



UNIVERSITY OF THE
WITWATERSRAND,
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Assessing the effectiveness of South African savanna biosphere
reserves in maintaining aquatic ecosystem structure and function

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A thesis submitted in fulfilment of the requirements for the Degree of Master of Science at

School of Animal, Plant and Environmental Sciences

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Declaration

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Submitted: 24 April 2019

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Abbreviations and Acronyms

BR – Biosphere Reserve

FFG – Functional Feeding Group

MGB – Magaliesberg Biosphere Reserve

MRB – Marico Biosphere Reserve

NMDS – Non-metric multidimensional scaling

SASS – South African Scoring System

SE – Standard error

Un - Unprotected

WTB – Waterberg Biosphere Reserve

Abstract

Biosphere reserves are sites established by UNESCO (United Nations Educational, Scientific and Cultural Organization) as scientifically significant. They are not formally protected areas; however, the label promotes a landscape management structure that encompasses sustainable development. The principle of sustainable development recognises the interconnectedness that exists between land use and freshwater resources which results in the interconnectedness of challenges experienced by both aspects. Therefore, land management has an impact on freshwater resources such as headwater streams which play a vital role in riverine processes and have a significant influence on the health of downstream ecosystems. This study investigated the structure and function of headwater streams in the context of Biosphere Reserve (BR) land management. The ecosystem health status and community structure of headwater streams in these reserves was compared to that of headwater streams in adjacent, unprotected sites. Land cover, physico-chemical and macroinvertebrate community data were collected from each stream (6 sites in the Magaliesberg biosphere reserve, 5 in the Marico biosphere reserve, 4 in the Waterberg biosphere reserve and 6 unprotected sites), with macroinvertebrates assessed using a combination of rapid bio-assessment (SASS5) and trophic group classifications. Unprotected sites did not have a single A/B category site, indicating all unprotected headwater streams were modified to some degree. Marico BR, Magaliesberg BR and Waterberg BR all had A/B categorised sites. Category C and D/E/F sites were present in all the BRs but the proportion was highest in the unprotected sites. A total of 6 of the sampling points were in core (conservation priority) areas, however not a single one was unmodified and natural (A category). There was no statistically significant difference between the SASS5 assessment indices of biosphere and unprotected sites. Relative abundances of two Functional Feeding Groups (FFGs), namely filter feeders and

predators, were analysed to assess changes in trophic processes and structuring; neither one was significantly different across these management contexts. Although pH had significant correlations with some aquatic macroinvertebrate data, including family-level richness in the macroinvertebrates, there was no statistically significant difference in pH across the range of geology types or ecoregions sampled in the study. This suggests variation in stream pH may be due other processes like acid deposition from urban and mining areas. The overall lack of significant difference in land-cover between BRs and unprotected sites meant that the effects of differing land-cover management practices on the structure of macroinvertebrates assemblages could not be fully explored. Nonetheless, both rapid bio-assessment of river health and comparative analysis of FFGs suggest that the ecological health of headwater streams inside biosphere reserves is improved in comparison to that of adjacent unprotected streams, apart from a few outliers. Therefore, South African savanna biosphere reserves are effective in maintaining aquatic ecosystem structure and function.

Keywords

Biosphere reserve, macroinvertebrates, aquatic health, functional feeding groups, management context, ecoregion

Chapter 1: Introduction

1.1 The importance of headwater streams

Headwater streams make up almost 80% of aquatic routes (they dominate channel networks) and they have a prominent role in the transport of organic matter, nutrients and energy, linkages between aquatic and terrestrial zones, and the ecology of flowing waters (Roy *et al.*, 2009; Callisto *et al.*, 2012). These streams are the key drivers of riverine processes and their habitats are spatially tied to larger streams as they continually provide essential resources such as food, nutrients, and detritus that enhance or degrade aquatic and riparian ecosystems downstream (Wipfli *et al.*, 2007).

Stream environments are a hierarchy of habitat sequences which are nested at multiple spatial scales; they are also exceptional ecological corridors when it comes to the dispersal of species in watersheds (Frissell *et al.*, 1986; Allan and Castillo, 2007). Headwater streams also provide ecological services (benefits people obtain from the natural environment and functioning ecosystems) like nitrogen sequestration, the promotion of primary production which leads to higher live biomass that results in more dead and decomposing biomass (which sustains consumers like fish and birds), habitat for endemic fauna and high-quality water (Bayley *et al.*, 1985; Kiffney *et al.*, 2003; Benstead *et al.*, 2009; Callisto *et al.*, 2012; Halvorson *et al.*, 2015). Headwater streams are characterised by large volumes of organic matter like detritus, wood, dissolved organic matter, invertebrates and other inputs from the riparian zone (Wipfli *et al.*, 2007). Energy and nutrient fluxes of these streams affect downstream communities through hydrological and ecological connections.

Headwater streams are source areas for biodiversity and thus greatly affect the ecology and biological integrity of streams in the lower reaches of their catchments (Wipfli *et al.*, 2007). Downstream habitats also receive different ecosystem services from headwater streams, which include water, nutrients like phosphorous and nitrogen, woody debris and food like invertebrate prey and organic matter (OM); therefore, headwaters streams are essential in the sustainability, productivity, and proper functioning of downstream ecosystems (Wipfli *et al.*, 2007).

The government of South Africa recognises the need to protect aquatic ecosystems, codified in legislation such as the National Water Act (Act 36 of 1998), that assists in setting target values for water quality (biological, chemical, hydrological, geomorphological and physico-chemical characteristics) and quantity (Department of Water Affairs and Forestry (DWAF), 2008; King and Pienaar, 2011). These target values are known as Resource Quality Objectives (RQOs) and they determine the level of protection that should be afforded to the particular water resource as it outlines how the water resource should be used (DWAF, 2008). It is important to protect and monitor aquatic ecosystems (Pollard and Du Toit, 2011), towards actively managing water resources.

1.2 Human impacts on streams

In order to ensure that management plans and decisions are adequately informed to guarantee the proper protection of the integrity of headwater streams, it is essential to understand the ecological importance of headwater streams and how land use activities can affect their structure and function (Gomi *et al.*, 2002). Headwater habitats play key ecological roles in

larger streams, however, riparian buffer protection of headwater streams is usually considered last (if at all), when land management plans are drawn and implemented (Wipfli *et al.*, 2007). This is a great concern as headwater streams and their riparian zones are not offered much protection from human activities (Wipfli *et al.*, 2007). Land use affects material transport downstream by altering flow regulation and channelization, which in turn alters the mechanisms through which water is stored in headwaters (Meyer and Wallis 2001).

Certain human activities like channelization and construction of dams directly affects streams, while land uses like land drainage, deforestation, urbanization and grazing indirectly affect streams (Wohl, 2006). Human activities within a stream channel alter the dynamics of aquatic and riparian communities, channel geometry, the volume and types of contaminants in the stream and the movement dynamics of the water and sediments (Wohl, 2006). Human activities within a watershed can directly and indirectly affect the quantity and transport of contaminants, sediment and water in a stream (Gomi *et al.*, 2002).

Human activities in or close to streams also affect macroinvertebrates which are used as a bio-monitoring tool and are thus important in terms of assessing the ecological state of lotic aquatic ecosystems (Dallas, 1995; Dickens and Graham, 2002). Macroinvertebrates are good indicator species for bio-assessment because they are visible to the naked eye, relatively easy to identify and they have a rapid life cycle, they are also a measure of habitat quality, quantity and diversity (Dickens and Graham, 2002). Macroinvertebrates also influence nutrient cycles, decomposition, translocation of materials and primary production; they are also a food source for numerous fish (Wallace and Webster, 1996). A loss of macroinvertebrate diversity may be an indication that the ecosystem is being polluted or negatively affected as they are indicators of stream degradation (Wallace and Webster, 1996).

This study used the South African Scoring System version 5 (SASS5) which is a rapid bio-assessment technique that uses macroinvertebrates as a proxy for measuring water quality (Dallas, 1995). Macroinvertebrates were also classified into Functional Feeding Groups (FFGs) which describes their most likely predominant feeding behaviour that is used to relate community structure with ecosystem functioning (Palmer *et al.*, 1993; Uwadiae, 2010). The macroinvertebrates were used in this study also because they are suitable for assessing the impacts of development (Dickens and Graham, 2002).

Human dominated land use (unnatural land modification) is characterised by increased relative abundance of pollution tolerant taxa, decreased diversity and overall abundance of macroinvertebrates, and alterations in how invertebrate feeding groups are distributed (Whiting and Clifford, 1983; Hall *et al.*, 2001). Decreased invertebrate diversity has adverse consequences for the whole ecosystem because it affects resource acquisition, decomposition, and rates of primary production (Jonsson and Malmqvist, 2000).

Urbanization is a land use activity that directly and indirectly affects streams. When urbanization is in its initial phases, the amount of sediments that is washed into streams significantly increases (Wohl, 2006), this leads to less percolation and infiltration into the soil, enhanced runoff, and input of road salts, metals and oils into water bodies (Moore and Palmer, 2005). After constructions are completed, the sediment discharge decreases and the water inflow in the streams increases due to artificial surfaces that do not allow water to percolate through them. This results in higher peaked flood hydrographs (potential for more frequent flooding), bank erosion, contaminants from urban areas, and the modification of aquatic and riparian habitats and channel geometry (Wohl, 2006). Agricultural activities like tilling and livestock grazing lead to soil erosion, runoff of nutrients, fine sediments and

pesticides into streams (Moore and Palmer, 2005). Such land uses can result in the modification of stream ecosystem processes, which can be studied by investigating the ecological response of macroinvertebrate assemblages (Paul and Meyer, 2001).

The biodiversity in freshwater ecosystems is threatened by human activities through the combined effects of different stressors such as overfishing, habitat degradation, and the introduction of alien species, flow regulation and pollution (Revenga *et al.*, 2005; Ormerod *et al.*, 2010). Consequently, a larger number of freshwater species are under threat (some even on the brink of extinction), in comparison to terrestrial and marine species – an indication that human freshwater practices are unsustainable (Dudgeon, 2010). Historic declines of freshwater species are underestimated, because shifting baselines (lack of past information and conditions resulting in distorted accepted norms for the condition of the natural environment) are hardly taken into account when assessing species loss over the years (Humphries and Winemiller, 2009; Soga and Gaston, 2018). The combination of environmental stressors will lead to further extinctions (Dudgeon, 2010).

Climate change and the resulting environmental changes will be exacerbated by the rising human population and higher water consumption which are the trajectories of the Anthropocene (Dudgeon, 2010). Although the relationship between biodiversity and the functioning of freshwater ecosystems has not been adequately established to allow for detailed templates of how freshwater ecosystems should be managed when faced with biodiversity loss, the practical approach would be to use the precautionary principle and avoid or minimise further losses (Hector *et al.*, 2001). The manner in which humanity uses and relies on most ecosystem services is unsustainable, which if continued may result in significant and mostly irreversible loss of the diverse life forms on earth (Dudgeon, 2010).

Macroinvertebrate richness has been shown to be affected by land use (Moore and Palmer, 2005). Prominent human threats that affect the structure of macroinvertebrate communities in streams include: deforestation, non-point pollution, overfishing, introduction of alien species, habitat degradation, urbanization, channelization and the cultivation of land (Moore and Palmer, 2005; Revenga *et al.*, 2005; Wohl, 2006; Ormerod *et al.*, 2010; Callisto *et al.*, 2012). The negative impacts that land use have on the ecology and ecosystems of rivers and streams are an environmental concern worldwide (Moore and Palmer, 2005). One of the initiatives that have been established to protect natural resources and the environment is UNESCO's Man and the Biosphere (MAB) programme.

1.3 History and function of UNESCO biosphere reserves

UNESCO launched the "Man and the Biosphere (MAB)" programme in 1970 after it held a Biosphere Conference in Paris in 1968 (Batisse, 1982). At the Biosphere Conference it was declared that it is necessary to promote interdisciplinary approaches to ensure that the conservation and utilization of land and water resources are not in opposition, but rather go hand-in-hand (Pool-Stanvliet, 2013). The vision of MAB is to reach a point where people act collectively and responsibly to build progressive societies that are in harmony with the biosphere by being conscious of how they interact with the planet (Cárdenas Tomažič *et al.*, 2016). The Man and the Biosphere (MAB) programme advocates for the establishment of biosphere reserves in the biogeographical provinces of different parts of the world which would also be used to improve the relationship between people and the environments they live in (Batisse, 1982).

Biosphere reserves are sites that are used to understand the interactions and manage changes between ecological and social systems, like the management of biodiversity and how to prevent conflicts between these systems (Cárdenas Tomažič *et al.*, 2016). These reserves encompass coastal, marine, freshwater and terrestrial ecosystems, and they promote solutions that reconcile the sustainable use of resources with the conservation of biodiversity (Pool-Stanvliet, 2013). National governments nominate and have jurisdiction over the biosphere reserves that are in the country, even though their status is recognized internationally (Batisse, 1982).

Biosphere reserves have three interrelated zones (core area, buffer zone, transition area) that aim to fulfil three functions that are mutually reinforcing and complement each other (Batisse, 1997; Cárdenas Tomažič *et al.*, 2016). The core area consists of a strictly protected ecosystem that makes a significant contribution to the conservation of species, genetic variation, ecosystems and landscapes. The buffer zone borders the core areas; it is used for activities that are ecologically friendly, including practices that emphasize scientific research, monitoring, education and training (Cárdenas Tomažič *et al.*, 2016). The transition area is where most activities are allowed, but it also fosters economic and human development that is sustainable ecologically and socio-culturally (Cárdenas Tomažič *et al.*, 2016).

In 1983 the first international biosphere reserve congress was held in Minsk, Belarus and the second one was held in Seville, Spain in March 1995 where the Statutory Framework of the World Network of Biosphere Reserves (WNBR) and the Seville Strategy for Biosphere Reserves were established (Pool-Stanvliet, 2013). These documents provide a common platform for developing biosphere reserves as they define the criteria, procedure and principles for the designation of biosphere reserves (Robertson-Vernhes, 2007). According

to the Seville Strategy, biosphere reserves are supposed to promote and demonstrate a balance between the biosphere and people (Batisse, 1997).

The concept of a biosphere reserve is to combine three functions; conservation (of ecosystems, landscapes, species and genetic variation), sustainable development (promoting ecologically and culturally sustainable economic development), and logistical support (research, education, monitoring and training) (Batisse, 1997). In the Twenty-first Century, the vision for Biosphere Reserves emphasises that they are capable of being models for sustainable development and platforms that can be used to reconcile people and nature (Pool-Stanvliet, 2013). Biosphere reserves are not formal protected areas; however, the label promotes a landscape management structure that encompasses sustainable development (Robertson-Vernhes, 2007).

In the early 1990s, The UNESCO MAB programme was introduced to South Africa as it was thought to be an appropriate holistic approach to conservation focussing on the Fynbos Biome in the Western Cape (Pool-Stanvliet, 2013). After the April 1994 first democratic elections in South Africa, the country was accepted back into the international arena and that is when it joined international conventions, including the UNESCO's Convention on Biological Diversity and the World Heritage (Pool-Stanvliet, 2013). South Africa and UNESCO signed an agreement in 1995, and it was introduced to the MAB programme with the first official biosphere reserve being the Kogelberg Biosphere Reserve designated in 1998 (Stanvliet *et al.*, 2004a).

The MAB programme is a tool that can be used to implement provincial policies and to support governmental goals such as climate change adaptation, sustainable development and environmental education and training (German Commission for UNESCO, 2015). In South Africa, agencies use different but similar programmes to manage the environment at a landscape-scale, such as World Heritage Sites, trans-frontier conservation and other biodiversity initiatives (Pool-Stanvliet, 2013). These landscape initiatives usually take priority above UNESCO biosphere reserves, but the aims and objectives of these landscape management mechanisms are similar (Pool-Stanvliet, 2013). However, the concept of biosphere reserves embraces the main principles of key landscape-scale management initiatives which are; promoting sustainable development and socio-environmentally attuned living practices, and thus addressing some of the challenges that landscape managers are faced with today (Stanvliet et al., 2004b).

Even though the MAB programme has been active in South Africa for more than two decades, the concept is not sufficiently known and supported in the country (Pool-Stanvliet, 2013). It is in line with modern landscape management because it seeks to balance ecological requirements with the economic needs of communities that live in these areas (Cárdenas Tomažič *et al.*, 2016). This gives it great potential for bridging political and administrative boundaries and increasing the practicality of sustainable development (Pool-Stanvliet, 2013).

In South Africa, biosphere reserves are often perceived as a tool for preventing unwanted development, but there are other benefits as well (Pool-Stanvliet, 2013). The concept of biosphere reserves does not focus on just biodiversity conservation but also on the ecological, social and cultural dynamics of the area that they are located in (Pool-Stanvliet, 2013).

Biosphere reserves are not just for protecting natural space, like other initiatives do. They do

not seek to separate protected areas from the broader biogeographical landscape; rather, they are managed as part of the wider landscape (Pool-Stanvliet, 2013). Another benefit of biosphere reserves is that they have an Ecosystem Approach, and they are part of the WNBR, so they are recognised internationally and open doors for accessing expertise, setting it up to receive funding from different international institutions (Lotze-Campen *et al.*, 2008).

Biosphere reserves recognize that intact ecosystems are pivotal in having healthy water bodies and therefore, management structures are encouraged to invest in restoring ecosystems which result in better ecosystem services (German Commission for UNESCO, 2015).

Through the principle of sustainable development and the recognition of the interconnectedness that exists between land use, ecosystem services and freshwater resources, biosphere reserves have a great potential to help manage freshwater resources because sustainable development emphasizes the interconnectedness of challenges such as water scarcity and pollution (German Commission for UNESCO, 2015). They have the capacity to develop environmentally sound management plans that can ensure that freshwater resources are protected and that they are well taken care of, to safeguard the continuity of ecosystem services that they provide.

The participation of active and willing stakeholders (scientists, politicians, volunteers, and local residents) also plays a vital role in the planning and implementation of management plans of biosphere reserves (Schultz *et al.*, 2011; Stoll-Kleemann and Welp, 2008). Therefore, as part of conserving ecosystems, biosphere reserves should, to an extent, preserve the ecosystem function of the headwater streams in them. In this context of the purpose of biosphere reserves, this study assesses how effective the Magaliesberg, Marico and Waterberg biosphere reserves are in maintaining aquatic ecosystems within them.

1.4 River Health Monitoring and Bio-assessment in South Africa

In 1997 the Department of Water Affairs and Forestry (DWAF) introduced the South African River Health Programme (RHP), which is an environmental monitoring programme that focusses on quantifying, assessing and recording information regarding riverine ecosystems and their ecological state (Roux, 2001). In 2016 the River Health Programme (RHP) was replaced by the River Eco-status Monitoring Programme (REMP) which has the same basic aims as the RHP, with just a few modifications (Department of Water and Sanitation (DWS, 2016)). This programme was initiated in an effort to better manage water resources and improve the monitoring capability of the country, and its design was in the same level as biomonitoring programmes in other parts of the world, like Britain and Australia (Hohls, 1996; Roux, 1997).

Although chemical and physical characteristics of rivers in South Africa have been monitored long before biological monitoring was introduced into the country (Dallas, 1995), biological monitoring (the use of biota as an indication of the health status of rivers), was introduced after the resource based-approach Receiving Water Quality Objectives (RWQO) policy was adopted in the country (DWAF, 1991; Dallas, 1995). Biological monitoring has been proven to be a more reliable measure compared to physical and chemical parameters, as it is more sensitive to environmental processes and conditions (Warren, 1971). Biological assessment of water quality can be measured using different types of biota (like fish, diatoms, macroinvertebrates), however, benthic macroinvertebrates are very useful for assessing biological integrity because they are one of the most sensitive groups of biota in aquatic ecosystems, they are readily sampled and are relatively non-mobile (Dallas, 2000).

Since the inception of RHP in the early 1990s a number of indices have been developed and refined, these indices are currently used as part of the River Eco-status Monitoring Programme (REMP) (which replaced RHP) (Cawood and Vos, 2016). The abiotic indices are: Physico-Chemical Driver Assessment Index (PAI), Geo-Morphological Driver Assessment Index (GAI) and Index for Habitat Integrity (IHI), and the biotic indices are: Riparian Vegetation Response Assessment Index (VEGRAI), Macro-Invertebrate Response Assessment Index (MIRAI), and Fish Response Assessment Index (FRAI) (Kleynhans *et al.*, 2008; Cawood and Vos, 2016).

While biotic indices based on environmental tolerances can provide information on overall ecosystem integrity, macroinvertebrate taxa can be used to provide insight into how a community is structured and organized (Boyero and Bailey, 2001). Therefore, macroinvertebrate assemblages were assessed using the rapid bio-assessment technique known as the South African Scoring System (SASS) (Dallas, 2000). SASS is a field-based rapid bio-assessment method that uses benthic macroinvertebrates to provide a measure of water quality and general river health (Dallas, 1995). This method was designed for lotic systems and it works very well in different biotopes, it is also suitable for assessing the impacts of development, and evaluating temporal and spatial trends of the ecology and the ecological state of aquatic ecosystems (Dickens and Graham, 2002).

SASS was developed by Chutter (1994) and the currently used version 5 (SASS5) is the ISO accredited refinement of SASS (Dickens and Graham, 2002). Data collection using the SASS5 methodology entails using a standard 1mm mesh kick net (with a 30cm square frame) to collect macroinvertebrates in the “stones in current”, “marginal vegetation” and “gravel, sand and mud” biotopes following the steps set out in Dickens and Graham (2002).

A SASS5 scoring sheet which has ‘sensitivity weighting’ assigned to each taxon (at family level) based on the sensitivity or resistance to perturbations and pollution (of that particular family) is then used to record the macroinvertebrates found in each biotope (Dickens and Graham, 2002). The Number of Taxa and the Average Score Per Taxon (ASPT) which are calculated based on the taxon found in each biotope are then used to generate three metrics which are used to interpret the water quality of the given site. Even though bio-assessment has been proven to be a very useful and efficient tool for assessing river health, it does not explicitly test ecosystem function or the integrity of ecosystem processes which are very important in the structure and function of these aquatic systems.

1.5 Assessing River Structure and Function

One of the most well-known and useful models used for assessing and understanding the ecology of rivers and streams is a conceptual framework first developed by Vannote *et al.* (1980), called the River Continuum Concept (RCC) which characterizes pristine running water ecosystems (Statzner and Higler, 1985). The RCC represents a synthesis of different areas of thought including: the linkage between streams and their terrestrial surroundings, the holistic approach, material cycling in open systems, the integration of community ecology principles and biotic interactions (Minshall *et al.*, 1985). The RCC is therefore a synthetic concept that presents streams as ecosystems, however, before the RCC several researchers emphasized the connectivity between fluid hydraulics and geomorphology, and they also recognized that there are shifts in community structure along the different sections of a river (Newbold *et al.*, 1981). Some focussed on fish and macroinvertebrates but viewed the

relationship as a series of individual zones, instead of an integrated continuum (Minshall *et al.*, 1985).

Hynes (1963) showed how stream dynamics depend on or are affected by terrestrial detritus (allochthonous), and described how important the stream-land linkage is. He showed that the terrestrial setting (grassland, desert, forest or taiga) of a stream affects the stream ecosystem processes that occur in that particular setting. Streams get the majority of their energy from the terrestrial systems which provide them with organic matter (Cummins, 1974). This led to the conclusion that aquatic primary production and terrestrial plant debris are important sources of simple carbon compounds and that they complement each other in terms of providing a variable food base for lotic consumers (Minshall *et al.*, 1985).

From headwaters to mouth, there is a continuous gradient of the physical conditions in a river system (Vannote *et al.*, 1980). This gradient prompts populations' responses which result in consistent patterns in terms of the way in which organic matter is loaded, transported, utilized and stored along the river (Vannote *et al.*, 1980). In essence, it creates a continuum of biotic adjustments in the river and it assumes that the way stream communities are structured and function is dependent on with the mean state of the physical system (Statzner and Higler, 1985). Therefore, the physical conditions of a channel influence the characteristics of the producer and consumer communities found in it (Vannote *et al.*, 1980).

The RCC has been widely criticised due to some of its assumptions, however, the main criticism is that it was developed for natural and undisturbed stream ecosystems (Vannote *et al.*, 1980), which are almost non-existent today. Nonetheless, this framework is useful because it classifies macroinvertebrates into functional feeding groups (Statzner and Higler,

1985). Functional feeding groups (FFG) of macroinvertebrates are a classification system that was introduced by Cummins and collaborators (Cummins, 1973; Cummins and Klug, 1979; Cummins *et al.*, 2005). This classification system is not based on taxonomy but on the morpho-behavioural mechanisms that macroinvertebrates use to acquire food (Cummins *et al.*, 2005), meaning that macroinvertebrates are categorised into FFGs in accordance with their feeding mechanism or behaviour (Table 1).

Table 1. Feeding Functional Group (FFG) categories and their associated food resources (adapted from Cummins *et al.*, 2005).

Functional groups	Feeding mechanism	Dominant food resources	Particle size range of food (mm)
Shredders	Chew gouge wood or live vascular plant tissue or conditioned litter	CPOM	>1.0
Filtering collectors	Filter particles from the water column (suspension feeders)	FPOM	0.01 – 1.0
Gathering collectors	Gather loose particles in depositional areas or ingest sediment (deposit feeders)	FPOM	0.05 – 1.0
Scrapers	Graze stems of rooted aquatic plants or rock and wood	Periphyton	0.01 – 1.0
Predators	Capture and engulf prey or tissue, ingest body fluids	Prey	>0.5

Note: CPOM = Coarse Particulate Organic Matter; FPOM = Fine Particulate Organic Matter

FFGs describe the most likely predominant feeding behaviour and they indicate the type of food available and how it is distributed in a given ecosystem; they are thus useful in determining the roles of different organisms in an ecosystem (Palmer *et al.*, 1993). They relate community structure with ecosystem functioning because their relative abundance affects the ecosystem (Uwadiae, 2010). A single set of macroinvertebrate samples that has been taken appropriately can be used to estimate ecosystem characteristics (Cummins *et al.*, 2005). FFGs may provide more insight into ecosystem processes underlying a given stream

macroinvertebrate community primarily because the spatial and temporal changes that happen in a system result in shifts in the species structure (Hawkins and Sedell, 1981). Hence, processes in streams can be understood by classifying macroinvertebrates into FFGs because the composition of macroinvertebrate assemblages are directly related to aquatic processes –which play a major role in ecosystem function (Uwadiae, 2010).

Important attributes of an ecosystem can be evaluated using FFG ratios because the measurements of relative proportions of FFGs capture the changes in ecosystem attributes that impact their nutritional resources base (Merritt *et al.*, 1996; Cummins *et al.*, 2005). Therefore, FFGs can be used as surrogates for ecosystem attributes such as: balance between autotrophy and heterotrophy; linkage between riparian Coarse Particulate Organic Matter (CPOM) inputs and food webs in the stream; relative abundance of Fine Particulate Organic Matter (FPOM) in transport (suspended load) compared to the FPOM deposited in sediments; and the geomorphic stability of the channel (Cummins *et al.*, 2005). The functional analysis of macroinvertebrates has been developed, refined and applied over the past three decades (Cummins *et al.* 2005). This technique is sensitive to the impacts of land-use in the watershed and riparian zone of a stream because it is based on the morpho-behavioural characteristics of macroinvertebrates; which is directly linked to their food acquisition modes (Merritt *et al.*, 1996).

This method is less time consuming than species-level diversity assessments, because instead of studying different taxa, collections of small groups of organisms can be studied based on how they function and process energy. Functional feeding groups simplify community data and they reduce the variability caused by taxonomic and structural complexities of systems

(Hawkins and Sedell, 1981). This assists in terms of the ability to recognize trends and patterns and to also understand natural ecosystems better.

Although FFGs are categorised based on the mechanisms that they use to obtain food and the particle size(s) of the food (Smith *et al.*, 2004), food resource categories of stream invertebrates are integrated and cannot always be clearly distinguished from each other (Cummins and Klug, 1979). They are separated based on microbial and biochemical characteristics, and correspond with the morpho-behavioural adaptations of invertebrate FFGs (Merritt *et al.*, 1996), however, these morpho-behavioural mechanisms sometimes lead to the ingestion of different kinds of food.

Functional feeding group assignments at the family-level, which is the lowest taxonomic level provided by the SASS5 protocol, are not always consistent and certain macroinvertebrate taxa (e.g. Baetidae) can be assigned to more than one FFG (Palmer *et al.*, 1993). As there is insufficient data on South African stream communities to assign FFGs to all families of macroinvertebrates, this research focussed only on predators and filter-feeders, using the taxon trophic assignments of Walsh (2008), which were based on South African streams.

Predators were a focus of this study because ecosystem processes and shifts can be detected in the distribution and abundance of predators (Estes *et al.*, 2011). Predator-prey ratios are a fundamental aspect of analysing the functionality of systems because community data from different environmental systems (terrestrial, marine and freshwater) show that predator richness is proportional to prey richness (Warren and Gaston, 1992). Prey affects predators through the food web (trophic cascades), and ecosystem dynamics are non-linear, meaning

that predators are important in understanding the top-down forcing of a system (Estes *et al.*, 2011), therefore, predators are a valuable aspect in terms of understanding the processes of an ecosystem. Prey niches (increased prey types increase predator niches), and energy ratios (the amount of energy available at each trophic level is proportional to the richness) are another way of understanding predator-prey ratios (Warren and Gaston, 1992). Predators indicate the overall food chain bioaccumulation, therefore all the processes that affect the aquatic macroinvertebrate community are reflected in predators (Estes *et al.*, 2011).

Filter feeders were looked into because they indicate the quantity and quality of suspended fine particulate organic matter (FPOM), as they feed on it (Wallace and Webster, 1996). Suspended fine particles make a great contribution to the energy and nutrient cycles of streams; however, they can also be comprised of pollutants (Whiles and Dodds, 2002). A study in the Kansas River drainage, north-eastern Kansas (United States), showed that filter feeders' abundance have a significant correlation with seston (suspended particles) concentrations, and that this relationship is affected by human activities (Whiles and Dodds, 2002).

Aims and Objectives

The main question addressed in this study is: do South African savanna biosphere reserves (Magaliesberg, Marico and Waterberg), have greater ecosystem integrity compared to adjacent, non-protected streams? The aim of this study was to assess the effectiveness of these South African savanna biosphere reserves in maintaining aquatic ecosystem health, structure and function. The objectives of this study were to assess the differences within and outside the biosphere reserves in terms of; the physico-chemical characteristics and land-cover differences; river health (by using the South African Scoring System version 5), and by comparing community structure (taxon assemblage) and trophic organisation (using FFG ratios). It is hypothesised that the physico-chemical characteristics, land-cover (natural vs transformed); river health, community structure and trophic organisation of the headwater streams within biosphere reserves will be improved when compared to the unprotected streams.

Chapter 2: Materials and Methods

2.1 Study sites

A total of 21 sites were sampled and categorised based on management context, catchment, ecoregion and geology type using land-use classifications within the riparian buffer (Fig. 1; Table 2). The sites were either managed by one of the three biosphere reserves (Waterberg, Magaliesberg or Marico), or they were unprotected; this categorization was the primary context used in this study. The sites were also categorised into ecoregions namely; Western Bankenveld, Waterberg and Bushveld. An ecoregion is an area that has homogenous species, dynamics, environment and natural communities (Olson and Dinerstein, 1998). The ecoregion classification system that was used, describes the three ecoregions as explained below (Kleynhans *et al.*, 2005). The Waterberg ecoregion vegetation is dominated by Bushveld types (especially the Waterberg Moist Mountain Bushveld), and the terrain is dominantly table lands with moderate to high relief. It has a generally moderate mean annual precipitation (coefficient of variation = 20 – 35%), with a low to medium drainage density.

The Western Bankenveld ecoregion has a varied topography that ranges from lowlands and mountains to closed hills and mountains with a moderate to high relief. Different Bushveld and Grassland types are found in this ecoregion, but the most dominant vegetation type is Mixed Bushveld. It has a low-to-moderate mean annual precipitation (coefficient of variation = 20 – 35%), with a moderate (but low in parts) drainage density. The Bushveld ecoregion mainly consists of plains with low relief, and the most dominant vegetation type is Mixed Bushveld, followed by other types of Bushveld (Clay Thorn and Waterberg Moist Mountain Bushveld). It has a moderate to low mean annual precipitation (coefficient of variation = 25 –

35%), with a low drainage density. The rainfall season for these three ecoregions is early to mid-summer, and their mean annual temperature is 14 to 22°C.

The Waterberg area is characterised by sandstone (Pool-Stanvliet, 2013), while the Magaliesberg area is mainly characterised by quartzite (followed by shale and dolomite) (Hall and Steart, 1905), and the streams in the Magaliesberg area have been shown to be naturally acidic (Bricker and Rice, 1989; Taylor *et al.*, 2009). Therefore the sites were also analysed based on geology to assess whether the pH levels of the sites would significantly differ based on geology. Lastly, the sites were categorised into catchments to give a hydrological context; overall, the site selection was limited by accessibility to sampling points and trying to balance the number of sites sampled in each management context.

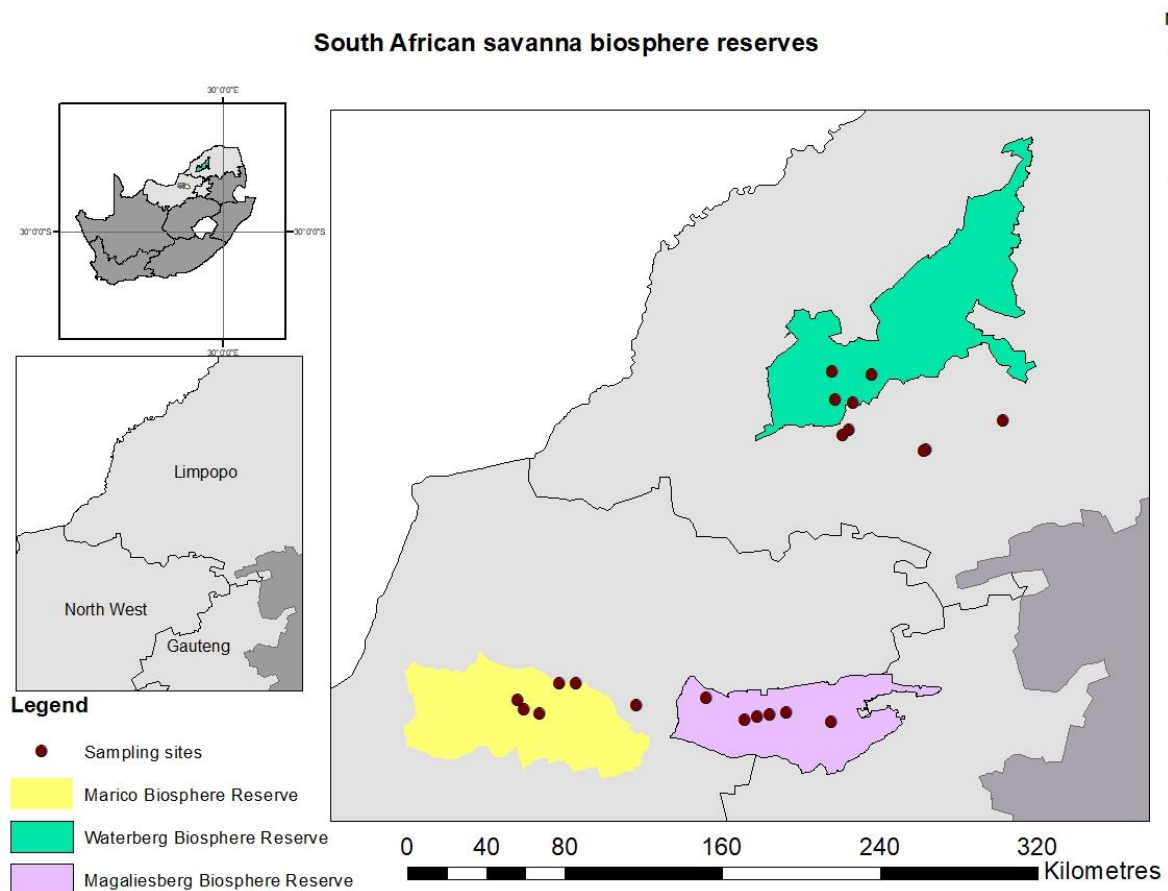


Figure 1. South African savanna biosphere reserves

Table 2. Sampling site categories used and the number of sites assigned to each.

Management context	Catchment	Ecoregion	Geology
Marico BR (5)	Groot Marico (5)	Western Bankenveld (13)	Transvaal, Rooiberg, Griqualand-west (8)
Waterberg BR (4)	Croc West (7)	Waterberg (6)	Rustenburg, Lebowa, Rashoop (4)
Unprotected (6)	Mokolo (8)	Bushveld (2)	Waterberg, Soutpansberg, Orange River (9)
Magaliesberg BR (6)	Mogalakwena (1)		

2.1.1 Waterberg Biosphere Reserve

The Waterberg Biosphere reserve lies between latitude 27° 30'S and 28° 40'S and longitude 23° 10'E and 24° 40'E (De Klerk, 2003). It includes twenty-six rural villages of the Mogalakwena Municipality in Brakensberg, and part of the Marakele National Park (De Klerk, 2003). The core areas of the biosphere reserve cover 121 249 hectares, the buffer zone covers 146 157 hectares and the transition zone covers around 150 000 hectares, totalling up to approximately 417 406 hectares (De Klerk, 2003). The Waterberg Biosphere Reserve was designated in March 2001 by UNESCO, after 7 years of negotiations between private landowners and rural African communities: it was the first biosphere reserve in the northern region of South Africa (Pool-Stanvliet, 2013; German Commission for UNESCO, 2015). This biosphere reserve is located in northern Limpopo Province in the Bushveld district (Fig. 2). The Waterberg escarpment is characterised by sandstone, and is sparsely populated (Pool-Stanvliet, 2013). Nature-orientated activities are the main drivers of the economy in the biosphere reserve, with a great focus being placed on environmental education (Pool-Stanvliet, 2013).

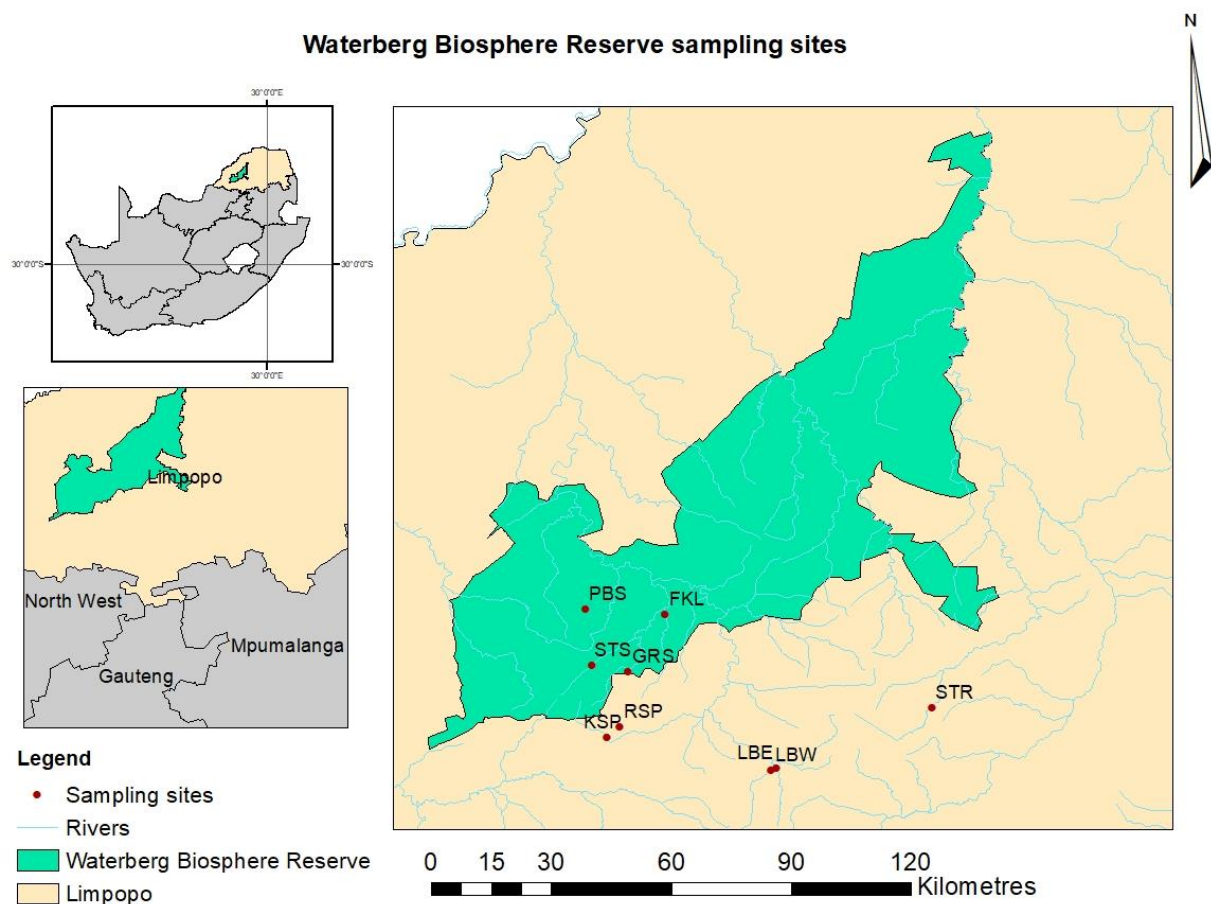


Figure 2. Waterberg Biosphere Reserve

2.1.2 Magaliesberg Biosphere Reserves

The Magaliesberg Biosphere Reserve was established in 2015 and is situated between Pretoria and Johannesburg (Fig. 3). The core area(s) cover 58,212 ha, the buffer area(s) cover 109,561 ha and the transition area(s) cover 190,097 ha, the entire biosphere reserve surface area (terrestrial and marine) covers 357,870 ha (UNESCO, 2017). This site lies on the boundary between the sub-Saharan savanna and the Central Grassland Plateaux (Cárdenas Tomažič *et al.*, 2016). It has a rich biodiversity which includes 443 bird species that make up 46.6% of the total bird species that are found in the sub-region of Southern Africa (Hirschauer, 2016; Cárdenas Tomažič *et al.*, 2016).

The biosphere reserve has unique natural features and is rich in cultural heritage; it is part of the ‘Cradle of Humankind’, which forms part of the World Heritage Site that houses 4-million year-old history of the human origin and evolution (Cárdenas Tomažič *et al.*, 2016). This region has more than 260 000 residents, and it is adjacent to major urban infrastructure (Hirschauer, 2016). This urban infrastructure impacts the economy as it is dominated by mining, tourism, urban development and agriculture (Cárdenas Tomažič *et al.*, 2016). The aim of the biosphere’s management plan is to encourage conservation and promote sustainable farming practices and tourism amongst initiatives such as water saving and solar power (Cárdenas Tomažič *et al.*, 2016).

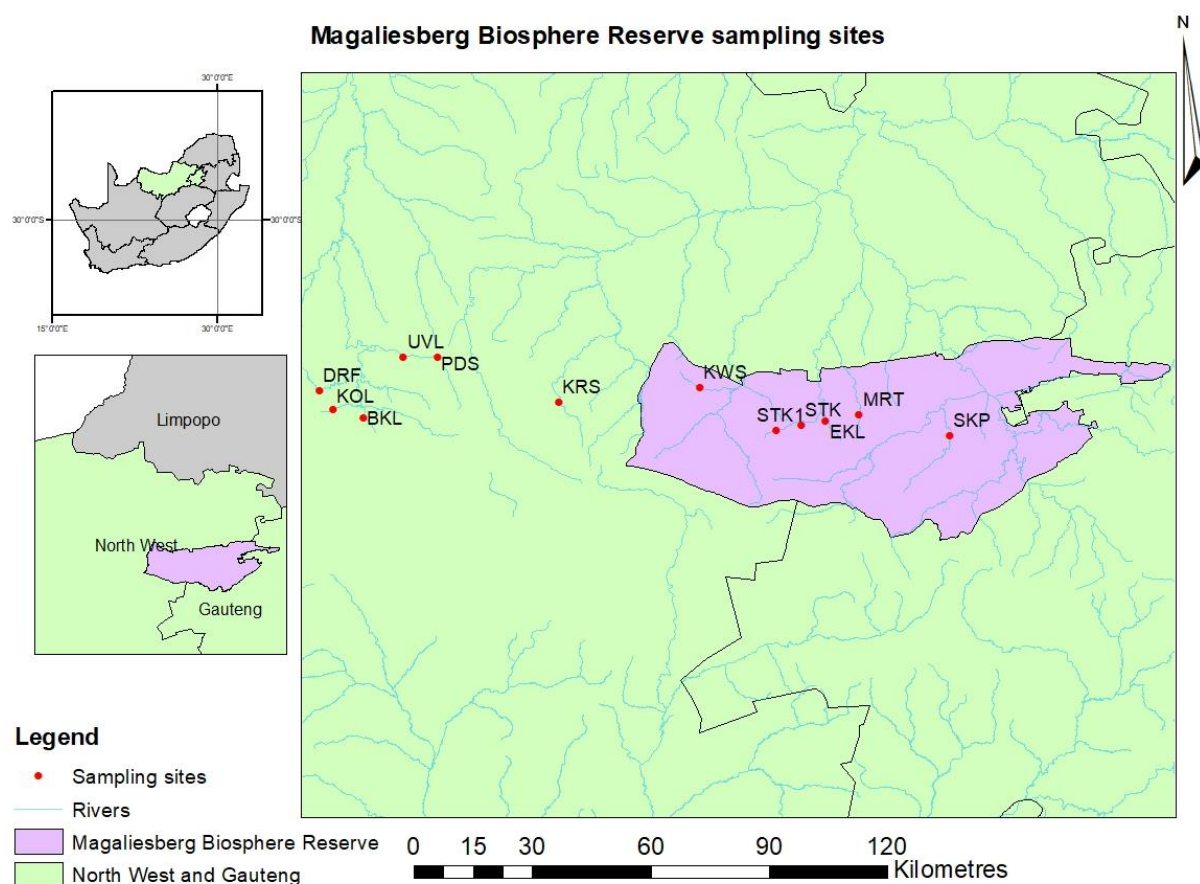


Figure 3. Magaliesberg Biosphere Reserve

2.1.3 Marico Biosphere Reserve

The Marico Biosphere Reserve was formally designated on 25 July 2018 by UNESCO (Fig. 4). The core area(s) cover 21,499 ha, buffer zone(s) cover 64,350 ha, and the transition zone(s) cover 361,419 ha, therefore the entire biosphere reserve covers an area of 447,268 ha. (UNESCO, 2019). The Marico Biosphere reserve was advocated for by; the Marico River Conservation Association (MRCA), Provincial Department of Rural, Environmental and Agricultural Development (READ) and local landowners. The Marico biosphere reserve has a unique freshwater ecosystem that includes the Marico, Molemane and Molopo river systems (UNESCO, 2019). The ecosystem of the reserve is characterised by a dolomitic system and wetlands, it also has vast grassland and savannah areas that support vulnerable plant species (UNESCO, 2019).

The main economic activities include subsistence livestock farming, agriculture, game ranching, and tourism, and there are 73 species of mammal that are part of the endemic fauna (UNESCO, 2019). Some of the sites that were initially assigned to the “Unprotected” management context during sampling were reassigned to the Marico BR (as sampling was done before the designation of the BR) prior to data analysis. This is justified by the fact that the conservation value of an area and the ecosystems in it are assessed and recognized as being worthy of protection, before the establishment of a biosphere reserve.

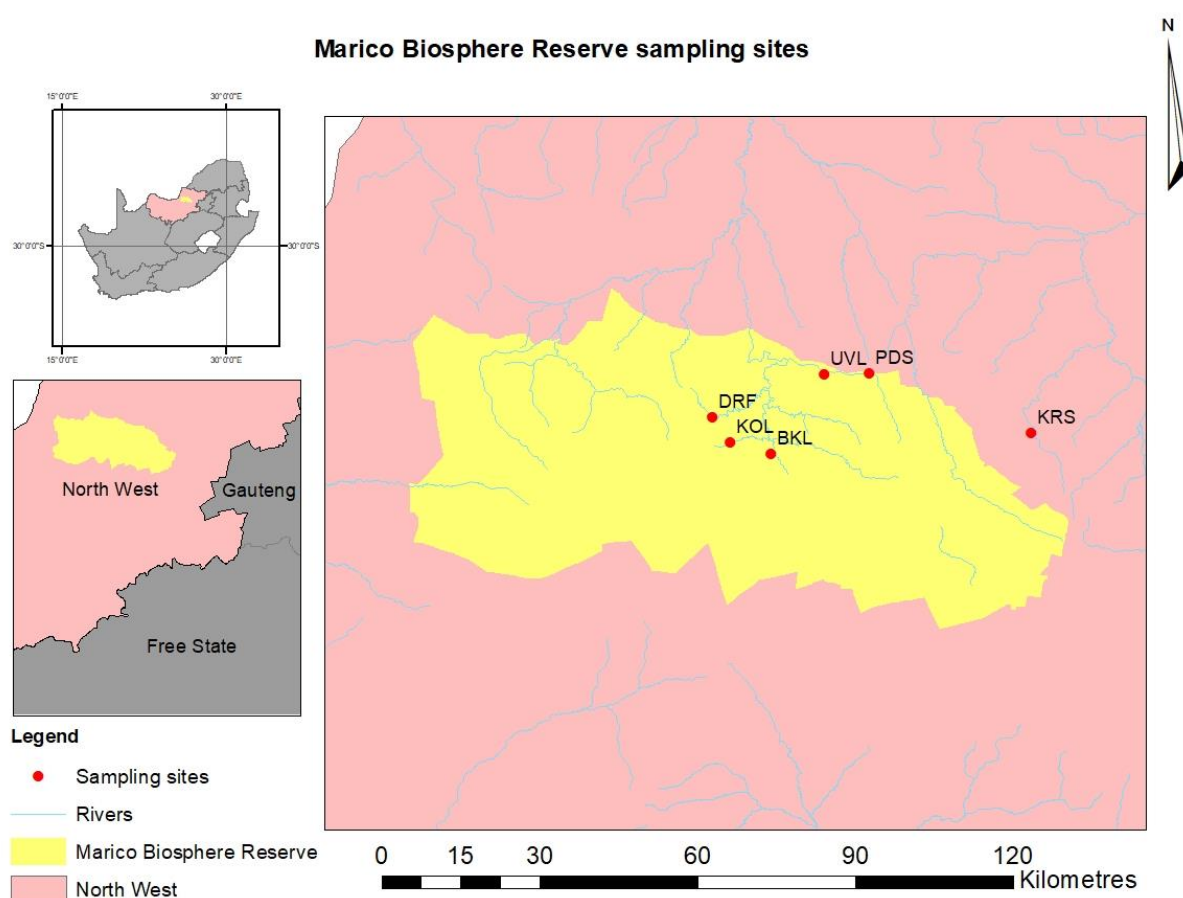


Figure 4. Marico Biosphere Reserve

2.2 Experimental design and protocol

2.2.1 Data collection and materials

Data collection was done from September to October 2017 (once-off), between 8am and 4pm. However, all sites were sampled in the same season (spring) to ensure that temperature variance amongst sites was minimal. Water samples were taken and tested in the laboratory for nitrate, ammonia and phosphorous, using a DR900 Multiparameter Portable Colorimeter which is auto-calibrated using company-supplied reagents (Hach, 2017). These chemicals in

particular were analysed to investigate the effect that cultivated land-use may have on the water quality, as they have been associated with agricultural run-off (Sharpley *et al.*, 1987; Heathwaite and Johnes, 1996; Tilman, 1999; Savci, 2012).

A range of physico-chemical data were collected, including turbidity which was measured using a HI-98703 Turbidity Meter which was calibrated to 0.1 and 15 NTU (Hannainstruments.co.uk, 2018), along with temperature, conductivity, pH and dissolved oxygen which were measured using a YSI multi-parameter physico-chemical probe which was calibrated in the lab (with oxygen re-calibrated for altitude in each biosphere reserve) (Xylem, 2018). Discharge was measured across a stream cross-section at each site using a FlowTracker Handheld ADV (auto-calibrated) (Acoustic Doppler Velocimeter) (Xylem-analytics Asia, 2018).

The SASS5 method was used to collect overall qualitative aquatic macroinvertebrate community data. The macroinvertebrates for SASS5 were collected in “stones in current”, “marginal vegetation” and “gravel, sand and mud” biotopes following Dickens and Graham (2002), all three biotopes were available and sampled at all sites. The sample procedure used (and described below) for SASS5 was conducted by following guidelines set out in Dickens and Graham (2002). For all three biotopes a 1mm mesh net (with a 30cm square frame) was used to collect the data. For the stones-in-current biotope, the stones were kicked for approximately two minutes (stones-out of current kicked for one minute) and turned over against each other so that the invertebrates can be dislodged into the net. For the Gravel, Sand and Mud (GSM) biotope, the gravel (small stones less than 2cm), sand grains (<2mm diameter) and mud, silt and clay particles (<0.06mm diameter) were be stirred by scraping or shuffling with feet, while simultaneously sweeping the net to catch dislodged biota over the

disturbed area for 1 minute. For the vegetation biotope, the net was used to swoop 2m of marginal vegetation (in-current and out-of-current), and 1m² of aquatic vegetation (in sites where it was available).

In addition to the three biotopes, hand-picking and visual observation of specimens that may have been missed by the sampling procedure was done for approximately 1 minute. The extra taxa recorded was added to the SASS5 sheet under the associated biotope. Each biotope sample was washed down to the bottom of the net; then the net was inverted and tipped into a white flat-bottomed tray. The samples were immersed by adding clean water to the tray, loose debris, stones and leaves were then removed (after checking that there are no organisms clinging to them). Then the macroinvertebrates were identified to family level using the *Aquatic Invertebrates of South African Rivers - Field Guide* (Gerber and Gabriel, 2002).

The identified macroinvertebrates were marked on the SASS5 scoring sheet, in order to provide a SASS5 sensitivity weighting for each sampling site, in the field. Low sensitivity weightings are assigned to taxa that are less sensitive (resistant), and high sensitivity weightings to taxa that are more sensitive or susceptible to pollution (Dallas, 2000). A total score is calculated by summing up the sensitivity weightings of each taxon (identified) to provide the SASS score, and then the Average Score Per Taxon (ASPT) is calculated by dividing the Total Score (SASS score) with the number of taxa (total number of taxa identified) at each site ($ASPT = \text{SASS score} / \text{number of taxa}$) (Dallas, 2000). This calculated SASS score and the ASPT are then used to obtain an ecological category using biological bands in Dallas (2007).

Four macroinvertebrate samples (from the stones in current biotope or Gravel, Sand, Mud (GSM), if the former was not available) per site were collected using a surber sampler. The surber sampler used had a medium frame (quadrat size = 25 x 25cm, sampling size = 6cm²) made from aluminium alloy with nylon screens (collar) attached to a 1mm net (on the inner brass ring) (Natural History Book Service, 2019). The end of the net was fitted with a removable filter (1mm mesh size), and a 250ml bottle which was used to temporarily store the macroinvertebrates before preserving them in 70% ethanol. The surber sampler was used to collect quantitative benthic samples for analysing numerical ratios of the macroinvertebrate communities including FFGs. Both the SASS kick-net samples and the quantitative benthic (surber) samples were placed into vials and preserved in 70% ethanol for laboratory verification of macroinvertebrate identification to family level using Gerber and Gabriel (2002) and enumeration of FFGs. In the laboratory, Functional Feeding Groups were distinguished by creating a list of families which were assigned preliminary FFGs, following Walsh (2008).

2.3 Data analyses

Abiotic physico-chemical data and land-cover data were compared using a non-parametric Kruskal Wallis (rank sum) Test, to assess if there is a significant difference between the different spatial groupings (management context and ecoregions). ESRI ArcMap 10.3 was used to generate land cover maps (Databases: Ruraldevelopment.gov.za - National Geospatial Information office, Dwa.gov.za - Resource Quality Information Services: Department of Water and Sanitation, South African National Biodiversity Institute (SANBI) Biodiversity GIS, and South African National Space Agency (SANSA)), for each of the sites, by creating

a 1km buffer around each sampling point and streams in the upstream catchment (Appendix C). The 2013-2014 South African National Land-Cover Dataset (Department of Environmental Affairs E-GIS) was used to create a layer of a river polygon of each stream, which was used to buffer and clip a 1km area of the upstream catchment of the sampling points. Then the meta-data of the 1km buffers was used to calculate the land-cover percentage of each land-cover class. Geology, catchment and ecoregion shapefiles (derived from the above mentioned datasets) were over-layed on the Land-Cover Dataset and classified accordingly.

Buffers included all tributaries upstream of sampling points, to ensure that land cover classes are adequately accounted for. The South African Protected Areas Database (SAPAD) and the South African Conservation Areas Database (SACAD) from the Department of Environmental Affairs Environmental Geographical Information Systems (E-GIS), were used to assign sampling sites to the relevant zones (core, buffer and transition). The land-cover classes analysed are natural (grassland, woodland/open bush, thicket/dense bush, low shrub-land) and transformed (cultivated commercial fields). Statistica version 13.3 (TIBCO Statistica, 2017) was used to create correlation matrix (Pearson and Spearman) to analyse correlations between aquatic macroinvertebrate data and physico-chemical data.

Geographic differences in aquatic macroinvertebrate data were analysed by using Primer version 5 software (Clarke and Warwick, 2001) to create cluster dendograms and nonmetric multidimensional scaling (NMDS) of the kick-net and surber macroinvertebrate data. These analyses were used to characterise the differences in community structure between sites, and also to assess the variation in overall community structure at a family level. The classification clustering method was used because it is widely employed in ecological studies and it takes

into account that there is a possibility that classification entities can be members of more than one group (overlapping) (Boesch, 1977).

Nonmetric multidimensional scaling (NMDS) was also used, which is an ordination technique that focusses on numerical optimization, and is well suited for a wide variety of data because it does not assume that there are linear and modal relationships (Legendre and Legendre, 1998). This is in contrast to other ordination methods such as principal components analysis and reciprocal averaging; it produces better ordinations due to it using the space of the same dimensions as the simulated data (Fasham, 1977; Legendre and Legendre, 1998). The calculations for making the algorithm for NMDS is complex, but the principle behind it is simple; it simply represents a matrix of dissimilarities by mapping or producing an ordination of the samples in a space of m dimensions (Fasham, 1977). NMDS assumes that inter-sample dissimilarity and inter-sample distance relationships in the ordination space should be ranked in order; to ensure that there is a good ordination; hence the more similarities between samples, the closer they will be in the ordination space (Fasham, 1977). NMDS has thus been used to characterise the differences in community structure between sites.

The abundance-per-taxon data from four surber samples collected at each site were averaged in order to compare mean abundance per site. For kick-net data, the three biotopes (Stones-in-current, Marginal Vegetation and Gravel-Sand-Mud) data were merged into a taxon list per site. The surber data was square-root transformed and the SASS data was transformed using a Presence/Absence standard (Anderson *et al.*, 2006), then they were standardised where a Bray-curtis similarity matrix was created before running the Cluster and Non-metric multidimensional scaling (NMDS) analyses on the data. Cluster analyses produced plot

dendograms clustered by group average and their respective NMDS ordinations were also analysed.

SASS scores and the Average Score Per Taxon (ASPT) of the different sampling points were analysed using means ($\pm 1SE$) and by running one-way ANOVA analyses (as they were normally distributed) on the data to investigate the differences between them. Ecological categories were also analysed because it is important to take into consideration the geographical and longitudinal variations of South African rivers when interpreting SASS data (Dallas, 2007). Ecological categories are guidelines that have been developed to assist with SASS data interpretation; they were generated using the SASS5 Score and ASTP values of reference sites (from different spatial groups around South Africa) to generate biological bands, based on Dallas and Day (2007). The ecoregion-specific biological bands of Dallas (2007), were used to analyse the ecological categories in this thesis, and for the purpose of this thesis the categories were analysed as clusters: A/B (Natural to good), C (fair), and D/E/F (poor to critically modified).

Functional feeding groups were also analysed as they are an informative proxy for assessing aquatic health and they are potential ecological structure indicators (Boyero and Bailey, 2001; Uwadiae, 2010; Armon and Hänninen, 2015). Functional feeding groups that were analysed in this study are predators and filter feeders. These particular FFGs were chosen because there was relatively high confidence in assigning them to all taxa at the taxonomic level provided by SASS (class and family), compared to FFGs like grazers and scrapers which have a greater intra-family variation, to reliably assign one FFG or another to each

family present (Palmer *et al.*, 1993). FFGs were categorised as predators vs non-predators and filter feeders vs non-filter feeders.

Species that were generally recorded as having more than a single feeding behaviour were classified as non-predators, following the Warren and Gaston (1992) method of categorising predators and non-predators. The approach that Warren and Gaston (1992) used was that omnivores were treated as non-predators unless there was enough literature stating that the given omnivore's principle feeding behaviour was predatory. The same was done for categorising filterers and non-filterers where macroinvertebrates that had more than a single feeding behaviour were categorised as non-filterers. The taxon FFG classification of Walsh (2008) was used as a primary guide to assign FFGs, as that study was also based on a South African context (grassland and savanna streams).

Chapter 3: Results

3.1 Abiotic variables

3.1.1 Physico-chemical variables

Data were collected between 19 September 2017 and 17 October 2017. Abiotic variables (physico-chemical and chemical variables, land-cover data), were summarised into management context (Table 3) and ecoregions (Table 4) in terms of their mean ($\pm 1\text{SE}$). The physico-chemical variables measured were: discharge, temperature, dissolved oxygen, conductivity and pH. The chemical variables (analysed in the laboratory) were phosphorus, nitrate and ammonia, and the dominant land-cover classes analysed are natural (grassland, woodland/open bush, thicket/dense bush, low shrub-land) and transformed (cultivated commercial fields). Discharge (Q) m^3/s mean ($\pm 1\text{SE}$) was lowest in Unprotected sites and the Waterberg, 0.01 (0), and highest in the Marico BR 0.09 (0.05) (Table 3). The highest mean ($\pm 1\text{SE}$) of cultivated commercial fields (transformed land-cover type) was in Unprotected sites, 9.82 (2.64), and the lowest was in Magaliesberg BR, 2.93 (0.86).

Table 3. Mean (± 1 SE) values of abiotic (physico-chemical, chemical, and land-cover data) variables based on management context (Marico BR = 5, Unprotected = 6, Magaliesberg BR = 6, Waterberg BR = 4).

Variable	Marico BR	Unprotected	Magaliesberg BR	Waterberg BR
Discharge (Q) m^3/s^*	0.09 (0.05)	0.01 (0)	0.04 (0.02)	0.01 (0)
Temperature $^{\circ}\text{C}$	18.14 (0.83)	19.63 (0.89)	18.18 (1.02)	23.55 (2.41)
Dissolved Oxygen %	71.72 (3.76)	64.07 (10.12)	74.73 (2.13)	80.68 (7.34)
Conductivity $\mu\text{S}/\text{cm}$	199.72 (75.32)	77 (26.81)	21.17 (3.94)	33.38 (7.17)
pH	7.328 (0.30)	6.82 (0.18)	6.58 (0.31)	6.6 (0.28)
Phosphorus mg/L	0.42 (0.09)	0.22 (0.07)	0.31 (0.11)	0.18 (0.03)
Nitrate mg/L	0.04 (0.03)	0.05 (0.05)	0.01 (0)	0.02 (0.02)
Ammonia mg/L^*	0.2 (0.02)	0.15 (0.04)	0.10 (0.02)	0.14 (0.03)
Grassland	18.25 (2.95)	7.78 (2.35)	9.62 (2.68)	4.64 (1.30)
Woodland/Open	37.17 (6.48)	16.04 (4.76)	19.77 (5.32)	9.44 (2.70)
Cultivated commercial fields	8.67 (2.96)	9.82 (2.64)	2.93(0.86)	8.82 (0.93)
Low shrubland	11.85 (1.82)	5.01 (1.53)	6.22 (1.77)	3.01 (0.83)
Thicket /Dense bush	5.39 (0.83)	2.31 (0.70)	2.95 (0.82)	1.38 (0.38)

Note: Variables that showed significant differences*

Table 4. Mean (± 1 SE) values of abiotic variables and dominant land-cover classes based on ecoregions (Western Bankenveld = 13, Bushveld = 2, Waterberg = 6).

Variable	Western Bankenveld	Bushveld	Waterberg
Discharge (Q) m^3/s	0.05 (0.02)	0.02 (0.01)	0.01 (0)
Phosphorus mg/L	0.36 (0.06)	0.14 (0.02)	0.18 (0.05)
Nitrate mg/L	0.04 (0.02)	0.02 (0.01)	0.02 (0.01)
Ammonia mg/L	0.16 (0.02)	0.07 (0)	0.13 (0.02)
Temperature $^{\circ}\text{C}$	18.52 (0.65)	17.6 (0.1)	22.63 (1.64)
Dissolved Oxygen %	67.56 (4.55)	71.4 (5.1)	82.17 (4.85)
Conductivity $\mu\text{S}/\text{cm}$	113.17 (35.89)	17.55 (2.95)	35.8 (9.1)
pH	7.06 (0.16)	6.01 (0.32)	6.60 (0.23)
Grassland	33.83 (4.05)	35.45 (3.49)	24.51 (6.86)
Woodland/Open bush	38.88 (5.03)	23.76 (2.59)	47.69 (13.32)
Cultivated commercial fields	8.00 (1.79)	0	7.30 (1.10)
Low shrubland	9.93 (1.97)	18.59	13.12 (4.63)
Thicket /Dense bush	6.4 (1)	17.87 (2.93)	6.22 (2.49)

Phosphorous and pH are normally distributed and they do not have a significant difference across management context (one-way ANOVA, $P > 0.05$). A non-parametric Kruskal Wallis Test was run on non-normal abiotic and land-cover data. Kruskal Wallis Test showed that there was no statistical difference ($p > 0.05$) between some abiotic variables across the different management contexts: nitrate, temperature, dissolved oxygen (%) and conductivity ($\mu\text{S}/\text{cm}$). However, there was a statistical difference for discharge (m^3/s) ($H_{3, 19} = 9.6$, $p = 0.02$) and ammonia ($H_{3, 21} = 8.37$, $p = 0.04$), across the different management contexts.

A non-parametric Kruskal Wallis Test was also run on non-normal abiotic variables across ecoregions. It showed that there was no statistically significant difference ($p > 0.05$) between abiotic variables across the ecoregions: nitrate (mg/L), ammonia (mg/L), temperature $^{\circ}\text{C}$, dissolved oxygen (%), conductivity ($\mu\text{S}/\text{cm}$) and discharge (m^3/s). As discharge was significantly different across management contexts, a Bonferroni post-hoc test (two-sided significance levels) was done to identify where the significant differences between the groups are. It showed that there is a significant difference between Marico BR and Unprotected ($p < 0.05$) in terms of discharge (Fig. 5).

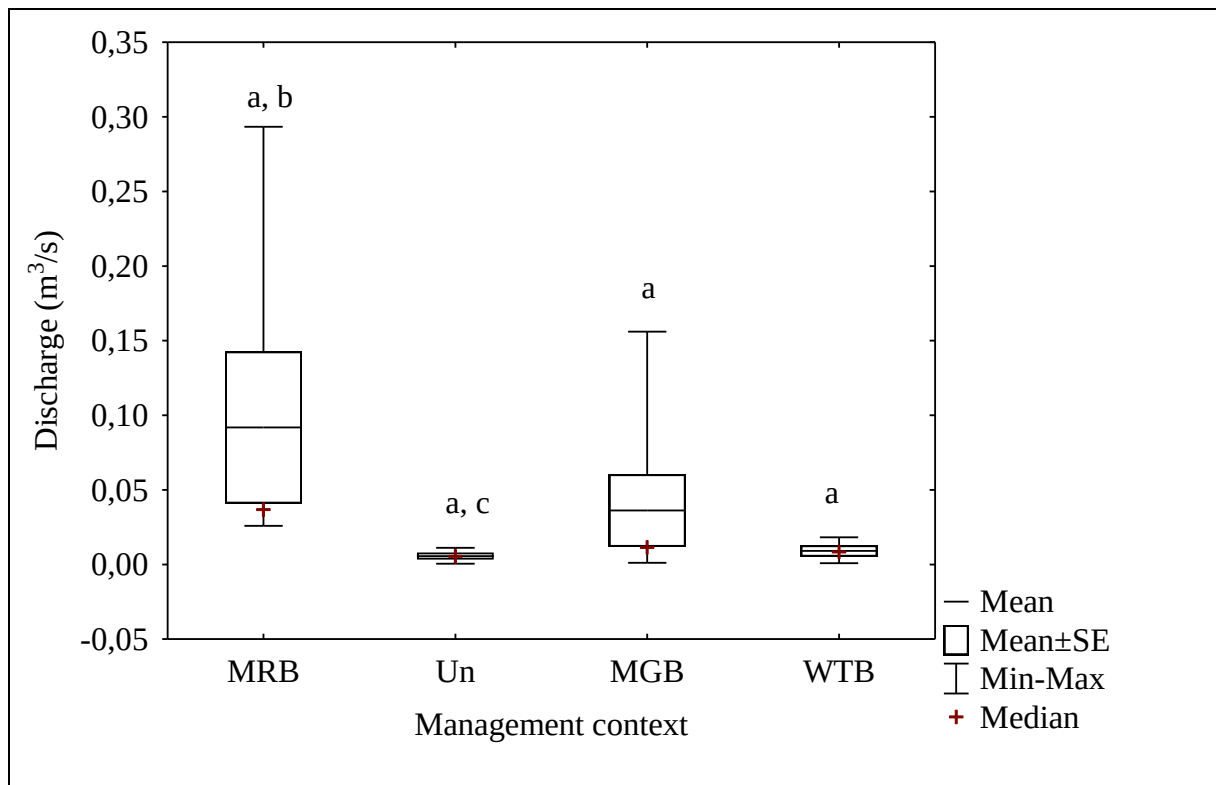


Figure 5. Mean ($\pm 1SE$) discharge across management contexts.

Note: MRB = Marico Biosphere Reserve, Un = Unprotected, MGB = Magaliesberg Biosphere Reserve, WTB = Waterberg Biosphere Reserve, W – B = Western Bankenveld, B = Bushveld, W = Waterberg

3.1.2 Land-cover analyses

The most dominant (highest mean ($\pm 1SE$)) land cover classes (grassland, woodland/open bush, cultivated fields/pivots/orchards, low shrub-land and thicket/dense bush), were correlated with abiotic variables and aquatic macroinvertebrate data, however there were no significant correlations between them. These dominant land-cover classes were compared across the different management contexts (Fig. 6). Grassland and thicket/dense bush were

most dominant in Magaliesberg BR, followed by Marico BR, Unprotected then Waterberg BR. Woodland/open bush was most dominant in Waterberg BR, followed by Unprotected, Marico BR and Magaliesberg BR. Cultivated commercial fields were most dominant in Unprotected, followed by Waterberg BR, Marico BR and Magaliesberg BR. Low shrub-land was most dominant in Waterberg BR followed by Marico BR, Magaliesberg BR and Unprotected.

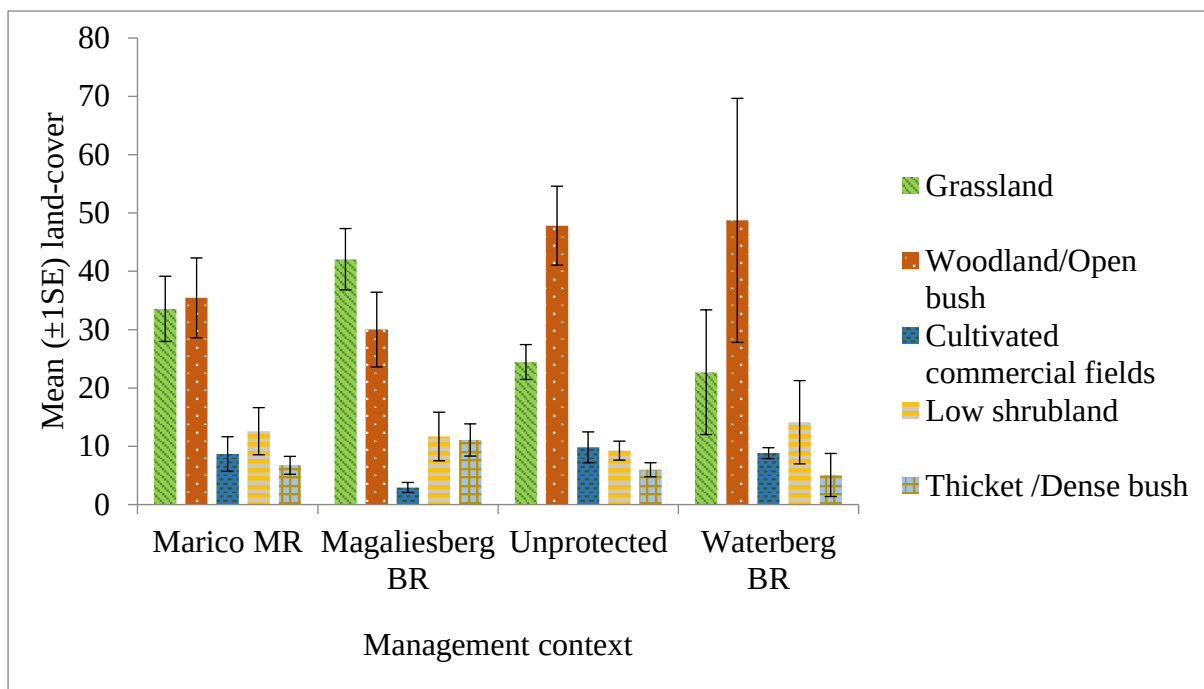


Figure 6. Mean ($\pm 1SE$) variation in dominant land-cover values (grassland, woodland/open bush, cultivated commercial fiends, low shrub-land and thicket/dense bush) across management contexts.

Low shrub-land was normally distributed, and it is not statistically significantly different between different management contexts (one-way ANOVA; $p > 0.05$). The remaining 4 main land-cover classes being: grassland, woodland/open bush, cultivated commercial fields and

thicket/dense bush are not normally distributed; therefore a non-parametric Kruskal Wallis Test was used to determine if they are significantly different across the management contexts. There is no statistical difference in grassland, woodland/open bush, cultivated commercial fields and thicket /dense bush across management contexts (Kruskal Wallis Test; $p > 0.05$). In terms of ecoregions, there is no statistical difference in, grassland, woodland/open bush, cultivated commercial fields, and thicket /dense bush, across ecoregions (Kruskal Wallis Test; $p > 0.05$).

There were no significant correlations between dominant land-cover classes and physico-chemical variables, except for between cultivated commercial fields and nitrate. They both have non-normal distributions therefore a Spearman rank correlation was performed, which revealed a significant correlation between them ($r_s = 0.60$, $p < 0.05$). Elevated levels of nitrate are mostly associated with greater cultivated commercial fields' land-cover; 51% of the variation in nitrate concentrations can be accounted for by the variation in cultivated commercial field's land-cover (%) (Fig. 7).

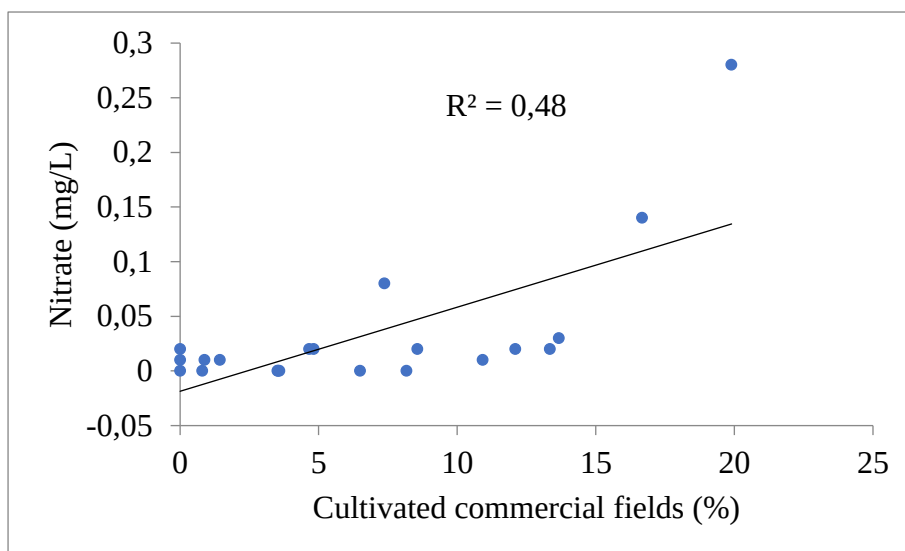


Figure 7. Relationship between nitrate concentrations and cultivated commercial fields land-cover.

3.2 SASS5 Rapid bio-assessment and Ecological Categories

The SASS scores of the different sites (Appendix B) were analysed based on management context (Fig. 8). The mean ($\pm 1SE$) SASS scores of the different management contexts are 106.75 (6.55) for Waterberg BR, 94.33 (16.48) for Magaliesberg BR, 93.17 (10.17) for Unprotected and 88.6 (10.2) for Marico BR. There was no statistically significant difference between the SASS scores of different management contexts (one-way ANOVA; $p > 0.05$).

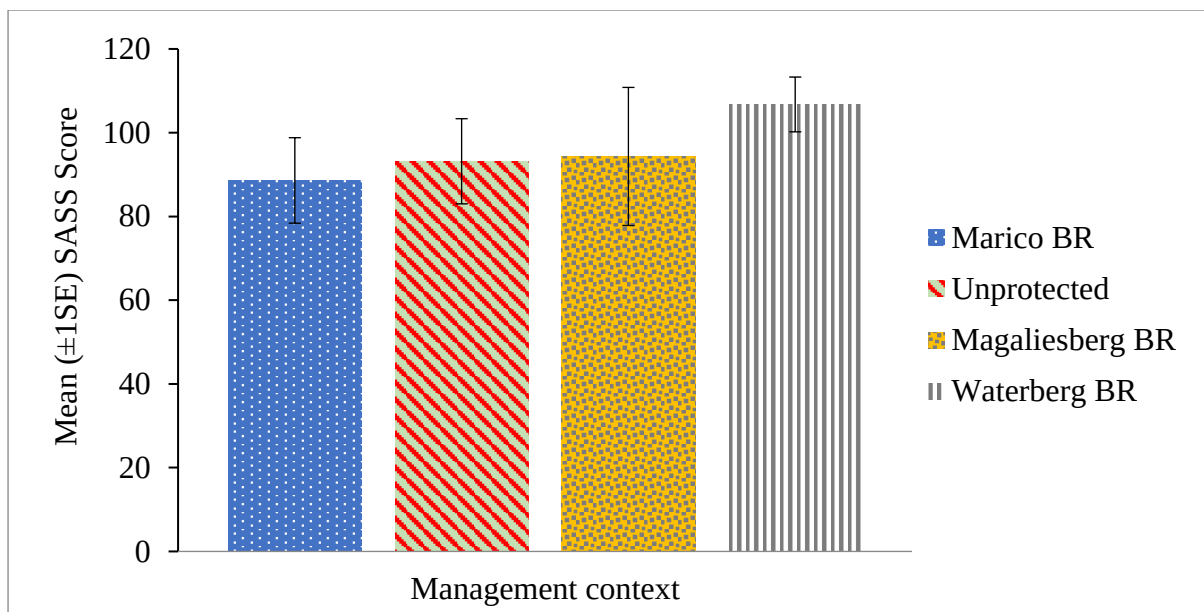


Figure 8. Mean ($\pm 1SE$) SASS scores across different management contexts.

Note: BR = Biosphere Reserve.

Aquatic macroinvertebrate community differences were also assessed by comparing the Average Score Per Taxon (ASPT) of the different management contexts (Fig. 9). The mean ($\pm 1SE$) ASPT of different management contexts are; Waterberg BR = 6.0 (0.30), Magaliesberg BR = 5.9 (0.12), Marico BR = 5.59 (0.37), and Unprotected = 5.31 (0.18).

There was no statistically significant difference between the ASPT of different management contexts (one-way ANOVA; $p>0.05$).

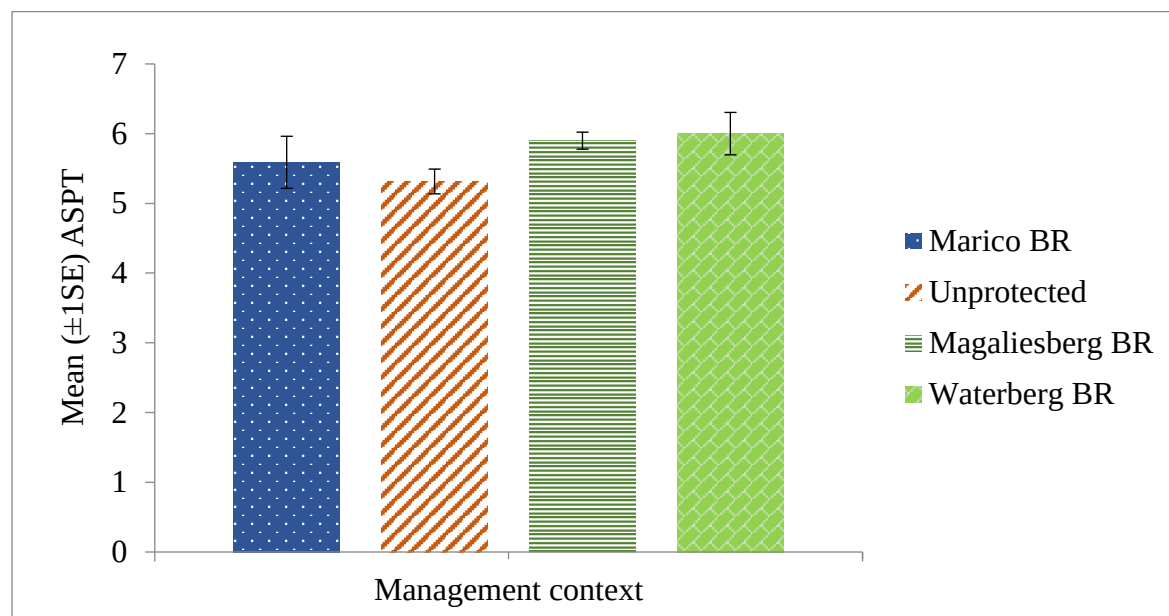


Figure 9. Mean (±1SE) Average Score Per Taxon across different management contexts.

The SASS scores of the different sites were analysed based on ecoregions (Fig. 10). The mean (±1SE) SASS scores of the different ecoregions are: 104.5 (7.38) for Waterberg, 93.08 (8.3) for Western Bankenveld and 79.0 (24.0) for Bushveld. There was no statistically significant difference between the SASS scores of different ecoregions (one-way ANOVA; $p>0.05$).

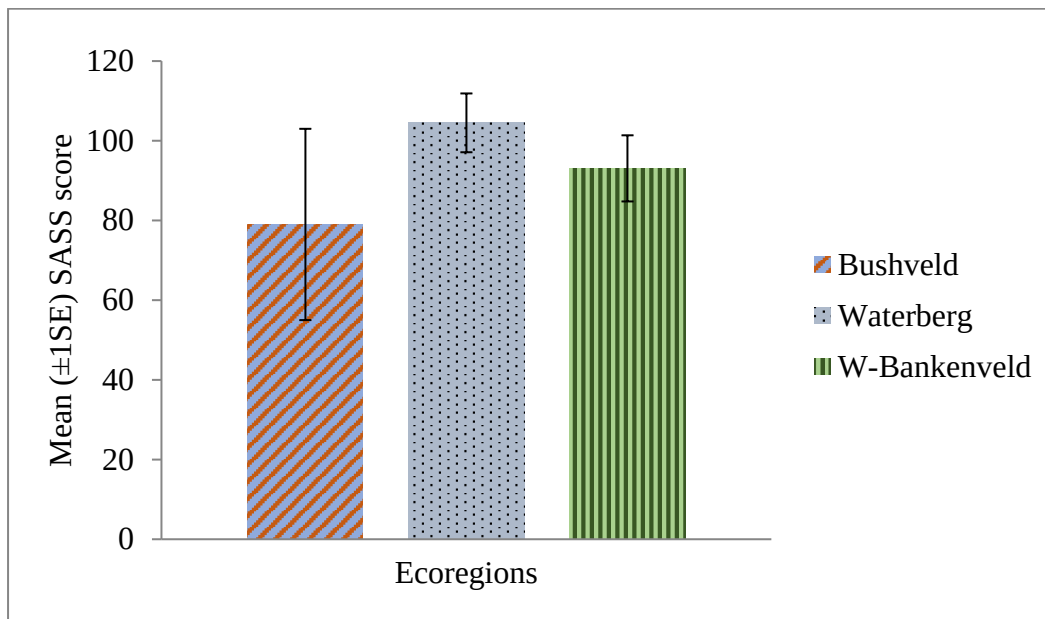


Figure 10. Mean ($\pm 1SE$) SASS scores across different ecoregions.

Aquatic macroinvertebrate community differences were also assessed by comparing the Average Score Per Taxon (ASPT) of the different ecoregions (Fig. 11). The mean ($\pm 1SE$) ASPT of different ecoregions are; Waterberg = 5.83 (0.24), W - Bankenvelde = 5.62 (0.18) and Bushveld = 5.61 (0.11). There was no statistically significant difference between the ASPT of different ecoregions (one-way ANOVA; $p > 0.05$).

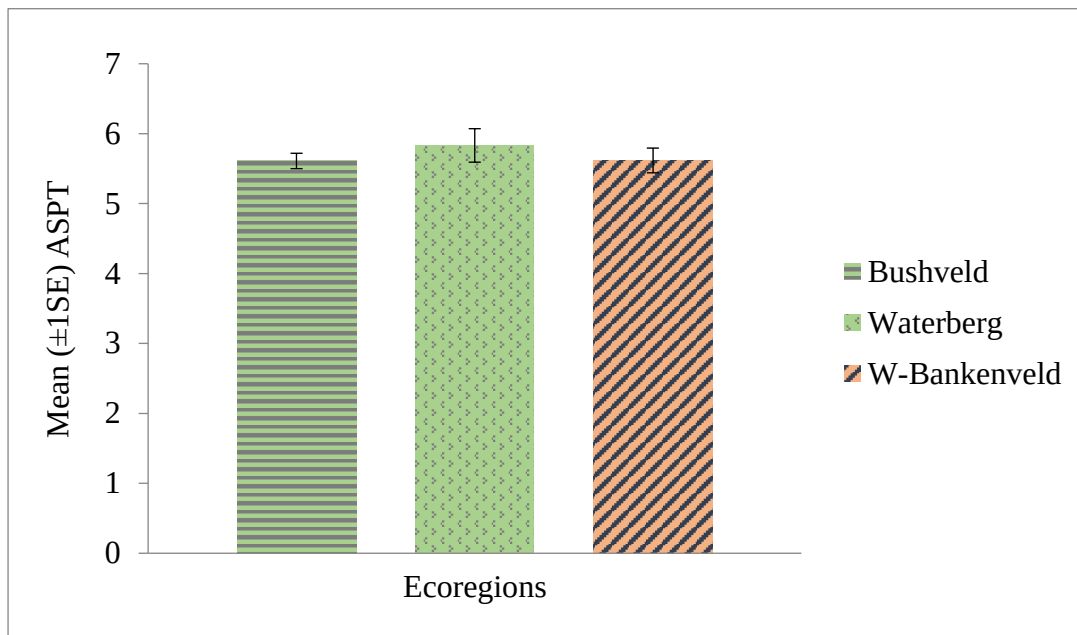


Figure 11. Mean (±1SE) Average Score Per Taxon (ASPT) across different ecoregions.

In terms of ecological categories, unprotected sites did not have a single A/B category site, indicating all unprotected headwater streams were modified to some degree (Fig. 12, see also Appendix A). Marico BR, Magaliesberg BR and Waterberg BR all had A/B categorised sites. The highest proportion of A/B sites was observed in Magaliesberg BR followed by the Marico and Waterberg BRs. Category C and D/E/F sites were present in all the BRs but the proportion was highest in the unprotected sites.

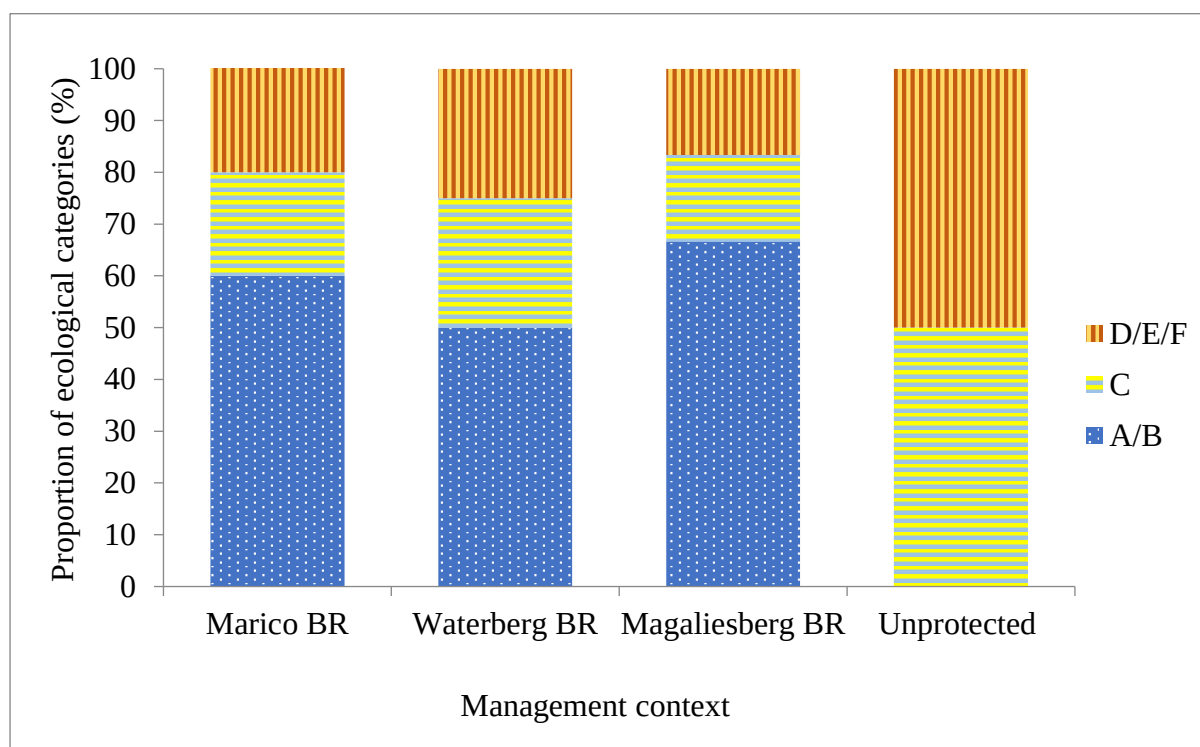


Figure 12. Proportional occurrence of river ecological categories across management context.

A total of 6 of the sampling points were in the core area, however not a single one was unmodified, natural (A ecological category) (Table 6). Two of the core sites were largely natural with a few modifications (B), one was moderately modified (C), 2 were largely modified (D) and one was critically modified (E/F). A total of 7 sites were in the buffer zone, ranging from A to C, 2 sites in the transition zone (C ecological category), and 6 unprotected sites (C to E/F).

Table 5. Site ecological categories and their management zones.

Sites	A	B	C	D	E/F
Core (6)		2	1	2	1
Buffer (7)	1	5	1		
Transition (2)				2	
Unprotected (6)			3	2	1
Total	1	7	6	5	2

The ecological categories of core sites ranged from B to E/F. The Magaliesberg BR has 3 core areas (two are B ecological categories and one site has a D ecological category), the Waterberg BR has two core sites (C and D ecological category), and the Marico BR has one core site (E/F ecological category) (Table 7).

Table 6. Locations and ecological categories of core sites.

Biosphere Reserve	Location	Site code	Ecological categories
Magaliesberg	Kgaswane Mountain Nature Reserve	KWS	D
	Magaliesberg Protected Natural Environment	MRT	B
	Fossil Hominid sites of SA: Cradle of Humankind	SKP	B
Waterberg	Welgevonden Game Reserve	STS	C
		PBS	D
Marico	Designated core area	DRF	E/F

3.3 Geographic differences of macroinvertebrate data

Cluster analysis show that out of 6 primary kick-net richness pairs of sites, only one pair has sites that belong to the same management context (Fig. 13 A). This pair has Unprotected sites which are LBE and STR, these two sites are 38.31km apart. Although LBE is spatially closer to LBW (1.35 km), it is 85.71% similar to STR, and is only similar to LBE at the 4th pair. This cluster suggests that there are great similarities in taxon richness prevalence between these two sites; however, this richness is not distinct across different management contexts. For example, there is an 84.44% similarity between STS and GRS, which are in different management contexts (Waterberg ecoregion and Unprotected, respectively).

This variability is also shown by the kick-net data richness in the Non-metric multidimensional scaling plot, where there is no clear separation between the management contexts (Fig. 13 B). When comparing similarity across ecoregions, there are three Western Bankenveld pairs, STR and LBE (85.71%) BKL and SKP (78.43%), KRS and EKL (60.86%). There is also one Waterberg pair STS and GRS which has an 84.44% similarity of kick-net richness data between the two sites (Fig. 14 A). Except for the KOL and KWS, the remaining sites have a >50% similarity, which indicates that although some sites belonging to the same ecoregion have greater similarities, overall, the kick-net aquatic macroinvertebrate richness is relatively variable across the ecoregions. The Non-metric multidimensional scaling plot shows that there is variability in the data across ecoregions as there is no clear separation between them (Fig. 14 B).

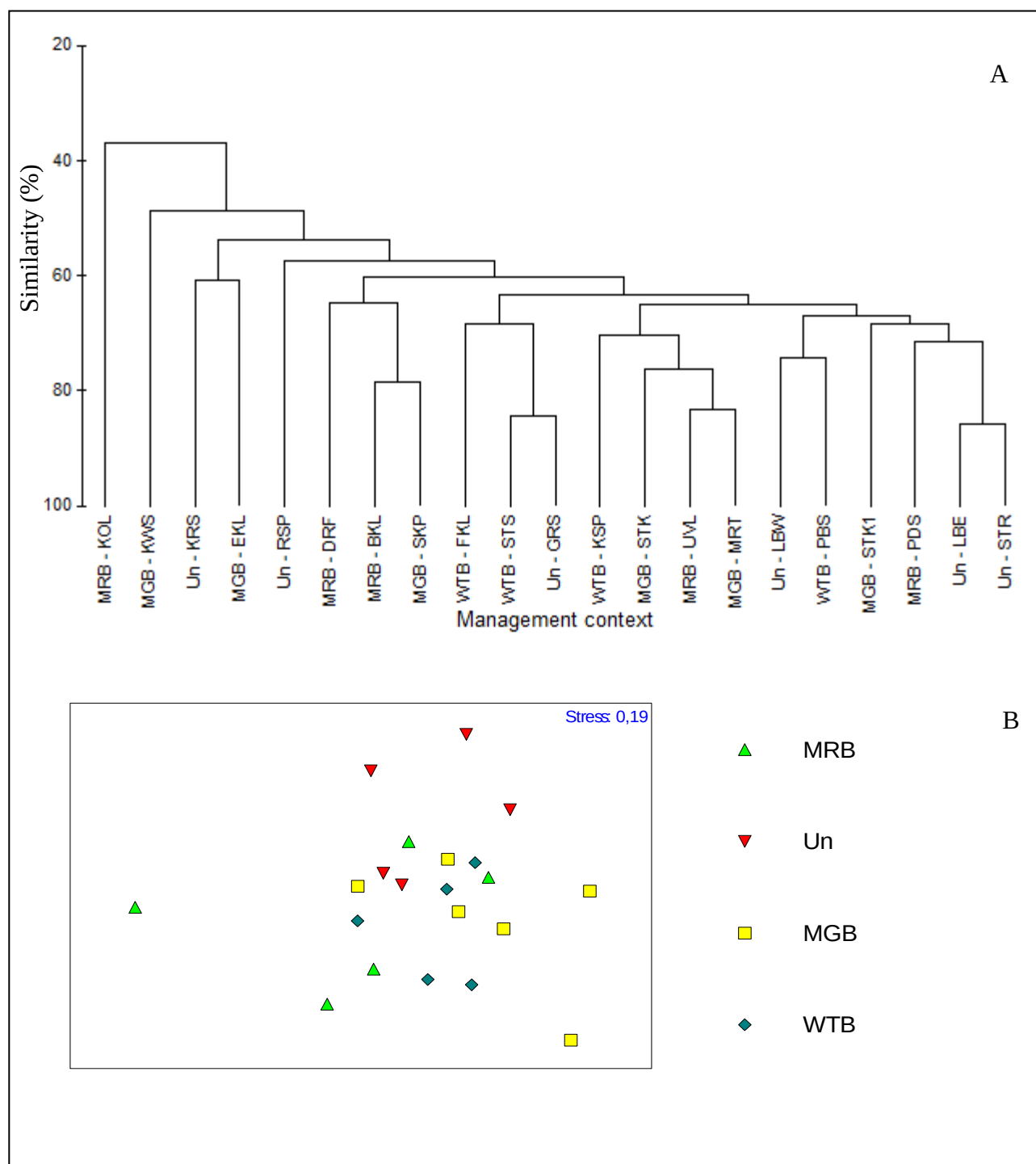


Figure 13. A) Cluster analysis of kick-net data based on management context. B) Non-metric multidimensional scaling (NMDS) analysis of kick-net data based on management context.

Note: Un- Unprotected, MGB– Magaliesberg Biosphere Reserve, MRB – Marico Biosphere Reserve, WTB - Waterberg Biosphere Reserve.

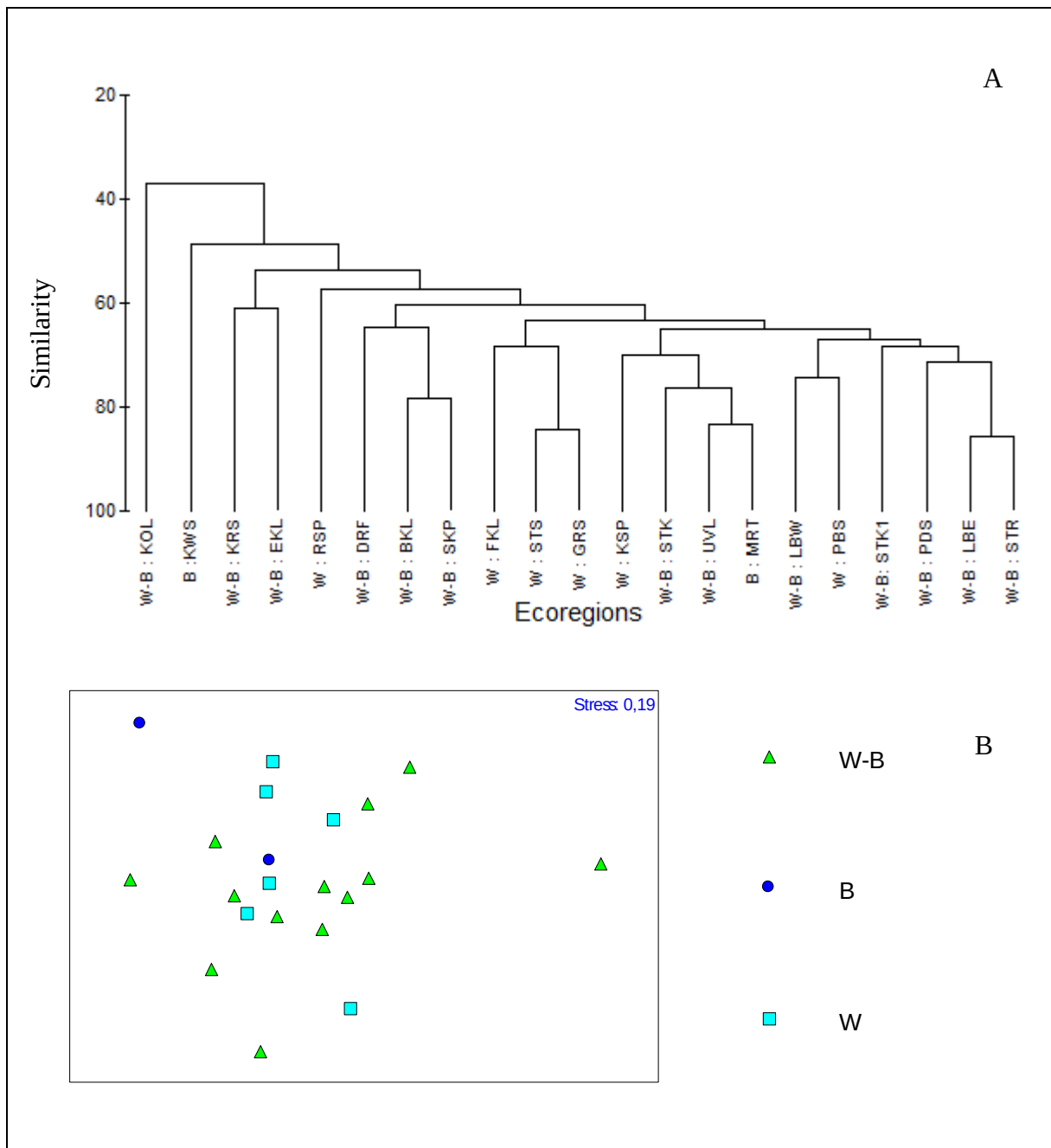


Figure 14. A) Cluster analysis of kick-net data based on ecoregions. B) Non-metric multidimensional scaling (NMDS) analysis of kick-net data based on ecoregions.

Note: W-B - Western Bankenveld, B – Bushveld, W - Waterberg.

Out of 6 primary quantitative (benthic) surber data pairs created by the cluster dendogram, only 2 pairs have an exclusive management context with a >70% similarity (Fig. 15 A). Unprotected pair; LBE and STR have a 78.6% similarity, Magaliesberg pair; EKL and STK have a 71.67% similarity. A total of 18 out of the 21 sites have a >50% similarity in terms of quantitative benthic (surber) data based on management context, and there is great variability and lack of distinction amongst the management contexts (Fig, 15 A and B). When comparing similarity across ecoregions, there are three Western Bankenveld pairs, LBE and STR (78.6%), EKL and STK (71.67%), PDS and KRS (70.66%); and one Waterberg pair FKL and GRS which have a 71.01% similarity in terms of quantitative benthic (surber) data (Fig. 16 A).

The distance between the sites of these pairs are 38.31km, 5.7 km and, 29.74km, respectively. BKL (W-B) and PBS (W) (more than 200km apart) have a 69.39% similarity, and KWS (B) and LBW (W-B) (more than 160km apart) have a 67.22% similarity. The physical distance between some sites is short and suggests that the geographic context (possibly geology and climate), could be the reason for the similarity in terms of aquatic health, however, this is nullified by the fact that some sites are very far apart but they also have similar characteristics. The NMDS analysis shows some separation between Western Bankenveld and the Waterberg ecoregions, however, there is still homogeneity amongst them (as shown by the similarity between a Bushveld site (KWS) and a Western Bankenveld site (LBW), despite the vast geographic distance between them (Fig. 16 B). Cluster and NMDS analysis of catchment categories (Groot Marico, Crocodile West, Mokolo) using surber and SASS data did not show any similarity clustering of sites in the same catchment, therefore the diagrams have not been included in the results.

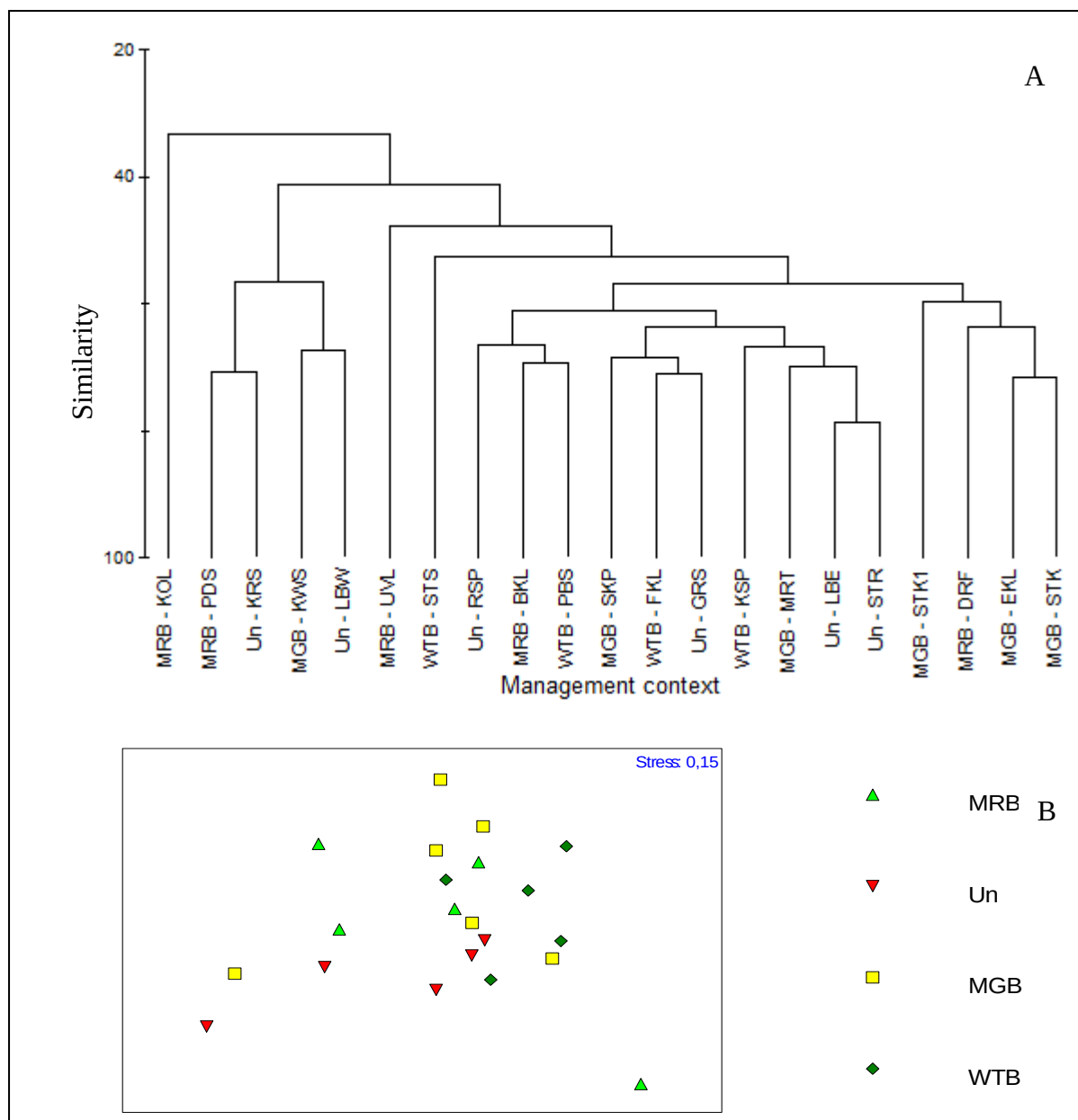


Figure 15. A) Cluster analysis of quantitative benthic (surber) data based on management context. B) Non-metric multidimensional scaling (NMDS) analysis of quantitative benthic (surber) data based on management context.

Note: Un- Unprotected, MGB– Magaliesberg Biosphere Reserve, MRB – Marico Biosphere Reserve, WTB - Waterberg Biosphere Reserve.

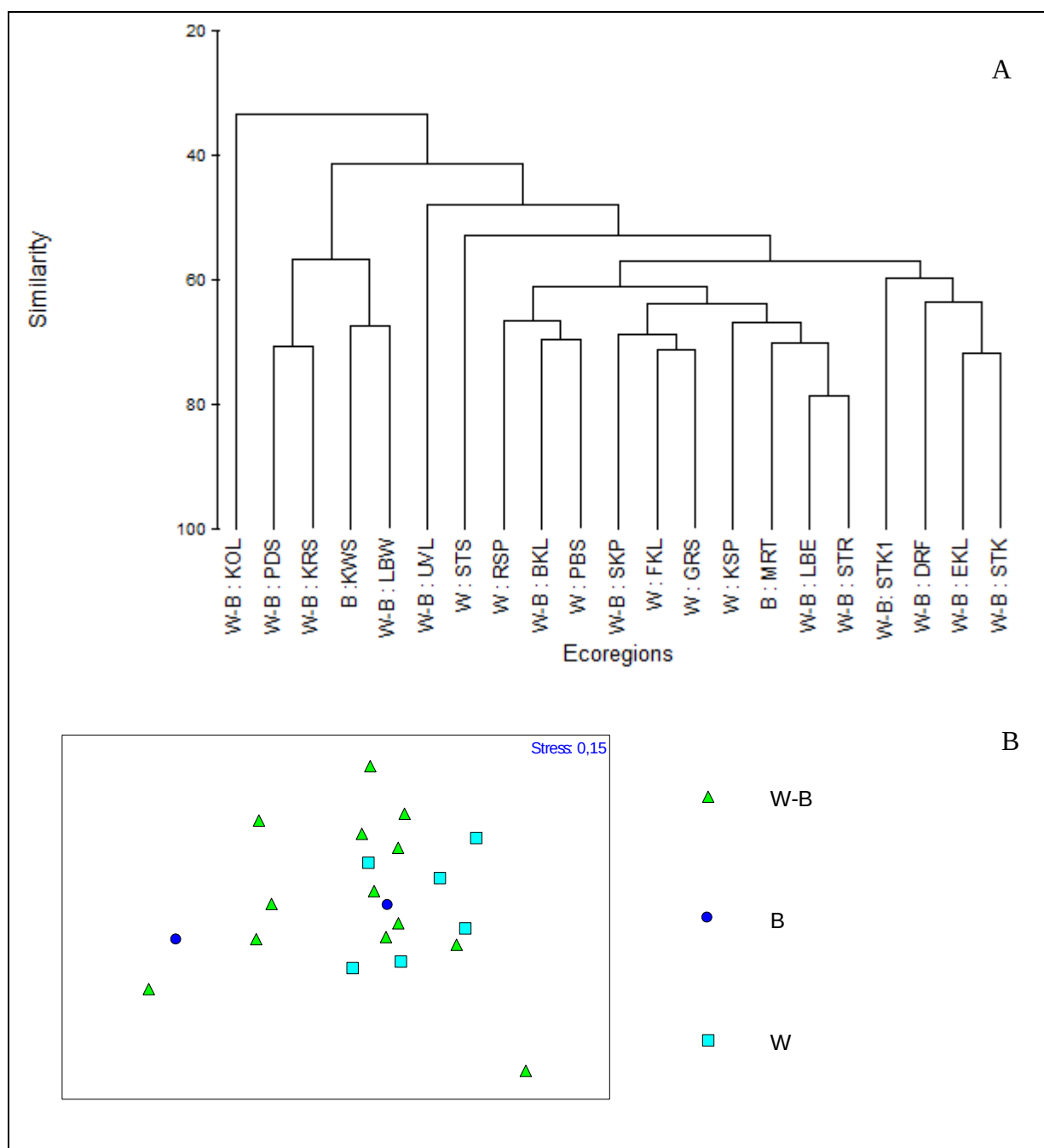


Figure 16. A) Cluster analysis of quantitative benthic (surber) data based on ecoregions. B) Non-metric multidimensional scaling (NMDS) analysis of quantitative benthic (surber) data based on ecoregions.

Note: W-B - Western Bankenveld, B – Bushveld, W - Waterberg.

3.4 Ecological structure indicators: Functional Feeding Groups (FFGs)

A Kruskal Wallis Test was run on the non-normally distributed predator and filter feeder ratios of the surber data. There was no significant difference ($p > 0.05$) across management contexts and ecoregions for: total predators (abundance); total non-filter feeders (abundance); total filter feeders (abundance); filter feeder families (diversity); surber predator/total abundance ratio and surber filter feeder / total abundance ratio. The mean ($\pm 1SE$) of total predators (abundance) was highest in Marico BR, 13.2 (9.52), and lowest in Unprotected, 3.67 (1.05). Total filter feeders (abundance) were highest in Unprotected 115 (66.33) and lowest in the Waterberg BR 3.25 (2.29) (Table 8).

Table 7. Mean ($\pm 1SE$) of functional feeding groups across management contexts and ecoregions.

Context	Total predators (abundance)	Total non-filter feeders (abundance)	Total filter feeders (abundance)	Filter feeder families	Surber predator/total abundance ratio	Surber filter feeder /total abundance ratio
Management context						
Marico BR	13.2 (9.52)	411.6 (136.27)	23 (9.04)	1.2 (0.37)	0.03 (0.02)	0.07 (0.03)
Unprotected	3.67 (1.05)	194.17 (44.83)	115 (66.33)	1.33 (0.21)	0.02(0.01)	0.26 (0.13)
Magaliesberg BR	8.33 (2.91)	199.67 (50.47)	30.17 (21.02)	1.33 (0.33)	0.04 (0.01)	0.15 (0.10)
Waterberg BR	12.25 (6.14)	141.25 (44.35)	3.25 (2.29)	1 (0.41)	0.09 (0.04)	0.02 (0.01)
Ecoregions						
Western bankenveld	8.92 (3.79)	303.77 (59.78)	62.69 (32.49)	1.23 (0.2)	0.03 (0.01)	0.15 (0.07)
Bushveld	7 (5)	123.5 (46.5)	81 (52)	1.5 (0.5)	0.03 (0.03)	0.39 (0.24)
Waterberg	9.5 (4.29)	131.67 (30.54)	3.67 (1.73)	1.17 (0.31)	0.08 (0.03)	0.03 (0.02)

Normally distributed functional feeding groups and their ratios were analysed using a one-way ANOVA. There was no statistically significant difference ($p > 0.05$) between total non-predators (abundance); predator families; non predator families; non-filter feeder families; kick-net predator family / total family ratio; surber predator family / total family ratio; kick-net filter feeder family / total family ratio and surber filter feeder family / total family ratio across management contexts and ecoregions.

3.5 Community functional trends along abiotic gradients

Abiotic variables and aquatic macroinvertebrate data were $\text{Log}_{10}+0.1$ transformed, so that relevant correlations between them can be determined. After the transformation, normally distributed abiotic data (phosphorous, ammonia, temperature, conductivity, and pH) were correlated with normally distributed aquatic macroinvertebrate data (SASS score, kick-net no. of taxa (abundance), ASPT, kick-net total families, surber total families, surber total abundance, surber non-predator abundance, surber predator families, surber non-predator families, surber non-filter feeder families, kick-net predator family/total family ratio, surber predator family / total family ratio, kick-net filter feeder family / total family ratio, surber filter feeder family / total family ratio).

There were several significant correlations ($p < 0.05$), namely between phosphorus and surber non-predator aquatic macroinvertebrate abundance, phosphorous and surber total abundance, conductivity and: surber non- predator families (diversity), surber non-filter feeder families (diversity) and surber total families (diversity) (Table 9). The pH readings had a significant correlation with surber predator families (diversity), surber non-predator families (diversity), surber non-filter feeder families (diversity) and surber total families (diversity) (Table 9).

Table 8. Pearson's correlation of abiotic variables and surber aquatic macroinvertebrate data (Marked correlations are significant at $p < 0, 05$ N=21).

Variable	Surber non-predator abundance	Surber predator families (diversity)	Surber non-predator families (diversity)	Surber non - filter feeder families (diversity)	Surber total families (diversity)	Surber total abundance
Phosphorus mg/L	0.61	NS	NS	NS	NS	0.61
Conductivity $\mu\text{S}/\text{cm}$	NS	NS	0.58	0.58	0.58	NS
pH	NS	0.57	0.57	0.61	0.64	NS

Note: Significant correlations are bold

There is a significant correlation between phosphorous and surber non-predator abundance, however, only 26% of the variation in surber non-predator abundance can be accounted for by the variation in phosphorous (Fig. 17 A). There is also a significant correlation between phosphorous and surber total abundance, but only 27% of the variation in surber total abundance can be accounted for by the variation in phosphorous (Fig. 17 B).

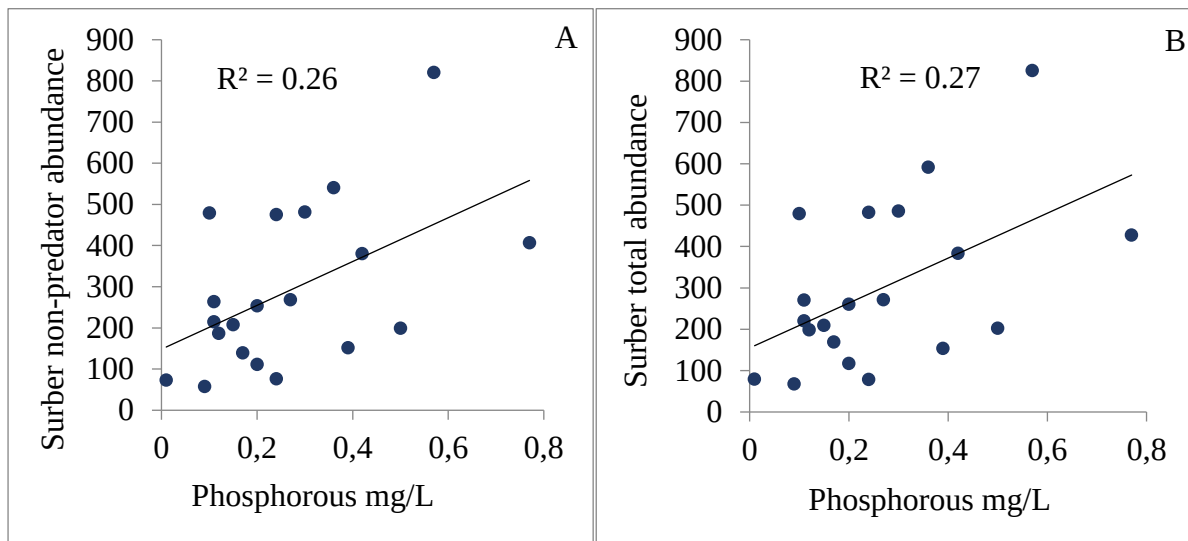


Figure 17. Scatter plots of phosphorous vs surber non-predator abundance (A), and phosphorous vs surber total abundance (B).

There is no statistically significant difference in pH across the geology types (one-way ANOVA; $p > 0.05$). Although surber total families and pH have a significant correlation, only 41% of the variation in surber total families can be accounted for by the variation in pH (Fig. 18).

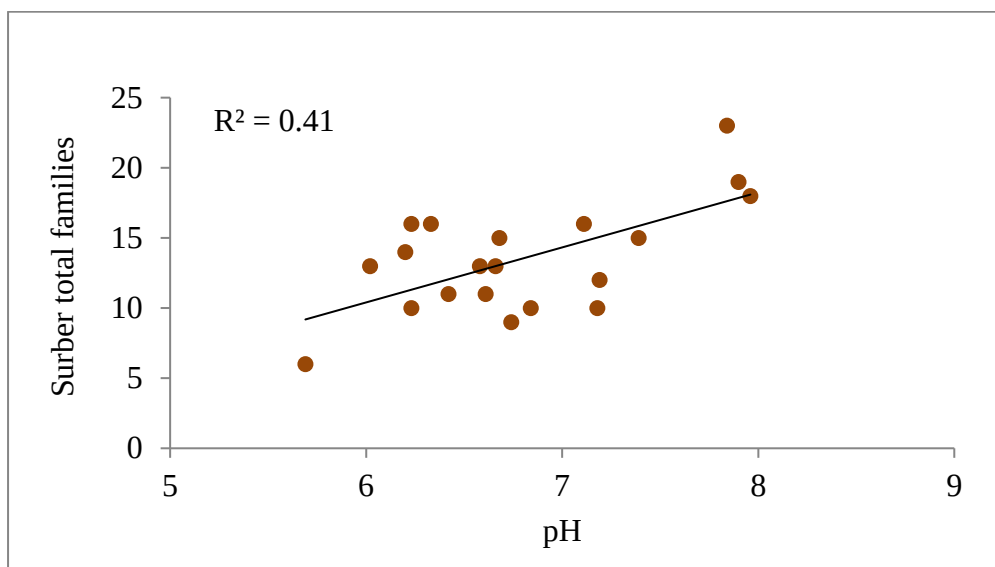


Figure 18. Surber total families and pH readings of different sites

The abiotic variables (discharge, dissolved oxygen and nitrate) and aquatic macroinvertebrate data (total predators, non-filter feeders, filter feeders, filter feeder families, surber predator/Tot. abundance ratio and Surber filter feeder /total abundance ratio) that were not normally distributed (after Log10+0.1 transformations) were correlated using the Spearman-rank correlation. The Spearman rank correlation, showed that there is a significant correlation ($p < 0.05$) between dissolved oxygen (%) and total filter feeders ($r_s = 0.49$), and 61% of the variation in total filter feeders (abundance) can be explained by the variation in dissolved oxygen (%) (Fig. 19).

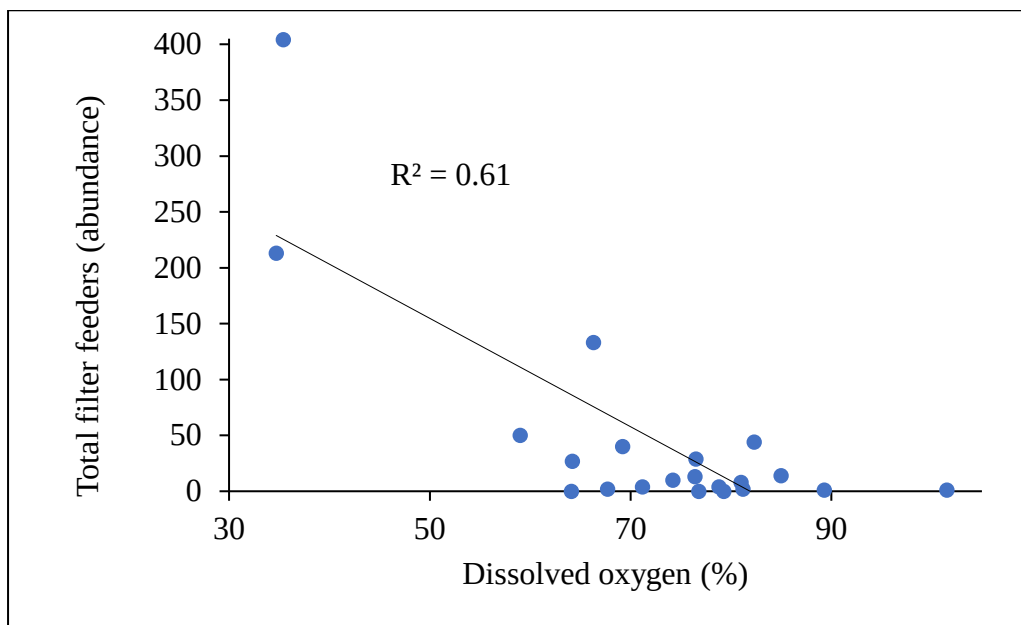


Figure 19. The relationship between total filter feeders (abundance) and dissolved oxygen (%).

Chapter 4: Discussion

This study aimed to assess whether South African savanna biosphere reserves (Magaliesberg, Marico and Waterberg) show signs of improved ecosystem integrity compared to adjacent, non-protected streams, and whether the reserves were measurably effective at managing aquatic ecosystems. The first objective was to investigate the physico-chemical and land-cover differences between sites within and beyond the biosphere reserves (and their effect on the structure of macroinvertebrates assemblages). The physico-chemical variables were largely homogenous across the management contexts and ecoregions, except for discharge and ammonia which were significantly different across the management contexts. The land-cover analysis indicated that there was no statistically significant difference in the dominant land-cover types (grassland, woodland/open bush, cultivated commercial fields and thicket /dense bush) across the management contexts. This overall lack of significance did not allow for an assessment of the effect of land-cover difference on the structure of macroinvertebrates assemblages.

The second and third objectives were to assess the differences between streams inside and outside the BRs in terms of river health (SASS5) and community structure (and trophic organisation) using FFG ratios. Although this study focused on only 2 FFGs, namely: predators and filter feeders (due to the limitation of lack of literature to accurately assign other FFGs), both the rapid bio-assessment of river health (SASS5) and FFGs suggest that the ecological health of headwater streams inside biosphere reserves is often, but not always, better than that of unprotected streams. This is also seen in that the proportion of sites with improved ecological condition is greater in BRs than in unprotected sites. Although evidence

for their effectiveness was not clear cut, these biosphere reserves do appear to maintain the ecosystem structure and function of the sampled headwater streams within them.

4.1 Abiotic variables

4.1.1 Physico-chemical variables

Most abiotic variables (nitrate, temperature, dissolved oxygen, conductivity and pH), were not significantly different across management context and ecoregions. There was a statistically significant difference between discharge and ammonia across different management contexts and none of the abiotic variables were different across ecoregions.

Discharge plays a major role in the processes and dynamics of headwater ecosystems as it directly affects the amount of Fine Particulate Organic Matter (FPOM) that is produced in headwater streams and exported to downstream (Cuffney and Wallace, 1989). Discharge affects the supply of energy sources (terrestrial and aquatic) like leaf litter from the riparian zone and algae, as well as the channel geometry (Sabo *et al.*, 2010). Discharge variation can cause drastic changes in leaf retention as higher discharge leads to less retentive efficiency to allochthonous inputs (Muotka and Laasonen, 2002). The variation in discharge and the frequency of intermittency are being modified by human activities and climate change all around the world and these changes affect the structure of river food webs and food chain length, depending on the flow variation, drainage area, ecosystem size, energy supply and flow variation of the particular system (Sabo *et al.*, 2010).

Unprotected sites had the lowest mean discharge; this could possibly be due to extensive water abstraction and channelization, given the accessibility and lack of formal protection for these streams. An example is the Hex River (Magaliesberg BR) which had originally been targeted as a sampling site, but was unusable because the river bed was dry. This was not due to the natural effect of the dry season, but rather due to water abstraction upstream of the accessible sampling point thus, there was no surface flow at the time of sampling.

4.1.2 Land-cover analyses

Land-cover within the 1km buffers that were created for the sites were variable; however, none of the dominant land-cover types (grassland, woodland, thicket/dense bush and cultivated commercial fields) were significantly different across the management contexts. However, mean cultivated commercial fields were (marginally) highest in Unprotected, followed by the Waterberg BR, which is the oldest savanna biosphere BR in South Africa. This is not surprising because farming has been part of the Waterberg area for decades (Boeyens *et al.*, 2009).

Grassland land-cover was most dominant in Magaliesberg BR, followed by Marico BR, Unprotected and Waterberg BR in order of diminishing land-cover. Cultivated commercial land-cover (fields/pivots/orchards) was most dominant in Waterberg BR followed by, Unprotected, Marico BR, and Magaliesberg BR. This suggests that Waterberg sites had a higher degree of agricultural intensification (as mentioned above) than the other two biospheres, although this was not reflected specifically in the physico-chemical variables.

4.2 SASS5 Rapid bio-assessment and Ecological Categories across management context

There was no statistically significant difference between the adjusted mean SASS scores and the adjusted Average Score Per Taxon (ASPT) of different management contexts. The mean SASS score was highest in the Waterberg BR, followed by Magaliesberg BR and Unprotected sites, the lowest score was in the Marico BR. The mean ASPT was highest in Waterberg BR, followed by Magaliesberg BR and Marico BR with the lowest being in the Unprotected context. The fact that there is no significant difference between the adjusted mean SASS scores and ASPT is an indication that the aquatic macroinvertebrate community structure and function is similar, irrespective of the management context across the sampled sites. There was no statistically significant difference between adjusted SASS score and adjusted ASPT of different ecoregions, denoting an overall homogeneity in river health.

While none of the Unprotected sites that were sampled had A/B (pristine to near pristine) category, some were only moderately altered (C category). Although most D/E/F category sites were Unprotected, these ecological categories were also found in the three BRs. The highest proportion of A/B sites were observed in the BRs, this suggests that all three biosphere reserves may be actively (or passively) preserving the natural and largely natural (A/B) class streams within them (especially the Magaliesberg BR), which cannot be said about the Unprotected sites.

When it comes to biosphere reserve zones, the core sites ranged from largely natural with a few modifications (B ecological category) to critically modified sites (E/F ecological category). These core sites are not necessarily ineffective as there are other factors at play,

like natural flow. For example, the sampled point in Kgaswane Nature Reserve (Magaliesberg BR) was closer to being a wetland than a stream (dense marginal vegetation and little flow) and this differing hydrology may have negatively impacted the SASS score. The buffer zone had an unmodified natural site (KSP – Upper Kaal Oog) as the only A category site in this study, which is in the Marico BR. The buffer zone also has the highest quantity of largely natural sites with few modifications (B ecological category). The transition zone had better categories compared to unprotected streams, this is an indication of the positive effect that BR management has; as transition zones are afforded the least protection compared to core and buffer zones. Core areas did not have better quality streams (ecologically) compared to buffer streams which suggests that zonation is not a consistent predictor of good stream health. This highlights that healthy streams are most often associated with zones recognised as having conservation value prior to the establishment of the BR (i.e. the core and buffer areas).

4.3 Geographic differences of macroinvertebrate assemblages

The cluster (similarities within) analyses of kick-net and surber data using both management context and eco-regions suggests that there is very little difference amongst the aquatic macroinvertebrate assemblages of these two spatial groupings. The NMDS analysis likewise shows no clear separation between the management contexts and ecoregions of both kick-net data and surber data. Kick-net and surber data showed that there is some variety in macroinvertebrate assemblage amongst the different sites. However, overall clear homogeneity was observed across the management contexts with minor separation in the

ordination space. Marico BR sites had good health despite the fact that it is a new biosphere reserve that had yet to be declared when the samples for this study were collected.

In terms of ecoregions, Western Bankenveld (13) had the most sites, followed by the Waterberg (6) and Bushveld (2). The analyses based on ecoregions may therefore not show a true representation of variation in physico-chemical data and aquatic macroinvertebrate communities within the different ecoregions due to the severely unequal distribution of sites in each ecoregion. Nonetheless, the results suggest that no sampled ecoregion within the greater savanna biome is obviously poorer in physico-chemical quality or community structure than the others. Therefore, comparisons between management contexts within the savanna biome ought not to be influenced by aquatic ecoregions.

4.4 Ecological structure indicators: Functional Feeding Groups (FFGs)

Functional Feeding Groups (FFGs) were also analysed as they are potential ecological structure indicators (Boyero and Bailey, 2001; Armon and Hänninen, 2015). Tests for variation in FFG (predator and filter feeder) ratios across management contexts and ecoregions did not show any significant differences. However, total predator abundance was highest in the Marico BR and lowest in Unprotected. This suggests that the Marico BR had the highest prey richness and overall trophic diversity, while unprotected sites had the lowest prey richness and overall trophic diversity; as predator-prey ratios of community data have shown that predator richness is proportional to prey richness (Warren and Gaston, 1992). Predators give an indication of the top-down forcing of a system (Estes et al., 2011), therefore, based on the prey niches phenomenon, because the Marico BR had the most predator niches, this suggests that it also had the most prey types (Warren and Gaston, 1992).

Total filter feeders (abundance) were highest in Unprotected and lowest in the Waterberg BR. Filter feeders indicate the quantity and quality of suspended fine particulate organic matter (FPOM), as they feed on it (Wallace and Webster, 1996). This suggests that the most FPOM was in Unprotected and the least in the Waterberg BR, as there is a significant positive correlation between filter feeders and the concentration of FPOM (Whiles and Dodds, 2002). The increased FPOM in unprotected sites might be as a result of added runoff from human activities like agriculture (the highest cultivated commercial fields mean was in the Unprotected context, Chapter 4.2), which might make them more vulnerable to pollution than biosphere streams.

4.5 Community functional trends along abiotic gradients

Correlations between abiotic variables and aquatic macroinvertebrate data were done in order to analyse the aquatic macroinvertebrate community functional trends along abiotic gradients. There was a significant correlation between phosphorous and total non-predators and also between phosphorous and surber total abundance. Phosphorous promotes primary production which leads to higher live biomass; this then results in more dead and decomposing biomass which leads to more FPOM (Bayley *et al.*, 1985; Benstead *et al.*, 2009; Halvorson *et al.*, 2015). The FPOM serves as a food source for filter feeders, resulting in more filter feeders (due to the abundance of food) and ultimately more predators in the ecosystem. This is in agreement with the hypothesis that there is a positive correlation between predators and non-predator invertebrates (Hawkins and Sedell, 1981); suggesting that an increased filter feeder abundance will result in an increase in predator abundance.

There was a significant correlation (negative relationship) between dissolved oxygen and total filter feeders in this study. This negative relationship may exist because filter feeders eat FPOM, which when abundant (and depending on the content e.g. detritus) decreases the amount of dissolved oxygen in the system. An increased level of organic matter can negatively affect water quality as it increases the biological oxygen demand (BOD) through bacterial decomposition and therefore decreases and at times depletes the dissolved oxygen in the system (Whiles and Dodds, 2002).

There were also positive relationships between pH and predator families, non-predator families, non-filter feeder families and surber total families. This indicates that pH has an effect on the distribution of macroinvertebrates as it affects their physiology (and mortality) and speed of leaf litter composition (food source) (Sutcliffe and Carrick, 1973). Most of the sites were moderately acidic (pH 5.8-7.2) according to the scale of Simpson *et al.* (1985). Although there was no statistically significant difference in pH across the geology types, pH has significant correlations with a few FFG counts, and surber total families (diversity). This suggests that pH plays a major role in the structure of savanna macroinvertebrate communities. It is very likely that the atmospheric deposition of acid through acid rain plays a major role in the pH readings of the Magaliesberg sites (Kgabi *et al.*, 2006). This is a concern, as even if the management of the Magaliesberg and Marico biosphere reserves puts in measures to protect and sustain the ecosystems of the streams, it will not alleviate the acidification of the streams, since the sources of acid deposition are outside the biospheres (e.g. platinum mines north of the Magaliesberg) (Ololade *et al.*, 2008).

There was also a positive relationship between nitrate and cultivated commercial fields. This suggests that the nitrates that are found in streams are mostly from the cultivated fields around the streams. The nitrates get washed into the streams through seepage, floods and other natural processes that take place. There is therefore a traceable effect that agricultural activities have on aquatic health, however, the recorded nitrate levels are way below the recommended 5 mg/L threshold (DWAF, 1996) and are therefore unlikely to be a major driver of ecosystem health in this study.

Conclusion

This study suggests that South African savanna biosphere reserves do not significantly enhance aquatic ecosystem integrity compared to unprotected rural streams in the savanna biome; however, they do make a difference in preserving key healthy rivers within the region. The first potential reason for the lack of major differences between biospheres reserves and adjacent unprotected areas is that both are located in rural areas (with minimum urbanization). This is beneficial in terms of the limitation or lack of certain types of pollution such as urban runoff and sewage spillage from wastewater treatment works. The rural context places the protected and unprotected streams on the same spectrum of likely impacts because the surroundings, activities and characteristics of the streams are similar. Although the sampled sites are predominantly located in rural land, some have been affected by channel modification, dams and weirs mostly for agricultural activities. These sites, however, did not have observed compromised invertebrate assemblages compared to those that did not have channel modifications (with the exception of the completely de-watered Hex River).

This trend can be seen in that the sampled streams, regardless of being in a biosphere reserve or not, seem to be affected mainly by the fertilizers from surrounding agricultural activities. Although there was no formal restriction on land use for the unprotected streams, they were not extensively polluted. Unprotected rural streams that were sampled were in relatively average health (B to D ecological categories) apart from Renostepoort (RSP), which was the only critically modified stream (E/F). This stream was not in a good state compared to the other sampled streams, however there was no obvious source of land-cover impact visible where the sampling was done. It is, however, possible that RSP dried up during a severe drought in 2015/2016, and that the relatively degraded state of this stream during sampling was a result of the unobserved historic effects of this climatic event. The 2015/2016 El Niño (a climatic event whereby the temperature of the surface waters of the tropical Pacific Ocean rises) was one of the most severe El Niño climate cycles on record (Siderius *et al.*, 2018). Therefore with it, South Africa (amongst other Southern African countries) experienced severe droughts (Siderius *et al.*, 2018). Limpopo (including the Waterberg) was one of the worst hit provinces in terms of drought (DWS, 2015), which could explain why Renostepoort (RSP) was the only critically modified stream (E/F).

The second potential reason why savanna biosphere reserves do not seem to significantly enhance aquatic ecosystem integrity compared to unprotected rural streams is that they do not have clear plans and goals in place that are being implemented by active stakeholders (except for the Magaliesberg BR). Having these in place can be used as a tool to monitor the progress and improvements or lack thereof, of the protection and sustainable use of the streams in the BRs. The Waterberg BR does not seem to have generated a measurable effect on rural grassland-savanna catchments that can distinguish its rivers from adjacent unprotected catchments, despite the fact that it was established 10 years ago. Although the Magaliesberg

BR has an active and enthusiastic managing board, we sampled too early in the life of the reserve (2 years old) for any river conservation initiatives to have been launched yet. The Marico BR was established shortly after sampling was done, therefore, just like the Magaliesberg BR, it has a great, but as yet unrealised, potential to plan and execute sustainable goals that will effectively protect headwater streams within them.

The trophic integrity and structure of macroinvertebrate assemblages inside and outside the biosphere reserves seem to be very similar. Despite increased agricultural intensification observed in the Waterberg BR, the nutrient loads and physico-chemical readings of the streams are way below the thresholds set by the South African Water Quality Guidelines for agricultural use: irrigation (DWAF, 1996). Overall, the land cover in these sites does not have a clear traceable effect on the physico-chemical variables and overall aquatic ecology, within the scope of this study.

SASS5 score results were not comparable with FFG ratios, mainly because there were no significant correlations between them. FFGs were also not particularly useful for understanding land-use impact on savanna stream ecosystems. This was because the FFGs and their ratios did not have any significant correlations with land-cover, therefore, in this context; FFGs were not a useful tool for studying the impact of land-use management on savanna head-water streams. This might be due to the problematic taxonomic uncertainty of assigning FFGs to South African families, given the limitation in available literature. In addition, the fact that only two FFGs (predators and filter feeders) were analysed, is also a limitation. More studies should be done to establish a South Africa-based taxon list of FFGs, so that they may be more effectively used as proxies for aquatic health studies.

Correlative data sometimes provides unexpected results; therefore, strong gradients can be used to make inferences about factors that affect the distribution of the particular pattern (Hawkins and Sedell, 1981). Although there were a lot of similarities between the sites; phosphorous, pH and discharge seem to be the most influential drivers of macroinvertebrate assemblages. These savannah BRs have a potential vulnerability to acid deposition and industrial intensification in the areas adjacent to them (like Rustenburg), which makes changes in river pH a topic worth pursuing in future research.

Savanna biosphere reserves are in relatively good health (excluding a few D category sites), though this cannot be attributed solely to their status as biosphere reserves. It is important to focus the attention of managers on the A/B class sites, as they should be given more protection to ensure that they remain un-impacted (or minimally impacted) by human activities. This is especially true in the new Marico BR, which has the potential to make an impact on the management of these headwater streams. Although South African savanna biosphere reserve zones do not uniformly protect the intended ecological health of streams from a zonation perspective (comparing river health from core to buffer to transition zones), they are a potentially useful tool in assessing the effectiveness of multi-tier management regimes on the aquatic health of headwater streams. In conclusion, apart from a few outliers, headwater streams in biosphere reserves are in good health relative to adjacent unprotected streams, and the continued existence of this management structure represents a net benefit for headwater stream integrity.

Acknowledgements

I would like to extend my appreciation to my supervisor, Dr Darragh Woodford for his patience, assistance and guidance. Thank you to the National Research Foundation (NRF), for funding this project. Thank you to the Endangered Wildlife Trust (special thanks to Jean-Pierre Le Roux), the North West Department of Rural Environment and Agricultural Development, and the Limpopo Department of Economic Development, Environment and Tourism for sampling permits and field assistance. Thank you to my fellow students: Tsedzuluso Mundalamo, Courtney Gardiner, Alexandra Carroll and Alexandra Evans, for assisting me with fieldwork. Thank you to Jonathan Levin and Gina Walsh for the extra mile assistance. A huge appreciation to my Lord Jesus Christ, without whom I would not have been able to complete this degree (I can do all things through Christ who strengthens me, Phil 4:13), and to my family (especially my mother Mrs M.J Dlamini) for being understanding and supportive.

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Appendices

Appendix A. Site names, codes, locality, context, eco-region, catchments, geology, class and zone of the sampling sites.

Name	Code	Locality	Context	Ecoregion	Catchment	Geology	Class	Zone
Upper Kaal Oog	KSP	-24.38399, 27.8848	MRB	Western Bankenveld	Groot Marico	Transvaal	B	Buffer
Bokkraal	BKL	-25.804019, 26.448579	MRB	Western Bankenveld	Groot Marico	Transvaal	B	Buffer
Upper Draai	DRF	-25.7409, 26.3481	MRB	Western Bankenveld	Groot Marico	Transvaal	D	Core
Uitvlugtspruit	UVL	-25.6668, 26.53992	MRB	Western Bankenveld	Groot Marico	Transvaal	C	Transition
Polkadraaispruit	PDS	-25.6664, 26.6166	MRB	Western Bankenveld	Groot Marico	Transvaal	C	Transition
Koster	KRS	-25.768323, 26.892510	Unprotected	Western Bankenveld	Croc West	Transvaal	D	Unprotected
Waterkloofspruit	KWS	-25.73488, 27.44498	MGB	Bushveld	Croc West	Transvaal	B	core
Eesterkloof	EKL	-25.810545, 27.501103	MGB	Western Bankenveld	Croc West	Rustenburg	B	Buffer
Kromrivier	STK	-25.82082, 27.44498	MGB	Western Bankenveld	Croc West	Rustenburg	B	Buffer
Maretlwane	MRT	-25.7979, 27.57718	MGB	Bushveld	Croc West	Rustenburg	B	Core
Sterkstroom WGV	STK1	-25.833038, 27.388694	MGB	Western Bankenveld	Croc West	Rustenburg	C	Buffer
Skeerpoort	SKP	-25.843498, 27.783818	MGB	Western Bankenveld	Croc West	Transvaal	B	Core
Grootfontein-KOL	KOL	-25.783931, 26.378600	WTB	Waterberg	Mokolo	Waterberg	A	Buffer
Renosterpoort	RSP	-24.50794, 27.86552	Unprotected	Waterberg	Mokolo	Waterberg	E/F	Unprotected
Loubad East	LBE	-24.600180, 28.216971	Unprotected	Western Bankenveld	Mokolo	Waterberg	C	Unprotected
Loubad West	LBW	-24.605097, 28.204697	Unprotected	Western Bankenveld	Mokolo	Waterberg	D	Unprotected
Sterk River	STR	-24.464276, 28.566783	Unprotected	Western Bankenveld	Mogalakwena	Waterberg	C	Unprotected
Platbos River	PBS	-24.244337, 27.789143	WTB	Waterberg	Mokolo	Waterberg	D	Core
Sterkstroom	STS	-24.36906, 27.80244	WTB	Waterberg	Mokolo	Waterberg	C	Core
Frikkiesloop	FKL	-24.25678, 27.96843	WTB	Waterberg	Mokolo	Waterberg	B	Buffer
Grootfonteinspruit	GRS	-24.53200, 27.83704	Unprotected	Waterberg	Mokolo	Waterberg	C	Unprotected

Appendix B. Macroinvertebrates recorded on the SASS5 sheets of the different sites.

Site code: KOL					
Date: 19/09/17					
Taxon	QV	S	Veg	GSM	TOT
ANNELIDA					
Oligochaeta (Earthworms)	1	1	1	1	A
CRUSTACEA					
Potamonautidae* (Crabs)	3	1			1
PLECOPTERA (Stoneflies)					
Perlidae	12				
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4		A	A	B
Leptophlebiidae (Prongills)	9	A			A
Tricorythidae (Stout Crawlers)	9	A			A
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10			A	A
HEMIPTERA (Bugs)					
Notonectidae* (Backswimmers)	3		1		1
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4	1	1		A
Philopotamidae	10	A			A
DIPTERA (Flies)					
Athericidae (Snipe flies)	10			1	1
Simuliidae (Blackflies)	5		1		1
SASS Score					80
No. of Taxa					12
ASPT					6,7

Site code: BKL					
Date: 19/09/17					
Taxon	QV	S	Veg	GSM	TOT
TURBELLARIA (Flatworms)	3	A			A
CRUSTACEA					
Potamonautidae* (Crabs)	3			1	1
PLECOPTERA (Stoneflies)					
Perlidae	12	A	A	1	A
EPHEMEROPTERA (Mayflies)					
Baetidae 2 sp	6		A	A	B
Caenidae (Squaregills/Cainflies)	6	1			1
Leptophlebiidae (Prongills)	9	1		A	A
Tricorythidae (Stout Crawlers)	9	A		B	B
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10		1		1
Coenagrionidae (Sprites and blues)	4		A		A
Aeshnidae (Hawkers & Emperors)	8	A		A	B
Gomphidae (Clubtails)	6	A	A		A
HEMIPTERA (Bugs)					
Gerridae* (Pond skaters/Water striders)	5		1		1
Veliidae/M...veliidae* (Ripple bugs)	5		1		1
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4	1	1	A	A
Philopotamidae	10		A	A	A
COLEOPTERA (Beetles)					
Gyrinidae* (Whirligig beetles)	5	1	A		B
DIPTERA (Flies)					
Athericidae (Snipe flies)	10	1			1
Chironomidae (Midges)	2	A	A	A	B
Muscidae (House flies, Stable flies)	1			1	1
Simuliidae (Blackflies)	5	1	A		A
Tabanidae (Horse flies)	5		A	A	A
SASS Score					128
No. of Taxa					21
ASPT					6,1

Site code: DRF					
Date: 20/09/2017					
Taxon	QV	S	Veg	GSM	TOT
TURBELLARIA (Flatworms)	3	A			A
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	A	A	A	B
Caenidae (Squaregills/Cainflies)	6	A	B	A	B
Tricorythidae (Stout Crawlers)	9	A	B	A	B
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4		A	A	A
Aeshnidae (Hawkers & Emperors)	8	A			A
Libellulidae (Darters/Skimmers)	4		A	1	A
HEMIPTERA (Bugs)					
Belostomatidae* (Giant water bugs)	3			A	A
Nepidae* (Water scorpions)	3		1		1
Notonectidae* (Backswimmers)	3			A	A
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4	A			A
Philopotamidae	10	A			A
COLEOPTERA (Beetles)					
Gyrinidae* (Whirligig beetles)	5		1	B	B
DIPTERA (Flies)					
Chironomidae (Midges)	2		A	A	B
Muscidae (House flies, Stable flies)	1			A	A
Psychodidae (Moth flies)	1		1		1
Simuliidae (Blackflies)	5	A			A
Tabanidae (Horse flies)	5			1	1
Tipulidae (Crane flies)	5	1			1
SASS Score					85
No. of Taxa					19
ASPT					4,47

Site code: UVL					
Date: 21/09/2017					
Taxon	QV	S	Veg	GSM	TOT
CRUSTACEA					
Potamonautidae* (Crabs)	3			A	A
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	A		A	B
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10	A			A
Coenagrionidae (Sprites and blues)	4		A		A
Aeshnidae (Hawkers & Emperors)	8	1			1
Gomphidae (Clubtails)	6			A	A
TRICHOPTERA (Caddisflies)					
Cased caddis:					
Leptoceridae	6		1		1
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5		1		1
Gyrinidae* (Whirligig beetles)	5	A	A	A	B
DIPTERA (Flies)					
Chironomidae (Midges)	2		A	A	B
Culicidae* (Mosquitoes)	1		1	1	A
Dixidae* (Dixid midge)	10		1		1
Simuliidae (Blackflies)	5	A	A		B
SASS Score					69
No. of Taxa					13
ASPT					5,31

Site code: PDS					
Date: 21/09/2017					
Taxon	QV	S	Veg	GSM	TOT
ANNELIDA					
CRUSTACEA					
Potamonautidae* (Crabs)	3	A	A	A	B
EPHEMEROPTERA (Mayflies)					
Baetidae 1 sp	4	A	A		B
Caenidae (Squaregills/Cainflies)	6		1		1
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10		A		A
Coenagrionidae (Sprites and blues)	4		1	A	A
Gomphidae (Clubtails)	6	A		1	A
HEMIPTERA (Bugs)					
Hydrometridae* (Water measurers)	6		1		1
Veliidae/M...veliidae* (Ripple bugs)	5			A	A
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4	B		A	B
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5		A		A
Elmidae/Dryopidae* (Riffle beetles)	8	1			1
Gyrinidae* (Whirligig beetles)	5	A	B	A	B
DIPTERA (Flies)					
Dixidae* (Dixid midge)	10			1	1
Simuliidae (Blackflies)	5	A	A	A	B
Tabanidae (Horse flies)	5	1	1		A
Tipulidae (Crane flies)	5		1		1
SASS Score	#REF!				81
No. of Taxa	10				15
ASPT	#REF!				5,4

Site code: KRS					
Date: 22/09/2017					
Taxon	QV	S	Veg	GSM	TOT
TURBELLARIA (Flatworms)	3	1	1		A
ANNELIDA					
Oligochaeta (Earthworms)	1		1	A	A
CRUSTACEA					
Potamonautidae* (Crabs)	3		1		1
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4		A	B	B
Baetidae > 2 sp	12	B			B
Caenidae (Squaregills/Cainflies)	6	A	A	A	B
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4		A		A
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4	A		A	B
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5			A	A
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5	A	A	1	A
Chironomidae (Midges)	2	1		A	A
Dixidae* (Dixid midge)	10			1	1
Simuliidae (Blackflies)	5	B	B	B	B
GASTROPODA (Snails)					
Ancylidae (Limpets)	6	1	1		A
SASS Score					66
No. of Taxa					13
ASPT					5,1

Site code: KWS					
Date: 26/09/2017					
Taxon	QV	S	Veg	GSM	TOT
TURBELLARIA (Flatworms)	3			1	1
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4			1	1
Leptophlebiidae (Prongills)	9			1	1
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4	1	A		A
Aeshnidae (Hawkers & Emperors)	8		1		1
Libellulidae (Darters/Skimmers)	4			A	A
HEMIPTERA (Bugs)					
Gerridae* (Pond skaters/Water striders)	5		1		1
MEGALOPTERA (Fishflies, Dobsonflies & Alderflies)					
Corydalidae (Fishflies & Dobsonflies)	8			1	1
COLEOPTERA (Beetles)					
Gyrinidae* (Whirligig beetles)	5		1	A	A
DIPTERA (Flies)					
Simuliidae (Blackflies)	5	C		A	C
SASS Score					55
No. of Taxa					10
ASPT					5,5

Site: EKL					
Date: 27/09/2017					
Taxon	QV	S	Veg	GSM	TOT
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4			A	
Baetidae 2 sp	6	B			
Baetidae > 2 sp	12		B		B
Caenidae (Squaregills/Cainflies)	6	A	1		A
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4		1		1
Gomphidae (Clubtails)	6	1			1
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4	A			A
COLEOPTERA (Beetles)					
Gyrinidae* (Whirligig beetles)	5	1		A	A
DIPTERA (Flies)					
Chironomidae (Midges)	2	A		A	B
Dixidae* (Dixid midge)	10		1		1
Simuliidae (Blackflies)	5		1		1
SASS Score					54
No. of Taxa					9
ASPT					6,0

Site code: STK					
Date: 27/09/2017					
Taxon	QV	S	Veg	GSM	TOT
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4			A	A
Baetidae 2 sp	6	A	A		B
Caenidae (Squaregills/Cainflies)	6	1	A	1	A
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4		1	1	A
Aeshnidae (Hawkers & Emperors)	8	1			1
Gomphidae (Clubtails)	6	B		A	B
Libellulidae (Darters/Skimmers)	4	A	A	1	B
HEMIPTERA (Bugs)					
Gerridae* (Pond skaters/Water striders)	5		1	A	A
Veliidae/M...veliidae* (Ripple bugs)	5	1			1
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4		1		1
Hydropsychidae > 2 sp	12	A			A
Philopotamidae	10	A			A
Cased caddis:					
Leptoceridae	6		1		1
COLEOPTERA (Beetles)					
Gyrinidae* (Whirligig beetles)	5	1	A		A
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5			1	1
Dixidae* (Dixid midge)	10			A	A
Simuliidae (Blackflies)	5		A		A
SASS Score					105
No. of Taxa					17
ASPT					6,2

Site code: MRT					
Date: 28/09/17					
Taxon	QV	S	Veg	GSM	TOT
PLECOPTERA (Stoneflies)					
Perlidae	12	A			A
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	A	A		B
Baetidae 2 sp	6			A	A
Caenidae (Squaregills/Cainflies)	6		1		1
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10		1		1
Coenagrionidae (Sprites and blues)	4		A		A
Aeshnidae (Hawkers & Emperors)	8	1			1
Gomphidae (Clubtails)	6	A	1		A
Libellulidae (Darters/Skimmers)	4	1	1		A
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4	1			1
Philopotamidae	10	1			1
Cased caddis:					
Leptoceridae	6	A	A		A
COLEOPTERA (Beetles)					
Gyrinidae* (Whirligig beetles)	5	A	1	B	B
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5		1		1
Chironomidae (Midges)	2	1	1	A	A
Culicidae* (Mosquitoes)	1		A		A
Simuliidae (Blackflies)	5	A			A
Tabanidae (Horse flies)	5	1			1
SASS Score					103
No. of Taxa					18
ASPT					5,7

Site code: STK1					
Date: 28/09/17					
Taxon	QV	S	Veg	GSM	TOT
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	1			1
Caenidae (Squaregills/Cainflies)	6		1	A	A
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4		A		A
Gomphidae (Clubtails)	6	1	A	1	A
HEMIPTERA (Bugs)					
Notonectidae* (Backswimmers)	3		A		A
Veliidae/M...veliidae* (Ripple bugs)	5	1	A		A
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4			1	1
Cased caddis:					
Hydroptilidae	6			1	1
Leptoceridae	6		A		A
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5		A		A
Gyrinidae* (Whirligig beetles)	5		B		B
Helodidae (Marsh beetles)	12	1			1
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5	A			A
Dixidae* (Dixid midge)	10	A			A
Simuliidae (Blackflies)	5	1			1
SASS Score					86
No. of Taxa					15
ASPT					5,7

Site code: SKP					
Date: 29/09/17					
Taxon	QV	S	Veg	GSM	TOT
TURBELLARIA (Flatworms)	3	1	1	A	A
ANNELIDA					
Oligochaeta (Earthworms)	1			1	1
CRUSTACEA					
Potamonautidae* (Crabs)	3	A	1	1	A
PLECOPTERA (Stoneflies)					
Perlidae	12			1	1
EPHEMEROPTERA (Mayflies)					
Baetidae 2 sp	6	B		B	B
Baetidae > 2 sp	12		A		A
Caenidae (Squagills/Cainflies)	6	1	A		A
Leptophlebiidae (Prongills)	9	A	1	A	A
Oligoneuridae (Brushlegged mayflies)	15				
Tricorythidae (Stout Crawlers)	9	A	1	A	A
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10		1	1	A
Coenagrionidae (Sprites and blues)	4		A		A
Gomphidae (Clubtails)	6			1	1
HEMIPTERA (Bugs)					
Gerridae* (Pond skaters/Water striders)	5		1		1
Veliidae/M...veliidae* (Ripple bugs)	5		A		A
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4	A		A	A
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5		A	A	A
Gyrinidae* (Whirligig beetles)	5	1		A	A
DIPTERA (Flies)					
Athericidae (Snipe flies)	10	1		1	A
Ceratopogonidae (Biting midges)	5	1		A	A
Chironomidae (Midges)	2			1	1
Dixidae* (Dixid midge)	10	1	1		A
Muscidae (House flies, Stable flies)	1		1		1
Simuliidae (Blackflies)	5	A	A	A	B
Tabanidae (Horse flies)	5			A	A
Tipulidae (Crane flies)	5		1	1	A
SASS Score					163
No. of Taxa					26
ASPT					6,3

Site code: KSP					
Date: 17/10/17					
Taxon	QV	S	Veg	GSM	TOT
CRUSTACEA					
Atyidae (Freshwater Shrimps)	8				A
PLECOPTERA (Stoneflies)					
Perlidae	12	A		1	A
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	A		A	A
Caenidae (Squaregills/Cainflies)	6	1		1	A
Leptophlebiidae (Prongills)	9	1	1	1	A
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10		1		1
Gomphidae (Clubtails)	6	A			A
HEMIPTERA (Bugs)					
Gerridae* (Pond skaters/Water striders)	5		1		1
Veliidae/M...veliidae* (Ripple bugs)	5		A		A
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4			A	A
Philopotamidae	10	1			1
Cased caddis:					
Leptoceridae	6		1		1
DIPTERA (Flies)					
Chironomidae (Midges)	2		A	A	B
Simuliidae (Blackflies)	5	1	A		A
SASS Score					92
No. of Taxa					14
ASPT					6,6

Site code: RSP					
Date: 17/10/17					
Taxon	QV	S	Veg	GSM	TOT
TURBELLARIA (Flatworms)	3	1			1
CRUSTACEA					
Potamonautidae* (Crabs)	3		1		1
Atyidae (Freshwater Shrimps)	8		A	1	A
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	A	1	A	A
Caenidae (Squaregills/Cainflies)	6	1	1	A	A
Leptophlebiidae (Prongills)	9	A		1	A
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10			1	1
Coenagrionidae (Sprites and blues)	4		A		A
Gomphidae (Clubtails)	6		1	1	A
HEMIPTERA (Bugs)					
Veliidae/M...veliidae* (Ripple bugs)	5	1			1
TRICHOPTERA (Caddisflies)					
Cased caddis:					
Leptoceridae	6		1		1
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5			1	1
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5		A		A
Chironomidae (Midges)	2		A	A	B
Culicidae* (Mosquitoes)	1			1	1
SASS Score					77
No. of Taxa					15
ASPT					5,1

Site code: LBE					
Date: 18/10/17					
Taxon	QV	S	Veg	GSM	TOT
CRUSTACEA					
Atyidae (Freshwater Shrimps)	8	1	B	A	B
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	A			A
Baetidae 2 sp	6		B	A	B
Caenidae (Squaregills/Cainflies)	6	1		A	A
Leptophlebiidae (Prongills)	9	A		B	B
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10			1	1
Coenagrionidae (Sprites and blues)	4			A	A
Gomphidae (Clubtails)	6			1	1
HEMIPTERA (Bugs)					
Veliidae/M...veliidae* (Ripple bugs)	5		A	A	B
TRICHOPTERA (Caddisflies)					
Philopotamidae	10			1	1
Cased caddis:					
Hydroptilidae	6	A		1	A
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5	1		A	A
Gyrinidae* (Whirligig beetles)	5		A	1	A
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5	A			A
Chironomidae (Midges)	2		1	A	A
Culicidae* (Mosquitoes)	1			1	1
Dixidae* (Dixid midge)	10			1	1
Psychodidae (Moth flies)	1		1		1
Simuliidae (Blackflies)	5	1	A	A	B
Tabanidae (Horse flies)	5			1	1
SASS Score					113
No. of Taxa					20
ASPT					5,7

Site code: LBW					
Date: 19/10/17					
Taxon	QV	S	Veg	GSM	TOT
ANNELIDA					
Oligochaeta (Earthworms)	1	1		A	A
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4			1	1
Caenidae (Squaregills/Cainflies)	6	A	1	A	B
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4	1	A	A	B
Gomphidae (Clubtails)	6	1	1		A
Libellulidae (Darters/Skimmers)	4		A	A	A
HEMIPTERA (Bugs)					
Gerridae* (Pond skaters/Water striders)	5		1		1
Naucoridae* (Creeping water bugs)	7		A		A
Veliidae/M...veliidae* (Ripple bugs)	5		1	1	A
TRICHOPTERA (Caddisflies)					
Philopotamidae	10	A	A	A	B
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5		1	A	A
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5		1	1	A
Chironomidae (Midges)	2	A	A	A	B
Culicidae* (Mosquitoes)	1	A		A	A
Simuliidae (Blackflies)	5	B	B	A	B
SASS Score					70
No. of Taxa					15
ASPT					4,7

Site code: STR					
Date: 19/10/17					
Taxon	QV	S	Veg	GSM	TOT
TURBELLARIA (Flatworms)	3			1	1
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	A	1		A
Baetidae 2 sp	6			A	A
Caenidae (Squaregills/Cainflies)	6	A	A	1	A
Leptophlebiidae (Prongills)	9	A		A	A
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10	A	A	A	B
Coenagrionidae (Sprites and blues)	4		A		A
Libellulidae (Darters/Skimmers)	4		1		1
HEMIPTERA (Bugs)					
Naucoridae* (Creeping water bugs)	7		1		1
Veliidae/M...veliidae* (Ripple bugs)	5		A		A
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4	1			1
Philopotamidae	10	A	A	1	A
Cased caddis:					
Hydroptilidae	6			1	1
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5	1		1	A
Elmidae/Dryopidae* (Riffle beetles)	8	1		A	A
Gyrinidae* (Whirligig beetles)	5		A		A
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5		1	A	A
Chironomidae (Midges)	2	A	A	A	B
Culicidae* (Mosquitoes)	1	1			1
Simuliidae (Blackflies)	5	A	A	A	B
Tabanidae (Horse flies)	5			1	1
SASS Score					110
No. of Taxa					20
ASPT					5,5

Site code: PBS					
Date: 24/10/17					
Taxon	QV	S	Veg	GSM	TOT
CRUSTACEA					
Potamonautidae* (Crabs)	3			1	1
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4			1	1
Caenidae (Squaregills/Cainflies)	6	1		1	A
Leptophlebiidae (Prongills)	9	1	1		A
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4		A		A
Gomphidae (Clubtails)	6	A		A	A
Libellulidae (Darters/Skimmers)	4	A	A	1	A
HEMIPTERA (Bugs)					
Gerridae* (Pond skaters/Water striders)	5		1		1
Naucoridae* (Creeping water bugs)	7			1	1
Veliidae/M...veliidae* (Ripple bugs)	5		A	1	A
TRICHOPTERA (Caddisflies)					
Hydropsychidae 1 sp	4			A	A
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5			A	A
Gyrinidae* (Whirligig beetles)	5	B		A	B
Helodidae (Marsh beetles)	12		1		1
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5		A		A
Chironomidae (Midges)	2	A	1	A	A
Culicidae* (Mosquitoes)	1		1		1
Dixidae* (Dixid midge)	10			1	1
Simuliidae (Blackflies)	5		1		1
SASS Score					102
No. of Taxa					19
ASPT					5,4

Site code: STS					
Date: 25/10/17					
Taxon	QV	S	Veg	GSM	TOT
ANNELIDA					
Oligochaeta (Earthworms)	1		A		A
CRUSTACEA					
Potamonautidae* (Crabs)	3		1		1
PLECOPTERA (Stoneflies)					
Perlidae	12	1			1
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	1	1	A	A
Caenidae (Squaregills/Cainflies)	6		1	1	A
Leptophlebiidae (Prongills)	9	A	1		A
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4		A		A
Aeshnidae (Hawkers & Emperors)	8		1		1
Gomphidae (Clubtails)	6			A	A
Libellulidae (Darters/Skimmers)	4	1	1		A
HEMIPTERA (Bugs)					
Gerridae* (Pond skaters/Water striders)	5		A		A
Veliidae/M...veliidae* (Ripple bugs)	5	A	A	A	B
TRICHOPTERA (Caddisflies)					
Ecnomidae	8		1		1
Hydropsychidae 1 sp	4		1		1
Cased caddis:					
Hydroptilidae	6	1			1
Leptoceridae	6			A	A
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5	1	1		A
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5	1	1		A
Chironomidae (Midges)	2	1	1	A	A
Dixidae* (Dixid midge)	10		1		1
Simuliidae (Blackflies)	5			1	1
Tipulidae (Crane flies)	5			1	1
SASS Score					123
No. of Taxa					22
ASPT					5,6

Site code: FKL					
Date: 25/10/17					
Taxon	QV	S	Veg	GSM	TOT
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4	A		A	B
Baetidae 2 sp	6		A		A
Caenidae (Squaregills/Cainflies)	6		A	1	A
Heptageniidae (Flatheaded mayflies)	13		A		A
Leptophlebiidae (Prongills)	9		1	1	A
ODONATA (Dragonflies & Damselflies)					
Chlorocyphidae (Jewels)	10		1		1
Coenagrionidae (Sprites and blues)	4		1		1
Libellulidae (Darters/Skimmers)	4		1		1
HEMIPTERA (Bugs)					
Veliidae/M...veliidae* (Ripple bugs)	5	A	A	1	A
TRICHOPTERA (Caddisflies)					
Ecnomidae	8	1			1
Cased caddis:					
Leptoceridae	6	1	1	A	A
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5	1	1	1	A
Elmidae/Dryopidae* (Riffle beetles)	8			1	1
Gyrinidae* (Whirligig beetles)	5			1	1
DIPTERA (Flies)					
Athericidae (Snipe flies)	10		1		1
Ceratopogonidae (Biting midges)	5		1		1
Chironomidae (Midges)	2	1	A	1	A
SASS Score					110
No. of Taxa					17
ASPT					6,5

Site code: GRS					
Date: 26/10/17					
Taxon	QV	S	Veg	GSM	TOT
PLECOPTERA (Stoneflies)					
Perlidae	12	1	A	1	A
EPHEMEROPTERA (Mayflies)					
Baetidae 2 sp	6	A			A
Caenidae (Squaregills/Cainflies)	6	A		1	A
Leptophlebiidae (Prongills)	9	A			A
ODONATA (Dragonflies & Damselflies)					
Coenagrionidae (Sprites and blues)	4		A		A
Aeshnidae (Hawkers & Emperors)	8	1			1
Gomphidae (Clubtails)	6			1	1
Libellulidae (Darters/Skimmers)	4		1		1
HEMIPTERA (Bugs)					
Gerridae* (Pond skaters/Water striders)	5		1		1
Hydrometridae* (Water measurers)	6		A		A
Nepidae* (Water scorpions)	3		1		1
Veliidae/M...veliidae* (Ripple bugs)	5	A	A		A
TRICHOPTERA (Caddisflies)					
Ecnomidae	8	1	A	A	A
Hydropsychidae 1 sp	4	1	A		A
Cased caddis:					
Hydroptilidae	6	1			1
Leptoceridae	6	1	A	A	A
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5	1	1	A	A
Elmidae/Dryopidae* (Riffle beetles)	8			1	1
DIPTERA (Flies)					
Ceratopogonidae (Biting midges)	5	1	A	A	A
Chironomidae (Midges)	2	A	1	A	A
Simuliidae (Blackflies)	5	A	A	A	B
SASS Score					123
No. of Taxa					21
ASPT					5,9

Appendix C: 1 km buffer maps of the sampling sites

Figure C 1. Sterkstroom WGV (STK1) land-cover buffer, Magaliesberg Biosphere Reserve.

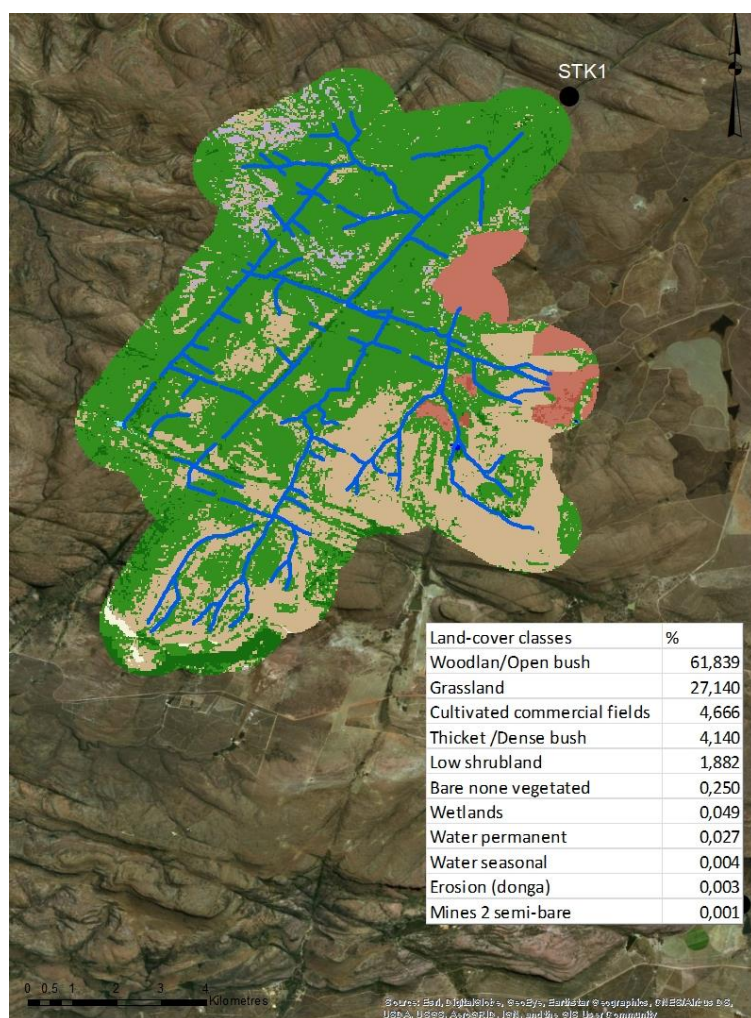


Figure C 2. Skeepoort (SKP) land-cover buffer, Magaliesberg Biosphere Reserve.

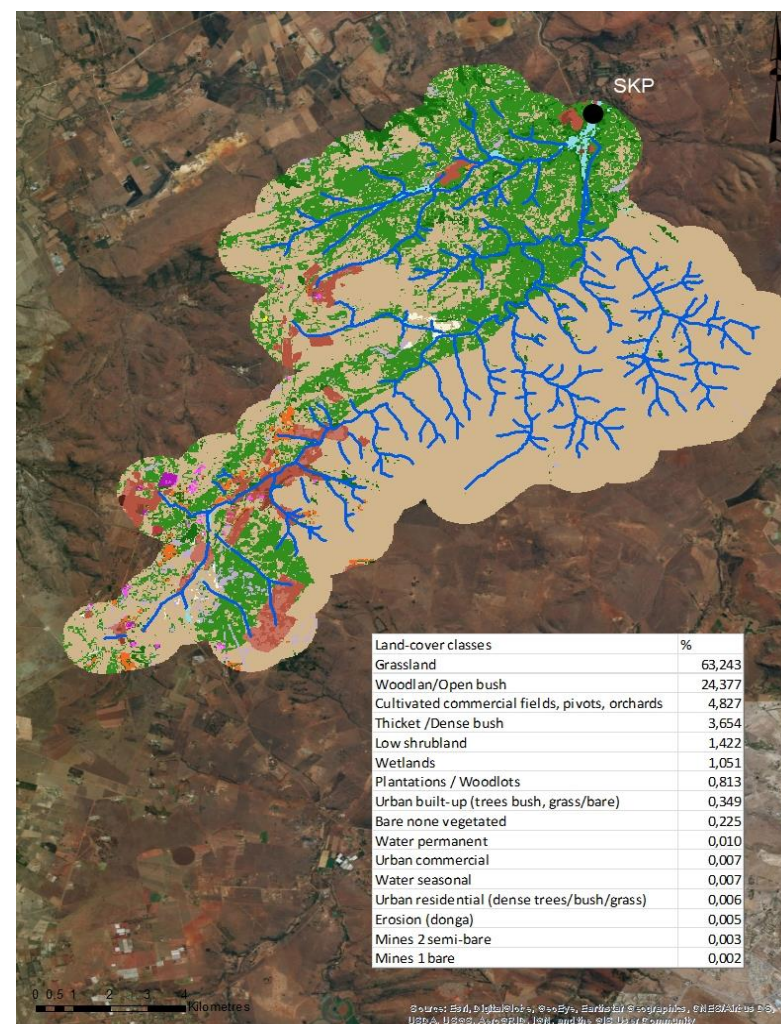


Figure C 3. Bokkraal (BKL) land-cover buffer, Marico Biosphere Reserve.

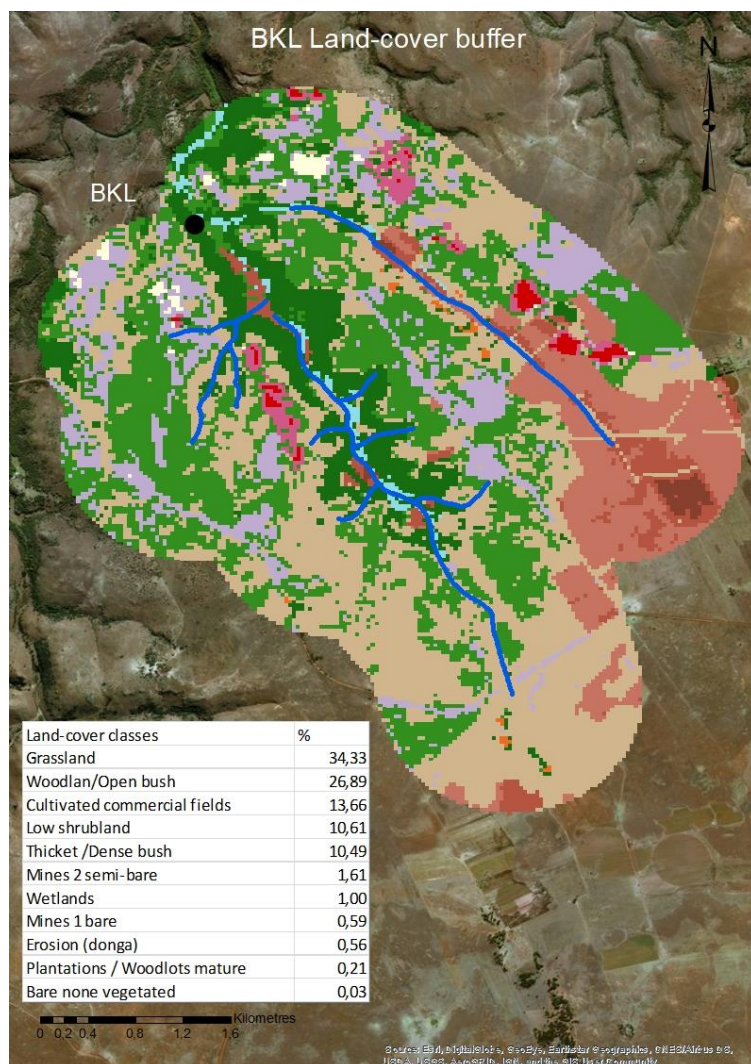


Figure C 4. Upper Draai (DRF) land-cover buffer, Marico Biosphere Reserve.

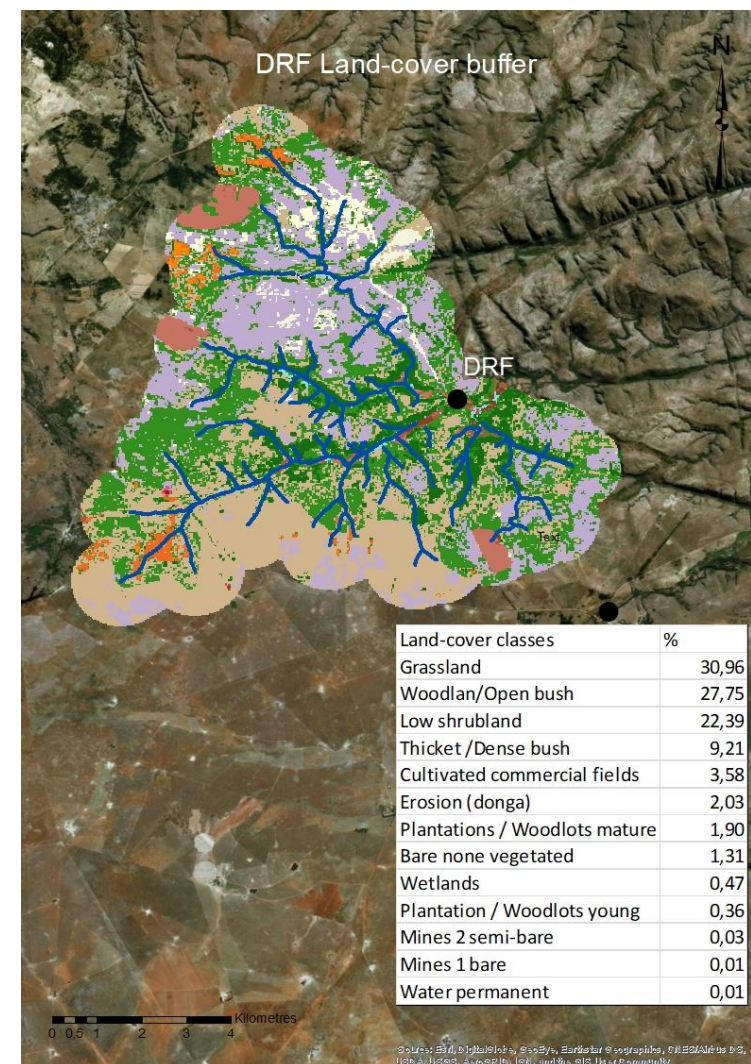


Figure C 5. Kliprivier – Grootfonteinspruit (GRS) land-cover buffer, Unprotected.

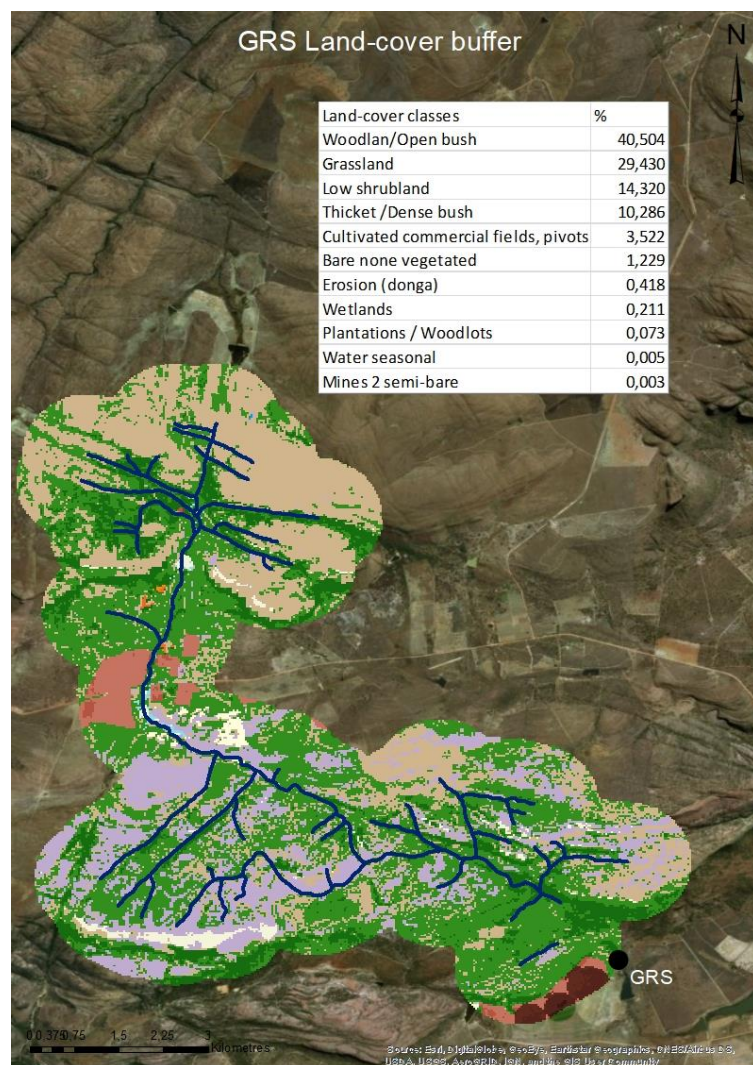


Figure C 6. Grootfontein – Kololo (KOL) land-cover buffer, Waterberg Biosphere Reserve.

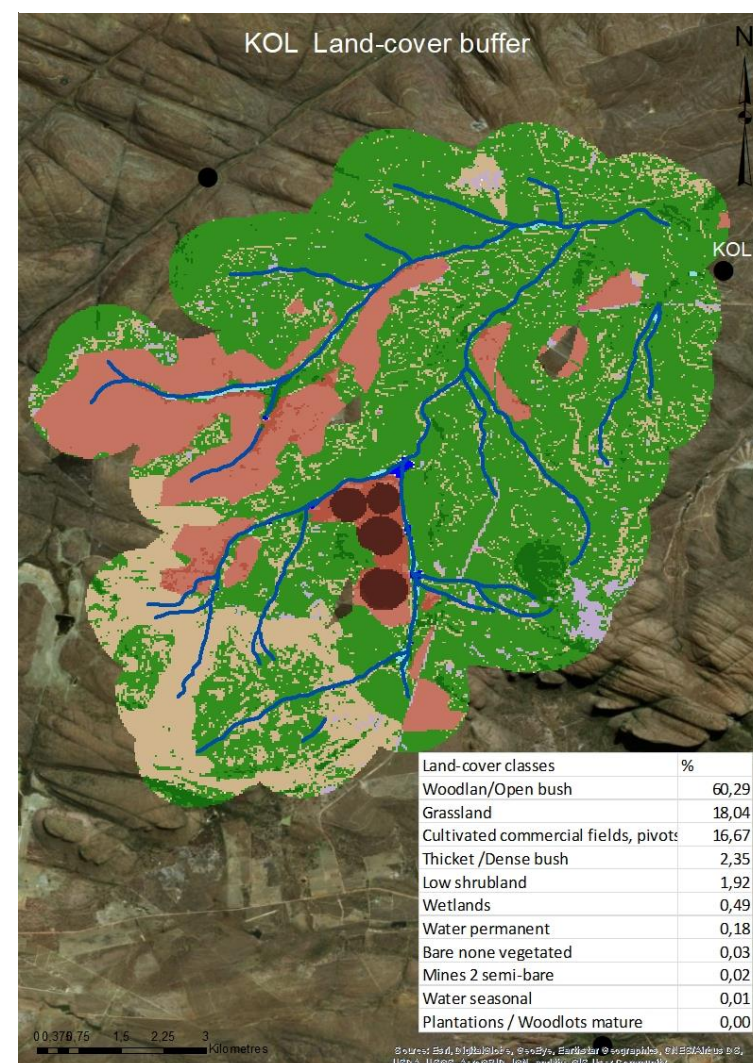


Figure C 7. Koster (KRS) land-cover buffer, Unprotected.

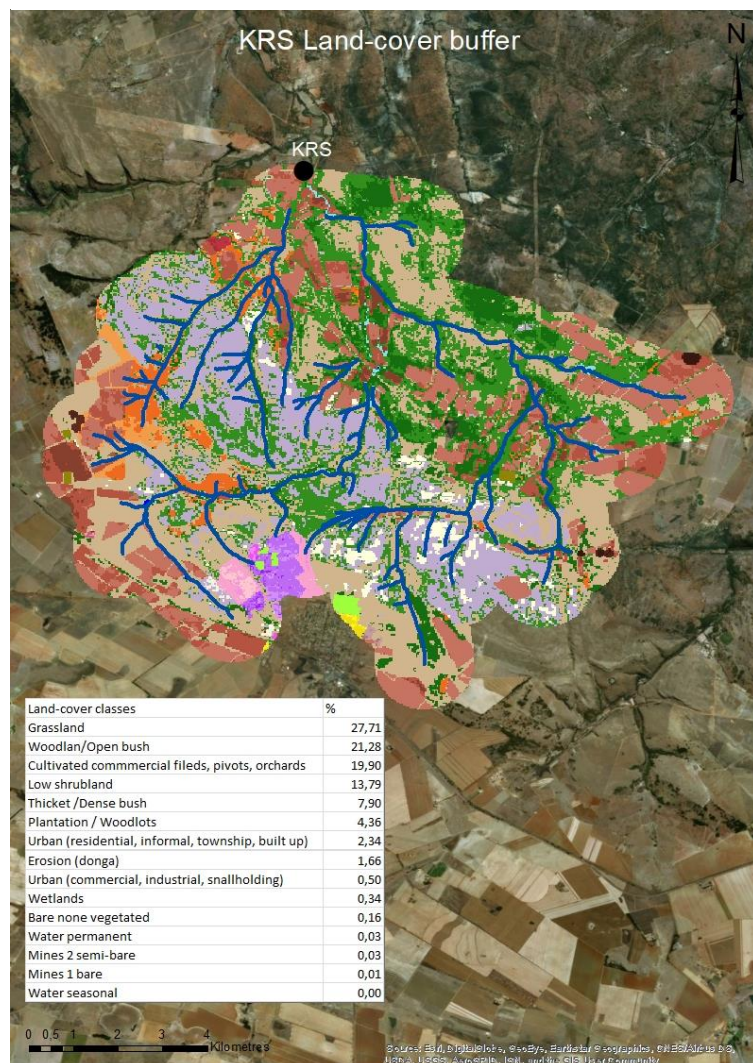


Figure C 8. Maretlwane (MRT) land-cover buffer, Magaliesberg Biosphere Reserve.

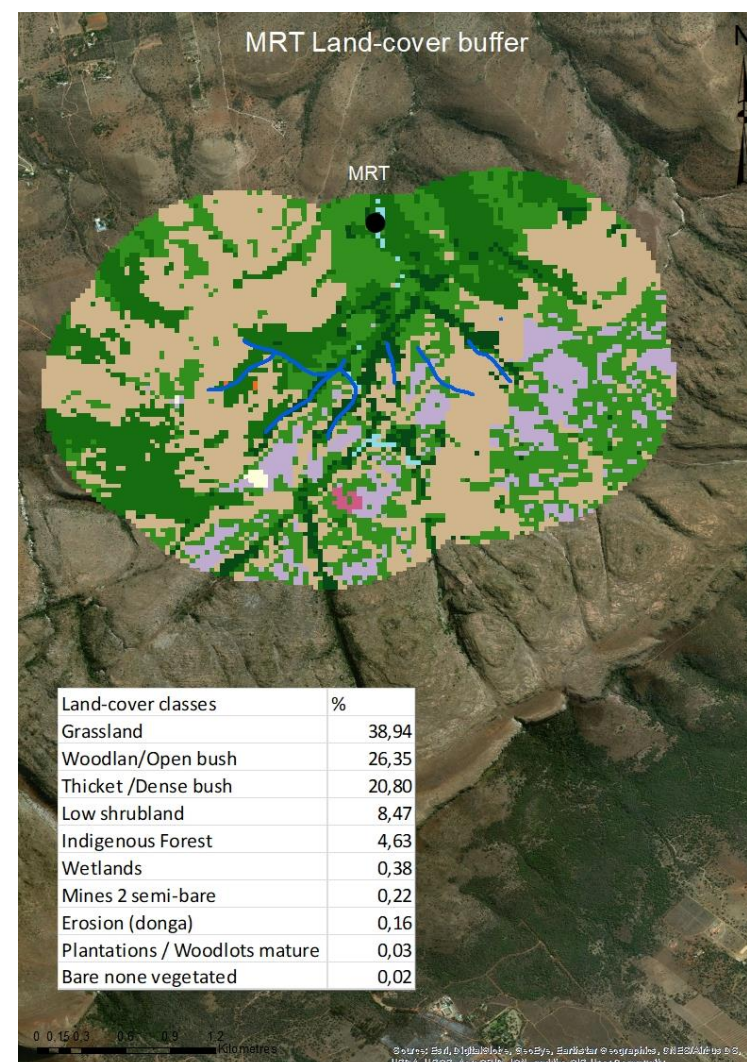


Figure C 9. Renosterpoort (RSP) land-cover buffer, Unprotected.

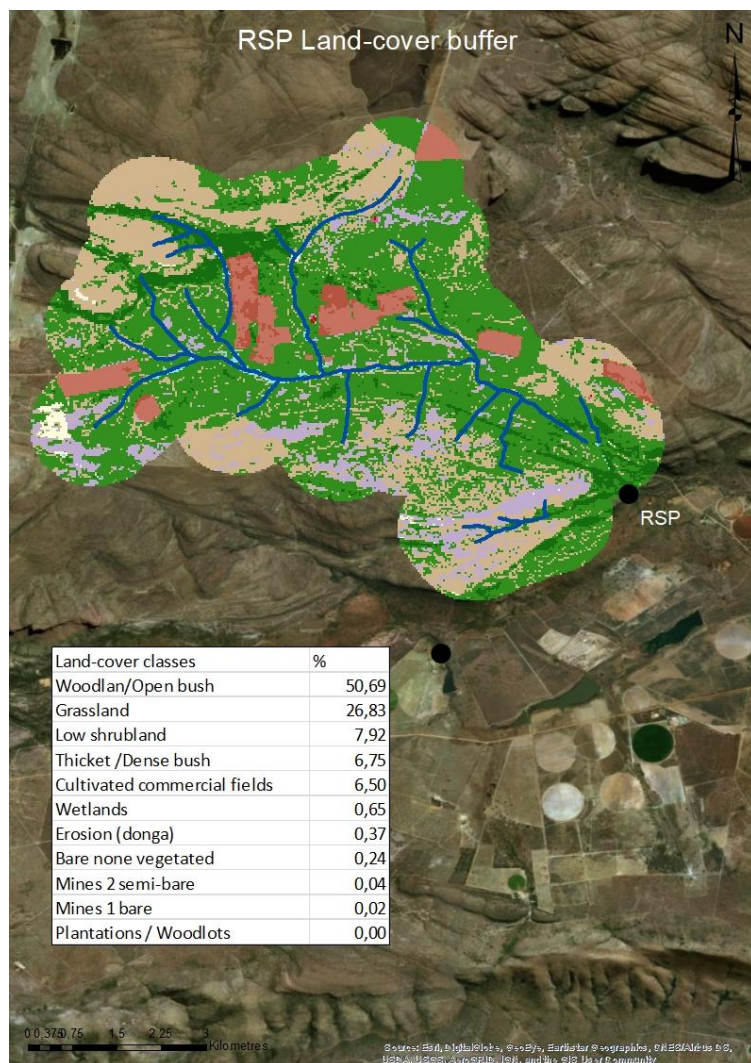


Figure C 10. Eesterkloof (EKL) land-cover buffer, Magaliesberg Biosphere Reserve.

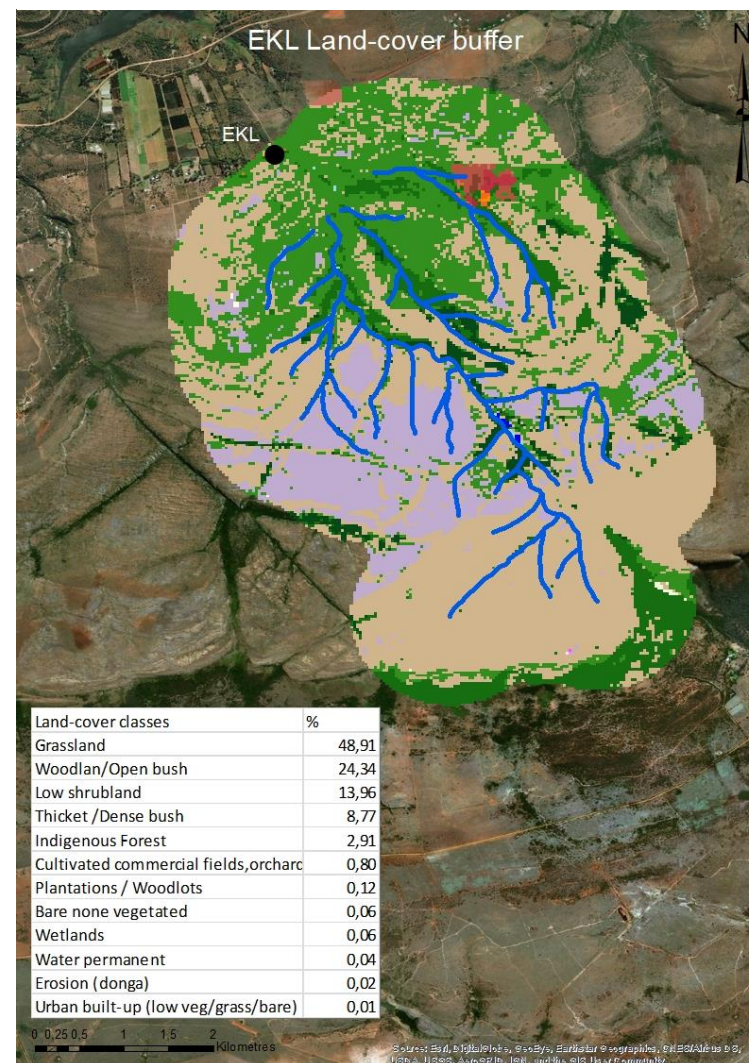


Figure C 11. Kromrivier (STK) land-cover buffer, Magaliesberg Biosphere Reserve.

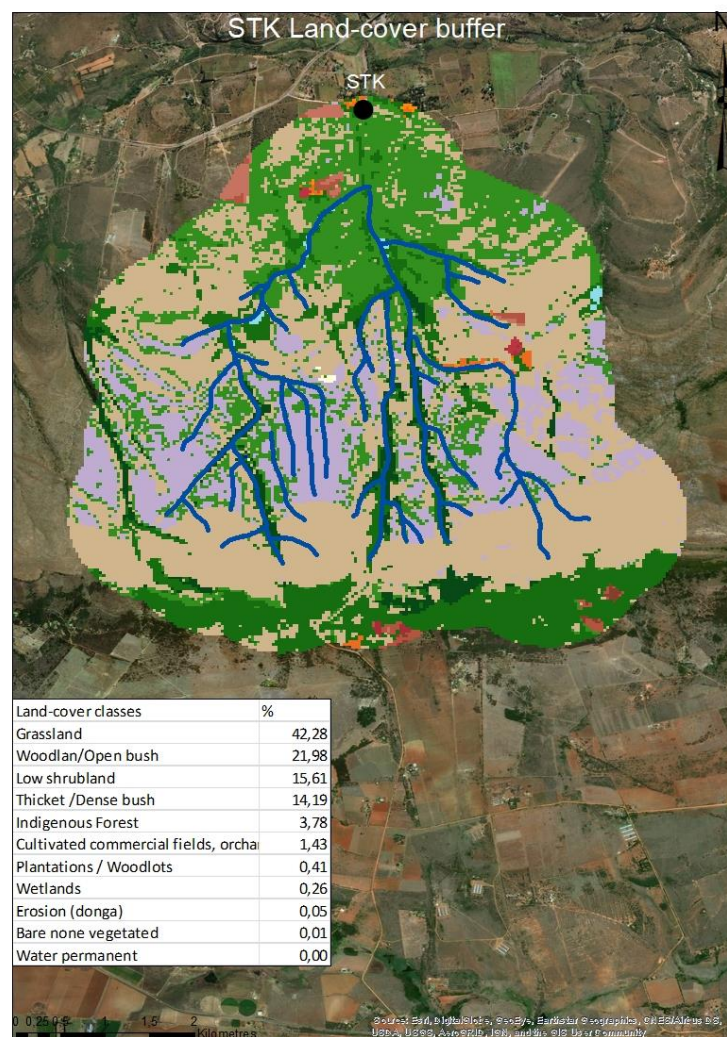


Figure C 12. Polkadraaispruit (PDS) land-cover buffer, Marico Biosphere Reserve.

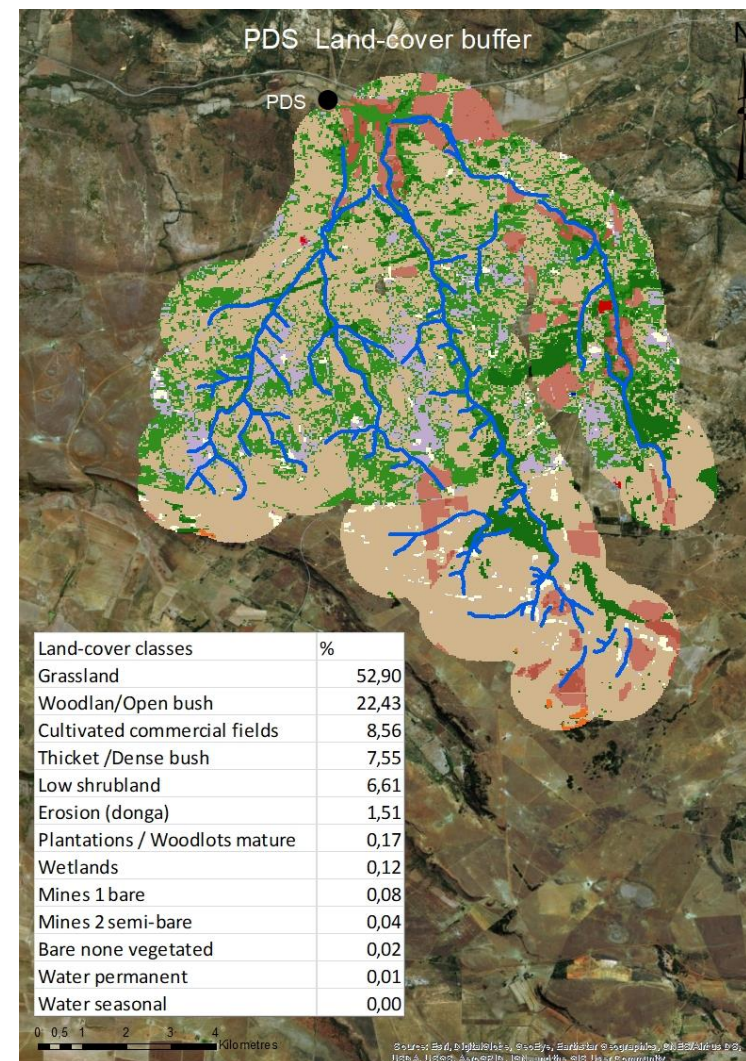


Figure C 13. Sterkstroom (STS) land-cover buffer, Waterberg Biosphere Reserve.

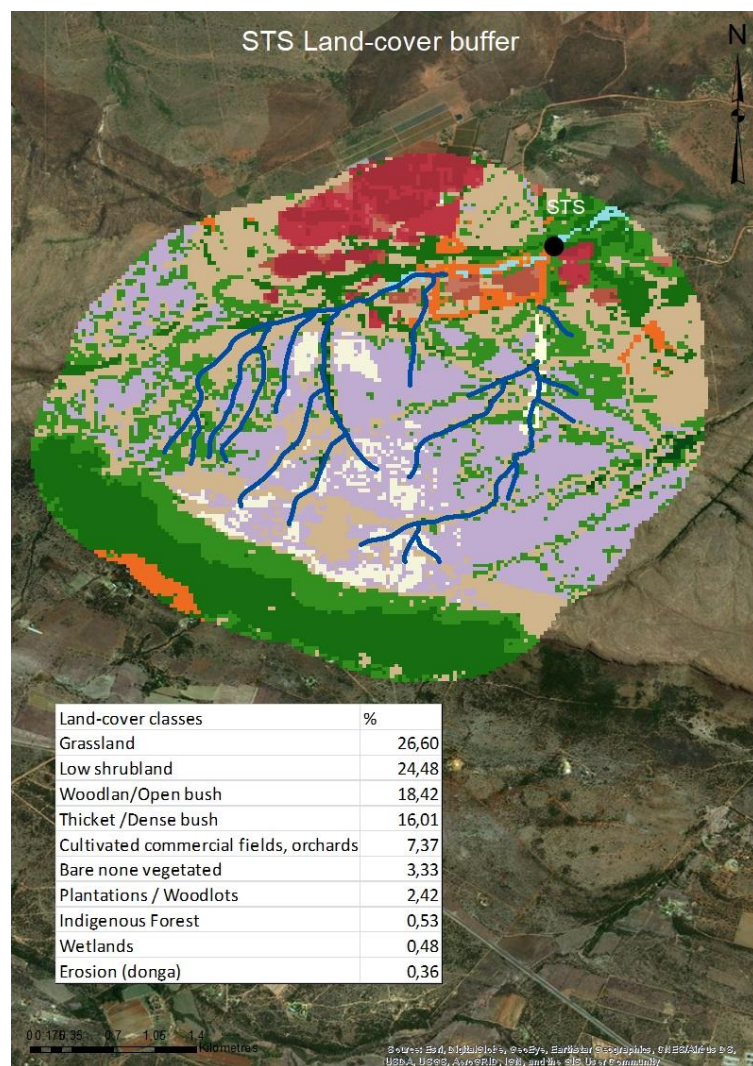


Figure C 14. Uitvlugtspruit (UVL) land-cover buffer, Marico Biosphere Reserve.

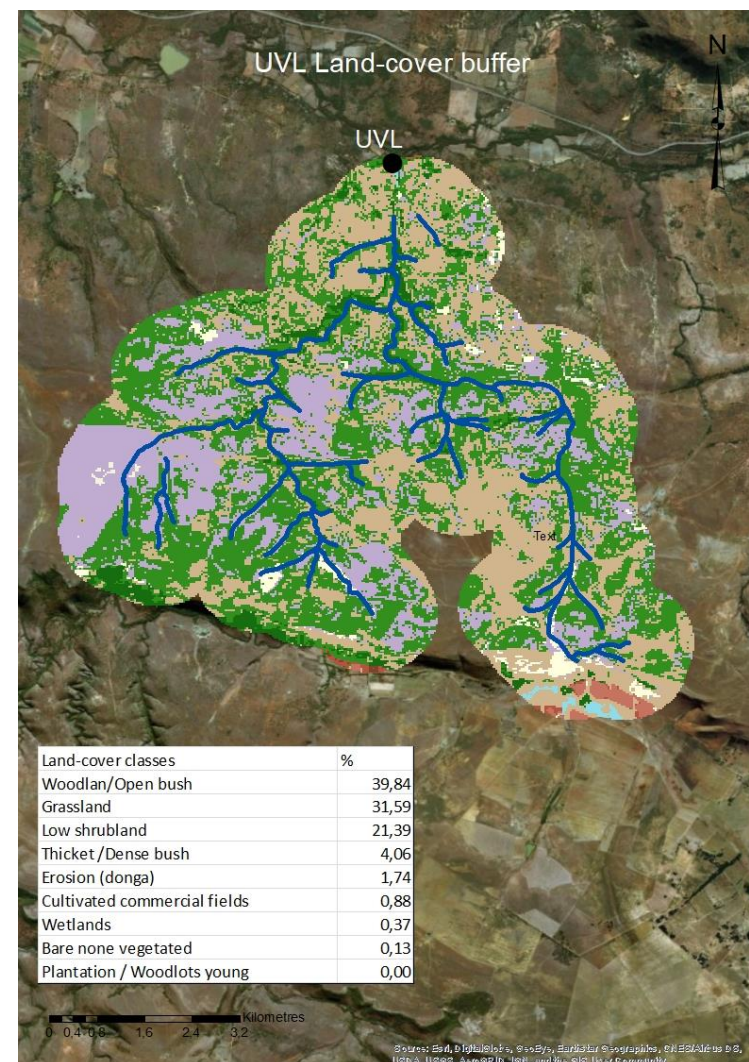


Figure C 15. Platbos River (PBS) land-cover buffer, Waterberg Biosphere Reserve.

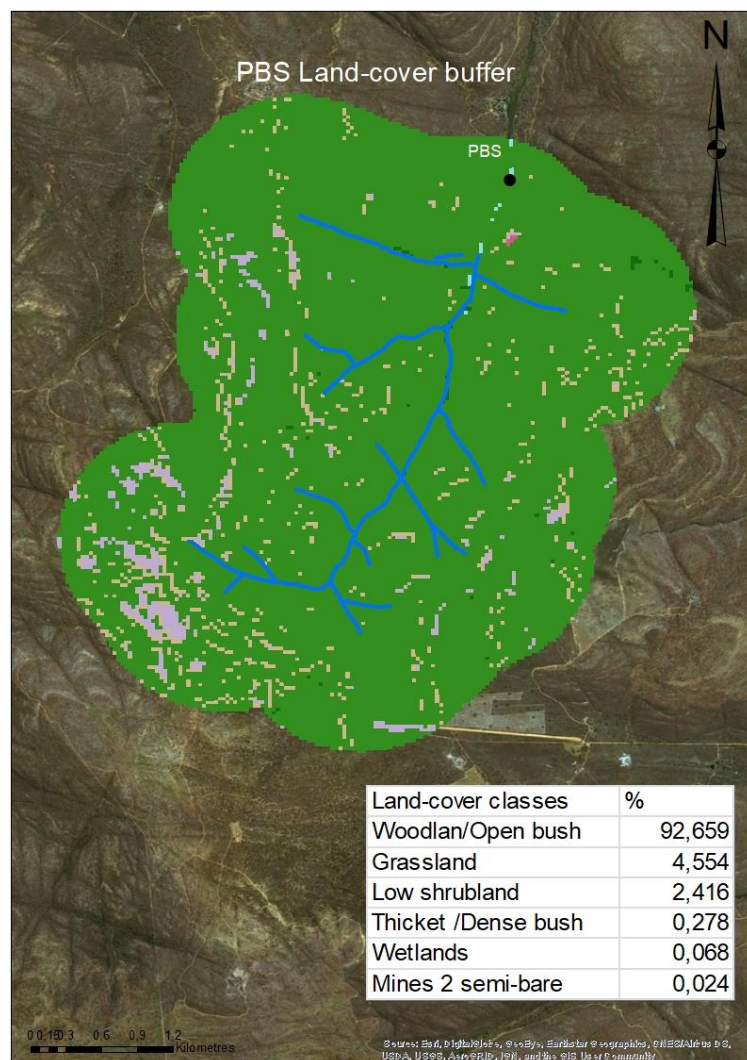


Figure C 16. Loubad West (LBW) land-cover buffer, Unprotected.

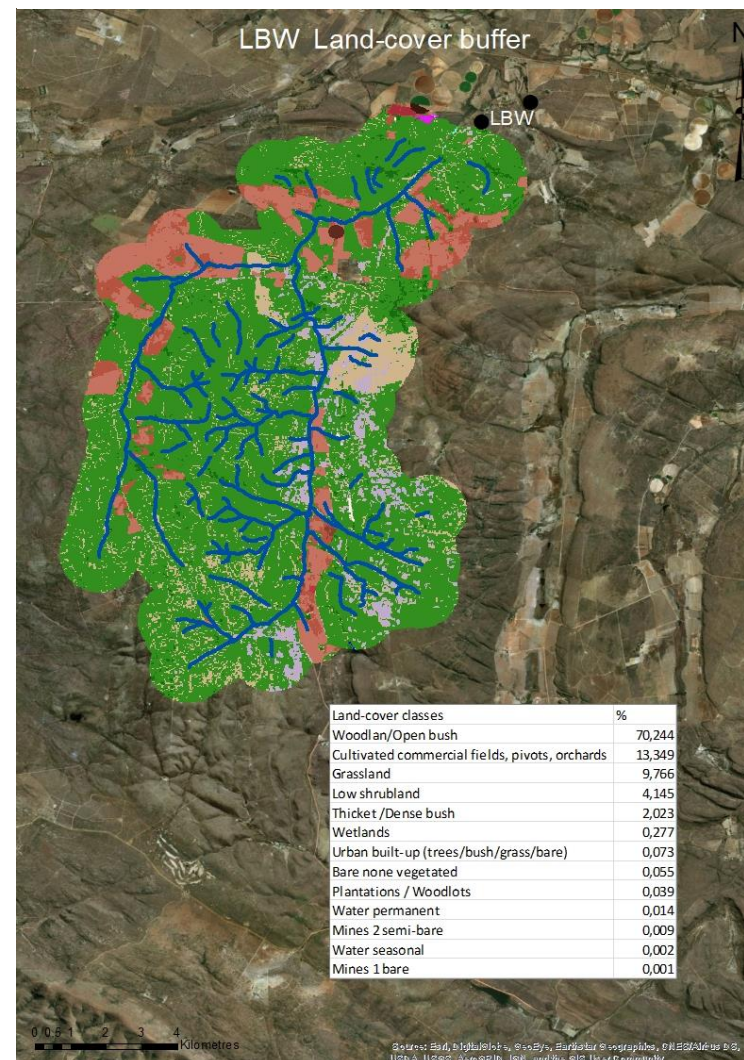


Figure C 17. Waterkloofspruit (KWS) land-cover buffer, Magaliesberg

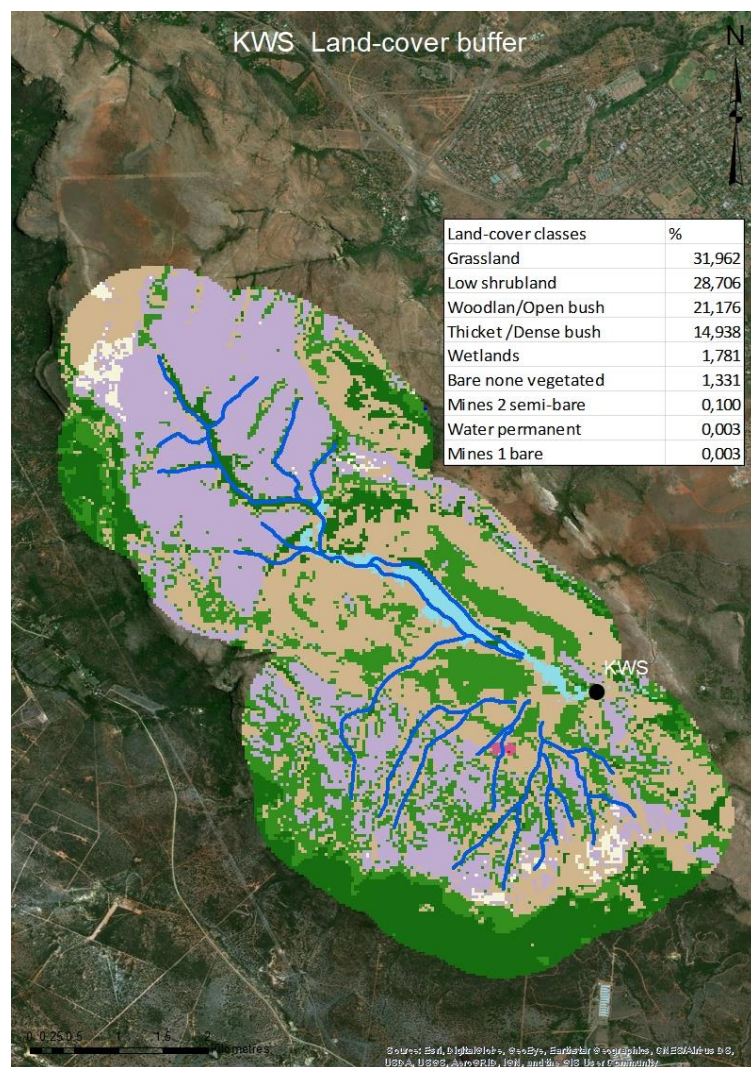


Figure C 18. Sterk River (STR) land-cover buffer, Unprotected.

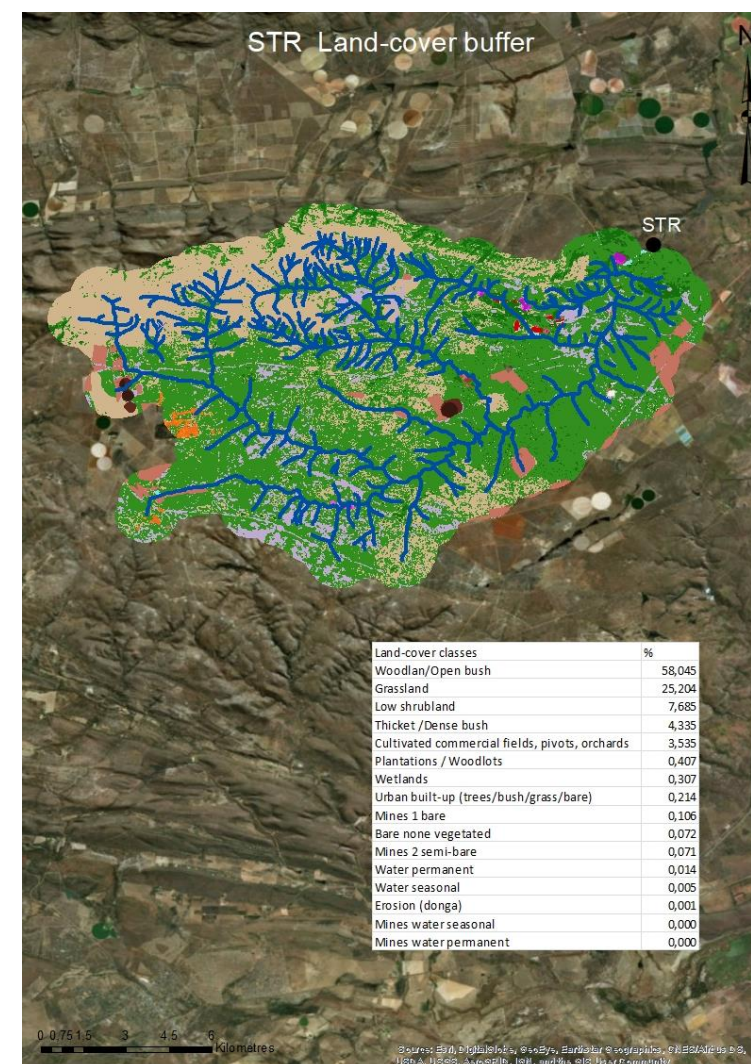


Figure C 19. Loubad East (LBE) land-cover buffer, Unprotected.

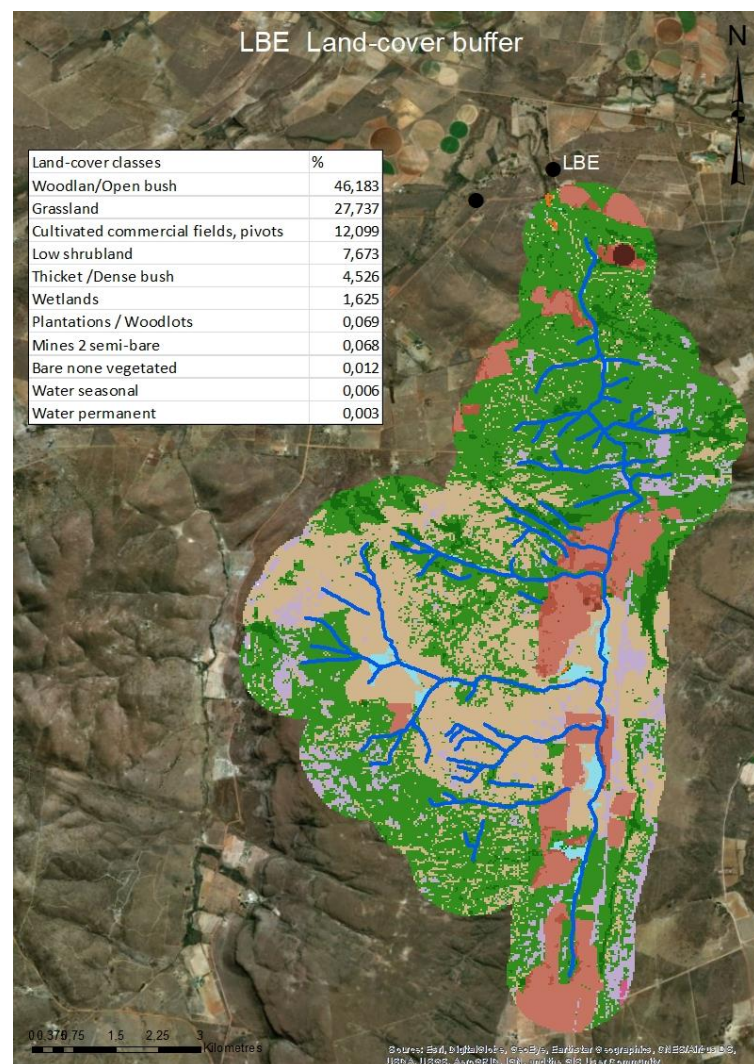


Figure C 20. Frikkiesloop (FKL) land-cover buffer, Waterberg Biosphere Reserve.

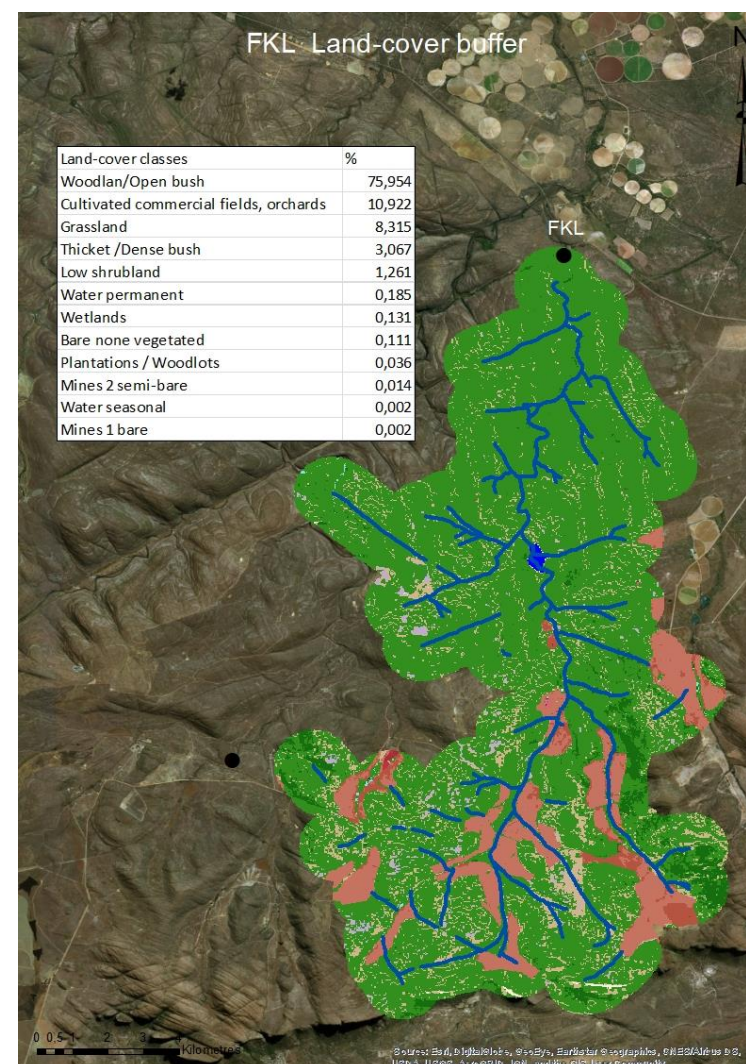


Figure C 21. Upper Kaal Oog (KSP) land-cover buffer, Marico Biosphere Reserve.

