoptera were the least abundant groups, representing only 4.92% and 2.07% of all samples collected.

Transect type ($\chi^2 = 24.97$, p < 0.001), as well as sampling station ($\chi^2 = 866.16$, p < 0.001) along a transect, both had significant impacts on the overall observed species richness. The interaction between position and transect type was also significant ($\chi^2 = 67.4$, p < 0.001). Similar patterns emerged when Shannon Evenness values were used, both position and transect type had significant impacts on species richness

(χ^2 =27.07, p < 0.001 and χ^2 =24.5, p < 0.001 respectively), yet the interaction between position and transect type were not significant (χ^2 =17.84, p = 0.21).

3.3.1 Environmental drivers

The model selection procedure showed aspect, average plant height, elevation and total vegetation cover as important environmental variables. All had significant impacts on overall species richness across all sampled sites (Table 3.1). Average height, elevation and total vegetation cover, all had positive relationships with overall species richness. Species richness along the CC-G transects had a significantly positive relationship with average vegetation height (Table 3.1). Aspect, average vegetation height and vegetation cover were all selected for on the OC-G transects, and were all significant, with average vegetation height and vegetation cover both having negative relationships with the observed species richness (Table 3.1). The contrasting responses along the OC-G and CC-G transects to vegetation cover are likely due to very bare sites in the CC transects, forcing a positive response in the model. Although vegetation cover was the only environmental variable selected for along the OC-CC transects, the observed effects were not significant (Table 3.3). Average vegetation height had a significantly negative effect on species richness along the F-CC transects (Table 3.1). Species richness along the F-G transects was positively associated with average vegetation cover (Table 3.1).

3.3.2 Spillover and contrast

When the entire assemblage of foliage dwelling insects was considered, both sampling station (Pseudo-F = 2.97, p < 0.001) and transect type (Pseudo-F = 4.83, p < 0.001) were significant in terms of the species composition observed. There was no evidence for significant interaction between the position and transect type (Pseudo-F = 1.01, p = 0.39). PERMANOVA tests indicated that significant differences amoung the species composition of the different stations in the different biotope types exist (Appendix 3.2). The only similarities between biotopes were between the OC and CC biotopes, and although largely similar, the following three combinations were significantly different: CC60 – OC30, OC30-CC30 and OC60-CC60 (Appendix 3.2). Overall, the PERMANOVA results showed that overall assemblages of the four biotopes differed significantly (Appendix 3.3).

The distance based linear models conducted gave similar results to that of PERMANOVA. Insect assemblage responses of the four biotopes grouped in accordance with biotope type. Grasslands were separate from forests and plantation biotopes, yet there was some similarity between plantation and forest in terms of insect assemblages. Responses between the two plantation biotopes (CC and OC) showed a high degree of similarity (Table 3.2). Grass and total heterogeneity, as well as vegetation cover, were highly significant drivers of the grassland assemblages. While total heterogeneity and vegetation cover were not important for the indigenous forest or plantation biotopes (Figure 3.2).

Sampling station was a non-significant environmental variable across the entire sampled assemblage (Table 3.2).

3.3.3 Biotope fidelity

188 species were associated with the four biotopes or their combinations. 69 species with indigenous forests and were classified as stenotopic forest species, 83 species with grasslands and were classified as stenotopic grassland species, three Hemiptera species were associated with closed canopy plantations. Eight species were associated with open canopy plantations consisting of three Hemiptera species, two Diptera, two Braconid wasp species and one Coleoptera species. Of the 18 individual GLMMs investigating the effect of position on species richness of the different species types along appropriate transects (Figures 3.3 – 3.6), only three indicated that position had no effect (Table 3.3). These were cultural species along CC-G and F-CC transects, and the ubiquitous species along OC-CC transects. All other model combinations indicated that position had a significant role (Table 3.3). Along F-G transects, grassland species were significantly lower at the G15 stations than at the G60 stations (Figure 3.3), while forest species were significantly higher at F60 stations than at the F15 stations (Figure 3.4). Furthermore, ubiquitous species along F-G transects, were surprisingly higher in species richness at F60 stations. Along the F-CC transects, forest species were significantly higher in species richness at the CC15 stations than they were at the CC60 stations (Figure 3.4). When considering the environmental variables driving the assemblages of these species groupings, grass heterogeneity and vegetation cover are significant in many instances. Interestingly, the only time grass heterogeneity has a negative impact on species richness, it is when considering that of the forest associated species (Table 3.4). The distance based linear models conducted on the species groups show a wide range of environmental variables that are responsible for the observed assemblages patterns (Table 3.2).

To determine the role of contrast and how a biotope fidelity impacts a species ability to spillover, PERMANOVA results indicated that most assemblages of all species showing biotope associations were significantly different across all biotope types, suggesting contrast between biotopes does play a role. The only assemblages not significantly different were the grassland stenotopic species in OC and CC plantations, as well as there being no significant difference between the assemblages of forests and CC plantations. Forest stenotopic species assemblages in the OC and CC plantations did not differ significantly (Appendix 3.3). CAP graphs (Figure 3.7), show distinct groupings of forest and grassland stenotopic species assemblages (Figure 3.7A and 3.7B respectively). The remaining biotope assemblages overlapped, perhaps suggesting spillover of stenotopic species among biotopes to which they show no form of fidelity.. Cultural species (Figure 3.7C), form no distinct assemblages, while ubiquitous species form a distinct grassland assemblage, while the other biotope assemblages were mixed (Figure 3.7D).

3.4 Discussion

Patches of remnant natural vegetation are essential for the maintenance of insect diversity across this timber production landscape, as distribution of species in agroecosystems varies (Brockerhoff et al. 2008). Natural grasslands and forest patches were significantly more diverse in insect species than the open and closed canopy eucalyptus plantations. Closed canopy plantations and indigenous forests had intermediate contrast, as also shown by (Peyras et al. 2013; Frost et al. 2015), and there was evidence here of spillover at the interface between these two biotopes. Cultural species are able to spillover from open canopy plantations into grasslands, but not from closed canopy plantations. Open-canopy plantations allow for some pioneer grasses to establish, reducing contrast between grasslands, and facilitating movement of cultural insect species out of these younger plantations and into the surrounding grasslands.

3.4.1 Environmental factors as drivers of biodiversity

The roles of landscape and habitat heterogeneity are pivotal for the maintenance of biodiversity (Crous et al. 2014b; Slancarova et al. 2014; Fahrig 2017). In complex landscapes comprising of multiple biotope types, there is a suite of environmental factors driving biodiversity patterns (Steffan-Dewnter & Tscharntke 2000; Rand et al. 2006; Loreau et al. 2013), as was the case here. I found that a range of vegetation-related environmental factors was significant, with either vegetation cover or vegetation height, or both, positively affecting species richness along transects, as well as the species richness of the various species fidelity groups across this landscape. With increasing cover or height, microhabitats and essential resources are provided, allowing more species, generalist and specialist, to persist.

3.4.2 Spillover and contrast

The biotopes here present a highly contrasting landscape mosaic. As previously shown for the epigaeic assemblages, abrupt changes occurred among the foliage-dwelling insect assemblages sampled in the four biotopes. There was no gradual change among overall assemblages the further away from the edge samples are taken. Each biotope presents different habitat conditions, and different environmental drivers were important for different biotope assemblages. With sharp contrast between biotopes, such as that between grasslands and indigenous forests, different micro-habitats and thermal environments are present (Reis & Sisk 2004; Boetzl 2016; Evans et al. 2016). Ant assemblages change significantly across grassland – forest ecotones due to fundamental differences in both microclimates, and micro-habitats, present in these two highly contrasting biotope types (Dröse et al. 2019). The contrast in this production landscape arises from the structural differences (Peyras et al. 2013) between grassland, forest, and plantation age classes. Each biotope has distinct environmental factors driving the insect assemblages and consequently, species assemblages are comprised of species that are best suited to each particular biotope (Rand et al. 2006).

Driven often by resource availability and population density, spillover requires that the species are able to forage or persist to a degree in neighbouring biotopes (Loreau et al. 2013). For this to occur, essential resources, such as food or host species, need to be present close to the home biotope (Rand et al. 2006). Lower contrast between biotopes, through similarities in structure, allows for biotope edges to be more permeable to species, and therefore to spillover as species are able to access resources beyond their natal biotope (Didham & Lawton 1999; Collinge & Palmer 2002; Inclán & Marini 2015; Evans et al. 2016). Overall, OC and CC species assemblages are almost indistinguishable from one another. This suggests that contrast between younger and older plantations is low as a result of few structural dissimilarities existing between the two age classes – younger trees are smaller, and their canopies have not closed as in the mature tree stands. Contrast between biotopes is not always dichotomous – either high or low- but can vary along a gradient (Peyras et al. 2013). This results in the boundaries between the two plantation age classes to be permeable to insect assemblages, and thereby allowing for multi-directional spillover between the two.

Interestingly, as with the epigaeic insect assemblages, species richness appears to be higher in CC plantations stations closest to the indigenous forests. Furthermore, there appears to be some overlap of assemblages in OC and CC plantations and indigenous forests. Due to similarities in the environmental drivers of these biotopes, lower contrast and higher permeability between these three biotope types can be inferred. The distribution of forest patches is driven the interaction between topology and the prevailing winds which form fire refugia in which forests become established (Luger & Moll 1993). The exclusion fire from this system by the presence of plantations, allows for expansion of secondary forest growth at the edges. This is perhaps one reason for the observed increases in species richness at the edges of the CC plantations, and for the overlap between assemblages. The expansion of forest plant species into the plantation edges, provides more resources and appropriate micro-habitats, facilitating spillover of indigenous forest species by reducing the contrast and increasing the permeability between the two biotopes.

3.4.3 Biotope fidelity

Species displaying biotope fidelity were present in all four biotopes. Grasslands and indigenous forests supported the highest proportion of stenotopic species compared to open and closed canopy plantations. Semi-natural and natural patches in agroecosystems are essential for the maintenance of landscape-wide species diversity (Duelli & Obrist 2003). Surprisingly, there were eleven species that showed sole affinity towards open or closed canopy plantations, the majority of these species were either Diptera or Hemiptera. Given that plantation forestry has been present for > 50 years in this landscape, the foliage-dwelling insect assemblages in the plantations, have had time to become established. The reduced contrast across age classes of trees, enables spillover between tree stands, thereby facilitating maintenance of plantation-based diversity in this system. Interestingly, there were no species associated

with all four biotope types, and so no true generalist species is present, further evidence that this landscape is a highly contrasting one.

Grassland stenotopic species do not spillover into either plantation age class, nor into the indigenous forests. In fact, there is a significant decrease in species richness of grassland-associated species close to the forest edge. Structural changes along a grassland-forest ecotone results in changes to the thermal environments through shading and woody bush encroachment, micro-habitats and resource availability, effectively excluding open grassland specialists from this ecotone (Dröse et al. 2019). As only the grasslands specialists are being considered here, the significant reduction in species richness in the open and closed canopy plantations is to be expected. Afforestation of grasslands by exotic timber stands, significantly alters the physical environment and effectively excludes grassland specialist species as the correct environmental conditions these species require are not present (Reino et al. 2009; Dröse et al. 2019). Furthermore, bush encroachment due to altered fire regimes may pose a treat to the grasslands stenotopic species in this landscape, as is the case with butterfly species in a similar landscape. When grassland fire frequency decreases, the amount of woody vegetation increased, resulting in a butterfly species assemblage that resembles forest assemblages, yet is not as rich and therefore these woody areas contribute less to the overall conservation of species (Gaigher et al. 2018). Should woody bush encroachment become an problem in the study landscape, valuable grassland species will be lost, while some forest species may increase in abundance.

There was a significant increase in species richness of forest species in closed canopy plantations neighbouring indigenous forests. With similar structural characteristics, as well as microclimatic conditions being similar (Peyras et al. 2013; Frost et al. 2015), this boundary of low contrast is permeable to forest species. In a New Zealand system, spillover of generalist hymenopteran predators from pine plantations to native forest was greater than that of specialist parasitoid species. Since generalist species do not require specific prey to be present in the recipient biotope, the spillover of these species depends solely on the abundance of potential prey species, and dual-directional movement of these species between plantation and forest occurs as food resources are available in both biotopes (Frost et al. 2015). Here, due to the overlap of assemblages between indigenous forests and the two plantation age classes, as well as the increase in species richness of forest species at the sites closest to the forest edges, species spillover from indigenous forest into *Eucalyptus* plantations. In a similar system, species assemblages between pine plantations and indigenous forests mix due to similarities in the understory vegetation (Yekwayo et al. 2016b, 2017). With increasing rates of spillover, changing species interactions at the edges can have varied, and unpredictable ecosystem level consequences (Frost et al. 2015).

Cultural species from open canopy plantations, but not those in closed canopy plantations spillover into grasslands. Due to less shading in open canopy sites, some grasses have established. The grasses may,

in turn, reduce the contrast between the grasslands and plantations and allow for flow of plantation species, out of plantations and into grasslands. As the ubiquitous species group contained species associated with forests and grasslands, the higher species richness in these two biotopes, when compared to the open and closed canopy plantations is expected, as more species show associations to the natural biotopes in this timber production landscape.

3.5 Conclusions

As with many production landscapes, natural, or semi natural, landscape features contribute significantly to the conservation and maintenance of indigenous biodiversity (Pryke & Samways 2012; Kietzka et al. 2015; van Schalkwyk et al. 2017). The same is true here, with the grasslands and indigenous forests being significantly higher in species richness and maintaining a complete assemblage of species across the landscape, with the exception of a few species which only occurred in the plantations. These ENs are diverse, providing essential habitat heterogeneity (Crous et al. 2013). *Eucalyptus* plantations dominate this landscape, and although not as diverse as the grasslands and indigenous forests, some foliage-dwelling species occur within them, with some species showing preference for the timber stands. Nevertheless, spillover between plantations of different ages does occur, suggesting that the re-establishment of assemblages post harvesting is most likely from other mature *Eucalyptus* plantations. The results here also suggest that greater vegetation cover and height promotes biodiversity within the plantations.

Contrast between the various biotope types impacts the degree to which species are able to spillover. Lower contrasting edges are more permeable, and allow for greater spillover, such as the boundaries between closed canopy plantations and indigenous forests, as well as the intermediate contrast between open canopy and grasslands, and which allows for cultural species to spillover into the grasslands. Spillover of biotope-associated species between biotopes was minimal, spillover of forest stenotopic species occurs into closed canopy plantations, while grassland stenotopic species do not spillover into the plantations or indigenous forests. Spillover of cultural species from open canopy plantation into grasslands occurs. In both cases, this is likely due to lower contrast and resources distributed along boundaries between biotopes, enabling species spillover. This suggests that having a mix of natural biotopes with open and closed canopies would not only promote biodiversity at the landscape level through their unique biodiversity value, but also promote biodiversity within the production patch through limited spillover.

3.6 References

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3.7 Tables and figures

Table 3.1: Results of model selection and models conducted on the species richness of all transects sampled as well as on the species richness of the individual transect types sampled. Chi square (X^2) values are shown (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). Abbreviations: CC- Closed canopy plantation, G – Grassland, OC – Open canopy plantation and F- Forest.

	Variable	Chai square	df	Species richness response
	Overall			
Overall	Aspect	154.97***	7	-
	Average plant height	28.52***	1	Positive
	Elevation	9.69**	1	Positive
	Vegetation cover	9.65**	1	Positive
	Transect types			
CC-G	Average vegetation height	424.63***	1	Positive
OC-G	Aspect	26.15***	5	-
	Average vegetation height	24.67***	1	Negative
	Vegetation cover	7.84**	1	Negative
CC-OC	Vegetation cover	2.42	1	Neutral
F-CC	Average vegetation height	29.53***	1	Negative
F-G	Vegetation cover	10.682**	1	Positive

Table 3.2: Results from DistLM following a step-wise selection procedure indicating the environmental variables, from the sequential model, significantly impacting the species assemblages sampled across the entire assemblage. Pseudo-F values are shown here (* p< 0.05; ** p< 0.01; *** p < 0.001).

	Aspect	Elevation	Slope	Average vegetation height	Vegetation cover	Distance to edge	Grass heterogeneity	Total heterogeneity	Total percentage explained variance
Overall species assemblage	2.2***	3.59***	2.73***	x	8.88***	x	15.77***	2.31***	15.04
Grassland stenotopic species	2.27***	4.68***	3.31***	x	13.38***	x	90.58***	2.45***	37.73
Forest stenotopic species	2.89*	4.26*	2.64*	x	18.82***	x	14.17***	x	17.7
Cultural species	x	3.31**	9.77***	x	7.76***	x	x	x	9.15
Ubiquitous species	x	7.84***	4.07***	x	5.1***	x	13.23***	2.72**	15.92

Table 3.3: Results from GLMM models investigating the impact of station position along a transect on the over all species richness of the five insect groupings. Chi square (X^2) values are shown (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). Abbreviations: CC- Closed canopy plantation, G – Grassland, OC – Open canopy plantation, and F- Forest.

Species type	Transect type	Effect of position X^2	df	Differences observed
Grassland	OC-G	335.75***	5	All G to OC stations
	CC-G	553.54***	5	All G to CC stations
	F-G	280.93***	5	G60 - G15; All F to G stations
Forest	F-G	240.67***	5	F60 - F15; All F to G stations
	F-CC	255.35***	5	CC 60 - CC 15; All F to CC stations
Cultural species	OC-G	17.11**	5	None
	CC-G	9.15	5	None
	F-CC	5.68	5	None
Ubiquitous species	OC-G	20.23**	5	OC30 - G15; OC30 - G30
	CC-G	15.23**	5	None
	CC-OC	3.46	5	None
	F-G	16.87**	5	F60 - F30
	F-CC	17.86**	5	None

Table 3.4: Results of model averaging conducted on the species richness of the the four species groupings, showing the response of species richness to environmental variables. Z values are shown (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$).

Species type	Variables	Estimate	SD of estimate	Z value	Confidence interval	Species richness response
Grassland	Average plant height	0.49	0.061	7.93***	[0.37; 0.61]	**Positive**
	Grass heterogeneity	1.35	0.05	25.95***	[1.25; 1.45]	**Positive**
	Vegetation cover	-0.27	0.077	3.50***	[-0.42; -0.12]	**Negative**
	Elevation	0.034	0.062	0.56	[-0.087; 0.16]	Neutral
Forest	Average plant height	-0.11	0.076	1.43	[-0.26; 0.04]	Neutral
	Grass heterogeneity	-0.99	0.096	10.28***	[-1.18; -0.8]	**Negative**
	Vegetation cover	0.24	0.067	3.56***	[0.11; 0.37]	**Positive**
	Elevation	0.2	0.097	2.01*	[0.005; 0.39]	**Positive**
Cultural	Average plant height	-0.14	0.17	0.83	[-0.54; 0.068]	Neutral
	Grass heterogeneity	-0.086	0.15	0.56	[-0.56; 0.13]	Neutral
	Vegetation cover	0.79	0.18	4.41***	[0.44; 1.15]	**Positive**
	Elevation	0.008	0.06	0.14	[-2.25; 0.4]	Neutral
Ubiquitous	Average plant height	-0.036	0.075	0.48	[-0.3; 0.07]	Neutral
	Grass heterogeneity	0.37	0.093	3.939***	[0.18; 0.55]	**Positive**
	Vegetation cover	0.12	0.11	1.11	[-0.018; 0.35]	Neutral

Figure 3.1: Map indicating sampling locations as well as the sampled farms. Green blocks indicate location of *Eucalyptus* plantations and symbols the midpoint of each transect.
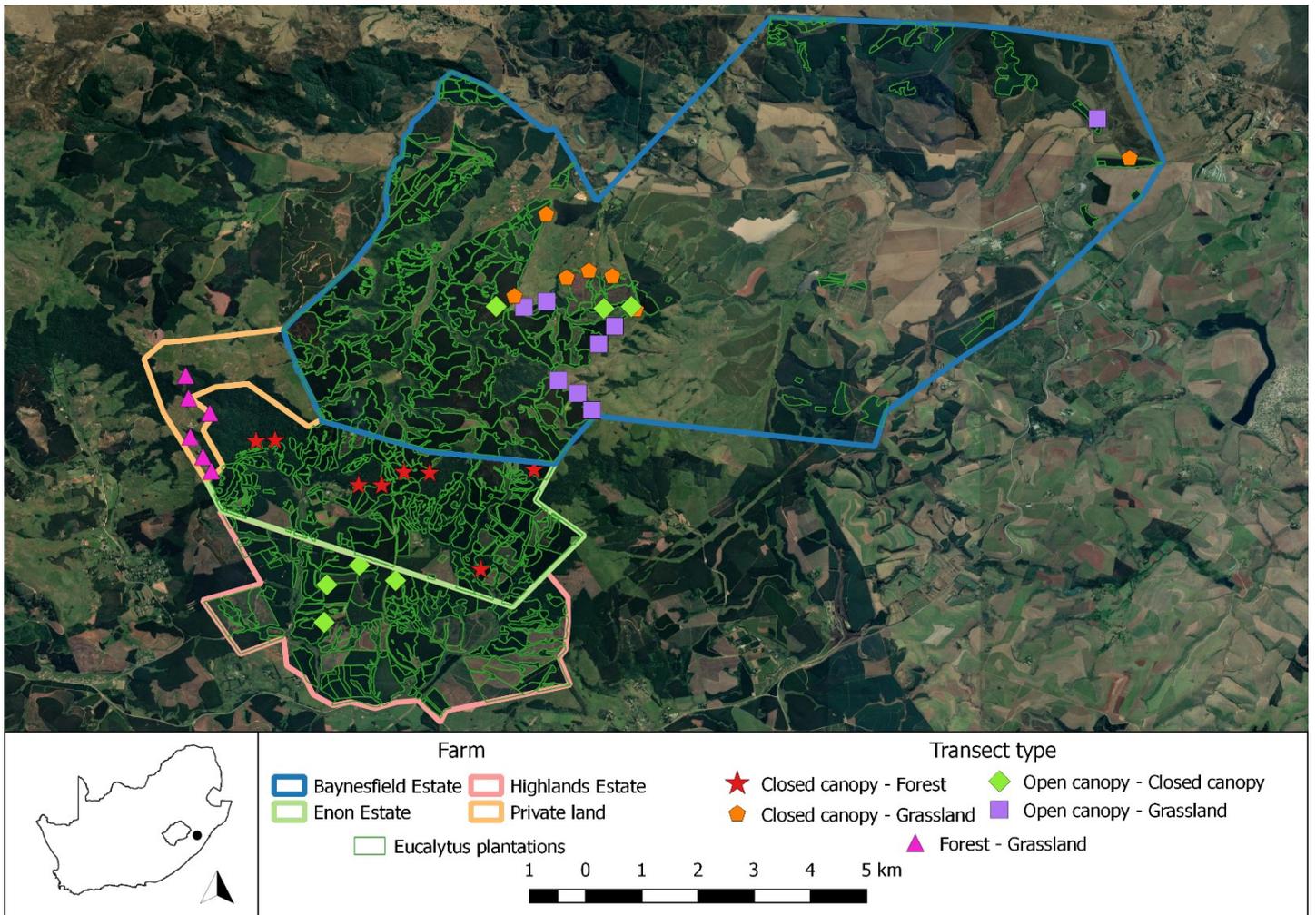
Satellite image from Google Earth (Image@2019 Maxar Technologies)

Figure 3.2: Similarity of overall species assemblages, categorized by biotope types; CC- Closed canopy plantation, G – Grassland, OC – Open canopy plantation and F- Forest. Environmental variables depicted are: Ghet -Grass heterogeneity, Thet – Total plant heterogeneity, VC – Vegetation cover, Aspect, Elevation and Slope.
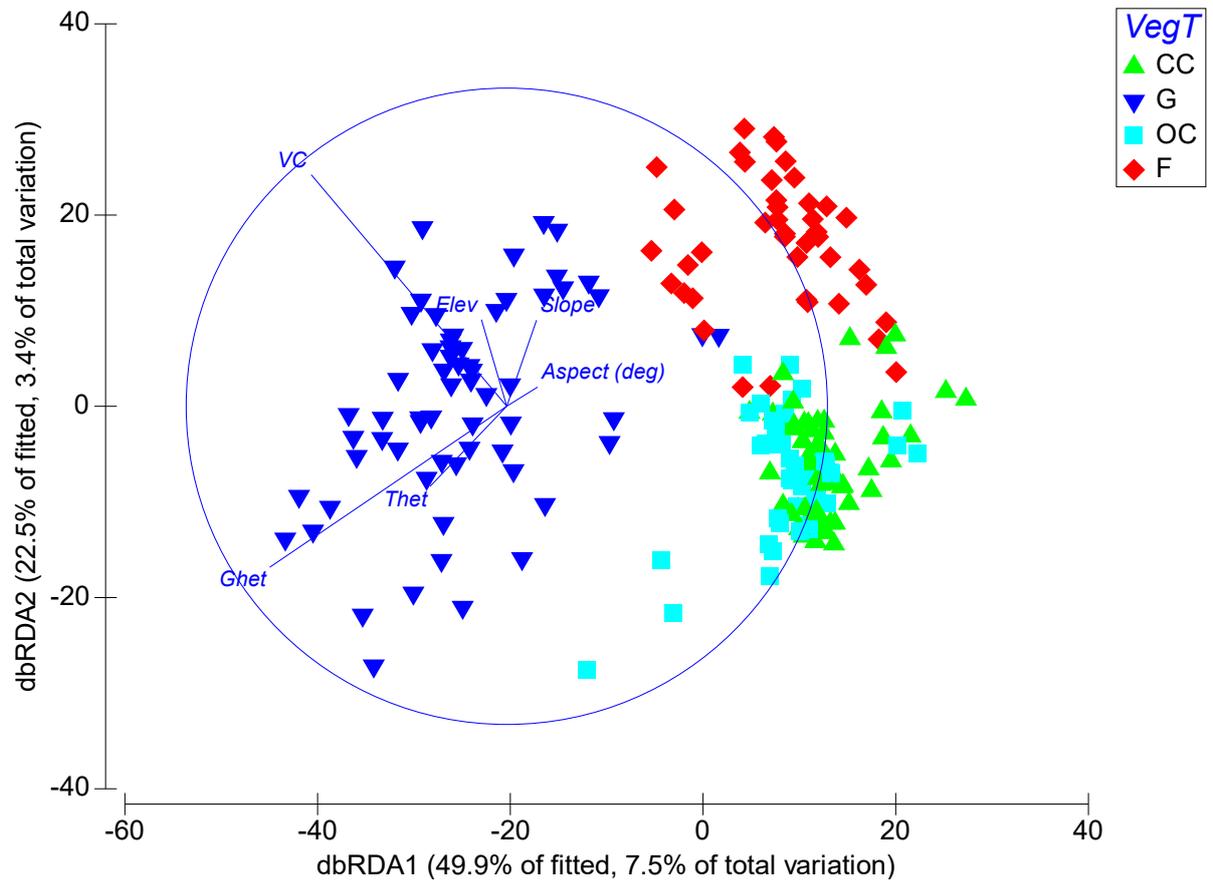
Figure 3.3: Grasslands species richness along three transect types, A) Grassland-open canopy transects, B) Grassland-closed canopy plantation transects, and C) Grassland-forest transects. Abbreviations: G –Grassland, OC – Open canopy and CC – Closed canopy.
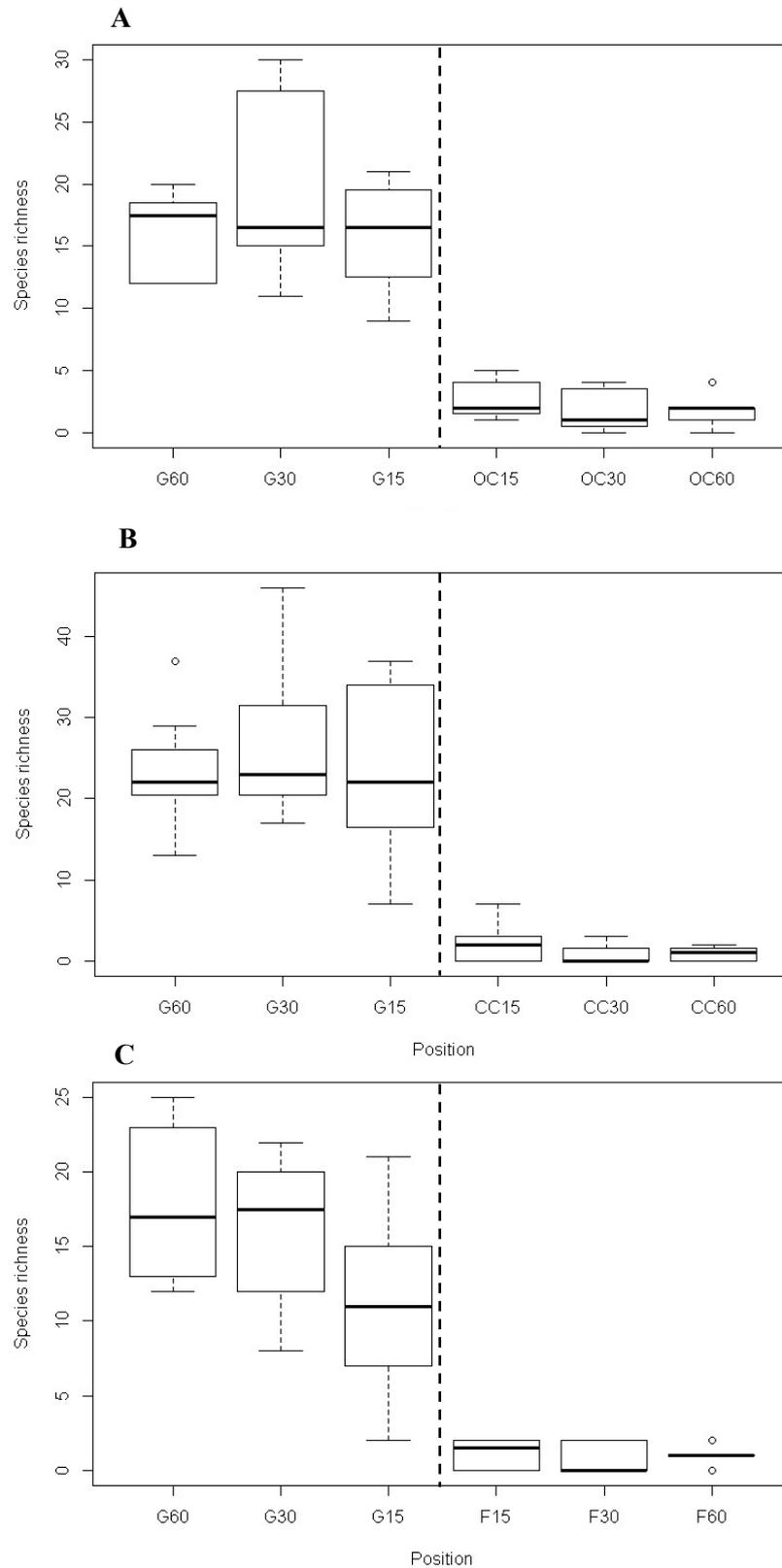
Figure 3.4: Forest species response across two transect types, A) Grassland-forest transects, B) Closed canopy plantation - forest transects. Abbreviations: G – Grassland, CC – Closed canopy plantation and F – Forest.
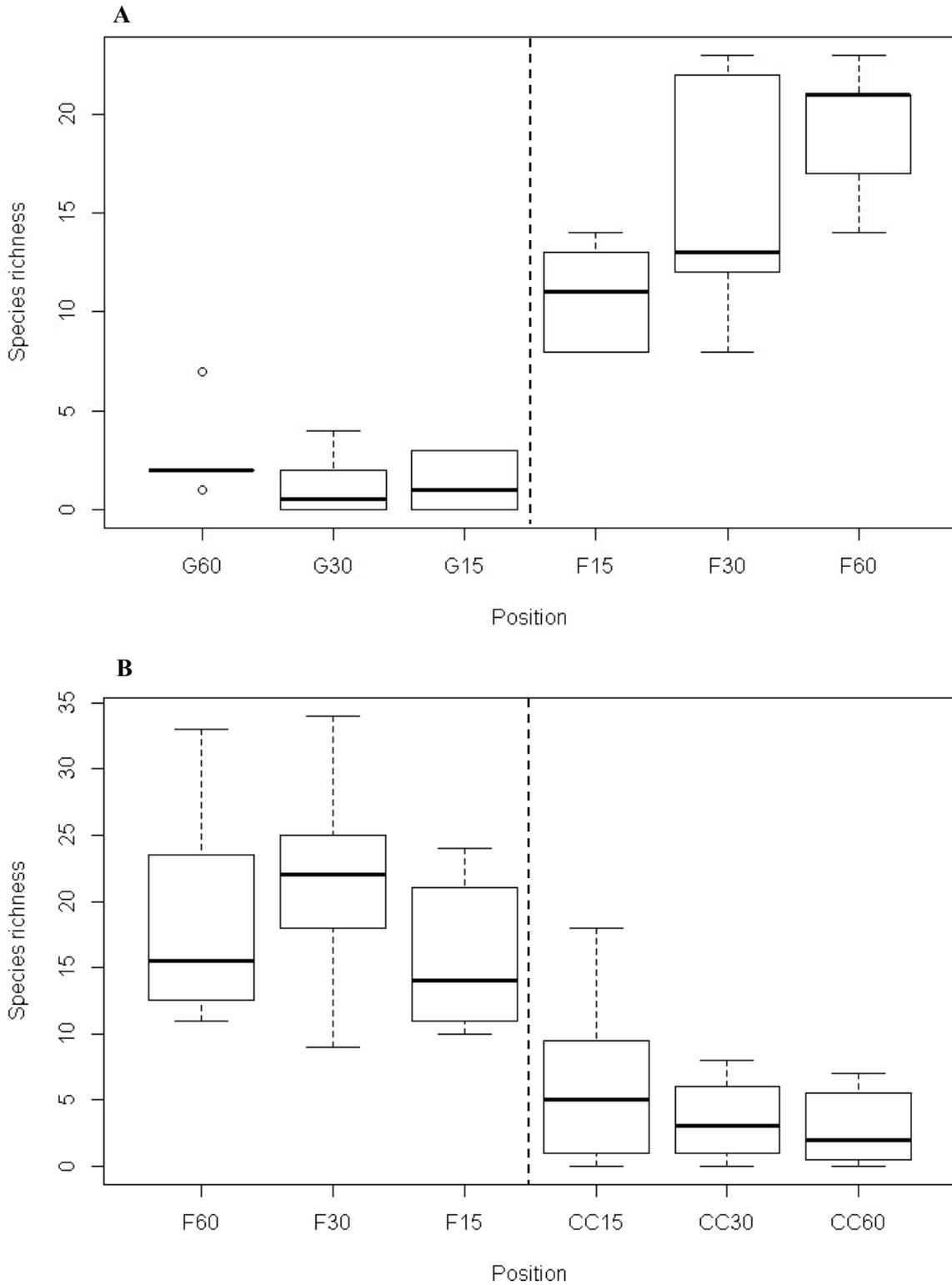
Figure 3.5: Cultural species responses across three transect types, A) Grassland open canopy plantation transects, B) Grassland-closed canopy plantation transects, and C) Forest-closed canopy plantation transects. Abbreviations: G – Grassland, OC – Open canopy plantation, CC – Closed canopy plantation and F – Forest.
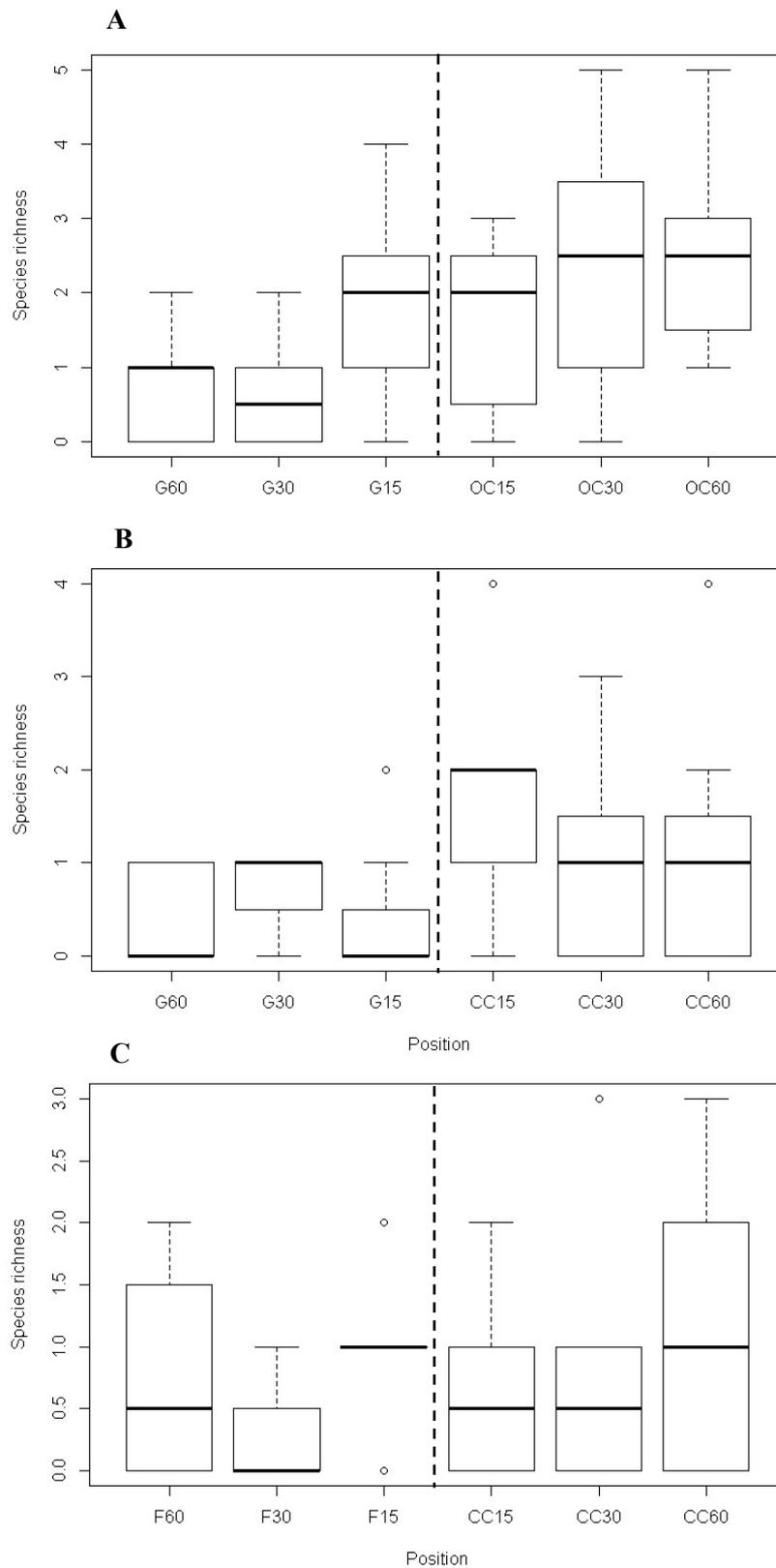
Figure 3.6: Ubiquitous species response along all 5 transect types, A) Grassland-open canopy plantation transects, B) Grassland-closed canopy plantation transects, C) Closed canopy-open canopy plantation transects, D) Grassland- forest transects, and E) Forest-closed canopy plantation transects. Abbreviations: G - Grassland, OC – Open canopy, CC - Closed canopy and F – Forest.
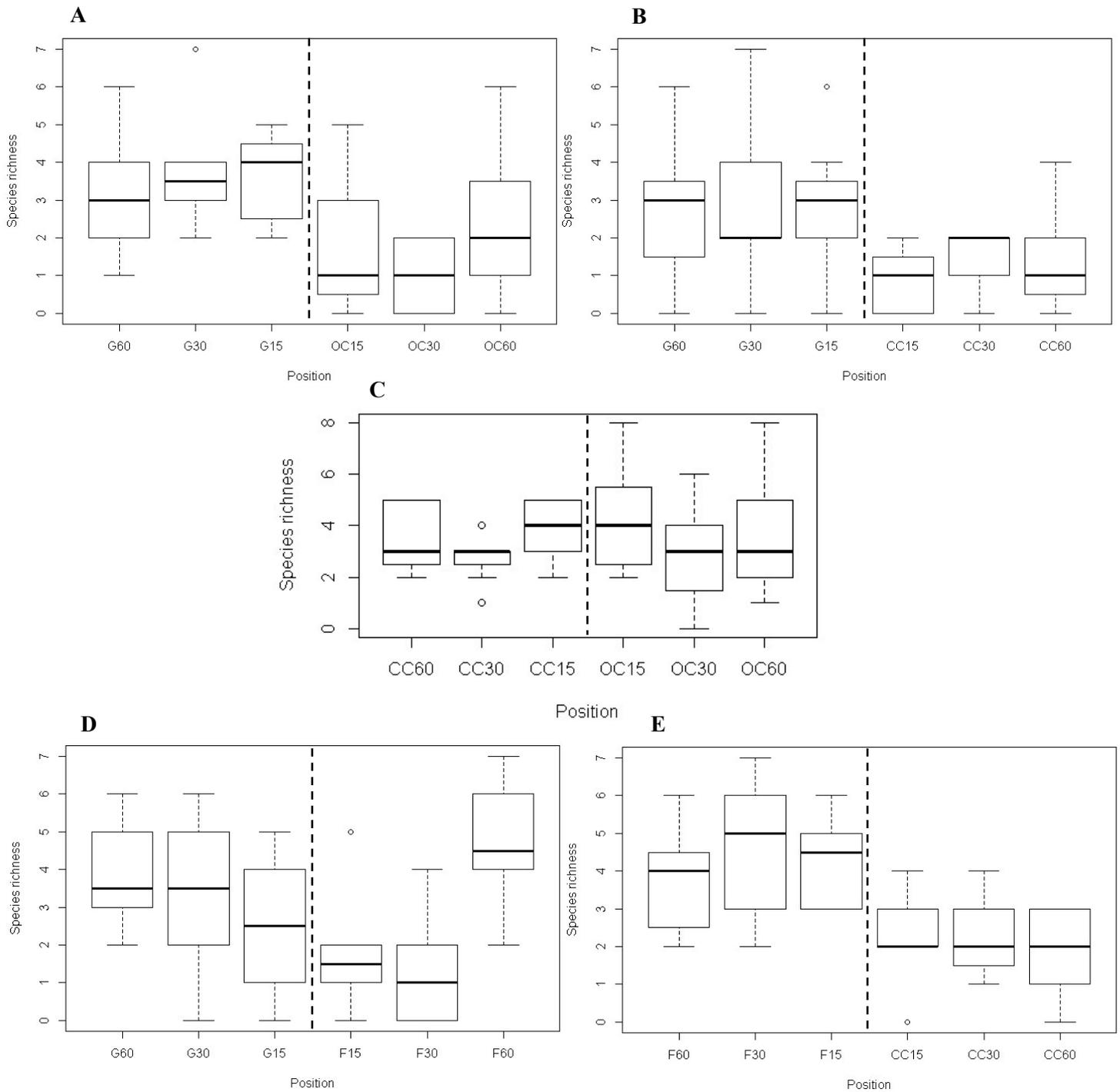
Figure 3.7: Canonical analysis of principal coordinates plots showing insect preference groups across the different biotope types. A) Forest stenotopic species, B) Grasslands stenotopic species, C) Cultural species and D) Ubiquitous species. Abbreviations used: G – grasslands, F – forests, CC–Closed canopy plantations, and OC – Open canopy plantations.
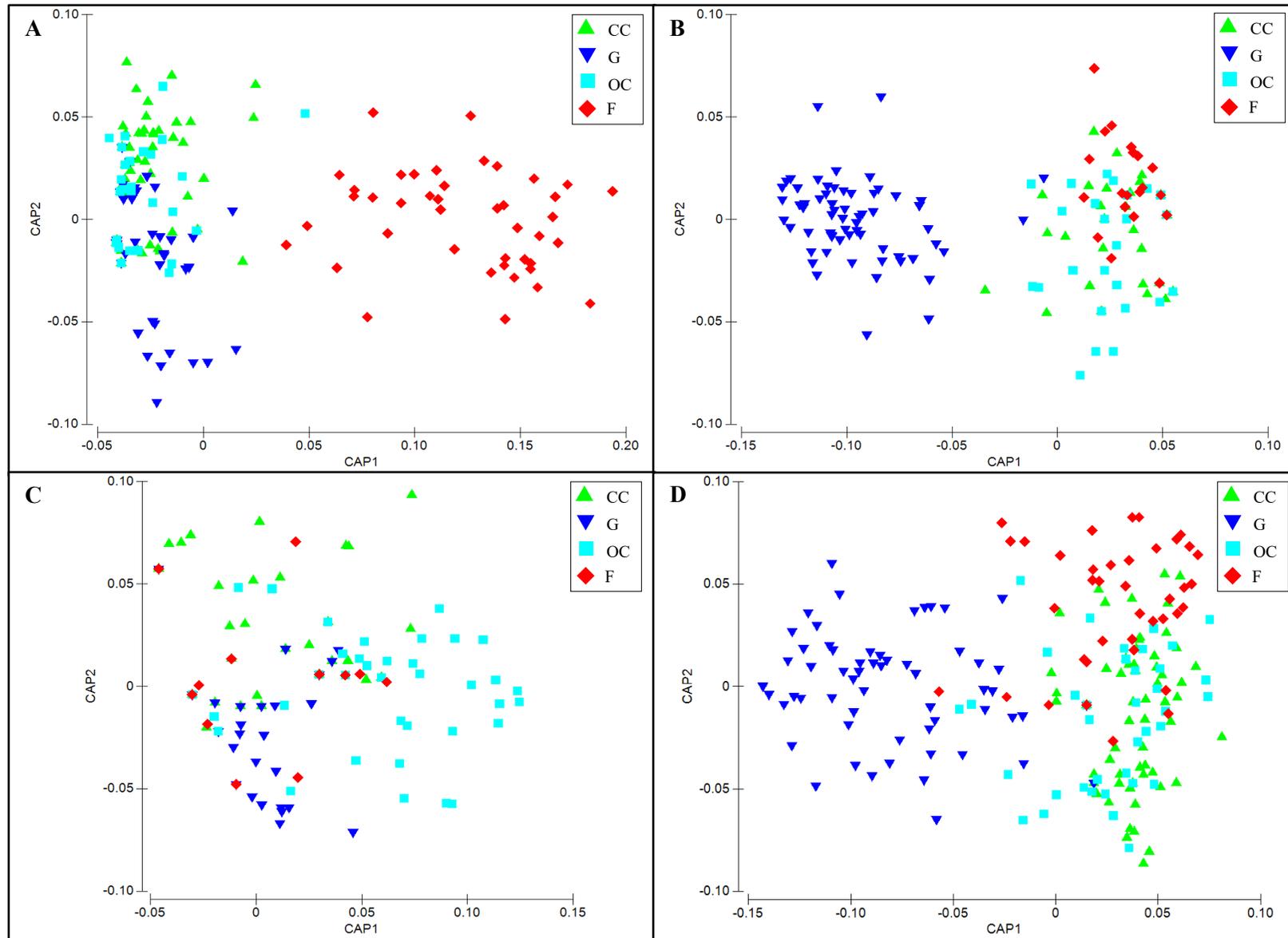
3.8 Appendices

Appendix 3.1: Speices accumulation curve of all individuals sampled across all sampling stations. Grey bars indicate 95% confidence interval across all sampling stations (n=216) irrespective of transect or biotope type.

Species accumulation curve
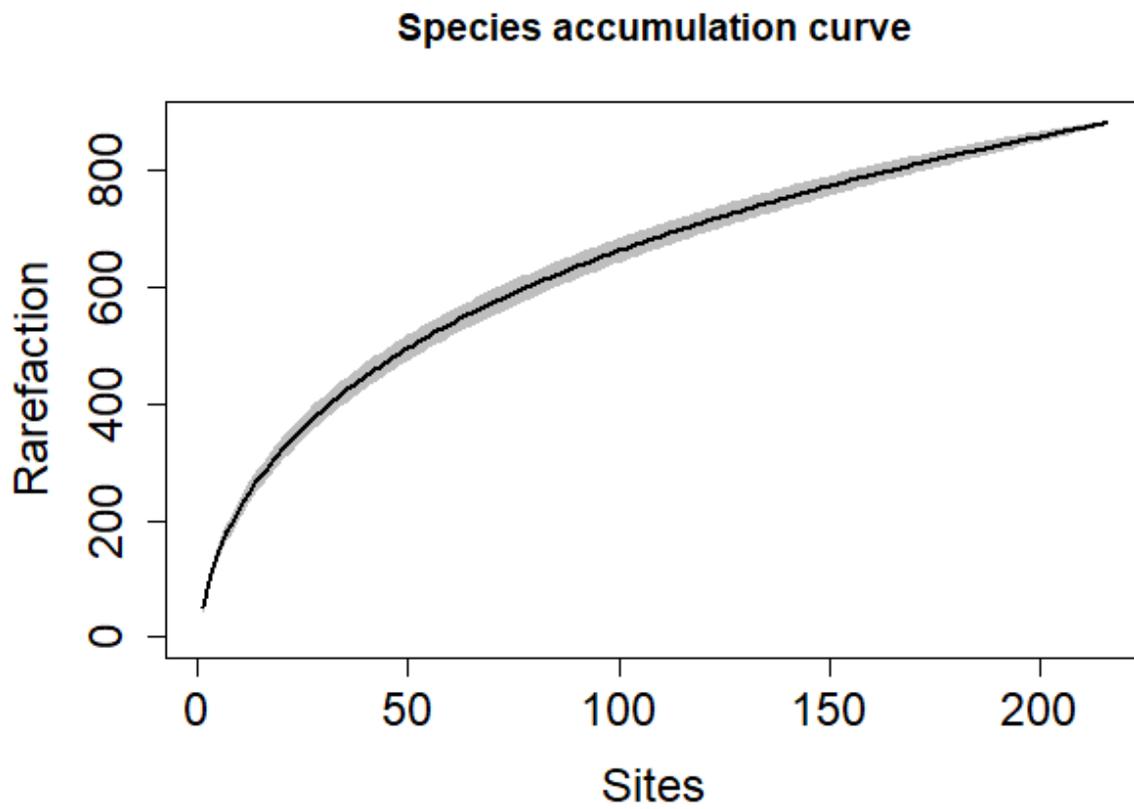
Appendix 3.2: Results from PERMANOVA posthoc tests showing t-values and the significant differences in insect assemblages at the various sampling stations of each transect type (* p < 0.05). Abbreviations: CC- Closed canopy plantation, G – Grassland, OC – Open canopy plantation and F- Forest. 60 – 60 m sampling station, 30 – 30 m sampling station and 15 – 15 m sampling station.

	CC15	CC30	CC60	F15	F30	F60	G15	G30	G60	OC15	OC30
CC30	0.67	-									
CC60	0.65	0.76	-								
F15	1.96*	1.90*	1.93*	-							
F30	1.94*	1.89*	1.88*	0.84	-						
F60	2.11*	2.06*	2.03*	0.86	0.97	-					
G15	2.31*	2.40*	2.48*	2.72*	2.82*	2.83*	-				
G30	2.38*	2.45*	2.52*	2.73*	2.83*	2.85*	0.75	-			
G60	2.39*	2.46*	2.52*	2.69*	2.80*	2.79*	1.00	0.88	-		
OC15	1.06	1.16	1.13	1.84*	1.93*	1.98*	2.06*	2.14*	2.14*	-	
OC30	1.13	1.17	1.18*	1.88*	2.00*	2.09*	2.11*	2.20*	2.21*	0.70	-
OC60	1.14	1.30*	1.27*	2.00*	2.07*	2.15*	2.18*	2.24*	2.22*	0.85	0.90

Appendix 3.3: Results from PERMANOVA posthoc tests showing t-values and the significant differences between the overall assemblage, as well as those of the of the four species groupings (*p< 0.05). Abbreviations: CC- Closed canopy plantation, G – Grassland, OC – Open canopy plantation and F- Forest.

	CC	OC	G
Overall species assemblage			
OC	1.73*		
G	4.09*	3.58*	
F	3.24*	3.26	4.68*
Grassland stenotopic species			
OC	**1.13**		
G	9.17*	7.23*	
F	**1.19**	1.54*	7.85*
Forest stenotopic species			
OC	**1.33**		
G	2.53*	1.75*	
F	6.30*	6.70*	7.30*
Cultural species			
OC	3.90*		
G	2.43*	4.54*	
F	2.51*	4.38*	2.15
Ubiquitous species			
OC	1.55*		
G	3.92*	3.17*	
F	2.76*	2.60*	3.75*

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Chapter 4:

Soundscapes among bush crickets vary in accordance to vegetation structural diversity and across the ecotones of a complex timber production landscape

"Even with insects,

Some can sing,

Some can't."

-Issa

Abstract

Many animal species have species-specific calls. The assemblage of calls from any one local area (soundscape) can be recorded and used for rapid, non-invasive, and accurate assessment of the various species present. As the landscape changes, so does the assemblage of songs. Ecotones, areas of transition between adjacent biotopes, support a mix of species from both biotopes. As acoustic species adapt to the acoustic space within biotopes, the soundscape across a landscape mosaic should reflect changes in the physical structure of a landscape. Similarities should exist in both the assemblages, and soundscapes of the ecotones, where transition from one biotope to another occurs. Using calls of bush crickets (Orthoptera, Tettigoniidae), I describe the soundscape of a complex mosaic landscape, consisting of timber production areas and natural grasslands as well as indigenous forest patches. I then characterise the complexity of the soundscape using species richness, an acoustic activity index, and the total time of recorded calls. I also acoustically explore the role that ecotones play in promoting bush cricket diversity, and investigate whether there are compositional changes between the core biotopes and at these ecotones. I then divided the soundscape into central frequency bands to determine whether this landscape impacts the distribution of species based on call parameters, as well as to identify whether characteristic species and central frequency bands exist in the various biotopes. Eleven species of bush cricket were identified, with vegetation cover the most important factor driving their local distribution. The natural forest-grassland ecotone supported the most complex soundscape. Assemblage composition changed as the sampled biotopes become woodier. As a result, higher frequency call bands are lost from these woodier environments, likely from species having adopted calls that maximise transmission yet minimise attenuation in woody environments. Bush cricket species showed distinct associations with particular biotope combinations. Therefore, in an effort to conserve the full soundscape, natural habitat heterogeneity must be maintained, as species have adapted to the acoustic spaces in each biotope. Lastly, the plantations were suitable habitats for many species, providing functional connectivity across the landscape.

4.1 Introduction

Many animals rely on acoustic communication to convey information, and aid in inter- and intra-specific communication (Gasc et al. 2013; Lampe et al. 2014; Ross & Allen 2014). Species-specific calls are also produced to find and select mates (Jain & Balakrishnan 2011; Deb & Balakrishnan 2014). Furthermore, evidence also suggests that in yellow-bellied marmots (*Marmota flaviventer*), nosier calls indicate a higher parasite load (Nouri & Blumstein 2019). Species-specific calls further indicate the presence of a species at a site, even if the caller cannot be seen, allowing for both rapid, and accurate assessments of acoustically communicating species richness (Riede 1993, 1998; Nischk & Riede 2001).

Attenuation, the loss of energy in a call, is affected by the structure of vegetation through which a call is transmitted. Morton (1975) used bird calls to test the degree to which species calls attenuate in a variety of landscapes. From this work, the acoustic adaptation hypothesis was developed, stating that a species will have adapted its calls to best transmit in its natal biotope. The southern African endemic, bladder grasshoppers, are known to emit calls that travel up to 1.5 – 1.9 km from the caller. Through playback experiments of calls in both native and non-native vegetation types, Couldridge et al. (2004) were able to provide support for the acoustic adaptation hypothesis, that is, calls in native vegetation types are transmitted over larger distances than calls in non-native habitats, thereby maximizing the communication range of species in their native habitats. The environmental shaping of acoustic space therefore results in distinct soundscapes forming across different biotopes (Morton 1975; Bormpoudakis et al. 2013). However, higher frequencies are more prone to the effects of attenuation (Morton 1975; Forrest 1994; Couldridge & van Staaden 2004).

Many parallels exist between ecoacoustics and landscape ecology (Pijanowski et al. 2011). Sounds across a landscape are not homogeneous, indeed, soundscapes can be highly heterogeneous. Soundscapes vary both temporally (Farina et al. 2011; Rodriguez et al. 2014; Grant & Samways 2016; Bradfer-Lawrence et al. 2019) and seasonally (Krause et al. 2011; Farina & Pieretti 2014), as well as with both landscape features (Mazaris et al. 2009; Tucker et al. 2014) and vegetation characteristics (Jain & Balakrishnan 2011; Bormpoudakis et al. 2013; Farina & Pieretti 2014). Rural landscapes are significantly more dominant in terms of biophony, than urban landscapes, where anthropogenic noises dominate and have a negative impact on native bird species richness (Joo et al. 2011). Acoustic responses can also be applied to climate change monitoring (Krause & Farina 2016). Furthermore, acoustic monitoring of dry lowland coastal forests in Tanzania, have proved successful in differentiating between intact natural forests, and previously logged and degraded forests (Sueur et al. 2008).

Acoustic behavioral responses to landscape alteration are not static. For example, habitat fragmentation can drive the development of bird 'dialects'. Birds learn their calls, and as a result of isolation among populations, idiosyncrasies can develop in the calls of individuals in the various populations. Given enough time, the divergence in calls could lead to the formation of reproductive barriers (Laiolo 2010).

Furthermore, grasshoppers, when exposed to high levels of anthrophony as nymphs, produce calls as adults that are higher in frequency (Lampe et al. 2012), as well as calls with altered syllable-pause ratios (Lampe et al. 2014). It is thought that these call modifications avoid signal masking by background noise (Lampe et al. 2014). As a result, ecoacoustics offers a complementary (Tucker et al. 2014), reliable, and non-invasive method where sounds in a landscape can be used to monitor changes across a landscape in relation to anthropogenic disturbance (Sueur & Farina 2015).

A number of insect orders produce sounds, with Hemiptera and Orthoptera being the most noticeable producers of insect sound in many landscapes (Nischk & Riede 2001). Hemiptera are largely diurnal and dominate the daytime soundscape (Farina et al. 2011), with a shift to orthopteran dominated soundscapes at night, consisting largely of cricket (Gryllidae) and bush crickets (Tettigoniidae) calls (Riede 1993; Nischk & Riede 2001). Orthoptera produce species-specific calls as long distance signals to attract mates (Couldridge & van Staaden 2004; Jain & Balakrishnan 2011). Using these calls, distribution (Thompson et al. 2019), species richness (Riede 1998) and abundances (Fischer et al. 1997) of orthopteran assemblages can be accurately assessed.

Orthoptera are sensitive to ecological condition. The composition of German grasshopper assemblages changed at the ecotone between dry-grassland and the surrounding agricultural land due to increased nutrient input from the surrounding fields (Fischer et al. 1997). Microhabitat changes at ecotones (Didham & Lawton 1999), due to novel interactions of the biotic and abiotic components of a landscape, drive the differential responses of species at biotope edges. As a result, forest-grassland edges comprise of a mix of both forest- and grassland-inhabiting beetle species (Ewers & Didham 2008). Similarly, ant assemblage composition changes significantly across forest-grasslands ecotones (Dröse et al. 2019). Permeability of the edges between biotopes is dependent on the contrast, or structural similarity, between biotope types (Ries et al. 2004), and the higher the contrast between biotopes, the less spillover occurs between them (Inclán & Marini 2015). The direction of spillover at an edge is largely driven by the availability of resources at an edge, yet responses to these resources is species dependent (Reis & Sisk 2004).

Bush crickets are highly cryptic, nocturnal, and difficult to sample (Riede 1998; Grant & Samways 2016; Thompson et al. 2019). Yet successful and accurate monitoring of species in this family is possible through various acoustic methods. Examples range from identifying morphologically indistinguishable species from one another (Nityananda & Balakrishnan 2006), to mapping responses of bush cricket species to transformed landscapes (Grant & Samways 2016; Thompson et al. 2019). They are prime candidates for acoustic assessments. Calls can be learnt and identified by a trained listener (Diwakar et al. 2007), and readily identified to species level, as well as to an acoustic species level when taxonomies are scant, this is based on the characteristic of each species' specific call (Riede

1998). Furthermore, bush crickets are sensitive to habitat condition as are grasshoppers (Fischer et al. 1997; Bazelet & Samways 2011, 2012).

Ecological networks (ENs) are areas of interconnected remnant habitat heterogeneous (Bazelet & Samways 2012; Crous et al. 2014a; Yekwayo et al. 2016) that span transformed landscapes. The remnants form conservation corridors (Samways et al. 2010) throughout the timber production landscape of South Africa (Samways & Pryke 2016). Conserving about 500 000 ha of mostly natural habitat, these ENs effectively extend protected areas into these production landscapes (Samways & Pryke 2016). Due to the complexity of these mosaic landscapes, natural biotopes, such as grasslands and indigenous forests, occur adjacent to stands of exotic commercially grown *Eucalyptus* trees, allowing for a study that is diverse in both biotope types, and one that encompasses both natural and artificial ecotones. It is therefore possible to acoustically characterize the soundscapes, using a sensitive taxon such as the Tettigoniidae, of both natural, and transformed biotopes, as well as the resultant ecotones formed between these components of a complex landscape mosaic.

In this study, I address the following aims and hypotheses: 1) To determine how the complexity of the bush cricket soundscape changes across this mosaic landscape. This was done for bush cricket species richness, as well as by using an acoustic activity index and, calculating the total length of calls at each site and biotope. I also identified occupied central frequency bands at the site and biotope levels. As Tettigoniidae are both highly cryptic (Riede 1998; Grant & Samways 2016; Thompson et al. 2019), and rely on bushes and trees for camouflage and sound casting (Grant & Samways 2016), it is expected that the natural forests will have the most complex bush cricket soundscapes. 2) To ascertain if there are compositional changes in the bush cricket assemblages across the biotopes, and to determine whether the ecotone regions allows for overlap between assemblages or whether these zones of transition have unique species. Ecotones are areas of transition and species overlap (Ewers & Didham 2008; Dröse et al. 2019), and so it is hypothesized that the ecotones here will have novel assemblages of species from both neighboring biotopes. 3) Finally, I characterize the soundscapes by identifying key species and frequency bands associated to each biotope. If the acoustic adaptation hypothesis holds true, species will associate more with natural biotopes, than the transformed areas, as they have adapted to these natural areas (Morton 1975), whereas plantations that have been present in this system for some 50 years are not a historic feature.

4.2 Methods

4.2.1 Study area and design

This study was conducted in the Baynesfield, Richmond and Byrne Valley areas of Kwa-Zulu Natal (29° 48' 24.14" S; 30° 14' 4.4" E), South Africa. A mosaic landscape, dominated by exotic timber production, remnant patches of Southern Mistbelt Forest and Midlands Mistbelt Grassland (South

African National Biodiversity Institue 2018) exist between production areas. Study sites were located on private land, as well as two timber production estates.

Sixty sites in seven target biotopes were identified (Figure 4.1), four core biotopes, where sites were located 60 m from the edge, and three ecotone biotopes, where sites were situated directly at the interface of two biotopes. Core biotopes identified for sampling were: indigenous forest (F, n = 8), natural remnant grassland (G, n = 10), closed canopy plantations (CC, n = 10), consisting of mature *Eucalyptus* trees with a closed canopy, and open canopy plantation (OC, n= 8), where *Eucalyptus* trees were 2–5 years old. Three ecotones identified for sampling were: forest-grassland ecotone (FG, n = 8), grassland-plantation ecotone, between plantation of both types (GP, n = 8), and closed canopy- forest ecotones (CCF, n = 8). Both OC and CC plantations were used in the GP ecotones due to limited distribution of plantation age classes in the landscape. Sampling effort was uneven across the biotopes due to technical issues, for example, battery failure or only half the frequency spectrum being recorded, resulting in incomplete sampling at a few sites which were excluded from the study.

4.2.2 Bush cricket sampling

Sampling was conducted from March-May 2018. Four SM2+, and three SM4 recorders (Wildlife Acoustics, USA) were used concurrently. Recorders were fitted with omnidirectional microphones (SMX-II omnidirectional microphone for the SM2+ recorders, and SMM-A2 omnidirectional microphone for the SM4 recorders), and all had a frequency response range of 20 – 20 000 kHz. Each recorder was programmed to record for 5 min, every hour. For the purpose of this study, only recordings at 19h00, 21h00, 23h00, 01h00, 03h00 and 05h00 were analysed. Recorders were placed at a site for four consecutive nights, allowing for simultaneous sampling of the seven identified biotopes at any one time, resulting in 24 recordings per site. After which, recordings were downloaded, and recorders redeployed at seven new sites. This was done until all sites were sampled. Overall, 1 440 recordings were generated, containing 120 hrs of soundscape recordings, two hours from every site.

Sampling rate for all recordings was set to 48 kHz, and the gain of the microphones set to six to enable faint, high frequencies to be recorded. All recordings were saved as uncompressed wave format files (.wav). Recorders were placed on naturally occurring landscape features (i.e. rocks and trees), or clearings in vegetation, enabling them to be higher than the surrounding grass or understorey vegetation, this prevented excessive attenuation of calls by surrounding vegetation. Ambient temperatures at the time of recording were measured and saved by each recorder. Due to safety concerns, and the challenging nature of entering indigenous forests at night, no nocturnal fieldwork was conducted at the sites, although bush crickets were sampled at a nearby forestry station to collect both reference calls and specimens to aid later signal identification. As calls are unique to each species, even those species which were not sampled at the forestry station can be identified as acoustic species.

4.2.3 Vegetation and environmental variables

At each site, a graduated pole was dropped a hundred times, i.e. at every meter along a transect to measure both plant height and percentage plant cover. The number of plant structural classes (forbs, shrubs, dead vegetation, as well as bare ground and rock) intercepted by the pole at each point, as well as the maximum height, were recorded. By adding all hundred points together, percentage plant cover could be determined, as well as the average plant height at each site. Plant structural heterogeneity was calculated by adding together the different structural classes present at a site.

4.2.4 Signal analysis

Using Raven Pro 1.5 (Cornell Labratories, USA), all recordings were visualised as a spectrogram spanning 0 – 24 kHz. By listening, and visually identifying the call patterns of each signal, bush cricket species and morphospecies were identified. This was done using calls isolated from individuals caught at the forestry station as well as from calls provided by PBC Grant. Each individual call that was identified was measured, and the following information extracted: begin, end, and delta time of each call, central frequency of each call (the frequency that represents 50% of all energy in the call), as well as bandwidth of the call. These parameters, as well as the visual patterns of the calls aided in their identification. For each species, the mean central frequency was calculated.

Species richness was determined for each recording, as well as for each site. Absolute abundance cannot be accurately determined from single channel recordings, thus the total time of calls at a site was calculated by summing each delta time measurement for each measured call. The total time of all the calls of each species at a site was also calculated. An acoustic Activity Index (AI) developed by Miller (2001) to determine bat activity and abundance and, successfully used earlier on bush crickets (Thompson et al. 2019), is an additional measure where an abundance estimate can be determined. By determining the presence of a species in each of a site's 24 recordings, it is possible to determine the number of recordings in which each species was identified. This was then repeated for each species at each site, and these presence values were then added together to determine the overall AI of a site.

Central frequency bands were delineated by dividing the frequency values between 5 kHz and 17 kHz, the range in which the central frequencies of all the identified species were located, into 2 kHz bands. Six bands were delineated, 5 – 7 kHz; 7- 9 kHz; 9 – 11 kHz; 11 – 13 kHz; 13 – 15 kHz and, 15 – 17 kHz. For each site, number of bands occupied, as well as species richness within each band was determined. To do this, each species central frequency was assessed, and depending on its value, assigned to a specific band. For example, site A has a total species richness of 2, species X has a central frequency of 6 kHz, it is assigned to the first band, and if species Y has a central frequency of 14 kHz, it is assigned to the fifth band. Site A therefore has two frequency bands occupied, but a species richness of 1 in both band one and band five. In addition to band occupancy and species richness, total time of all species calls per frequency band was calculated.

4.2.5 Statistical analysis

Sampling adequacy was determined by plotting the species accumulation curve. In addition the expected species richness and Chao2 estimator (Chao 1987) across all sixty sites was calculated. These analyses were conducted in *Vegan* (Oksanen et al. 2018) in *R* (R Core Team 2018). A mantel test was performed in *R* using *Ade4* (Dray & Dufour 2007), and showed that there was no spatial autocorrelation of the data (Mantle test = -0.055, p = 0.87). However, a random spatial variable was still included in all linear models to account for the clumpy spatial distribution of sites (Bolker et al. 2009). A general linear mixed effects model (GLMM) with Laplace approximation, and fitted with a Poisson distribution, was used to determine whether there was a significant impact of temperature on the species richness observed in all 1 440 recordings. The variable 'farm' (a three factor spatial categorical variable describing the ownership of the land the sites were located on) was included as a random effect. The package *lme4* (Bates et al. 2015) was used to conduct the model. As there was no significant impact of temperature ($\chi^2 = 0.29$, p > 0.05), all 24 recordings per site were pooled for further analysis.

Species richness, total call time at a site, as well as total AI of a site, were three separate measures used to assess the complexity the soundscape of each sampled site. Five environmental variables pertaining to vegetation structure and cover, as well as topology, were investigated as these were hypothesized to be important for bush cricket diversity. Slope, elevation, average vegetation height, vegetation cover, and structural heterogeneity were used, and showed no correlation. Model averaging, using the package, *MuMin* (Barton 2019), was used for both species richness and call time. All possible GLMM model combinations are automatically generated using the dredge function, and then the resultant AICc values compared. Those models whose AICc differed by < 2, were then averaged. P values and confidence intervals were used to then determine which environmental variables had a significant effect on the response variable. Poisson distribution was used for species richness, and binomial for total call time. In both cases, Laplace approximation was used, and 'Farm' was used as the random variable. As only one model was selected for the total AI values, a standard linear mixed effect model (LMM) was run for the total AI values, using the package *lme4* (Bates et al. 2015). Here, a global model was created, followed by models where successive variables were removed. The resultant models were then compared to the global model to determine which environmental variables were significant. Where significance was indicated between any of the main effects, Tukey post hoc comparisons were run. Mixed effects models were used to investigate the influences of biotope types on species richness, total call time, and total AI values. Once again, GLMMs and LMMs following the same procedures outlined above were used. A GLMM was used for species richness and for the number of occupied central frequency bands. A LMM was used for total call time data, which was near normal following the Anderson-Darling normality test (A = 0.81, p = 0.033) and the Quantile-Comparison Plot. Furthermore, these data did not follow a binomial distribution, and normal distribution performed best based on AIC

value. A LMM, with the same parameters, was used to investigate the influence of biotope type on total AI.

Compositional changes in bush cricket assemblages were investigated using Permutational multivariate analysis of variance (PERMANOVA) (Clark & Warwick 2001) and Primer-6 (Clark & Gorley 2006). Assemblages were characterised by species presence at a site, total call time per species at a site, as well as the species-specific AI values at a site. The same procedures were followed for all three assemblage descriptors. Firstly, data was square root transformed, and a Bray-Curtis similarity matrix was calculated. PERMANOVAs were then run to investigate how the bush cricket assemblages differed between the seven sampled biotopes. Unrestricted permutation of the raw data, as well as 9999 permutations, were used in these PERMANOVAs. Secondly, using the Bray-Curtis similarity matrix, distance based linear models (distLM) were used to determine significant environmental drivers. Normalised environmental drivers were used, as was a step-wise selection procedure in the models, final models were selected based on AICc values. To investigate how composition of the various central frequency bands changed between biotopes, central frequency bands were treated as species and the same PERMANOVA and distLM procedures were followed. Composition of the bands were characterised by presence of central frequency bands at a site, as well as the total length of calls in a band at a site.

To identify species associated to the various biotopes, *indicspecies* (Caceres & Legendre 2009) in *R* was used. Multi-level pattern analysis was used to determine any associations between species and the various biotope types. Both species presence in a biotope, and total time each species called in a biotope, were used as input values. The same was done with central frequency bands, to determine whether any were associated with biotopes, again, presence and time per band were used as input values.

4.3 Results

A total of 89 539 bush cricket calls and choruses were identified, belonging to 11 species (Table 4.1). The species accumulation curve reached an asymptote of 11 (Appendix 4.1), and Chao2 estimate indicated that 11 species (±0) were to be expected across the entire landscape. Across all 1 440 recordings, temperature at the time of recording did not have any significant impact on the species richness ($\chi^2 = 67.4$, p > 0.05).

4.3.1 Bush cricket soundscape complexity

Overall, vegetation cover and slope were the most significant environmental variables driving the response of bush crickets across this landscape. Species richness responded positively to vegetation cover, as did total call time and the acoustic activity measured at each site (Table 4.2). Slope negatively influenced total call time, as well as acoustic activity of a site (Table 4.2). Elevation was selected for, but did not significantly impact total call time measured at each site (Table 4.2). Species richness was not significantly impacted by biotope type (Table 4.3, Figure 4.2A), while acoustic activity was

significantly impacted by biotope type, with differences occurring between forest-grassland ecotones and sites in closed canopy plantations (Table 4., Figure 4.2B). There were many significant differences in total call time across biotopes (see Table 4.3 for detailed comparisons, and Figure 4.2C). Differences across biotope types become more defined when AI and total call time are used as response variables. There were no significant differences in number of central frequency bands occupied across different biotopes (Table 4.3, Figure 4.2D).

Grassland and the grassland-plantation ecotone were the only two biotopes in which high frequency callers were recorded (Figure 4.3). As biotopes become woodier (i.e. both plantation age classes, and indigenous forests), the fewer high frequency calls were recorded, as well as a reduced number of occupied bands relative to increased woodiness.

4.3.2 Bush cricket compositional changes

Distance based linear models indicated that vegetation cover, as well as elevation were significant drivers of species assemblages when characterised by species presence, call time, and acoustic activity (Table 4.2). PERMANOVA results indicated that there were significant differences in bush cricket assemblages for species presence, total call time, and acoustic activity (Appendix 4.2). There were similarities among assemblages of closed canopy plantations and forests, as well as forest-plantation ecotones. Grassland assemblages were similar to those of the grassland-plantation and grassland-forest ecotones. Closed canopy plantation assemblages were similar to those of the open canopy plantations. Distance based RDA plots corroborated these observations of overlap occurring between ecotone assemblages and neighbouring biotopes, but with significant differences between core areas of the grassland, forest, and both plantation age classes (Figure 4.4).

4.3.3 Central frequency bands and species-specific biotope preference

As with the compositional analyses, distance based linear models indicated that vegetation cover and elevation were significant drivers of distribution and occupancy of central frequency bands across this landscape (Table 4.2). PERMANOVA results showed similar patterns when either presence of central frequency bands, or length of all calls within each band, were analysed (Appendix 4.2). There were similarities between central frequency bands of closed and open canopy plantations, as well as between bands present in grassland, and between plantation-grassland and forest-grassland ecotones (Appendix 4.2).

Of the 11 species identified, seven showed distinct biotope preferences (Table 4.4). A single ecotone species was identified, and interestingly, no species showed a marked preference to any of the core biotopes. Where species showed association with core biotopes, they also were associated with one or more ecotones of that biotope type. Similarly, central frequency bands also indicated associations to the various biotopes (Table 4.5).

4.4 Discussion

Acoustic characterisation of a complex mosaic landscape is indeed possible, with bush crickets proving to be well suited for this. Species were recorded in all seven biotopes, including both young and mature plantation stands. This is interesting, as in a similar study in a similar landscape, *Eucalyptus* stands were found to be devoid of all acoustics species, except for a single cricket species (Grant & Samways 2016). Here, I found five different bush cricket species within the two plantation age classes, and these assemblages are also significantly different from the other biotopes. Similarly, ecotones also have characteristic species assemblages, the component species of which are also present in either both, or one, of the adjacent biotopes. In turn, the natural ecotone between indigenous grassland and forest is the most speciose of all biotope types, and therefore of conservation significance.

Bush cricket diversity across this landscape can be represented using species richness, Activity Index (AI) values, as well as total call time. In contrast, species richness, a traditional measure of diversity at a site (Gotelli & Colwell 2001), does not resolve differences in bush cricket diversity, with emergent patterns appearing only when either AI or total call time are used. AI considers both species richness and the number of recordings per site at which each species was recorded. This also avoids pseudoreplication, as only call presence is used in the calculations and not call number per recording. In turn, call abundance is not an accurate reflection of true abundance, as one species may call once in 5 minutes and another a hundred times, thereby skewing the data should the number of recorded calls be the sole value used. AI prevents this from occurring (Miller 2001; Thompson et al. 2019). Here, total call time was preferred as a better measure of true abundance, as it represents the total time that all species were calling, and not just presence at a site. As each measure characterises a different facet of the bush cricket soundscape, and similar patterns are resolved for each species, a combined approach using species richness, AI values, and total call time, is sufficient to characterise the complexity of the soundscape.

Irrespective of which measure was used, vegetation cover positively affected both diversity and assemblage composition across all sites and assemblages. Bush crickets rely on vegetation especially for camouflage (Grant & Samways 2016), call broadcasting sites (Arak & Eiriksson 1992), and for oviposition (Rentz 1988; Bailey & Rentz 1990). Following this, importance of vegetation cover and structure was expected. However, the negative influence of slope on total call time and AI was not expected. Among Himalayan bird species, individuals orientate themselves so that they call up-hill, facilitating call transmission (Hunter 1989), perhaps this the case here. Sharper gradients may impede call transmission of the individual bush cricket calls, and so in areas where steeper gradients are encountered, less bush cricket activity occurs.

The forest-grassland ecotone was not only the most speciose soundscape, but also the most complex bush cricket soundscape, as both total call time and AI were high. The naturally changing microclimates

between these two biotopes, as well as increase in woody vegetation and grasses close to the forest edge (Kotze & Samways 1999; Dröse et al. 2019), result in a natural increase in vegetation cover and heterogeneity between forest and grassland. As habitat heterogeneity is essential for maintenance of biodiversity (Crous et al. 2013; Slancarova et al. 2014; Fahrig 2017), these naturally complex edges, a mix of both grassland and forest vegetation types, are able to support a more complex bush cricket soundscape, composed of both grassland and forest species, and not only generalist species as in the plantations.

The *Eucalyptus* plantations are structurally more complex than the grassland, yet can be considered less so than indigenous forests, as the plantation trees are regularly spaced and mostly monocultures. The understorey in plantations is managed and virtually non-existent, except for some pioneer grass species in open-canopy plantations. This relatively open environment, allows higher frequencies to carry more than in natural forest. However, soundscapes between the two different plantations types are not significantly different, with the same bush cricket species occurring in both. This suggests that there are no differences in microhabitats between the two, and that bush cricket assemblages perceive the different plantation age classes as the same.

The species assemblages and soundscapes reflect this adaptation to the physical environment. Assemblages in the different biotopes did not form tight groups, but rather, species assemblages changed as the complexity of the environments increased. Even so, assemblages showed marked changes at the ecotones. For example, the grassland assemblages were not significantly different from the assemblages at the grassland-forest ecotone, yet this ecotone is significantly different from the assemblages inside the forest. Similarly, grassland assemblages, closed canopy plantations, and the grassland-plantation ecotone are also different. Again, this sharp turnover in assemblage composition directly reflects vegetation structure. Similar environments, such as plantations and forest support this contention, as there were no significant differences in assemblage composition of either biotope, or the intermediate ecotone formed between the two of them, with the species equally at home in both indigenous forests and adjoining plantation. Likely, these plantations are not barriers to movement of these insects across this landscape mosaic, and may even facilitate movement between patches of natural vegetation.

Although not representative of species, central frequency bands represent portions within the acoustic space where bush cricket species are active. As with the characteristic species, associations of bands to biotopes were the same when both presence of bands, and time spent calling in each band, were considered. As biotopes in this landscape become woodier (grass → plantation → indigenous forest), fewer central frequency bands were occupied, and those that were occupied, were lower-frequency bands. High-frequency calls are more prone to signal attenuation than lower frequency calls (Morton 1975; Forrest 1994; Couldridge & van Staaden 2004; Jain & Balakrishnan 2011). Here, there was a

shift in band associations when biotopes became progressively more open. This possibly resulted in bush cricket species, having adapted to the natural biotopes, showing higher frequency band associations with grassland and their ecotones. Grassland is characteristically an open, grass- and forb-dominant environment, while forest is complex, with saplings and shrubs making up the understorey layer and mature trees forming a closed canopy. This increase in structural complexity, increases the number of barriers to sound propagation, and as a result, higher frequency calls do not propagate well in the forests (Morton 1975; Forrest 1994).

Changes in the structural heterogeneity of the seven biotope types present unique acoustic environments, which in turn, shape species local distributions and thereby the resultant soundscapes (Bormpoudakis et al. 2013). Characteristic species of each biotope's soundscape could be determined using both species presence, and time spent calling, with no discernible differences. Generalist species of *Ruspolia*, *Plangia* (Grant & Samways 2016; Thompson et al. 2019) and *Conocephalus* (Grant & Samways 2016), were all here associated with either artificial edges, or with a range of biotope types. Interestingly, results indicate that *Conocephalus maculatus* is an artificial-edge specialist, characteristic of the plantation-grassland ecotone. This is contrary to the finding of Grant & Samways (2016), who found *C. maculatus* to be a generalist species inhabiting a wide range of biotope types, from wetlands, grasslands and indigenous forests. As this species does not show seasonal fluctuations in numbers (Bazelet & Naskrecki 2014a), perhaps the population size in this landscape is considerably smaller than the one sampled by Grant & Samways (2016), leading to less sampling instances. The call of this particular species is conspicuous and not easily mistaken acoustically, making this an unlikely reason for it to be found only in one biotope.

Specialist species, such as the endangered *Thoracistus semeniphagus* (Rentz 1988; Bazelet & Naskrecki 2014b), was found here to prefer grassland and forest edges. Interestingly, a similarly endangered close relative in the same genus, *Thoracistus thyraeus*, which was thought to occur only in grassland and weedy edges (Rentz 1988; Bazelet & Naskrecki 2014c), was here found in all the sampled biotopes, and not characteristic of any particular biotope. A third, commonly occurring member of the genus, *Thoracistus viridifer* (Rentz 1988; Bazelet & Naskrecki 2014d), was also acoustically identified, and found associated with open, less woody habitats of the grassland and grassland-forest ecotone, as well as the artificial grassland-plantation ecotone.

4.5 Conclusions

Acoustic monitoring in these production landscapes is both a straightforward method, as well as complementary to traditional assessment methods (Tucker et al. 2014), enabling not only the physical environment to be monitored, but also offers a window through which to observe how individual species perceive and interact with spatially different ecological conditions (Mazaris et al. 2009; Sueur & Farina 2015; Krause & Farina 2016; Mullet et al. 2017).

The importance of habitat heterogeneity for the maintenance of biodiversity is well known, and well-studied (Grant & Samways 2011; Crous et al. 2014b; Fahrig 2017; van Schalkwyk et al. 2017). Yet this is only one perceivable layer of the landscape. Soundscapes, and ecoacoustics, add an additional layer through which landscapes can be studied (Mazaris et al. 2009) and through which species response to habitat transformation can be understood (Mazaris et al. 2009; Joo et al. 2011; Sueur & Farina 2015; Krause & Farina 2016). By characterising the soundscape of this timber production landscape, I have shown that a relatively complex landscape maintains considerable habitat and environmental heterogeneity that, in turn, supports a wide range of diverse acoustic species. In addition to maintaining a wide array of species, conserving heterogeneity in this landscape ensures that soundscape heterogeneity is also conserved. Among this heterogeneity is a wide variety of ecotone types which support a diverse and complex bush cricket soundscape. Species composition varies with an increase in woodiness of the biotopes, indicating that species have adapted to the acoustic space available in each biotope. Importantly, maintenance and conservation of natural ecotones, such as grassland-forest in this timber production landscape is essential to maintain the full complement of bush cricket species. Yet the plantation stands, surprisingly, maintain many species, and provide functional connectivity across this transformed landscape.

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4.7 Tables and figures

Table 4.1: Relative call abundance (% time) of each bush cricket (Tettigoniidae) species call in each biotope sampled, as well as the central frequency (CF) of each species call.

Species	Forest	Closed canopy plantation	Plantation forest ecotone	Plantation grassland ecotone	Forest grassland ecotone	Grassland	Open canopy plantation	CF (kHz)
Conocephalus maculatus				6.18		0.39		15.02
Conocephalus sp. 1	52.06	40.01	57.12	0.22	5.21	2.07	21.98	11.23
Plangia sp. 1	9.05	5.12	5.01		0.45	0.17	3.09	12.34
Ruspolia sp. 1				6.37	4.37	18.25		9.23
Tettigonniidae sp. 1				2.28				14.14
Tettigonniidae sp. 2						0.38		13.88
Tettigonniidae sp. 3				0.21	0.02		0.31	9.65
Tettigonniidae sp. 4	0.06		0.04					10.03
Thoracistus semeniphagus				0.28	0.03	6.91		9.01
Thoracistus thyraeus	38.83	42.81	26.37	6.76	44.49	16.44	40.51	6.45
Thoracistus viridifer		12.07	11.45	77.70	45.42	55.40	34.11	13.53

Table 4.2: Showing the pertinent environmental variables determined by model averaging for total species richness and total call time across all sites, as well as the results for the standard model procedure conducted on the acoustic activity across all sites (* p < 0.05; ** p < 0.01; *** p < 0.001). Finally multivariate results from distLM conducted on the entire bush cricket assemblage and central frequency band are presented (* p < 0.05). x Denotes variables not included in the particular model.

	Test statistics	Vegetation cover	Elevation	Slope
	Univariate responses (mixed models)			
Species richness (GLMM, model averaging)	Estimate	0.1		
	SD	0.15		
	z value	1.98*	x	x
	Confidence interval	[0.003; 0.62]		
	Relationship	Positive		
Call time (GLMM, model averaging)	Estimate	4.08	-0.63	-1.83
	SD	1.08	0.85	0.85
	z value	3.7***	0.74	2.11*
	Confidence interval	[1.92; 6.25]	[-2.86; 0.4]	[-3.54; -0.13]
	Relationship	Positive	Neutral	Negative
Acoustic activity (LMM, standard procedure)	χ^2	5.57*		7.41**
	df	1	x	1
	Relationship	Positive		Negative
	Multivariate responses (distance based linear models)			
Species presence	Pseudo F	10.88*	6.74*	2.43
	Explained cumulated variation		27.83	
Species call time	Pseudo F	2.25*	6.34*	x
	Explained cumulated variation		21.21	
Species acoustic activity	Pseudo F	9.81*	6.77*	2.55
	Explained cumulated variation		26.87	
Central frequency band presence	Pseudo F	13.66*	11.0*	3.27
	Explained cumulated variation		35.89	
Central frequency band time	Pseudo F	9.71*	6.49	x
	Explained cumulated variation		23.09	

Table 4.3: Results of models investigating the effect of biotope on species richness, acoustic activity, total call time, and number of central frequency bands occupied in each biotope (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Abbreviations: FG- Forest-grassland ecotone, GP- Grassland-plantation ecotone, CCF- Closed canopy plantation-forest ecotone, CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland and F- Forest.

	Model type	χ^2	df	Differences observed
Species richness	GLMM	7	6	None
Acoustic activity	LMM	16.12*	6	FG-CC**
Call time	LMM	32.48***	6	GP – CC*** FG – CC*** G – CC** GP – CCF* FG – CCF** OC – GP** OC – FG**
Central frequency bands	GLMM	9.46	6	None

Table 4.4: Indval results indicating bush cricket species with strong associations to each biotope (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Abbreviations: FG-Forest-grassland ecotone, GP- Grassland-plantation ecotone, CCF- Closed canopy plantation -forest ecotone, CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland and F- Forest.

Biotope combinations	Values	*Conocephalus maculatus*	Tettigoniidae sp. 1	*Thoracistus semeniphagus*	*Thoracistus viridifer*	*Ruspolia* sp. 1	*Plangia* sp.1	*Conocephalus* sp. 1	Total species associated
GP	Species presence	0.50**							1
	Species call time	0.50**							1
GP + OC	Species presence		0.53**						1
	Species call time		0.53**						1
FG + G	Species presence			0.48*					1
	Species call time			0.48*					1
GP+ FG + G	Species presence				0.72***	0.60***			2
	Species call time				0.72**	0.60*			2
F + CC+ CCF + FG+ OC	Species presence						0.63***	0.42*	2
	Species call time						0.63***	0.42*	2

Table 4.5: Indval results indicating central frequency bands with strong associations with particular biotopes (* p < 0.05, ** p < 0.01, *** p < 0.001). Abbreviations: FG- Forest-grassland ecotone, GP- Grassland-plantation ecotone, CCF- Closed canopy plantation-forest ecotone, CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland, and F- Forest.

Biotope combinations	Values used	9-11 kHz	11-13 kHz	13-15 kHz	15-17 kHz	Total bands associated
GP	Band presence				0.5*	1
	Band call time				0.5*	1
GP+ FG + G	Band presence			0.75***		1
	Band call time			0.75***		1
GP + FG + G + OC	Band presence	0.53**				1
	Band call time	0.53**				1
F + CC + CCF + FG + OC	Band presence		0.66***			1
	Band call time		0.66***			1

Figure 4.1: Map showing location of the sampled farms and recording locations as indicated by the symbols. Green polygons indicate the location of *Eucalyptus* plantations.
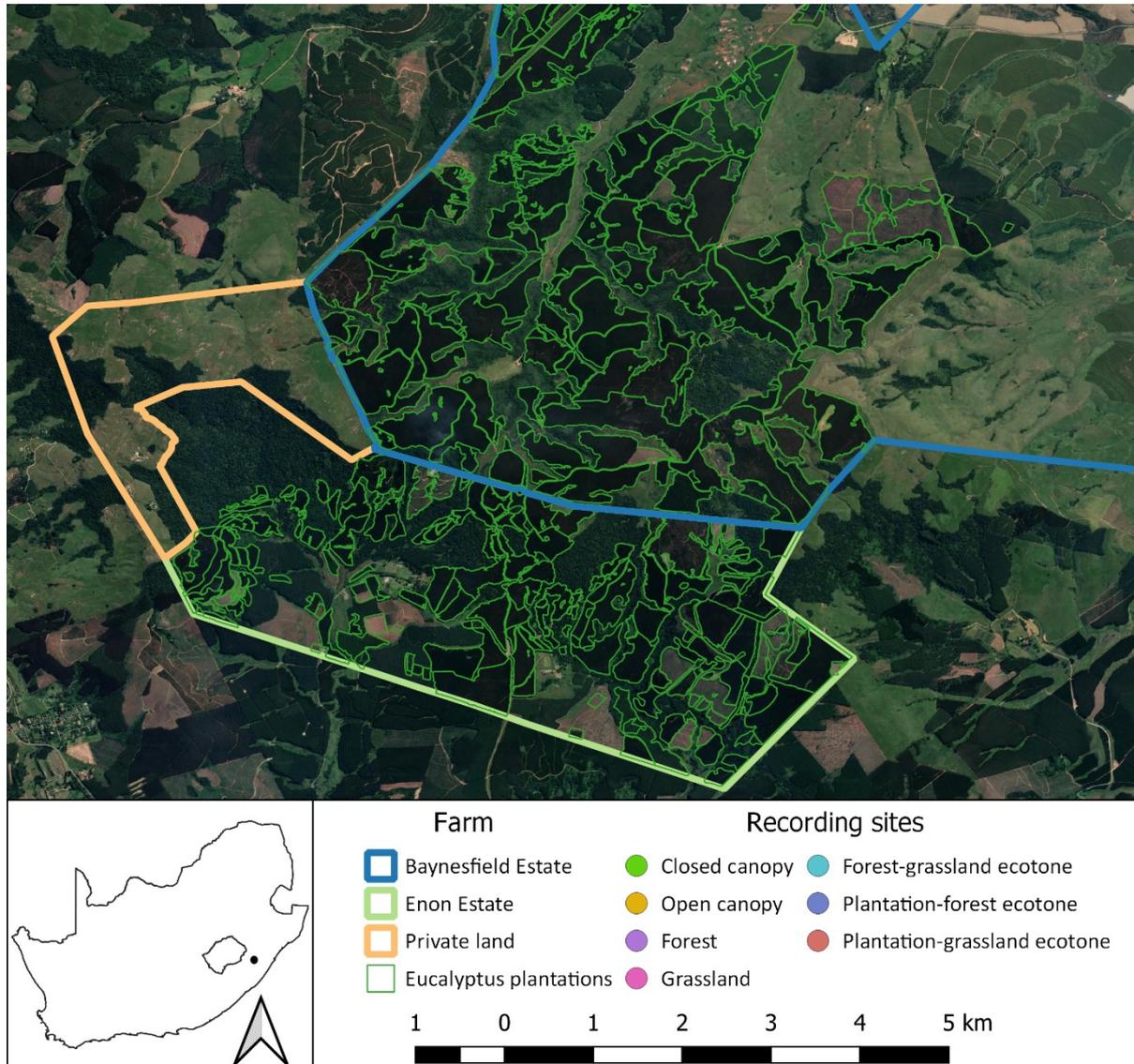
Satellite image from Google Earth (Image@2019 Maxar Technologies)

Figure 4.2: Changes across biotopes, in A: bush cricket species richness; B: Acoustic activity and, C: Total call time and, D) Number of occupied central frequency bands. Abbreviations: FG- Forest-grassland ecotone, GP- Grassland-plantation ecotone, CCF- Closed canopy plantation-forest ecotone, CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland and F- Forest.
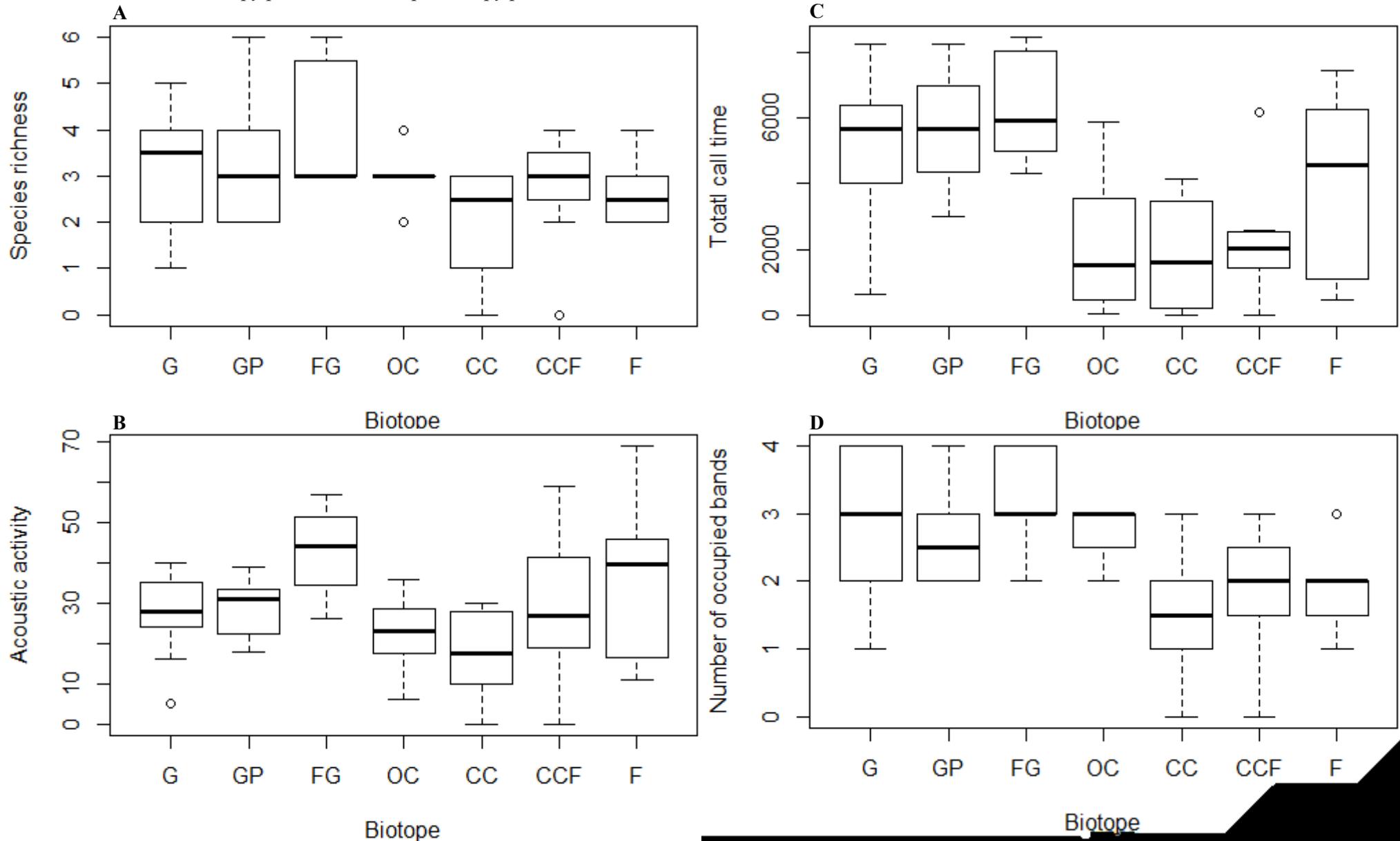
Figure 4.3: Illustration of occupied central frequency bands in each biotope based on the species that occur in biotope. Abbreviations: FG- Forest-grassland ecotone, GP- Grassland-plantation ecotone, CCF- Closed canopy-forest ecotone, CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland, and F- Forest.

Frequency bands		G	GP	FG	OC	CC	CCF	F
	15-17 kHz	x	x					
	13-15 kHz	x	x	x	x	x	x	
	11-13 kHz	x	x	x	x	x	x	x
	9-11 kHz	x	x	x	x		x	x
	7-9 kHz							
	5-7 kHz	x	x	x	x	x	x	x
		Open	**Open-woody**		**Woody**			

Figure 4.4: Distance based RDA plots showing bush cricket species assemblage responses to the different sampled biotopes. A: Species presence, B: Species call time and C: Species acoustic activity. Abbreviations: FG- Forest-grassland ecotone, CCG- Grassland-plantation ecotone, CCF- Closed canopy plantation-forest ecotone, CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland and F- Forest.

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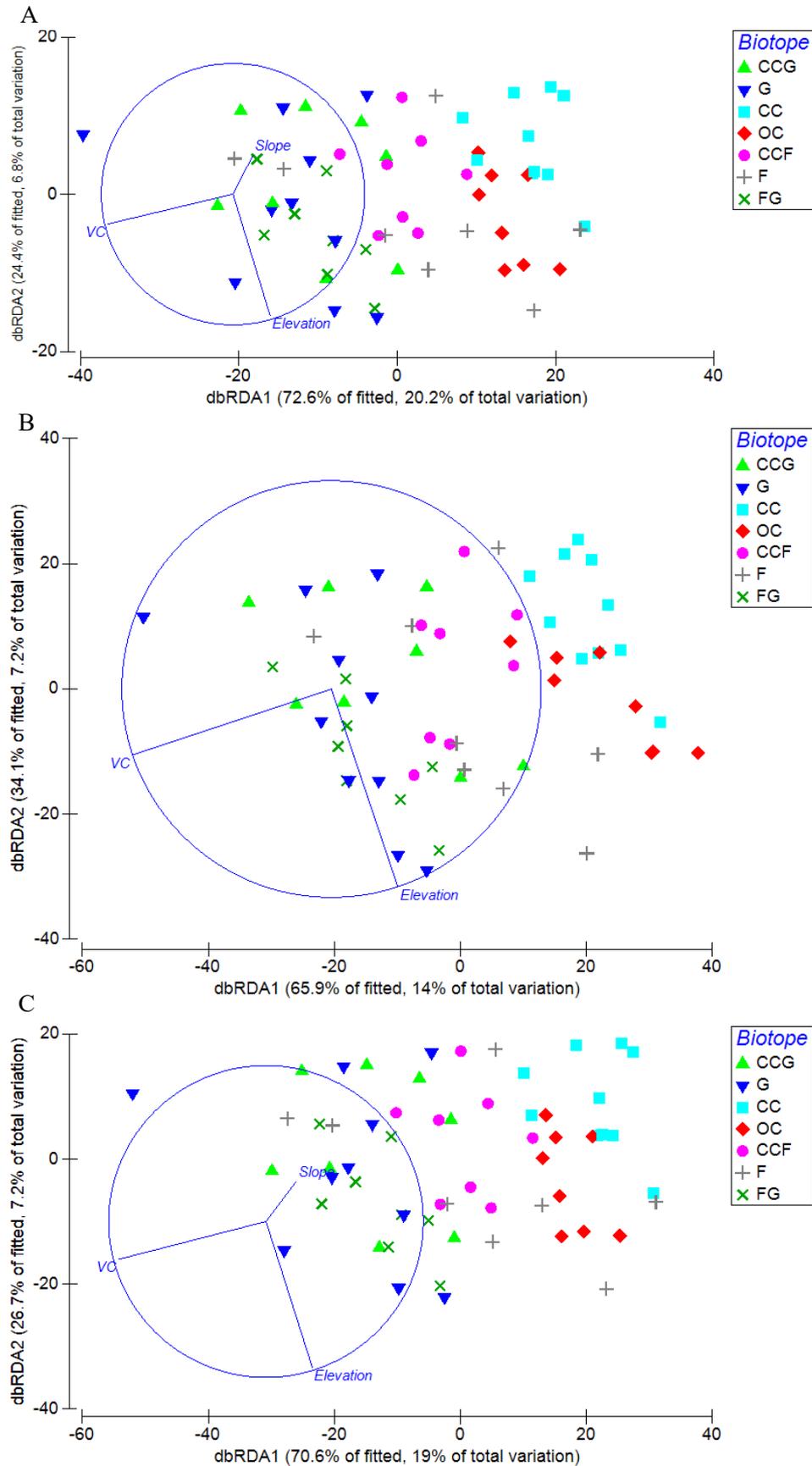
4.8 Appendices

Appendix 4.1: Species accumulation of all bush cricket species identified in all recordings. Grey area indicates 95% confidence interval.

Species accumulation curve
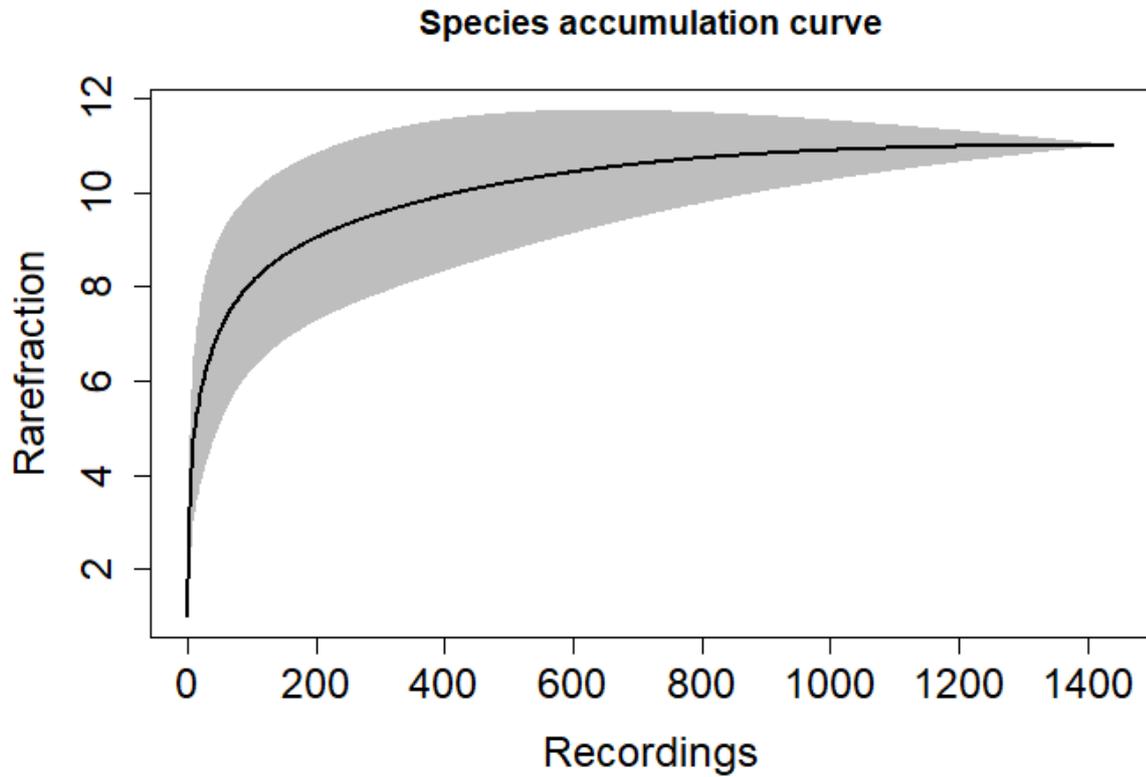
Appendix 4.2: PERMANOVA posthoc test results showing t-values and indicating significant differences between the bush cricket assemblages characterised by species presence, total species call time, species acoustic activity, as well as the presence and total call time of each central frequency band (* p < 0.05). Abbreviations: FG- Forest-grassland ecotone, GP- Grassland-plantation ecotone, CCF- Closed canopy-forest ecotone, CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland, and F- Forest.

	F	G	CC	OC	CCF	GP
Species presence						
G	3.94*					
CC	**1.45**	2.93*				
OC	1.79*	2.65*	**1.52**			
CCF	**0.69**	2.97*	**0.85**	**1.31**		
GP	5.03*	**1.18**	3.43*	2.98*	3.65*	
FG	3.62*	**0.98**	2.42*	**1.71**	2.43*	1.76*
Species total call time						
G	3.28*					
CC	**1.18**	2.28*				
OC	1.61*	2.04*	**0.83**			
CCF	**1.14**	2.66*	**0.79**	**1.21**		
GP	5.01*	**1.35**	3.12*	3.01*	3.91*	
FG	3.44*	**1.35**	2.23*	2.13*	2.78*	2.7*
Species acoustic activity						
G	3.68*					
CC	**1.38**	2.61*				
OC	1.64*	2.33*	**1**			
CCF	**1.05**	2.73*	**0.85**	**1.14**		
GP	5.74*	**1.38**	3.61*	3.33*	3.92*	
FG	3.62*	**1.4**	2.41*	2.00*	2.50*	2.51*
Central frequency band times						
G	4.03*					
CC	**1.26**	2.72*				
OC	1.72*	2.43*	**0.85**			
CCF	**0.844**	3.08*	**0.65**	**1.17**		
GP	5.86*	**1.62**	3.41*	3.40*	4.12*	
FG	3.75*	**1.69**	2.34*	2.27*	2.84*	2.90*
Central frequency band presence						
G	4.9*					
CC	**1.45**	3.70*				
OC	20.3*	2.8*	2.06*			
CCF	**0.78**	3.19*	**0.25**	**1.54**		
GP	60.3*	**1.15**	4.15*	4.08*	3.78*	
FG	4.4*	**1.12**	2.87*	**1.95**	2.50*	2.42*

Chapter 5:

Acoustic response of a threatened bush cricket species (Tettigoniidae: *Thoracistus thyraeus*) to a complex production landscape mosaic

"Grasshopper's song in

moonlight – someone's

survived the flood."

-Issa

Abstract

South Africa is a mega-diverse country, in which 169 species of bush crickets occur, two thirds of which are threatened endemics. Among these is the Endangered Inflated Seedpod Shieldback (*Thoracistus thyraeus*), a charismatic species, easily identified acoustically. Thought to be confined to grasslands, it is threatened by habitat transformation, especially by plantation forestry. Ecological networks of remnant grassland and forest conservation corridors within timber production areas of South Africa presents an opportunity to determine the response of singing *T. thyraeus* in a complex landscape mosaic of grassland and *Eucalyptus* plantations. I identified its preferred biotopes, as well as important environmental drivers of its distribution, and investigated potential competition from co-occurring bush cricket species to determine whether acoustic niche partitioning occurs in the bush cricket assemblage. *T. thyraeus* was recorded in all seven sampled biotopes, with average vegetation cover and height driving this distribution. No acoustic competition was found to occur between *T. thyraeus* and other sympatric bush cricket species, as a result of acoustic niche partitioning between species. Where species call in the same central frequency band, differences in call structures appear to prevent acoustic interference between species. *T thyraeus* is well conserved in this modified landscape, and likely not Endangered. It is a good candidate to promote insect conservation as well as acoustic heterogeneity in this complex production landscape, as *T thyraeus* it is charismatic and easily identifiable acoustically.

5.1 Introduction

South Africa, is a mega-diverse country supporting three global biodiversity hotspots: Succulent Karoo, Cape Floristic Region, and Maputaland-Pondoland-Albany hotspots (Myers et al. 2000; Mittermeier et al. 2004; Mittermeier et al. 2011). Bush crickets are diverse within South Africa (Bazelet et al. 2016), with an estimated 169 species, of which two thirds are thought to be endemic (Picker et al. 2004). Congruency exists between these biodiversity hotspots and hotspots of bush cricket (Tettigoniidae) diversity and endemism (Bazelet et al. 2016). Due to available information on individual species, 133 have been assessed according to the IUCN Red List assessment criteria (Bazelet et al. 2016; Thompson et al. 2017), of which all threatened species, those assessed as vulnerable (VU), endangered (EN), and critically endangered (CR), are endemic to the region (Thompson et al. 2017). Furthermore, this taxon appears to be representative of the threat status of ecosystems. Threatened ecosystems in the Western Cape, South Africa, appear to contain more sensitive, and threatened, bush cricket species (Thompson et al. 2017).

One genus of bush crickets, *Thoracistus*, is particularly interesting. All species within the genus are endemic to South Africa, and the males have an enlarged pronotum that extends over the tegmina, and almost covering the entire abdomen, although such a pronotal extension also occurs in other genera. The exact function of this expanded pronotum is unknown, but may function to amplify male calls. Male calls are used to distinguish between species in the genus (Rentz 1988), as is the case for many bush crickets (Nityananda & Balakrishnan 2006). Restricted to the southern and eastern parts of South Africa, members of this genus often occur in groups in grasslands and along wooded edges, as well as some species being present in disturbed habitats (Rentz 1988). The threat status of the eight species within this genus range from Least Concern (Bazelet & Naskrecki 2014d) to Critically Endangered (Bazelet & Naskrecki 2014b,c).

Thoracistus thyraeus (Inflated Seedpod Shieldback) (Figure 5.1) is one of the most distinctive members of the genus. It has the largest pronotum extension (Rentz 1988). The male song consists of short rapid bursts carried over many metres, is highly distinctive in the field and in audio recordings. Until recently, *T. thyraeus* was known from only three locations, although I have also located it in the Richmond and Baynesfield region of KwaZulu-Natal, South Africa (Chapter 4). It is thought that *T. thyraeus* is restricted to grasslands and pasturelands (Bazelet & Naskrecki 2014c), although the type specimen was collected from alongside a weedy road verge (Rentz 1988). It is red-listed as Endangered, and thought to be highly sensitive to habitat transformation as a result of agricultural activities, including plantation forestry (Bazelet & Naskrecki 2014c). Thus, the distribution of this species across a landscape with plantation and natural elements, should be limited to areas of naturally occurring vegetation, and not include the transformed areas characterised by plantation forestry.

Natural areas of this region are maintained between the forestry areas through an interconnected network of conservation corridors and patches in the form of large-scale ecological networks (ENs) (Samways & Pryke 2016). The interconnected nature of the ENs brings both natural patches and production lands together to form a complex landscape mosaic. Various transition zones between different natural habitats, as well as transitional areas between transformed and natural habitat patches exist within the ENs. The responses of invertebrate diversity, as well as their assemblages, to these transitional zones are both dependent on contrast between vegetation types (Yekwayo et al. 2017; Chapters 2 and 3), as well as on species traits (Chapter 3). Indigenous forest and grasslands have highly distinct arthropod assemblages, yet where contrast is low, between forests and mature plantations, some spillover occurs at the edges (Chapters 2, 3 and 4).

Many bush crickets are edge-tolerant, unlike acridid grasshoppers, whose abundance often increases with distance from edge (Bieringer & Zulka 2003; Samways 1977a). Bush cricket assemblage composition changes as the sampled biotopes become woodier, with the edges between the different biotopes supporting species from both adjacent biotopes (Chapter 4).

The acoustic adaptation hypothesis postulates that higher frequencies are attenuated in dense vegetation (Morton 1975), such as forests, and so higher frequency calls are more likely in more open environments, such as grasslands. A second hypothesis, put forward by Krause (1993), is that of the acoustic niche. Here, it states that the frequency spectrum is divided into bands, or niches, which can only be occupied by one species at one time (Krause 1993; Mullet et al. 2017). Crickets can partition the frequency spectrum, and when released from competitive pressure, species will occupy a larger frequency range (Schmidt et al. 2013). Furthermore, in theoretical cricket and bush cricket assemblages where all calls are evenly distributed in time and space, incidences of masking and call interference is high between the species. Yet, when a soundscape is dominated by one species, less masking and interference occurs, likely due to more acoustic space being available (Balakrishnan et al. 2014). Therefore, the distribution of acoustically communicating species in a landscape is potentially driven by two factors: competition for acoustic space occurring between species (Krause 1993; Schmidt et al. 2013; Balakrishnan et al. 2014), as well as the role that the landscape plays in filtering calls (Morton 1975; Couldridge & van Staaden 2004).

Here I aim to observe and contextualise the response of *T. thyraeus*, a charismatic and easily identifiable species, to the complex landscape mosaic in this semi-transformed production landscape. To do this, I address the following objectives: 1) to determine which landscape elements *T. thyraeus* occupies, as little is known about this species' distribution, although it is thought to be highly sensitive to transformation, and therefore not likely to occur in plantations or on their edges, even when adjacent to natural vegetation, 2) to determine which environmental variables drive the distribution of *T. thyraeus* across this landscape. As bush crickets respond positively to woodier vegetation for camouflage (Grant

& Samways 2016), oviposition (Rentz 1988; Bailey & Rentz 1990) and call sites (Arak & Eiriksson 1992), environmental variables relating to vegetation structure are likely to be important drivers, and 3) to investigate inter-specific competition between *T. thyraeus* and co-occurring species. As many species are sympatric, I expect that there will be evidence for frequency niche partitioning between species. However, where species occupy the same central frequency band, call interference and acoustic competition is likely, and I expect to see evidence of species having adapted calls that avoid call interruption or interference.

5.2 Methods

5.2.1 Study area and design

This study was undertaken in the Richmond, Byrne Valley and Baynesfield areas of KwaZulu-Natal, South Africa (29° 48' 24.14" S; 30° 14' 4.4" E). This is a complex mosaic landscape comprising commercially-grown timber, mainly *Eucalytus* spp., interspersed with patches of remnant patches of Southern Mistbelt Forest and Mistbelt Grasslands (South African National Biodiversity Institue 2018). Sampling sites were on private land, as well as two forestry estates (Figure 5.2).

Ecological networks (ENs) conserve an estimated 500 000 ha of natural, and semi-natural habitat of varying conservation value, within the timber production regions of South Africa (Samways & Pryke 2016). Not only essential for the maintenance of habitat heterogeneity (Bazelet & Samways 2011; Crous et al. 2014; van Schalkwyk et al. 2017), these ENs ensure that the full complement of species is conserved across these mosaic landscapes (Bazelet & Samways 2012; Crous et al. 2013).

Sixty sites were selected for this study. Seven biotopes were used for this study: three ecotones and four core biotopes. Core biotopes were grassland (G, n = 10), indigenous forest (F, n = 8), open canopy *Eucalyptus* plantations (OC, n = 8) where trees were 2-5 years old, and finally, closed canopy plantations (CC, n = 10), where trees were mature and the canopy closed. Ecotones were: 1) natural and between grassland and forest (FG, n = 8), 2) between *Eucalyptus* of both ages and grasslands (GP, n = 8), and 3) between closed canopy plantation and indigenous forest (CCF, n = 8). Sampling effort across the different biotopes was uneven due to the spatial distribution and availability of sampling sites, and some technical problems.

5.2.2 Bush cricket sampling

Sampling was conducted form March-May 2018. Concurrent recordings were made using three SM4 song meters, and four SM2+ song meters (Wildlife Acoustics, USA). All were fitted with omnidirectional microphones (SMX-II omnidirectional microphone for the SM2+ recorders, and SMM-A2 omnidirectional microphone for the SM4 recorders), and all had a frequency response range of 20-20 000 hz. Each recorder was scheduled to record for 5 min every 2 hrs, on the hour, between 19h00 pm and 05h00 am, resulting in six recordings per night per site. Seven sites, one in each biotope, were sampled simultaneously for four consecutive nights, resulting in 24 five-min recordings, or 2 hours of

sound samples, from each site. After four nights, recorders were collected, recordings downloaded and then the recorders redeployed at the following seven sites. In total, 1 440 individual recordings were collected, over a total sample time of 120 hours.

Sample rate for the recordings was set to 48 000 Hz, and gain of the microphones set to 6, enabling the faint high frequency bush cricket calls to be sampled. All sound files were saved as uncompressed wave files (.wav). Attenuation of the calls within the recordings was minimised by placing the recorders on naturally occurring landscape features such as rocks, trees, or in clearings. Ambient temperature at the time of recording was measured, and stored, by the individual song meters. Due to the challenging nature of entering and navigating through the indigenous forests at night, no nocturnal sampling of specimens was undertaken, and so individuals were sampled at a nearby forestry station, enabling voucher specimens to be collected and the calls to be recorded for signal identification. Acoustic species were identified when no specimen was collected.

5.2.3 Vegetation and environmental variables

At each site, plant height and cover, as well as the number of plant structural classes (forbs, shrubs, dead vegetation, and percentage bare ground, rock and stones), was determined, using a graduated pole that was dropped 100 times while walking along a transect parallel to the edge. Height and number of plant classes (grass, shrub, forb and dead vegetation) intercepted by the pole were recorded, allowing for average height and vegetation cover to be determined, as well plant structural heterogeneity to be calculated by summing the number of plant structural classes identified at each individual sample point.

5.2.4 Signal analyses

Spectrograms, between 0-24 kHz, of all recordings were generated using Raven Pro 1.5 (Cornell Laboratories, USA). Individual bush cricket calls were identified by listening, as well as visually identifying, call patterns in the spectrograms. Calls were identified from the field, as well as from call recordings provided by PBC Grant. Individual calls were identified, and the following measurements made: begin, end, and length of each call, as well as the centre frequency and bandwidth. These parameters, as well as the visual structure of each call, were used to identify the species. Mean central frequency was calculated for each species.

As temperature did not influence species richness of recordings (Chapter 4), recordings were pooled. For each site, an Acoustic Activity Index (AI) for each species was calculated (Miller 2001; Thompson et al. 2019) to replace abundance values, as abundance cannot be accurately determined from single channel recordings. Total call time of all identified species at each site were also calculated. The soundscape between 5-17 kHz was delineated into 6 x 2 kHz bands; 5-7 kHz, 7-9 kHz, 9-11 kHz, 11-13 kHz, 13-15 kHz, and 15-17 kHz. This range was chosen as the central frequencies of all identified bush cricket species were within it. Species were then assigned to the various bands depending in which band their mean central frequency occurred.

5.2.5 Statistical analyses

In Chapter 4, sampling was found to be sufficient across the study area. Spatial autocorrelation for acoustic activity, and call time, of *T. thyraeus* was determined by conducting a Mantel test in *R* (R Core Team 2018) using *Ade4* (Dray & Dufour 2007). As there was evidence for spatial autocorrelation in both acoustic activity (Mantel test = 0.19, p < 0.05), and call time (Mantel test = 0.022, p < 0.05), a random spatial variable was included in all models to account for spatial distribution of sites (Bolker et al. 2009). This random effect was 'farm', consisting of the three land holdings (either estate or private land).

To determine *T. thyraeus* population distribution across the mosaic landscape, both acoustic activity, and total call time at each site were used, as both values successfully characterised bush cricket diversity (Chapter 4). Generalised linear mixed effects models (GLMMs) were used to investigate the distribution of *T. thyraeus* in terms of acoustic activity and call time, across the seven sampled biotopes. Models were run using the package *lme4* (Bates et al. 2015), and fitted with a Poisson distribution and Laplace approximation. Where significance was indicated, Tukey post-hoc comparisons were run to determine which pairs were significantly different at the 5% level.

To determine which environmental drivers shaped population distribution of *T. thyraeus* across the landscape, five non-correlated environmental variables were investigated: slope, elevation, average vegetation height, vegetation cover, and structural heterogeneity. Model averaging using the package *MuMin* (Barton 2019), was used for the acoustic activity data. Here, all possible GLMM combinations of the five environmental variables are automatically generated, then resultant AICc values are compared, and those whose AICc values differ by <2, are then averaged. GLMMs were calculated with Laplace approximation and Poisson distribution to obtain P values, and confidence intervals. A standard GLMM procedure, outlined above and using the package *lme4* (Bates et al. 2015), was used for the call time data, as model averaging only selected one model when conducted on the call time data. For this, data were transformed to a ratio to fit a binomial distribution, and a GLMM model including all five environmental values, was created. This was then compared against models where focal variables are removed, thereby determining which models have a significant impact on the call time of *T. thyraeus*.

To assess whether inter-specific competition between bush cricket species directly impacts the acoustic calling behaviour of *T. thyraeus*, the seven species identified as indicator species in Chapter 4 were used here, and only those species that occurred at more than eight sites were included. Four species were selected: *Thoracistus viridifer, Conocephalus* sp. 1, *Ruspolia* sp. 1 and *Plangiai* sp. 1. Individual GLMMs were run following procedures outlined above, where acoustic activity, and call time of *T. thyraeus* were compared to corresponding acoustic activity and call time of the selected species. For example, a negative relationship between either the AI or call time of *T. thyraseus* and the companion

species would be indicative of direct competition between the two, and vice versa for a positive relationship. Acoustic activity models were run with Laplace approximation and fitted to Poisson distribution, while binomial distribution was used for call time models.

5.3 Results

Thoracistus thyraeus was recorded at 41 of the sixty sampled sites, and was present in every biotope type. Biotope type significantly impacted both acoustic activity and call time of *T. thyraeus*, with the forest-grassland ecotone having the highest AI, as well as longest call times (Figure 5.3). AI was significantly higher in the forest-grassland ecotone compared to in closed canopy plantation, grassland, and forest biotopes. The AI of the forest-grassland ecotone was higher than in both closed canopy-forest and grassland-plantation ecotones (Table 5.1). Call times in the forest-grassland ecotone were significantly longer than in closed and open canopy plantation and grassland (Table 5.1).

A range of environmental variables influenced the acoustic activity of *T. thyraeus*. Average vegetation height, elevation, and vegetation cover all positively influenced AI of the species, while slope negatively influenced it (Table 5.2). Both average height and elevation positively affected the species call time (Table 5.2).

Thoracistus viridifer, *Conocephalus* sp. 1, *Ruspolia* sp. 1 and *Plangia* sp. 1 were the only other species which occurred at >8 sites, and whose AI and call time values were modelled against those of *T. thyraeus* (Table 5.3). Only in the comparison with AI of *Conocephalus* sp. 1, was there an increase in AI of *T. thyraeus* (Table 5.3). Otherwise, neither AI nor call time of co-occurring species had any significant impact on AI or call time of *T. thyraeus*.

In this mosaic landscape, *T. thyraeus* was sympatric with eight of the ten identified bush cricket species (Table 5.4). When considering central frequency bands, *T. thyraeus* was the sole occupant of the 5-7 kHz band. While four species occupied the 9-11 kHz band, at any given site, only a maximum of two of the four possible species co-occurred. In the 11-13 kHz band, two species, *Plangia* sp. 1 and *Conocephalus* sp. 1, were often within acoustic range of each other. The 13-15 kHz band was dominated by *T. viridifer*, along with two seldom-encountered species, Tettigoniidae sp. 1 and 2. The sole occupant of the highest band, 15-17 kHz, was the edge specialist *Conocephalus maculatus* (Table 5.4).

5.4 Discussion

Thoracistus thyraeus was identified in all seven sampled biotopes, indicating that this species is not restricted only to grasslands (Bazelet & Naskrecki 2014c) or wooded edges (Rentz 1988) as previously suggested. It is likely that the original Red List assessment of Endangered is overly cautious, and that *T.* thyraeus is in fact not threatened. No competitive interactions between *T. thyraeus* and other sampled bush crickets were found, suggesting that the various species rely on different niche space within the environment based on use of different frequency bands.

5.4.1 Population distribution of Thoracistus thyraeus *across the landscape*

 Thoracistus thyraeus occurred in all seven sampled biotopes, and both AI and total call time indicate that the grassland-forest ecotone is the most active area for the species. This ecotone is characterised by changing microclimates, as well as increasing woody vegetation and grasses towards the forest edge (Kotze & Samways 1999; Dröse et al. 2019), resulting in an increase in vegetation cover and heterogeneity. The increase in habitat complexity at the interface between grassland and indigenous forest provides a wide variety of potential resources for *T. thyraeus* (Crous et al. 2013; Slancarova et al. 2014; Fahrig 2017). This suggests that plant species composition was not the driver of *T. thyraeus*, but rather amount of dense vegetation present. Field observations of *T. thyraeus* at the forest station support this, as the species was recorded calling from a dense patch of invasive bracken and other weedy species. Call time in grassland, open and closed canopy plantations and the grassland-plantation and closed canopy plantation-forest ecotones were the same. Although these biotopes are different structurally, they lack weedy vegetation as along the grassland-forest edges. *T. thyraeus* is not restricted to grasslands alone, and as a result, the original Red List assessment of Endangered is likely not accurate. In addition, the species is able to persist within the *Eucalyptus* plantations and is likely not a habitat specialist, and can maintain a viable population within a transformed landscape.

5.4.2 Environmental drivers of Thoracistus thyraeus *population distribution across the landscape*

Vegetation characteristics are known to be drivers of bush cricket diversity, with many species depending on vegetation as oviposition sites (Rentz 1988; Bailey & Rentz 1990), calling sites (Arak & Eiriksson 1992), and to ensure effective camouflage (Grant & Samways 2016). Average vegetation height and elevation were significant drivers of AI and call time, with average vegetation height an additional driver of *T. thyraeus* AI. Elevation is perhaps significant, due to the location of the sampled forest edges, which were located at the higher elevations. The significance of average vegetation height, and cover, agree that it is woody vegetation at the forest edges that drive the population distribution of *T. thyraeus*. Both plantation age classes had considerably less understorey than indigenous forest, and lower *T. thyraeus* AI and call time. The understorey within the indigenous forest allows *T. thyraeus* to penetrate deeper into to these natural areas.

5.4.3 Interspecific competition between Thoracistus thyraeus *and co-occurring species*

Acoustically communicating species are able to prevent acoustic masking, through temporal, spatial and frequency partitioning (Brumm & Slabbekoorn 2005). Further separation is possible when species call in different seasons, or time of the day (night vs. day), as well as by avoiding calling at the same time as another species, or by structuring calls to fall in the pauses in another species song. In a multispecies chorus of three cricket and two bush cricket species, call temporal structure, frequency, and intensity provides acoustic space and prevents species from masking each other, enabling effective communication among individuals within each species in the chorus (Jain et al. 2014).

Thoracistus thyraeus was recorded singing with all but two of the eleven sampled bush cricket species, especially within the species-rich forest-grassland ecotone, where *T. thyraeus* was most active. Coexistence between species is possible when each species uses a different microhabitat, as is the case with *Tettigonia caudata* and *Te. viridissima* (Schirmel & Fartmann 2013). Here, comparisons between *T. thyraeus* and the four other abundant species, *T. viridifer*, *Conocephalus* sp. 1, *Ruspolia* sp. 1 and *Plangia* sp. 1, indicated no significant impact on the acoustic behaviour of *T. thyraeus*. *Conocephalus* sp.1, did indicate a significant positive relationship with the AI of *T. thyraeus*, although this relationship is likely due to *Conocephalus* sp. 1 being indicative of the woodier habitats, including forests and the forest-grassland ecotones (Chapter 4). The relationship between *T. thyraeus* and *Conocephalus* sp. 1 is likely due to both species responding similarly to the distribution of forests and the forest-grassland ecotones, which supports the theory of microhabitat preference preventing interference. However, transformation of the landscape can lead to this preference breaking down and one species greatly altering the song of another, especially the song of *Platycleis falx* greatly affecting that of *P. intermedia* in France (Samways 1977a).

There is evidence for acoustic niche partitioning (Krause 1993), and here the songs of the various species were distributed across different central frequency bands, leading to *T. thyraeus* apparently not in any direct acoustic competition with any of the other bush cricket species as it is the sole occupant of the 6-9 kHz band. Although this may not remain the case, if species distributions change in the future, new species may arrive at the sites and compete directly with *T. thyraeaus* for acoustic space. Furthermore, it is not possible to determine how historic competition in the past has shaped the current community.

Intraspecific competition may occur between male *T. thyraeus* individuals, especially at forest-grassland edges where calling activity is highest. In the case of *Te. viridissima*, males of this species compete for calling sites at the expense of safety and sound transmission, as they call from atop bushes where they are at higher predation risk, but their calls will travel further. There is a trade-off between natural and sexual selection, with males calling from deeper in the bushes more able to avoid predators but their calls are attenuated more (Arak & Eiriksson 1992).

The most speciose band was 9-11 kHz, although only two species were ever calling together, suggesting competition between species, with not just habitat suitability driving assemblage composition. Interestingly, the 11-13 kHz band was only occupied by two species, *Plangia* sp. 1 and *Conocephalus* sp. 1, which often occurred together within a site. Since they fall in the same frequency band, acoustic masking of the one species, by nature of the acoustic niche hypothesis should occur (Krause 1993), yet these species are often recorded together. The answer instead, may lie in the temporal structures of the individual species calls. *Conocephalus* sp. 1 produces a long buzzing call that is on average 24.4 sec long, with long breaks between calls. While *Plangia* sp. 1 produces many short rapid pulse-like calls

that average about 0.4 sec in length. Although *Plangia* sp. 1 does call at the same time as *Conocephalus* and risks potential masking, it also calls in between *Conocephuls* sp. 1 calls which will allow for conspecific females to identify the male calls. Calling between co-occurring species calls has been observed before in bush cricket species, as a way for species to avoid acoustic masking (Samways 1977b; Jain et al. 2014).

5.5 Conclusions

The charismatic and acoustically conspicuous *T. thyraeus* is not as ecologically constrained as originally thought, and occurs in a wide range of biotopes alongside many other bush cricket species. Originally known from three locations, this study adds to the known distribution of a species belonging to an understudied taxon. Naturally occurring edges between grasslands and forest are of particular conservation significance for bush cricket diversity, likely due to the increased woodiness and structural heterogeneity in these transition zones. As with other bush cricket species, plant height and cover are important drivers of *T. thyraeus* presence, with maintenance of natural vegetation layers in this production landscape being beneficial to the species' conservation. Vegetated areas and edges could support populations of *T. thyraeus* within the *Eucalyptus* timber plantations in this landscape. Indeed, this red-listed species is well conserved in this timber-dominated landscape mosaic, showing the value of ecological networks of conservation corridors for local conservation.

The original conservation assessment of *T. thyraeus* is likely overly conservative, as it was based on three known locations. As this study has shown, the distribution of the species is likely not constricted to natural grasslands, and is not hampered by commercial forestry, the actual conservation status of *T. thyraeus* is likely better than that implied by the Red List assessment. Furthermore, this bush cricket is potentially an umbrella species for local bush cricket diversity, as it was recorded alongside nine other bush cricket species, yet this would need to be formally assessed and tested for. As its song is so distinctive and easily detected by human ear, *T. thyraeus* It could easily be used by local land managers to identify high conservation value ecotones and other sites, especially those rich in structural heterogeneity.

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5.7 Tables and figures

Table 5.1: Results of generalised linear models investigating effect of biotope type on species acoustic activity and total call time of *Thoracistus thyraeus* (* p < 0.05; ** p < 0.01; *** p < 0.001). Abbreviations: FG- Forest-grassland ecotone, GP- Grassland-plantation ecotone, CCF- Closed canopy plantation-forest ecotone, CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland, and F- Forest.

	Model type	χ^2	df	Differences observed
Acoustic activity	GLMM	79.8***	6	FG-CC*** FG-CCF** FG – GP** OC- GP * FG-F*** G-FG*** OC-G*
Call time	GLMM	19.91**	6	FG-CC* G-FG* OC-FG*

Table 5.2: Environmental drivers of *Thoracistus thyraeus* determined by model averaging for acoustic activity, and by following a standard modelling procedure for call time across all sampled sites (* p < 0.05; ** p < 0.01; *** p < 0.001).

	Test statistics	Average height	Elevation	Slope	Vegetation cover	Structural heterogeneity
Acoustic activity (GLMM, model averaging)	Estimate	0.72	0.8	-0.47	-0.26	
	SD	0.1	0.16	0.122	0.13	
	Z value	7.24***	5.07***	3.82***	1.97*	x
	Confidence interval	[0.52;0.91]	[0.49;1.12]	[-0.71; -0.23]	[-0.53; -0.002]	
	Relationship	Positive	Positive	Negative	Positive	
Call time (GLMM, standard procedure)	χ^2	6.31*	15.42***	0.02	0.21	0.013
	df	1	1	1	1	1
	Relationship	Positive	Positive	Neutral	Neutral	Neutral

Table 5.3: Results of generalised linear mixed effects models investigating whether the co-occurrence of a species at a site has a direct influence on either the acoustic activity or total call time of *Thoracistus thyraeus* (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Species pair combinations	Measurement	χ^2	df	Response
Thoracistus thyraeus* and *Thoracistus viridifer	Acoustic activity	3.34	1	Neutral
	Call time	0.9	1	Neutral
***Thoracistus thyraeus* and *Conocephalus* sp. 1**	Acoustic activity	11.49***	1	Positive
	Call time	0.0002	1	Neutral
***Thoracistus thyraeus* and *Ruspolia* sp.1**	Acoustic activity	2.73	1	Neutral
	Call time	0.23	1	Neutral
***Thoracistus thyraeus* and *Plangia* sp. 1**	Acoustic activity	1.87	1	Neutral
	Call time	0.07	1	Neutral

Table 5.4: Description of the bush cricket species central frequency bands, and time in seconds of each species calling at each site in each biotope.

Biotope	5 – 7 kHz	9 – 11 kHz				11 – 13 kHz		13 – 15 kHz			15 – 17 kHz
	Thoracistus thyraeus	*Thoracistus semeniphagus*	*Ruspolia* species. 1	*Tettigoniidae* species 4	*Tettigoniidae* species 3	*Plangia* species 1	*Conocephulus* species 1	*Thoracistus viridifer*	*Tettigoniidae* species 1	*Tettegoniidae* species 2	*Conocephalus maculatus*
Grassland			2503					2829		129	201
			1500					6767			
	10		3839			87				65	
	8	73	166					7020			
	646	16	1412			1		367			
	321	749						2853			
	3662	1757						5178			
	2559						224	981			
	1281	973					844	2608			
Grassland-plantation ecotone			900					3954	1036		229
			1076					6508			33
								2685			2548
	1543							3022			
	1							4188			
	1157		300		56			6773			
	370		618		39	1	98	5286			
		125			3			2894			
Forest-grassland ecotone	2880						199	1254			
	4793					0.3		866			
			300		12			5735			
	2860	8	300			129	1070	7			
	2719	9	922			31	661	1444			
	4023					6		3713			
	2664					60		5682			
	2670		700			5	718	4382			
Open canopy plantation	388				3	142	702				
	224					7	1577				
	31					15					
	142				27	320					
					4	9		2873			
	338				19	32					
	4012						1332	542			
	1735						116	2370			
Closed canopy plantation								2105			
	140					68		75			
						207					
						61					
	2269					161	1715				
						32	3420				
	3152					24	532				
	1690					10	1320				
	483					361	239				
Closed canopy plantation-ecotone						45	2000				
	1646					160	165				
	2083					6	3627	488			
						11	870	1593			
	228					69	910				
	189					250	1244				
	647			8		370	1566				
Forest						360	4987				
	600					430					
	1422					354	5691				
						29	1095				
	38					276	144				
	4920					576					
	3305			18		508	3262				
	2067					348	1386				

Figure 5.1: *Thoracistus thyraeus*, Inflated Seedpod Shieldback.

Photographed by Dr. Claudia Hemp, and reproduced with permission.

Figure 5.2: Map showing location of the sampled farms and recording locations as indicated by the symbols. Green polygons indicate the location of *Eucalyptus* plantations.
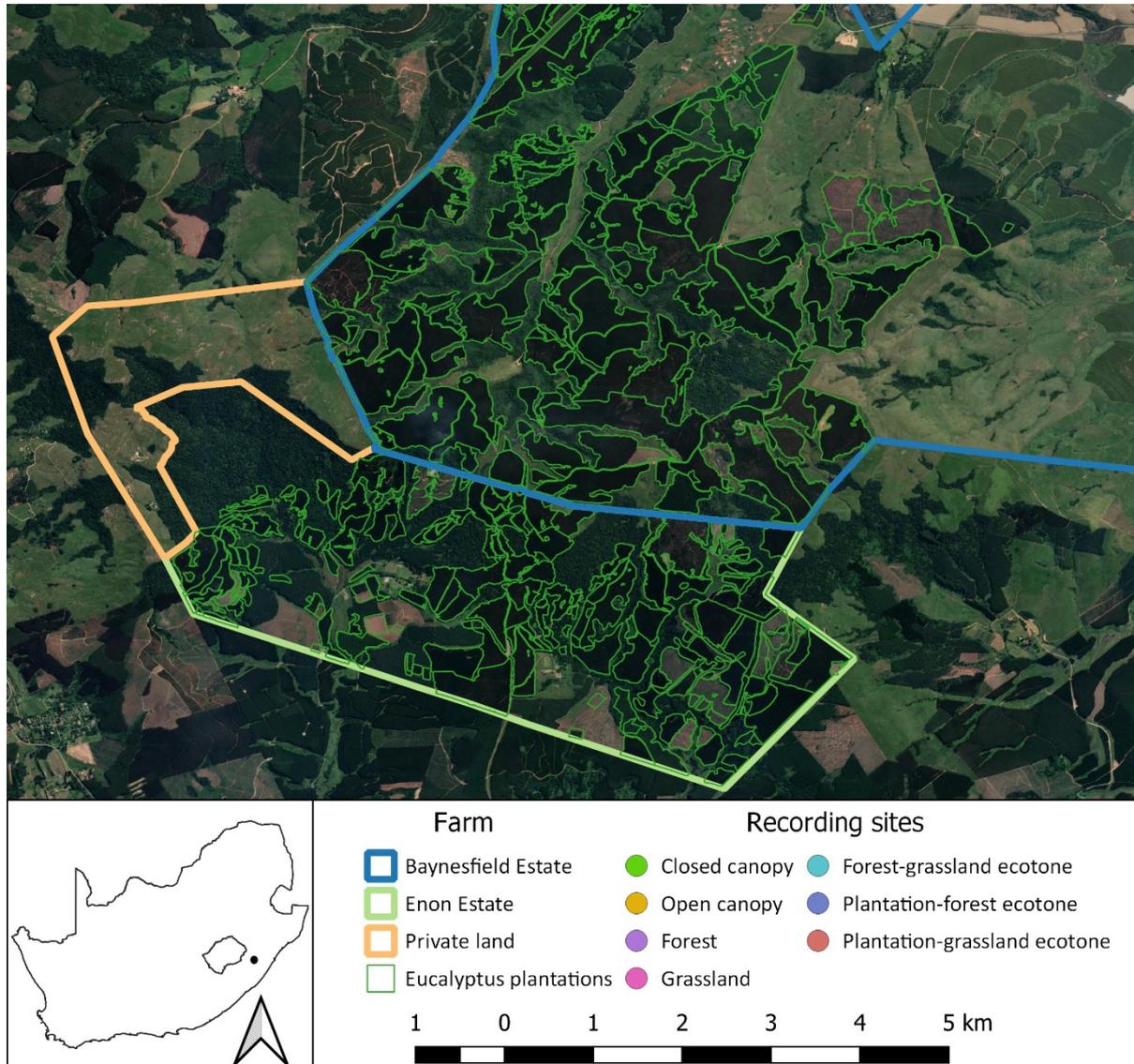
Satellite image from Google Earth (Image@2019 Maxar Technologies)

Figure 5.3: Changes in the acoustic activity (A), and call time (B), of *Thoracistus thyraeus* across all seven sampled biotopes. Abbreviations: FG- Forest-grassland ecotone, GP- Grassland-plantation ecotone, CCF- Closed canopy plantation-forest ecotone, CC- Closed canopy plantation, OC- Open canopy plantation, G- Grassland, and F- Forest.
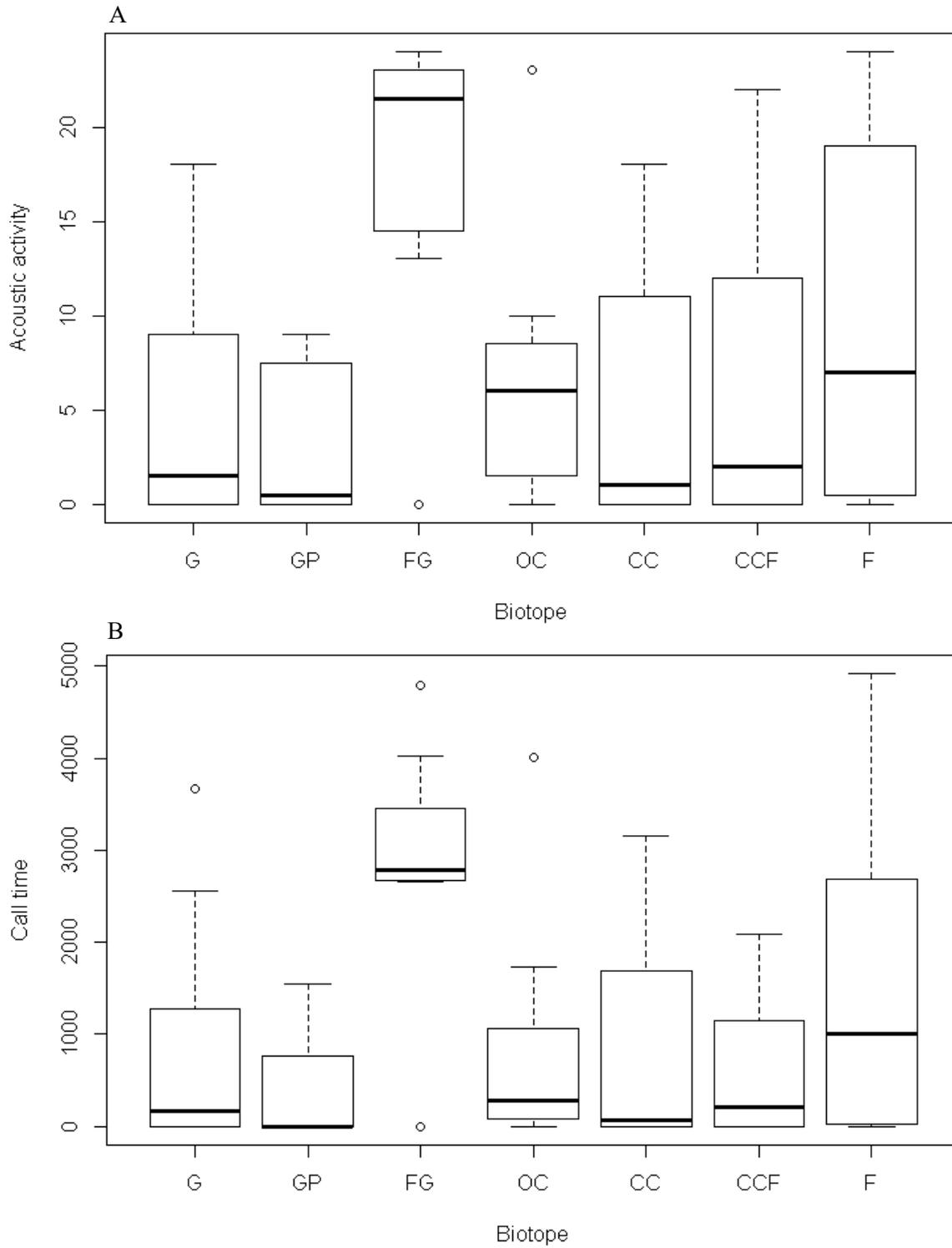
Chapter 6:

General discussion

"Insects

on a bough,

floating down river-

Still singing."

-Issa

overal6.1 Summary of findings

Here, I show that naturally occurring habitat patches and biotopes in KwaZulu-Natal timber forestry landscape are invaluable for the conservation of the full complement of insect biodiversity, which complements other findings from this landscape (Yekwayo et al. 2016, 2017), and in other production landscapes (Duelli & Obrist 2003; Phalan et al. 2011). The natural patches, indigenous forests and grasslands, are the most species rich, and form the most distinct assemblages for the epigaeic, foliage, and acoustic assemblages sampled here. Ecological contrast between various natural and anthropogenic biotopes drives local distribution of species and species assemblages in this complex system.

6.1.1 Environmental drivers of insect diversity

Many environmental drivers of species richness and assemblage composition were identified. Vegetation related environmental drivers were common across all sampled assemblages (epigaeic, foliage, and bush crickets), average vegetation height and cover most notably so. In addition, foliage-dwelling insect assemblages responded to the diversity of grasses. Increasing vegetation height, cover, and diversity results in a more diverse array of resources available for species to utilise. With this resultant increase in resource heterogeneity, a wider range of species can be supported (Ries et al. 2004; Tscharntke et al. 2008; Slancarova et al. 2014). Conversely, the transformation of grasslands through afforestation changes this natural heterogeneity (Bremer & Farley 2010), and results in the reduced insect diversity observed within the plantation mosaic studied here.

6.1.2 Contrast and spillover in the landscape

Both epigaeic and foliage-dwelling insect assemblages responded to changing contrast in a similar manner. As these results are based on a multi-taxon approach, observations are a robust indicator of the patterns occurring across the landscape in a wide array of insects. The sampled grasslands maintained distinct assemblages adapted to the particular ecological conditions present (Dröse et al. 2019). As a result, grasslands contrast greatly with the other sampled biotopes – forest and both plantation age

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classes, and therefore grassland assemblages and species are not able to spill over to any degree into these other biotopes. This great contrast prevented spillover to such a degree that the distribution of grassland assemblages was not solely as a response of biodiversity to habitat transformation, but also the result of the species themselves being adapted to particular habitats in which they were sampled (Duelli & Obrist 2003; Rand et al. 2006; Gaigher et al. 2015).

Interestingly, the anthropogenic edge between the indigenous forests and closed canopy plantations appears to be beneficial to indigenous forest insect species. There is evidence for some spillover occurring here in both the epigaeic and foliage-dwelling insect assemblages. The presence of understorey forest species along the edges of these plantation boundaries likely reduces the contrast at the interface and facilitates the observed spillover (Yekwayo et al. 2017). Determining species biotope fidelity confirmed this observation, as it is indeed the species displaying fidelity towards the indigenous forests that significantly increased the observed species richness along the plantation edges. Edges, and the resultant edge effects, are often thought to have negative impacts on biodiversity, although in many instances, positive responses to edges have also been noted (Ries et al. 2004; Ries & Sisk 2004). Foliage and epigaeic insect assemblages respond well to the artificial edges between forests and plantations.

The lowest contrast occurred between the open and closed canopy plantations. Epigaeic and foliage-dwelling insect assemblages in the two plantation age classes were indistinguishable from one another. This was likely due to similar environmental conditions present in both. Insect species, both epigaeic or foliage species, are therefore able to establish in one plantation age class, and then spillover into the other, thereby providing resilience to the insect assemblages within the transformed plantation areas within this landscape provided a mix of age classes is present. Considering that these timber stands have been present in the landscape for > 50 years, it is likely that insect assemblages within the mosaic have had time to become established.

6.1.3 Acoustic monitoring

Acoustic monitoring of this landscape has been shown to be a successful and efficient method with which to timeously identify and locate acoustically communicating bush cricket species in this complex landscape. Similar patterns to the epigaeic and foliage-dwelling assemblages emerged. The additional samples recorded in the ecotones between the various biotopes helped to determine the transition occurring between bush cricket assemblages. Bush cricket assemblages changed rapidly at these ecotones, with assemblages between the core biotopes being highly distinct, while the ecotone assemblages contained species from both biotopes. The forest-grassland ecotone proved to be important for overall bush cricket diversity and activity, as well as for the Endangered species *Thoracistus thyraeus*, due to the increased habitat heterogeneity along these naturally occurring edges. Bush crickets, as with many other orthopterans, are sensitive to ecological conditions (Riede 1998; Bazelet

& Samways 2011; Matenaar et al. 2015; Thompson et al. 2017), and are an appropriate taxon to resolve differences in assemblages across complex landscape mosaics.

Furthermore, acoustic sampling highlighted the fact that landscape heterogeneity is more than the physical features present, and that heterogeneity exists within the soundscape as well (Mazaris et al. 2009; Laiolo 2010; Joo et al. 2011; Grant & Samways 2016). The acoustic space is also a resource which acoustically communicating species are able adapt to ensure that their calls transmit successfully (Morton 1975), as well as to avoid acoustic interference from other species (Krause 1993). The soundscape is interlinked with the landscape, and as a result, tracks the diversity of habitats and biotopes (Mazaris et al. 2009; Villanueva-Rivera et al. 2011; Bormpoudakis et al. 2013; Tucker et al. 2014), thus providing an additional layer through which biodiversity can be monitored across landscapes. Furthermore, *Thoracistus thyraeus,* an endangered bush cricket in this landscape, was found to occur in all seven sampled biotopes, contrary to what was initially thought (Rentz 1988, Bazelet & Naskrecki 2014), this species is not restricted to grasslands, but is well conserved in this modified landscape. In addition, *T. thyraeus* could possible be an umbrella species for bush cricket diversity in this landscape, but this would need to be formally investigated.

The adaptation of the individual species to their acoustic environment (acoustic adaptation hypothesis) is evident in this landscape, as higher frequency calls are not present in the woodier biotopes, landscape here is acting as a filter (Morton 1975), with species associating with biotopes that enable the most efficient call transition (Couldridge & van Staaden 2004). Direct acoustic competition between species was found to be a second factor driving the distribution of species (Samways 1977a; Krause 1993; Schmidt et al. 2013; Balakrishnan et al. 2014; Jain et al. 2014), as species locate themselves in local areas where acoustic masking and interference will not occur. Furthermore, where bush crickets do occur together and interference and masking are likely as both species are found in the central frequency band, the individual species show call adaption strategies to avoid this (Samways 1977b; Balakrishnan et al. 2014; Jain et al. 2014), in the case observed here, calls appear to be timmed so that calls of one species (*Plangia* sp. s) can be heard between calls of another species (*Conocephalus* sp. 1).

6.2 Conservation implications

6.2.1 Conservation of natural biotopes

The importance of indigenous biotopes, such as grasslands, forests and riparian zones in these managed ENs is well known (Samways et al. 2010; Crous et al. 2014; Kietzka et al. 2015; Samways & Pryke 2016; Yekwayo et al. 2016; van Schalkwyk et al. 2017). These regions maintain landscape-wide insect diversity (Yekwayo et al. 2016), and provide both habitat heterogeneity (Crous et al. 2013) and ecosystem services (Samways et al. 2010). This study provides further proof of the importance of these areas, without the grasslands and forests, highly dynamic and specialised insect assemblages will likely be locally lost. In addition, the environmental drivers identified as important for insect diversity suggest

that the maintenance of vegetation height and cover are essential for the maintenance of insect diversity in this landscape.

Although the expansion of plantation forestry has been largely halted in South Africa (Samways & Pryke 2016), these grasslands and indigenous forests are still at risk of degradation. For effective conservation and management of these biotopes, managers should identify the threats and perturbations present on their property. For example, if left unchecked, unmanaged cattle grazing can have lasting impacts on grassland biodiversity, especially grasshopper diversity (Joubert et al. 2016; Joubert-van der Merwe & Pryke 2018), as large tracts of the grasslands sampled here are grazed, this needs to be effectively managed. Furthermore, due to the importance of fire in regulating grassland species assemblages, appropriate fire regime management is essential (Gaigher et al. 2018) Regarding improvements in the conservation of the indigenous forests, harvesting of plant material and debarking of trees for the traditional medicine is endangering many forest tree and shrub species. This can result in changes to the natural succession indigenous forests (Chungu et al. 2007; Chirwa et al. 2008), leading to potential fragmentation and increases in negative edge effects, in turn disrupting insect assemblages. In addition, invasive plants, such as Bug Weed (*Solanum mauririanum*), have invaded some areas within the sampled forests. These species pose further risks to the indigenous forest systems and biodiversity (Olkers 2011) and must be removed (Olkers 2011; van Wilgen et al. 2012).

Contrast may be high at the natural edges that occur between the grasslands and indigenous forest, but due to increases habitat heterogeneity, these edges are incredibly important for bush cricket diversity. Occurring at the interface between the grasslands and forests, active management of the grasslands and indigenous forests will benefit not only the ecotone, but also the overall bush cricket assemblage in this landscape.

6.2.2 Managing contrast

Land sparing and land sharing are two strategies employed in an attempt to minimise the impact of agricultural activities on biodiversity. Land sharing occurs when agricultural production and conservation activities are in the same local space. Land sparing on the other hand, is when land is set aside for the sole purpose of conserving natural biodiversity while intensive agricultural activities take place on a different parcel of land. In many instances, land sparing is the preferred and most effective approach, as it protects specialist species (Phalan et al. 2011). As contrast drives the distribution of insect species within my study area, land sparing in the form of protection and management of the grasslands and indigenous forests is the best way to conserve landscape-wide species diversity.

What of land sharing? As contrast is such an important factor to consider, perhaps land sharing is possible. Along the plantation forest boundaries, contrast does appear to be lower, as species are able to spillover from the forests into the plantations, with spillover from the plantations into the forests observed. This spillover has been attributed to the mixing of forest plant species at the edges of the

plantations. In Brazil, efficient and cost-effective tropical forest restoration was achieved by intercropping valuable native pioneer and non-pioneer tree species with a single *Eucalyptus* species. After a number of years, the *Eucalyptus* trees can be harvested and sold, and in doing so, up to 44-75% of the restoration costs can be recouped. This had no significant impact on the diversity of native species post felling (Brancalion et al. 2019). Here in KwaZulu-Natal, if foresters in the area wanted to improve the conservation of forest insect species, perhaps lower contrast between plantations and forests could be utilised. Perhaps intercropping native tree species within the *Eucalyptus* plantations could be feasible. Not only could this improve insect species conservation, but these intercropped native species could perhaps be logged, and the timber sold. A caveat though, is that the indigenous trees species and *Eucalyptus* grow at different rates. In the original study, Brancoalion et al. (2019), this was not an issue as the *Eucalyptus* growth rate was used to aid in the restoration of indigenous forests.

The open and closed canopy plantations sampled here are not "green deserts", but rather support unique insect assemblages (Bremer & Farley 2010). Although the species supported by both plantation age classes are likely generalist in nature, the species present still provide valuable ecosystem services, from nutrient recycling and decomposition to soil aeration all of which benefit timber production. In order to maintain these insect assemblages, the low contrast between tree ages class can be utilised, connectivity between plantations of varying ages should be maintained by ensuring that, when harvesting, older plantations are maintained in areas alongside recently felled areas. In doing so, older plantations will be able to act as source populations for younger plantations and help in the reestablishment of insect diversity after felling.

6.2.3 Potential of acoustic monitoring

Acoustic sampling is known to provide rapid and accurate assessments of components of biodiversity, with the added advantage of being non-invasive (Sueur et al. 2008; Sueur & Farina 2015; Krause & Farina 2016). The 120 hours of recordings here took roughly a month to process, involving the identification and measurement of nearly 80 000 bush cricket calls. In comparison, the sample processing for chapters 2 and 3 spanned almost 11 months. The acoustic sampling proved to be less intensive, and invasive, than the sampling conducted for chapters 2 and 3, as the recorders are designed for long periods of unmanned deployment. As acoustic sampling is non-invasive (Sueur et al. 2008), these methods can be deployed in more ecologically sensitive areas, and can capture a wide array of information (Krause & Farina 2016), from both day and night choruses (Bradfer-Lawrence et al. 2019). Apart from song meters, very little specialised laboratory equipment is required for acoustic monitoring, and samples do not need to be stored in bulky containers or ethanol. Processing of recordings is not limited to the confines of a laboratory, as only a computer with the appropriate software are required. This enables acoustic monitoring of a landscape to move beyond the realms of science and scientists, and with adequate guidelines, could be a method implemented by conservation managers and foresters directly.

Although the number of species identified from the traditional methods is over a hundredfold that of the number of bush crickets identified acoustically (1137 vs 11 species), ecoacoustics still has value. When used in conjunction with traditional sampling methods, acoustic assessments add additional layers of ecological information that is not sampled by pitfall traps and sweep netting. Landscape heterogeneity is not limited to the physical features, but also includes the acoustic space and the resources provided i.e. sound transmission characteristics (Morton 1975) and acoustic niche availability (Krause 1993). By monitoring land- and soundscapes, the interaction of an individual species with and within their environment can be determined. The acoustic observations and assessments may not be as complete as when using multi-taxon approaches, but in acoustically communicating species, sound is essential, as it encodes information pertinent to the individuals and aids in both inter- and intraspecific communication (Gasc et al. 2013; Ross & Allen 2014). Therefore, ecoacoustic methods may not be as applicable to all ecological questions as traditional biodiversity monitoring methods, but when applied to species and assemblages where sound is an integral feature, it allows for in-depth understanding of critical features of a species ecology.

Bush crickets in the KwaZulu-Natal region, although hard to sample and locate visually, are perfect candidate taxa for acoustic sampling. The male songs are easily identified in spectrograms and detected in the field (Thompson et al. 2019). Orthoptera are known indictors of habitat quality (Riede 1998; Bazelet & Samways 2011; Matenaar et al. 2015), and as shown here in this study, bush cricket species show an affinity for various biotope types based on their adaptations to both the acoustic space and presence of other bush cricket species. Should disturbances occur in this landscape, acoustic monitoring can be used as a potential early warning system, by picking up changes occurring in the soundscape. For example, bush encroachment may cause forest dwelling bush crickets to become resident in grasslands, which in turn will alter the bush cricket soundscape recorded in these grasslands. Care needs to be taken as bush crickets are highly seasonal. Most species emerge in later spring/early summer as adults, with peak abundances occurring mid to late summer (Grant 2014; Thompson et al. 2019). As a result, a bush cricket based acoustic monitoring programme would be best suited to the summer months.

6.2.4 Thoracistus thyraeus *and a way forward*
Thoracistus thyraeus has a loud obvious call that carries many meters and is not difficult to locate either by humans (Rentz 1988) or with song meters. In fact, the human ear has been proved to be accurate in detecting and locating bush cricket calls in field, even though there are high-frequency components (Diwakar et al. 2007). In addition, this species has a very descriptive and appropriate common name: the Inflated Seedpod Shieldback (Bazelet & Naskrecki 2014), which aids in generating interest and concern in the eyes of the general public (Barua et al. 2012; Schlegel et al. 2015; Clausnitzer et al. 2017). Although likely not endangered, this species could be used to garner support and interest in the public domain for insect conservation in the area.

The largest production estates sampled here, Baynesfield and Enon, are under the management of NCT Forestry Co-operative LTD (NCT) and are both certified with the Forest Stewardship Council (FSC), and therefore subscribe to sustainable forestry practices. In addition to the management of these estates, Baynesfield is affiliated with the Oribi Working Group, which aims to conserve and monitor the populations of this endangered antelope, with breeding pairs occurring within the sampled grasslands. Furthermore, a portion of the sampled private lands sampled here can be found in the Minerva Nature Reserve, which is under the management of NCT. Conservation of biodiversity in this production landscape is therefore a priority for managers, and they likely would be open to a tool that enables the identification of areas that are of importance to insect diversity. This study has already successfully generated some awareness and interest in *T. thyraeus*, with for example, the forester at Enon, upon hearing about a population of *T. thyraeus*, immediately wanted to know how to manage the patch in which the species was located, to prevent any further disturbance to the population. Although anecdotal evidence, it is promising and indicative of success should *T. thyraeus* be used to encourage insect conservation in this landscape.

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Species list

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
Orthoptera	Acrididae	Sp. 1		1	1	2
		Sp. 2		1	1	2
		Sp. 3			1	1
		Sp. 4		1	1	2
		Sp. 5			1	1
		Sp. 6		1	1	2
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9		1	1	2
		Sp. 10		1		1
		Sp. 11		1		1
		Sp. 12		1		1
		Sp. 13		1		1
		Sp. 14		1		1
		Sp. 16			1	1
		Sp. 17			1	1
		Sp. 18			1	1
		Sp. 19			1	1
		Sp. 20			1	1
		Sp. 21			1	1
		Sp. 22			1	1
		Sp. 23			1	1
		Sp. 24			1	1
		Sp. 25			1	1
		Sp. 26			1	1
		Sp. 27			1	1
		Sp. 28			1	1
		Sp. 29			1	1
		Sp. 30			1	1
		Sp. 31			1	1
		Sp. 32			1	1
		Sp. 33			1	1
		Sp. 34			1	1
		Sp. 35			1	1
		Sp. 36			1	1
		Sp. 37			1	1
		Sp. 38			1	1
		Sp. 39			1	1
		Sp. 40			1	1
		Sp. 41			1	1
		Sp. 42			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 43			1	1
		Sp. 44			1	1
		Sp. 45			1	1
		Sp. 46			1	1
		Sp. 47			1	1
		Sp. 48			1	1
		Sp. 49			1	1
		Sp. 50			1	1
		Sp. 51			1	1
		Sp. 52			1	1
		Sp. 53			1	1
		Sp. 54			1	1
		Sp. 55			1	1
	Lentulidae	Sp. 1			1	1
		Sp. 2			1	1
	Tetrigidae	Sp. 1		1	1	2
		Sp. 2		1	1	2
		Sp. 3		1	1	2
		Sp. 4		1	1	2
		Sp. 5		1	1	2
		Sp. 6			1	1
		Sp. 7			1	1
	Tettigoniidae	*Thoracistus thyraeus*	1			1
		Thoracistus viridifer	1			1
		Thoracistus semeniphagus	1			1
		Conocephalus maculatus	1			1
		Conocephalus sp. 1	1			1
		Plangia sp. 1	1			1
		Ruspolia sp. 1	1			1
		Tettigoniidae sp. 1	1			1
		Tettigoniidae sp. 2	1			1
		Tettigoniidae sp. 3	1			1
		Tettigoniidae sp. 4	1			1
	Thericleidae	Sp. 1			1	1
		Sp. 2		1	1	2
		Sp. 4			1	1
		Sp. 5		1	1	2
Hemiptera	Achilidae	Sp. 1			1	1
		Sp. 2			1	1
	Adelgidae	Sp. 1		1		1
		Sp. 2			1	1
		Sp. 3			1	1

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 4			1	1
		Sp. 5		1	1	2
	Anthocoridae	Sp. 1			1	1
	Aphididae	Sp. 1		1	1	2
	Belostomatidae	Sp. 1		1		1
	Cercopidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6		1	1	2
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10			1	1
		Sp. 11			1	1
		Sp. 12		1	1	2
		Sp. 13			1	1
		Sp. 14			1	1
		Sp. 15			1	1
		Sp. 16			1	1
		Sp. 17			1	1
		Sp. 18			1	1
		Sp. 19			1	1
		Sp. 20			1	1
		Sp. 21			1	1
		Sp. 22			1	1
	Cicadellidae	Sp. 3			1	1
		Sp. 5		1		1
		Sp. 6		1	1	2
		Sp. 8		1	1	2
		Sp. 9			1	1
		Sp. 10		1	1	2
		Sp. 11			1	1
		Sp. 12			1	1
		Sp. 13			1	1
		Sp. 14			1	1
		Sp. 15			1	1
		Sp. 16			1	1
		Sp. 17			1	1
		Sp. 20			1	1
		Sp. 21			1	1
		Sp. 22			1	1
		Sp. 24			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 25			1	1
		Sp. 28		1	1	2
		Sp. 29		1	1	2
		Sp. 31		1	1	2
		Sp. 32			1	1
		Sp. 33		1	1	2
		Sp. 34			1	1
		Sp. 35		1	1	2
		Sp. 36			1	1
		Sp. 37			1	1
		Sp. 38			1	1
		Sp. 39			1	1
		Sp. 40		1	1	2
		Sp. 41			1	1
		Sp. 42		1	1	2
		Sp. 43		1	1	2
		Sp. 46			1	1
		Sp. 47			1	1
		Sp. 48			1	1
		Sp. 49			1	1
		Sp. 50			1	1
		Sp. 51			1	1
		Sp. 53			1	1
		Sp. 54			1	1
		Sp. 55			1	1
		Sp. 56			1	1
		Sp. 58		1		1
		Sp. 59		1	1	2
		Sp. 60			1	1
		Sp. 61		1	1	2
		Sp. 62			1	1
		Sp. 63			1	1
		Sp. 64			1	1
		Sp. 65			1	1
		Sp. 66			1	1
		Sp. 67			1	1
		Sp. 68			1	1
		Sp. 69			1	1
		Sp. 70		1	1	2
		Sp. 71			1	1
		Sp. 72			1	1
		Sp. 73			1	1
		Sp. 74		1	1	2
		Sp. 75		1	1	2

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 76		1	1	2
		Sp. 77		1	1	2
		Sp. 78			1	1
		Sp. 79			1	1
		Sp. 81			1	1
		Sp. 82			1	1
		Sp. 83		1	1	2
		Sp. 84		1	1	2
		Sp. 85		1	1	2
		Sp. 86			1	1
		Sp. 87		1	1	2
		Sp. 88			1	1
		Sp. 89			1	1
		Sp. 90		1	1	2
		Sp. 91		1	1	2
		Sp. 92		1	1	2
		Sp. 93			1	1
	Coreidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4		1	1	2
	Delphacidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 4			1	1
		Sp. 5		1	1	2
		Sp. 6			1	1
		Sp. 7		1		1
		Sp. 8			1	1
		Sp. 9		1	1	2
		Sp. 10			1	1
		Sp. 11		1	1	2
		Sp. 12			1	1
		Sp. 13		1	1	2
		Sp. 14			1	1
		Sp. 15			1	1
		Sp. 16			1	1
		Sp. 17		1	1	2
		Sp. 18			1	1
		Sp. 19			1	1
		Sp. 20			1	1
		Sp. 21			1	1
		Sp. 22			1	1
	Dictyopharidae	Sp. 1			1	1
		Sp. 2		1	1	2

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6		1	1	2
		Sp. 7		1	1	2
		Sp. 8			1	1
		Sp. 9		1	1	2
	Dinidoridae	Sp. 1			1	1
	Dipsocoridae	Sp. 1			1	1
		Sp. 2			1	1
	Enicocephalidae	Sp. 1			1	1
	Flatidae	Sp. 1			1	1
	Fulgoridae	Sp. 1			1	1
		Sp. 2			1	1
	Gerridae	Sp. 1		1		1
		Sp. 2		1		1
	Issidae	Sp. 1			1	1
		Sp. 2		1	1	2
	Lygaeidae	Sp. 1		1	1	2
		Sp. 2		1		1
		Sp. 3			1	1
		Sp. 4		1	1	2
		Sp. 5		1		1
		Sp. 6		1		1
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9		1	1	2
		Sp. 10		1		1
		Sp. 11			1	1
		Sp. 12		1	1	2
		Sp. 13		1	1	2
		Sp. 14		1	1	2
		Sp. 15			1	1
		Sp. 16		1	1	2
		Sp. 17			1	1
	Meenoplidae	Sp. 1		1	1	2
		Sp. 2		1	1	2
		Sp. 3			1	1
	Membracidae	Sp. 1			1	1
		Sp. 2		1	1	2
	Miridae	Sp. 1		1	1	2
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4		1	1	2

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 5			1	1
		Sp. 6		1		1
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10			1	1
		Sp. 11			1	1
		Sp. 12			1	1
		Sp. 13			1	1
		Sp. 14			1	1
		Sp. 15			1	1
	Pentatomidae	Sp. 1		1	1	2
		Sp. 2		1	1	2
		Sp. 3		1		1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6		1	1	2
		Sp. 7		1	1	2
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10			1	1
		Sp. 11			1	1
		Sp. 12			1	1
	Pyrrhocoridae	Sp. 1		1	1	2
		Sp. 2			1	1
		Sp. 3		1	1	2
		Sp. 4			1	1
	Reduviidae	Sp. 1		1	1	2
		Sp. 2		1		1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5		1	1	2
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8		1	1	2
		Sp. 10			1	1
		Sp. 11		1	1	2
		Sp. 12			1	1
	Rhopalidae	Sp. 1		1		1
	Saldidae	Sp. 1			1	1
	Scutelleridae	Sp. 1		1	1	2
		Sp. 2			1	1
	Tingidae	Sp. 1		1	1	2
		Sp. 2			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6		1	1	2
Coleoptera	Anobiidae	Sp. 1			1	1
		Sp. 2			1	1
	Anthicidae	Sp. 1		1	1	2
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5		1	1	2
		Sp. 6		1	1	2
		Sp. 7		1	1	2
		Sp. 8		1	1	2
		Sp. 9		1	1	2
		Sp. 10			1	1
		Sp. 11		1	1	2
	Apionidae	Sp. 1		1	1	2
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4		1	1	2
		Sp. 5			1	1
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10		1	1	2
		Sp. 11			1	1
		Sp. 12		1	1	2
		Sp. 13			1	1
		Sp. 14			1	1
	Bostrychidae	Sp. 1		1	1	2
		Sp. 2		1	1	2
		Sp. 3			1	1
		Sp. 4		1		1
	Bruchidae	Sp. 1			1	1
	Buprestidae	Sp. 1		1		1
		Sp. 2			1	1
		Sp. 3		1	1	2
		Sp. 4			1	1
		Sp. 5		1	1	2
		Sp. 6		1		1
		Sp. 7			1	1
		Sp. 8		1	1	2

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 9		1		1
	Cantharidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
	Carabidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1		1
		Sp. 4		1		1
		Sp. 5		1		1
		Sp. 6		1		1
		Sp. 7			1	1
		Sp. 8		1		1
		Sp. 9		1		1
		Sp. 10		1		1
		Sp. 11		1		1
		Sp. 12		1		1
		Sp. 13			1	1
		Sp. 14		1	1	2
		Sp. 15		1		1
		Sp. 16		1		1
		Sp. 17		1		1
		Sp. 18		1		1
		Sp. 19		1	1	2
		Sp. 20		1		1
		Sp. 22		1		1
		Sp. 24		1		1
		Sp. 25		1		1
		Sp. 26		1		1
		Sp. 27		1		1
		Sp. 28		1		1
		Sp. 29		1		1
		Sp. 30		1		1
	Cerembricidae	Sp. 1			1	1
		Sp. 2		1		1
		Sp. 3		1		1
	Chrysomelidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6			1	1
		Sp. 7		1	1	2
		Sp. 8			1	1
		Sp. 9			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 10			1	1
		Sp. 11			1	1
		Sp. 12			1	1
		Sp. 13		1		1
		Sp. 14			1	1
		Sp. 15			1	1
		Sp. 16			1	1
		Sp. 17			1	1
		Sp. 18			1	1
		Sp. 19			1	1
		Sp. 20		1	1	2
		Sp. 21		1	1	2
		Sp. 22			1	1
		Sp. 23		1		1
		Sp. 24		1	1	2
		Sp. 25		1	1	2
		Sp. 27			1	1
		Sp. 28		1	1	2
		Sp. 29			1	1
		Sp. 30			1	1
		Sp. 31			1	1
		Sp. 32			1	1
		Sp. 33			1	1
		Sp. 34		1	1	2
	Cleridae	Sp. 1		1		1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6			1	1
	Coccinellidae	Sp. 1		1		1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10			1	1
		Sp. 11			1	1
		Sp. 12			1	1
		Sp. 13		1	1	2
		Sp. 14			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
	Corylophidae	Sp. 1		1		1
	Cucujidae	Sp. 1			1	1
		Sp. 2			1	1
	Curculionidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1	1	2
		Sp. 4		1	1	2
		Sp. 5		1		1
		Sp. 6		1		1
		Sp. 7			1	1
		Sp. 8		1	1	2
		Sp. 9			1	1
		Sp. 10			1	1
		Sp. 11			1	1
		Sp. 12			1	1
		Sp. 13			1	1
		Sp. 14			1	1
		Sp. 15			1	1
		Sp. 16			1	1
		Sp. 17		1		1
		Sp. 18		1	1	2
		Sp. 19			1	1
		Sp. 20		1		1
		Sp. 22			1	1
		Sp. 23		1		1
		Sp. 24		1	1	2
		Sp. 25			1	1
		Sp. 26			1	1
		Sp. 27		1	1	2
		Sp. 28			1	1
		Sp. 29			1	1
		Sp. 31		1	1	2
		Sp. 33			1	1
		Sp. 34		1	1	2
		Sp. 35			1	1
		Sp. 36		1		1
		Sp. 37		1		1
	Discolomidae	Sp. 1		1	1	2
		Sp. 2		1	1	2
		Sp. 3		1	1	2
		Sp. 4		1		1
		Sp. 5		1	1	2
		Sp. 6		1	1	2
		Sp. 7		1		1

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
	Elateridae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1		1
		Sp. 4		1	1	2
		Sp. 5			1	1
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10		1	1	2
		Sp. 11			1	1
		Sp. 12		1	1	2
		Sp. 13		1		1
		Sp. 14		1		1
		Sp. 15		1		1
		Sp. 16		1		1
	Eucnemidae	Sp. 1			1	1
	Lycidae	Sp. 1		1		1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6		1		1
	Melandryidae	Sp. 1		1	1	2
		Sp. 2			1	1
		Sp. 3		1	1	2
	Meloidae	Sp. 1		1	1	2
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4		1	1	2
		Sp. 5		1		1
	Melyridae	Sp. 1			1	1
		Sp. 2		1	1	2
	Mordellidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
	Mycetophagidae	Sp. 1			1	1
		Sp. 2		1		1
	Paussidae	Sp. 1			1	1
	Phalacridae	Sp. 1		1	1	2
		Sp. 2		1	1	2
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5		1		1

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
	Ptilidae	Sp. 1		1		1
		Sp. 2		1		1
	Rhipiphoridae	Sp. 1			1	1
		Sp. 2		1	1	2
		Sp. 3		1	1	2
	Scarabaeidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1		1
		Sp. 4		1		1
		Sp. 5		1		1
		Sp. 6		1		1
		Sp. 7		1		1
		Sp. 9		1		1
		Sp. 10		1		1
		Sp. 11		1		1
		Sp. 12			1	1
		Sp. 13		1		1
		Sp. 14		1		1
		Sp. 15		1		1
		Sp. 16		1		1
		Sp. 17		1		1
		Sp. 18		1		1
		Sp. 19		1		1
		Sp. 20		1		1
		Sp. 22		1		1
		Sp. 23			1	1
		Sp. 24		1		1
		Sp. 25			1	1
		Sp. 26		1		1
		Sp. 27		1		1
	Staphylinidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1		1
		Sp. 5		1		1
		Sp. 6		1		1
		Sp. 7		1		1
		Sp. 8		1		1
		Sp. 9		1		1
		Sp. 10		1		1
		Sp. 11		1		1
		Sp. 12			1	1
		Sp. 13		1	1	2
		Sp. 14		1	1	2
		Sp. 15		1	1	2

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 16		1	1	2
		Sp. 17		1	1	2
		Sp. 18		1	1	2
		Sp. 19		1	1	2
		Sp. 20		1	1	2
		Sp. 21			1	1
		Sp. 22		1	1	2
		Sp. 23		1	1	2
		Sp. 24		1	1	2
		Sp. 25			1	1
		Sp. 26		1		1
		Sp. 27		1	1	2
		Sp. 28		1	1	2
		Sp. 29		1		1
		Sp. 30		1		1
		Sp. 31		1		1
		Sp. 32		1		1
		Sp. 33		1		1
		Sp. 34		1		1
		Sp. 35		1	1	2
		Sp. 36		1		1
		Sp. 37		1		1
		Sp. 38		1		1
	Tenebrionidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1		1
		Sp. 4		1		1
		Sp. 5		1	1	2
		Sp. 6		1		1
		Sp. 7		1		1
		Sp. 8		1		1
		Sp. 9		1	1	2
		Sp. 10			1	1
		Sp. 12		1	1	2
		Sp. 13		1		1
		Sp. 15		1		1
		Sp. 16		1		1
		Sp. 17		1		1
	Trogidae	Sp. 1			1	1
Diptera	Acroceridae	Sp. 1		1	1	2
		Sp. 2		1	1	2
	Anthomyiidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1	1	2

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 4			1	1
		Sp. 5		1		1
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8		1		1
	Asilidae	Sp. 1		1		1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5		1	1	2
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8		1	1	2
		Sp. 9			1	1
		Sp. 10		1		1
		Sp. 11			1	1
		Sp. 12			1	1
		Sp. 14		1	1	2
	Asteiidae	Sp. 1		1		1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
	Bibionidae	Sp. 1		1	1	2
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6			1	1
		Sp. 7		1	1	2
	Calliphoridae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1		1
		Sp. 4			1	1
		Sp. 5		1	1	2
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8		1		1
		Sp. 9		1		1
		Sp. 10			1	1
		Sp. 11			1	1
		Sp. 12		1	1	2

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 13			1	1
		Sp. 14			1	1
	Canacidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
	Celyphidae	Sp. 1			1	1
	Chironomiidae	Sp. 1			1	1
		Sp. 2			1	1
	Chyromyidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
	Clusiidae	Sp. 1			1	1
		Sp. 2		1	1	2
	Cryptochetidae	Sp. 1		1	1	2
		Sp. 1		1	1	2
		Sp. 2		1	1	2
		Sp. 3			1	1
		Sp. 4		1	1	2
	Diopsidae	Sp. 1			1	1
		Sp. 2		1	1	2
	Dolichopodidae	Sp. 1		1		1
		Sp. 2			1	1
	Drosophilidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4		1		1
		Sp. 5			1	1
		Sp. 6		1	1	2
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9		1	1	2
		Sp. 10			1	1
		Sp. 11		1	1	2
		Sp. 12		1	1	2
		Sp. 13		1	1	2
	Empididae	Sp. 1			1	1
	Fannidae	Sp. 1		1	1	2
	Glossinidae	Sp. 1			1	1
	Heleomyzidae	Sp. 1			1	1
	Lauxaniidae	Sp. 1			1	1
	Lonchaeidae	Sp. 1			1	1
		Sp. 2		1	1	2

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6			1	1
	Muscidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6			1	1
		Sp. 7		1	1	2
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10			1	1
		Sp. 11			1	1
		Sp. 12			1	1
		Sp. 13			1	1
		Sp. 14		1	1	2
		Sp. 16		1	1	2
		Sp. 17			1	1
		Sp. 18		1	1	2
		Sp. 19			1	1
		Sp. 20		1	1	2
		Sp. 21		1	1	2
		Sp. 22		1	1	2
		Sp. 23		1	1	2
		Sp. 24			1	1
		Sp. 25		1	1	2
		Sp. 26			1	1
		Sp. 27			1	1
		Sp. 28			1	1
		Sp. 29			1	1
		Sp. 30			1	1
		Sp. 31		1	1	2
		Sp. 32			1	1
		Sp. 33		1	1	2
		Sp. 34		1	1	2
		Sp. 35		1	1	2
		Sp. 36			1	1
		Sp. 37		1	1	2
		Sp. 38			1	1
		Sp. 39		1	1	2
		Sp. 40		1	1	2
		Sp. 41			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 42		1	1	2
		Sp. 43			1	1
		Sp. 44			1	1
		Sp. 45			1	1
		Sp. 46			1	1
	Mycetophilidae	Sp. 1		1	1	2
		Sp. 2			1	1
		Sp. 3		1	1	2
		Sp. 4		1	1	2
		Sp. 5		1	1	2
		Sp. 6			1	1
		Sp. 7			1	1
	Phoridae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1		1
		Sp. 4		1		1
		Sp. 5		1	1	2
		Sp. 6		1	1	2
		Sp. 7			1	1
	Piophilidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4		1	1	2
		Sp. 5			1	1
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8		1	1	2
		Sp. 9			1	1
		Sp. 10		1	1	2
		Sp. 11			1	1
		Sp. 12		1	1	2
		Sp. 13			1	1
		Sp. 14		1	1	2
	Rhagionidae	Sp. 1			1	1
	Sarcophagidae	Sp. 1		1	1	2
		Sp. 2			1	1
	Sciaridae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
	Sepsidae	Sp. 1			1	1
	Simuliidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 4			1	1
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10			1	1
		Sp. 11			1	1
	Sphaeroceridae	Sp. 1		1		1
		Sp. 2			1	1
		Sp. 3		1	1	2
		Sp. 4		1	1	2
		Sp. 5			1	1
	Syrphidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 4			1	1
		Sp. 5		1	1	2
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10		1		1
	Tachinidae	Sp. 1		1		1
	Tanyderidae	Sp. 1		1		1
	Tephritidae	Sp. 1		1	1	2
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5		1	1	2
		Sp. 6		1	1	2
		Sp. 7		1	1	2
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10		1		1
		Sp. 11			1	1
		Sp. 12		1	1	2
		Sp. 13			1	1
		Sp. 14		1	1	2
		Sp. 15			1	1
		Sp. 16		1	1	2
		Sp. 17			1	1
		Sp. 18		1	1	2
		Sp. 19			1	1
		Sp. 20		1	1	2
		Sp. 21		1	1	2

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 22		1	1	2
		Sp. 23			1	1
		Sp. 24		1	1	2
		Sp. 25		1	1	2
		Sp. 26			1	1
	Thanmaleidae	Sp. 1		1		1
	Tipulidae	Sp. 1		1		1
		Sp. 3			1	1
		Sp. 5		1	1	2
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8		1	1	2
		Sp. 9			1	1
		Sp. 10		1	1	2
		Sp. 11		1	1	2
		Sp. 12			1	1
		Sp. 13			1	1
	Ulidiidae	Sp. 1			1	1
		Sp. 2			1	1
Hymenoptera	Andrenidae	Sp. 1		1		1
	Anthophoridae	Sp. 1		1		1
	Apidae	Sp. 1		1		1
		Sp. 2			1	1
	Braconidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1	1	2
		Sp. 5		1	1	2
		Sp. 7		1		1
		Sp. 8			1	1
		Sp. 9			1	1
		Sp. 10			1	1
		Sp. 11			1	1
		Sp. 17			1	1
		Sp. 18			1	1
		Sp. 19			1	1
		Sp. 20			1	1
		Sp. 21			1	1
		Sp. 22			1	1
		Sp. 24			1	1
		Sp. 25		1	1	2
		Sp. 26		1	1	2
		Sp. 27			1	1
		Sp. 28			1	1
		Sp. 29			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 30			1	1
		Sp. 31			1	1
		Sp. 32			1	1
		Sp. 33			1	1
		Sp. 34			1	1
		Sp. 36			1	1
		Sp. 37			1	1
		Sp. 38			1	1
		Sp. 39			1	1
		Sp. 40			1	1
		Sp. 41			1	1
		Sp. 42			1	1
		Sp. 43			1	1
		Sp. 44			1	1
		Sp. 45			1	1
		Sp. 46			1	1
		Sp. 47			1	1
		Sp. 48		1	1	2
		Sp. 49		1	1	2
		Sp. 50			1	1
		Sp. 51			1	1
		Sp. 52			1	1
		Sp. 53			1	1
		Sp. 54			1	1
		Sp. 55			1	1
		Sp. 56			1	1
		Sp. 57			1	1
		Sp. 59			1	1
		Sp. 60			1	1
		Sp. 61			1	1
		Sp. 62			1	1
		Sp. 63			1	1
		Sp. 64			1	1
		Sp. 65			1	1
		Sp. 66			1	1
		Sp. 67			1	1
		Sp. 68			1	1
		Sp. 70			1	1
		Sp. 71			1	1
		Sp. 72		1	1	2
		Sp. 73			1	1
		Sp. 74			1	1
		Sp. 77			1	1
		Sp. 78			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 79			1	1
		Sp. 80			1	1
		Sp. 81		1	1	2
		Sp. 82			1	1
		Sp. 83			1	1
		Sp. 84			1	1
		Sp. 85			1	1
		Sp. 86			1	1
		Sp. 87			1	1
		Sp. 88		1	1	2
		Sp. 89		1	1	2
		Sp. 90			1	1
		Sp. 91			1	1
		Sp. 92			1	1
		Sp. 93			1	1
		Sp. 94			1	1
		Sp. 96			1	1
		Sp. 97		1	1	2
		Sp. 98			1	1
		Sp. 99			1	1
		Sp. 100			1	1
		Sp. 101			1	1
		Sp. 102			1	1
		Sp. 103			1	1
		Sp. 104			1	1
		Sp. 105		1	1	2
		Sp. 106			1	1
		Sp. 107			1	1
		Sp. 108		1	1	2
		Sp. 109			1	1
		Sp. 110			1	1
		Sp. 111		1		1
		Sp. 112			1	1
		Sp. 113			1	1
		Sp. 114		1	1	2
		Sp. 115		1		1
	Chalcidoidae	Sp. 1			1	1
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8		1	1	2

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 9		1	1	2
	Diapriidae	Sp. 1			1	1
		Sp. 2		1		1
	Evaniidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3			1	1
		Sp. 4			1	1
		Sp. 5			1	1
	Formicidae	Sp. 1		1	1	2
		Sp. 2		1	1	2
		Sp. 3		1	1	2
		Sp. 4		1	1	2
		Sp. 5		1	1	2
		Sp. 6		1	1	2
		Sp. 7		1	1	2
		Sp. 8			1	1
		Sp. 10		1		1
		Sp. 11		1		1
		Sp. 12		1	1	2
		Sp. 13		1	1	2
		Sp. 14		1	1	2
		Sp. 15		1	1	2
		Sp. 16		1	1	2
		Sp. 17		1		1
		Sp. 18		1	1	2
		Sp. 19		1	1	2
		Sp. 20		1	1	2
		Sp. 21		1	1	2
		Sp. 22		1	1	2
		Sp. 23		1	1	2
		Sp. 24		1		1
		Sp. 25		1		1
		Sp. 26			1	1
		Sp. 28		1	1	2
		Sp. 29		1	1	2
		Sp. 30		1	1	2
	Halictidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1		1
		Sp. 4			1	1
		Sp. 5			1	1
		Sp. 6			1	1
		Sp. 7			1	1
		Sp. 8			1	1

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
	Ichneumonidae	Sp. 1		1		1
		Sp. 2		1		1
		Sp. 3		1		1
		Sp. 4		1		1
		Sp. 6		1	1	2
		Sp. 7		1		1
		Sp. 8		1		1
		Sp. 9		1		1
		Sp. 11		1		1
		Sp. 12			1	1
		Sp. 14		1	1	2
		Sp. 15			1	1
		Sp. 16			1	1
		Sp. 17			1	1
		Sp. 18			1	1
		Sp. 19			1	1
		Sp. 20			1	1
		Sp. 22		1	1	2
		Sp. 23			1	1
		Sp. 25		1	1	2
		Sp. 26			1	1
		Sp. 27			1	1
		Sp. 28		1	1	2
		Sp. 29		1	1	2
		Sp. 30			1	1
		Sp. 31			1	1
		Sp. 32		1	1	2
		Sp. 33			1	1
		Sp. 34		1	1	2
		Sp. 35			1	1
		Sp. 36		1	1	2
		Sp. 37		1	1	2
		Sp. 38			1	1
		Sp. 39			1	1
		Sp. 40			1	1
		Sp. 41			1	1
		Sp. 42			1	1
		Sp. 43			1	1
		Sp. 44		1	1	2
		Sp. 45			1	1
		Sp. 46			1	1
		Sp. 47		1	1	2
		Sp. 48			1	1
		Sp. 49		1	1	2

Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 50			1	1
		Sp. 51		1	1	2
		Sp. 52			1	1
		Sp. 53		1	1	2
		Sp. 54			1	1
		Sp. 55			1	1
		Sp. 56		1		1
		Sp. 57			1	1
		Sp. 58		1	1	2
		Sp. 59			1	1
		Sp. 60			1	1
		Sp. 61		1	1	2
		Sp. 62		1	1	2
		Sp. 63		1		1
		Sp. 64		1		1
	Megachilidae	Sp. 1			1	1
		Sp. 2			1	1
	Mutillidae	Sp. 1		1	1	2
		Sp. 2			1	1
		Sp. 3			1	1
		Sp. 4		1	1	2
	Orussidae	Sp. 1		1		1
	Platygasteridae	Sp. 1			1	1
	Pompilidae	Sp. 1		1		1
		Sp. 2		1	1	2
		Sp. 3		1		1
		Sp. 4		1		1
		Sp. 5		1		1
		Sp. 6		1		1
		Sp. 7		1		1
		Sp. 8		1		1
		Sp. 10		1		1
		Sp. 11		1		1
		Sp. 12		1		1
		Sp. 13		1		1
		Sp. 14			1	1
		Sp. 15		1	1	2
		Sp. 16		1	1	2
		Sp. 17		1	1	2
		Sp. 18			1	1
		Sp. 19		1	1	2
	Sapygidae	Sp. 1		1		1
	Sphecidae	Sp. 2		1		1
	Vespidae	Sp. 1			1	1

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Order	Family	Species / morphospecies	Acoustic assemblage	Epigaeic assemblage	Foliage assemblage	Number of assemblages sampled in
		Sp. 2			1	1
		Sp. 3		1		1