Dung beetle assemblages on reclaimed coal mines in eMalahleni (South Africa), their environmental associations and tunnelling ability on compacted soils

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Disclaimer

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Dedicated to Zindi Steenkamp

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Abstract

Opencast coal mining operations are a major contributor to habitat destruction through the removal of soil, vegetation and fauna from an area. Habitat loss and fragmentation is known to adversely impact invertebrates because of their small size and limited dispersal range that also applies to local dung beetle assemblages. Dung beetles have the potential to aid in reclamation efforts through their beneficial activities in soil although there is little known about their assemblages on reclaimed mine land. Additionally, highly compacted soils are a feature of reclaimed mine sites that may pose a significant challenge to tunnelling dung beetles and may limit their remediation benefits. This study aimed to describe the local dung beetle assemblage in terms of species richness and abundance on reclaimed mine sites in comparison to reference areas (including cattle farms and a protected area). Furthermore, it aimed to determine if dung beetles can tunnel into compacted soils, and how these soils may influence their tunnelling depth as shown through penetration resistance measurements. Dung beetles were collected using standard baited pitfall traps from five reclaimed mined sites and three reference sites (two cattle farms and a Telperion Nature Reserve) from January 2015 to April 2017. Various abiotic factors that could influence the distribution of beetles were measured including soil bulk density, vegetation cover, humidity and soil texture. Dung beetle abundance was found to be significantly higher at the Telperion Nature Reserve than any other site ($F_{(7, 56)} = 8.613$, $p<0.05$). Species richness was found to be higher at reference sites than reclaimed sites with a single exception ($F_{(7,56)} = 17.61$, $p<0.05$). These differences were attributed primarily to the absence of dung on the reclaimed sites, and environmental differences in the soil and vegetation profile. Dung beetle assemblages on reclaimed mined sites were found to differ significantly from the reference sites (R=0.55, $p<0.05$). While increasing vegetation cover, sand percentage ($F_{(1, 30)} = 5.46$, $p<0.05$; $R^2 = 0.15$) and bulk density on sandier soils ($F_{(1, 30)} = 8.61$, $p<0.05$; $R^2 = 0.22$) were found to be affiliated with higher species richness. Increasing clay percentage on the other hand showed to be affiliated with a decreased species richness ($F_{(1, 30)} = 5.58$, $p<0.05$; $R^2 = 0.16$).

To determine the influence of a change in penetration resistance on dung beetle tunnelling ability, three beetle species were used, namely: *Onitis alexis* Klug, 1835, *Digitonthophagus gazella* (Fabricius, 1787) and *Euoniticellus intermedius* (Reiche, 1849). Five individuals of each species (at an approximately even sex ratio) were placed on 30 separate 1 kg cattle dung pats where they were left to tunnel for 14 days. Dung pats were place on the soil surface with a range of penetration resistance levels between 100 kPa and 5 000 kPa. *Digitonthophagus gazella* showed a negative correlation with increasing penetration resistance (p<0.05; R^2=0.65). While *Onitis alexis* tunnel depth showed no correlation to penetration resistance, *Euoniticellus intermedius* increased tunnel depth with increasing penetration resistance (p<0.05; R^2=0.35).

Although tunnelling depth was notably shallower than previously observed for the individual species, all three species could tunnel past the site average of 3 193 kPa as well as the equipment threshold value of 5 000 kPa. Live eggs and larvae were found in multiple brood balls that gives credence to the fact that dung beetles could complete their life cycle in reclaimed mined soils. Reclaimed mine sites supported a relatively high diversity of beetles in lower abundance and these results indicate the potential to improve assemblage structure with the incorporation of large herbivores on site. Differences in species richness between sites were attributed to vegetation cover, soil texture and bulk density differences between sites. Dung beetle species that were well adapted to mined sites were identified and recommended, should breeding be necessary to enhance their beneficial activities. Beetles active on mined sites occurred at great enough numbers to suggest that the population present in the area will be sufficient for rehabilitation efforts, provided dung becomes available.

Chapter 1

The coal mining sector in eMalahleni (South Africa) and how dung beetles can assist in reclamation efforts

1.1 Mining in South Africa

Platinum, gold, diamonds and coal are the commodities that structure the mining industry in South Africa and in turn, contribute significantly to the economy. The mining sector, one of the nation's largest employers with approximately half a million workers in its entirety, contributed more than R300 billion to the gross domestic product in 2016 (Chamber of Mines of South Africa, 2016). For the time being, coal remains arguably the most important mined commodity in South Africa.

1.2 Coal in South Africa

Coal alone, contributed more than R100 billion to the economy in 2016, dwarfing the contribution of even gold in the same period (Chamber of Mines of South Africa, 2016). At that time, 17% of South Africa's mining workforce were coal miners. In 2016, coal sales amounted to R112 billion with 70% of South Africa's energy needs being dependent on coal (Chamber of Mines of South Africa, 2016). Despite the rise in more environmentally sustainable alternatives of energy production through wind, solar and hydroelectrical methods, coal remains the world's primary energy source with an estimated 41% of energy needs met by means of coal combustion (World Coal Association, 2012).

The country's coal resources are located in the Ecca deposits that form one stratum of the Karoo Supergroup geological bodies (Aitken, 1994). Although coal deposits are found in both the Free State and KwaZulu-Natal, close to 83% of coal produced in South Africa originates from Mpumalanga, specifically near the Witbank/ eMalahleni city centre (Figure 1; Pinetown *et al.* 2007).

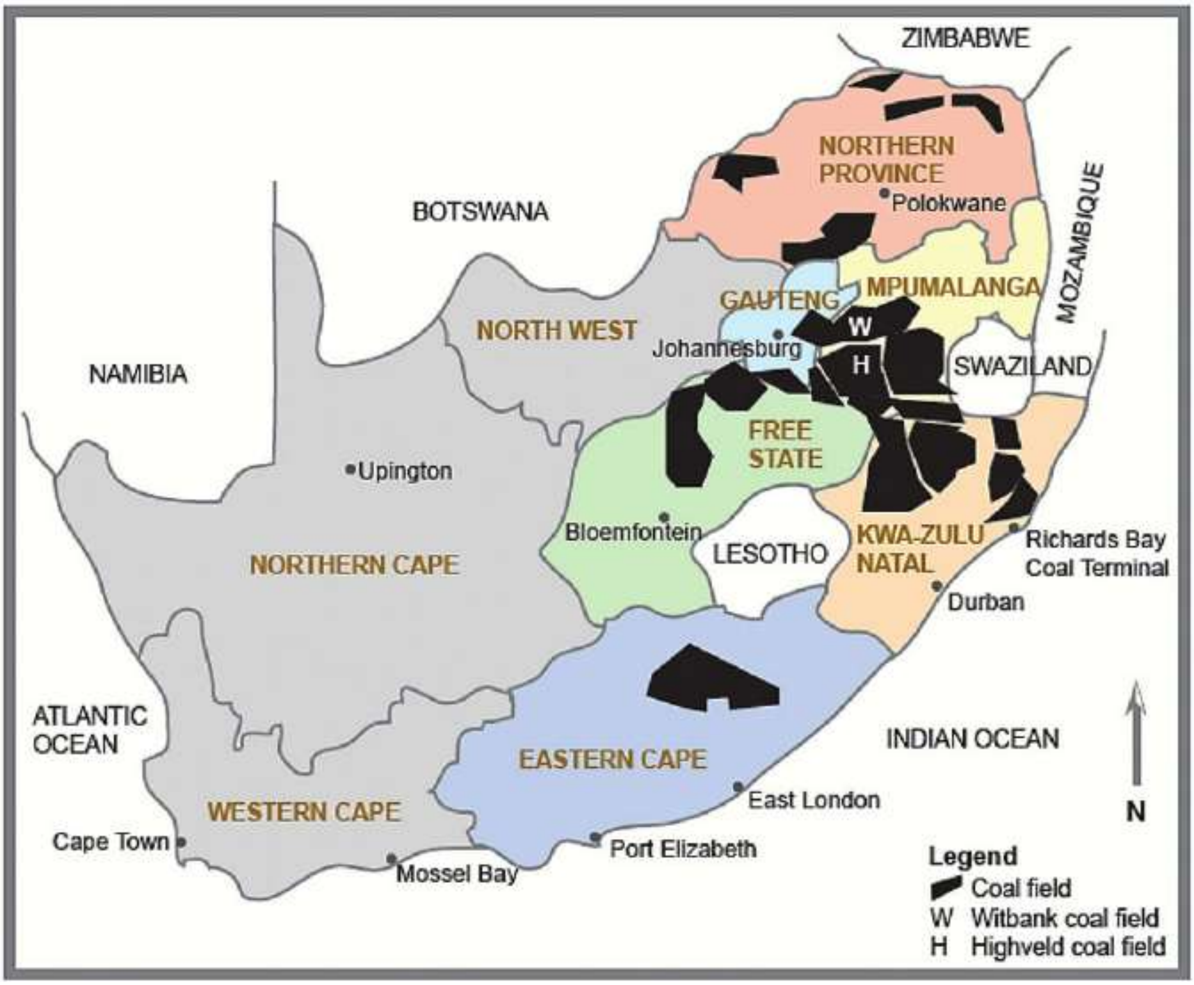

Figure 1: The coalfields of South Africa, highlighting the Highveld and Witbank areas as primary coal producers. Adapted from Pinetown *et al.* (2007).

1.3 Coal extraction

The method of coal removal is dictated by the subterranean seam of coal, its quality and its depth (Scott *et al.,* 2010). Various methods of coal extraction exist that are broadly categorised as either surface or underground mining. Approximately 40% of coal mining worldwide, is classified as surface mining and has significant consequences for the environment (World Coal Association, 2012. Opencast, surface mining is also the most commonly used practice in South Africa (World Coal Organisation, 2017). The approach to surface coal mining begins with the removal of vast quantities of soil (topsoil and subsoils) and rock, to expose the coal seams. The overburden (earth covering coal) is explosively fractured and removed. The coal is then extracted for further processing on site or at another facility.

1.4 Mine closure legislation

In the past, little to no consideration has been given to the rehabilitation of previously mined areas, mainly due to the lack of responsibility towards environmental and socio-economic factors regarding degraded lands (Limpitlaw *et al.* 2005). Historically neglected mined areas have led to a multitude of problems concerning surface disturbance, acid mine drainage, and pollution that are still contributing to ecosystem damage, decades after they have ceased operations (Bell *et al.*, 2001; Limpitlaw *et al.* 2005) . The destructive history of abandoned mines and continued degradation of lands by current operations have led to obligatory rehabilitation by law (Minerals and Petroleum Resources Act of 2012). This legislation has become increasingly important as the number of closed mines have increased in the last few years (Sorensen, 2009). Strict adherence to these best practice procedures may minimise the impact that mining operations have on the environment, economy and local communities (Limpitlaw *et al.* 2005).

Guidelines developed by the Chamber of Mines of South Africa and Coaltech, have stipulated that rehabilitation should aim to minimise the loss of productive land-use capability by restoring the area to its natural or pre-determined state (Tanner & Mohr-Swart, 2007). Additionally, the "Public Participation Process" of the Mineral and Petroleum Resources Development Act 28 of 2002 requires that the impacted land must be left in a condition that will be useable to society (Tanner & Mohr-Swart, 2007).

1.5 Impact on the environment

1.5.1 Habitat destruction

Open-cast coal mining has a devastating impact on local ecosystems. Habitats are lost by the removal of the soil that destroys the vegetation and kills or displaces the established fauna. This process also makes it difficult to rehabilitate the area after mining has ceased. Soils are stockpiled for extended time periods, even decades (Figure 2; Ghose *et al.* 1989; Sheoran *et al.* 2010).

Figure 2: Habitat destruction on an active mine site after coal mining operation with multiple stockpiles. Photo: Alexandra Howard.

During the process of soil removal, organically enriched topsoils are often mixed with infertile subsoils, decreasing its value for resurfacing. Furthermore, the soil is exposed to years of sunlight and rain that diminishes any microbes and nutrients from the stockpiles (Ghose, 2004).

1.5.2 Secondary effects of rehabilitation

Unfortunately, regardless of legislation, many operations fail to adhere to rehabilitation guidelines that lead to secondary effects on abandoned or "rehabilitated" lands (Sorensen, 2009). When reclamation is initiated, depleted coal seams are filled by fractured, waste coal and rock before being covered with homogenised topsoil.

The topsoil depth rarely complies with the proposed 60 cm minimum that is required for effective restoration for an arable land capability class and can be as shallow as 10 cm, or even absent depending on the protocol followed by the operation in charge or the topsoil resources available (Ghose, 2004). This leads to water filtering through to the waste coal layer, generating acid mine drainage (AMD) that can negatively impact groundwater resources (McCarthy, 2011). Through this process sulphuric acid is produced due to the reaction of oxygenated water and pyrite (McCarthy, 2011). Although pyrite is found in natural coal seams, the increased surface area that is created by fracturing coal, exponentially increases acid production on poorly managed mines (Bell *et al.* 2001). Acid accumulation can then adversely affect water, soil, vegetation and animals in the region (Ochieng *et al.* 2010).

Figure 3: Soil on reclaimed mined site (eMalahleni, South Africa) with presumably high clay content and no vegetation. Photo: Gustav Venter.

Heavy machinery coupled with the constant wetting and drying of the soil, also contributes to severely compacted soils on reclaimed sites (Truter *et al.* 2013). Unnaturally high compaction makes it extremely difficult for vegetation to establish, a process that is vital to the successful rehabilitation of the land (Figure 3; Bassett *et al*., 2005). The penetration resistant soils also affect soil biota and subsequent successional plant growth and animal establishment (Bengough *et al.* 2006; Jouquet *et al.* 2012).

Apart from restoring areas to a more natural state, the goal of rehabilitating areas generally also aim to use the areas for cattle farming or agriculture, both being rarely achieved or completely implemented (Limpitlaw *et al.* 2005).

1.6 Biological remediation of mined soils before dung beetles

Many efforts have been made in the past with varying degrees of success to rehabilitate soil and vegetation to a useable state. Although a variety of taxa such as ants have been used as bioindicators, few soil-dwelling organisms have been identified or utilised that effectively improve the physico-chemical properties of soil. A common method of rehabilitation found on coal mined areas is that of phytoremediation that uses common local grass species with a sufficient soil layer to facilitate nutrient cycling and successional change in vegetation (Salt *et al.* 1998). Bioremediation is generally reserved to microbes that enable the improvement of contaminated or degraded substrate such as soil or water and generally involves oxidation or reduction of polluting substances (Kensa, 2011). Another approach applied recently in a South African context, is through the application of arbuscular mycorrhizal fungi on coal dumps, that mutualistically aid in plant nutrient uptake and the biodegradation of coal (Cowan *et al.* 2016). In addition, earthworms have been used to increase topsoil fertility, redistribute soil nutrients and aid in the recycling of organic materials in reclaimed mined soils (Frous *et al.* 2007). The study by Frouz *et al.* (2007) showed that reclaimed mines that have a higher density of soil macrofauna, saw higher values for various aspects of soil fertility that could be attributed to production of coprolites and distribution of nutrients through their activities in the soil. Up to date the focus for mine reclamation using macro soil fauna has been on earthworms, without considering other organisms that could be equally, or more suited to improve soil conditions.

A large contributor to the soil ecosystem has been neglected in this aspect despite delivering a multitude of ecosystem services that could be directly beneficial to degraded mined soils. With a high diversity in Southern Africa that has been extensively researched, dung beetles are ideally equipped for soil reclamation on mined sites.

1.6.2 Dung beetle abundance in Southern Africa

Dung beetles (Scarabaeinae) are a diverse group with 12 tribes, more than 200 genera and approximately 5 700 species worldwide (Davis *et al.* 2008). Southern Africa alone boasts a dung beetle diversity of at least 760 species (Ferreira, 1969). As their name suggests, they are primarily coprophagous beetles that have other notable feeding strategies with some species being fungivores, detrivores, and even frugivores (Davis *et al.* 2008).

1.6.3 Factors that influence their regional occurrence

Dung beetles, follow a similar trend to other taxa in Southern Africa that decrease along the rainfall gradient from East to West (Davis, 2002). Rainfall has structured the primary differentiation of seven regional centres of dung beetle distribution that include the Highveld and bimodal, North-East mid-summer, Kalahari, Arid late summer, East Coast, and Winter bimodal rainfall region (Davis, 1997). Altitude, climate and vegetation also strongly influence the diversity of beetles in a region (Davis *et al.* 2008).

1.6.4 Factors that influence their local distribution

Variables that affect their distribution at a finer scale include soil, vegetation and dung (Davis, 2002). Their daily activity is affected by day to day fluctuations in temperature and rainfall and light intensity, with the majority of beetles being most active during wet and hot conditions (Davis *et al.* 2008).

Because the majority of dung beetles tunnel into soil to construct nests, soil type might affect their local distribution (Osberg *et al.* 1994). Soil type is dependent on particle size or texture, that determines the water drainage and retention abilities of the soil and in turn the resistance to penetration (compaction) it will provide (Davis *et al.* 2008). Soils that are deep and sandier tend to support the highest diversity, with specialist species occurring at either extreme (Davis *et al.* 2008).

The relationship between dung beetles and local vegetation is not so dependent on the diversity thereof, but rather the amount of shade and cover it provides, that in turn influences the temperature, humidity and light intensity in the microclimate (Davis, 1996). This has led to dung beetles being affiliated with shade, partial shade or unshaded habitats (Davis *et al.* 2008). Although there are specialists on both extremes, most beetles favour unshaded grasslands as a general rule.

The previously mentioned factors will have no meaning if an area is devoid of dung, their primary source of nutrients. Dung beetles are primarily affiliated with mammalian dung, with preferences depending on the size, water and fibre content of the dung coupled with the chemical composition thereof (Davis *et al.* 2008; Martin-Piera & Lobo, 1996). Many beetles are attracted to the dung of ruminants (larger droppings). Some beetles prefer the pellets of small herbivores, the dung of omnivores and carnivores or the larger dung pats of non-ruminants such as rhinos (Davis *et al.* 2008).

1.6.5 Breeding behaviour

Dung beetles are known to exploit dung in a few ways. Beetles that primarily reside within dung pats are referred to as endocoprids, while beetles that partition dung to be rolled away to a distant location are telecoprids (Halffter & Edmonds, 1982). These two interaction types are present in the minority of beetles as approximately 70% of beetles are paracorpids (tunnellers) in that they partition dung and bury it in tunnels directly below the dung pat (Figure 4; Halffter & Edmonds, 1982). There are some species that are referred to as kleptocoprids in that they steal the dung balls of other beetles under the ground or in transit (Davis *et al.* 2008).

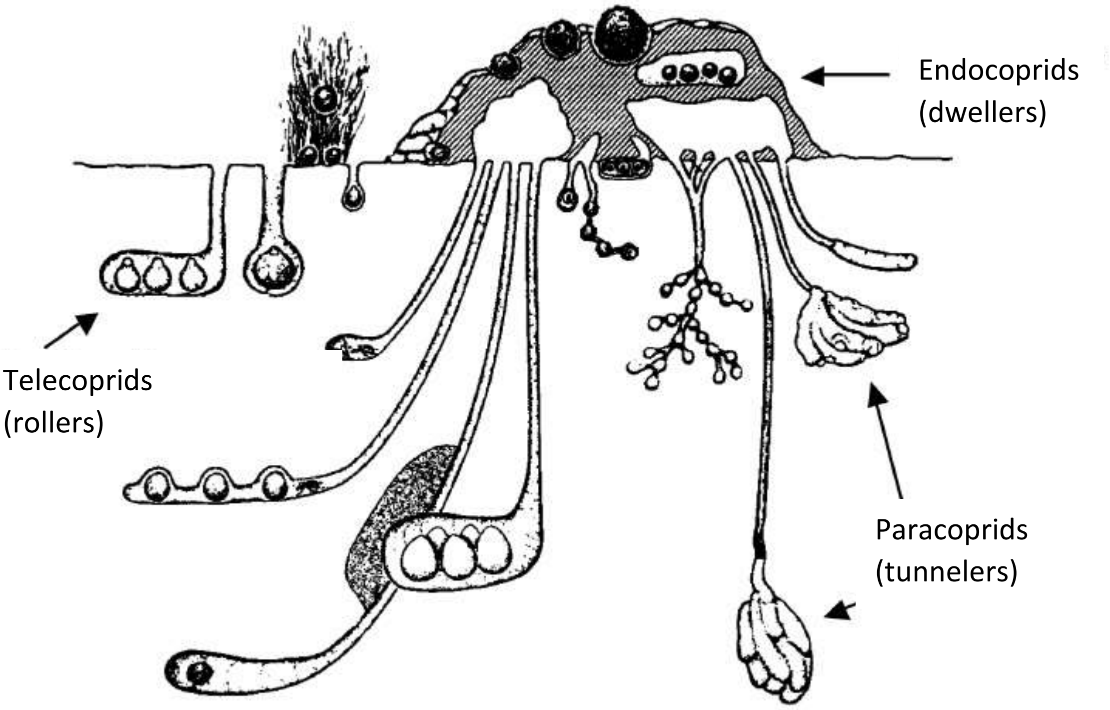

Figure 4: Basic illustration of three nesting behaviours based on dung utilisation (Taken from: Halffter & Edmonds, 1982).

1.6.6 Ecosystem services provided by dung beetles

The importance of dung beetles in agro-ecosystems has been shown in many instances as indicators of biological change and through the many ecosystem services that they provide.

Because of their graded sensitivity to habitat disturbance, relatively well-known taxonomy and ease of collection they have been identified as a valuable bioindicator group (Bicknell *et al.* 2014). In addition, scarabs have been recognised as a valuable taxon for evaluating and determining biodiversity patterns at a spatial and temporal scale (Davis & Scholtz, 2001; Favila & Halffter, 1997; Nichols *et al.* 2008).

Given that dung beetles have an intimate relationship with soil, they have also proven valuable in delivering many services that improve soil conditions and subsequently vegetation composition. Through their tunnelling activities in soil, dung beetles have been observed to increase water infiltration rates and reduce soil compaction (Brown *et al.* 2010). Their active incorporation of nutrient-rich dung into the soil profile has also been linked to increased productivity of grassland ecosystems (Bang *et al.* 2005). All of the previously mentioned factors along with their active bioturbation of soils, improve the hydrological and physico-chemical properties thereof (Bang *et al.* 2005; Nichols *et al.* 2008). Other notable services include secondary seed dispersal in which dung beetles disperse and bury seed-laden dung, and reduce dung breeding pests through their removal of dung from the soil surface (Shepherd & Chapman, 1998; Waterhouse, 1974). The removal or dispersal of dung can be beneficial as it controls dung breeding pests (Waterhouse, 1974). This was most famously demonstrated in Australia where flies took advantage of the dung produced by the introduction of cattle by European settlers in 1788 (Hughes *et al.* 1978; Scholtz *et al.* 2009). Native dung beetles were specialised on the marsupial droppings that were small, dry and distinct to that of cattle manure (Scholtz *et al.* 2009). In 1967 South African dung beetles were released in Northern Australia, with four genera becoming successfully established within three years (Waterhouse, 1974). New research by Slade *et al.* 2015 indicates that dung beetles even reduce greenhouse gases through (mainly methane emissions from dung pats) their removal and burial of dung.

Many of the ecosystem services that result from dung beetle activity directly address the challenges associated with soil quality and plant growth on reclaimed mine land. This makes them potential candidates to be considered for use as biological agents in the process of reclamation. However, prior to this study, dung beetles had not been considered for this purpose.

1.7 Study aims

The purpose of the study was to inform various aspects of a long-term project that aimed to determine the viability of using dung beetles as a complementary method of improving reclaimed lands after mining operations have ceased. The impact of opencast coal mining in eMalahleni, on dung beetle assemblages has not been determined. Nor has there been a comparable study undertaken in the area to establish dung beetle assemblages on farms or disturbed areas that may be reflective of pre-mining conditions. In addition, no study has specifically been conducted to determine the influence of a soil compaction gradient on dung beetle tunnelling ability on mined or unmined soils.

This study aimed to:

i) Describe dung beetle assemblage structure in terms of abundance and species richness across multiple reclaimed coal mined sites and compare these sites to reference sites in eMalahleni, South Africa.

ii) Determine small scale environmental differences between sites that may account for assemblage divergence;

iii) Identify key species that may be indicative of reclaimed sites and may be beneficial to use in mass breeding and release programmes should local abundance and diversity be lacking, and;

iv) Determine if increasing penetration resistance (compaction) in reclaimed mine soils will influence burrowing depth and ability of three dung beetle species commonly used in mass breeding that naturally occur at the study sites.

Chapter 2

How dung beetle assemblages (Coleoptera: Scarabaeinae) are affected by environmental factors across reclaimed mined sites in eMalahleni (South Africa)

2.1 Introduction

Habitat loss and fragmentation, and subsequent loss of biodiversity is becoming more common and severe due to the constantly increasing human population, and our propensity to exploit natural resources (Vitousek *et al.* 2008). Our growing global population (currently at more than 7.6 billion) demands greater quantities of water, food and power supplies, that in turn drive landscape transformation for agriculture and mining (Bell *et al.* 2001; Tilman, 2001). Due to these factors and their influences on climate change, loss of diversity in the last 300 years has exponentially exceeded that which has been documented for the same timeframe in earth's geological record (Dirzo & Raven, 2003). The negative effects of increasing fragmentation present themselves through the primary loss of biodiversity that includes decreasing levels of species abundance and richness, altered distribution patterns and reduced genetic diversity of populations across all taxa (Ehrlich, 1988; Reid *et al.* 2005). A large contributor to fragmentation and habitat loss is that of coal mining, specifically, the opencast method.

Surface coal mining operations have a destructive effect on soil and vegetation and contribute to air and water pollution that result in a multitude of secondary effects present long after operations have ceased (McCarthy, 2011; Truter *et al.* 2013). As part of South Africa's primary coal producing region, collieries in eMalahleni (Mpumalanga Province) are known to have significant effects on the local environment, despite efforts to restore land once the coal deposit has been depleted (Bell *et al.* 2001).

When land is restored much attention is given to the vegetation and the large vertebrates (especially mammals), whilst other contributors are often neglected. It is well established that fragmentation and habitat destruction is of more significant threat to invertebrates due to their reduced ability to disperse over larger areas (Scholtz *et al.* 2009; Tscharntke *et al.* 2002). Dung beetles (Scarabaeinae) are no exception to this as they have been identified as indicators of environmental change and are subsequently sensitive to these changes (Bicknell *et al.* 2014). Dung beetle assemblages are known to be affected by fragmentation and habitat loss that leads to lowered species abundance, diversity and evenness in an area (Davis & Scholtz, 2004; Estrada *et al.* 1998; Hutton & Giller, 2003). Unfortunately, previously mined lands, demonstrate issues that may unfavourably impact dung beetle communities. Most organisms are primarily affected by the removal of vegetation, related habitat and food sources from an area. These organisms could potentially recolonise such sites once resources are restored (Brändle *et al.* 2000; Mrzljak & Wiegleb, 2000). Dung beetles are exposed to a multitude of problems on mined areas due to their dependency on soil, vegetation and dung (Davis, 1996; Davis *et al.* 2013; Nealis, 1977).

Both paracoprid (tunnelling) and telecoprid (rolling) dung beetles are dependent on soil type and texture that influence the water retention abilities thereof (Hanski & Cambefort, 1991; Barkhouse & Ridsdill-Smith (1986)). Dung beetles show differential affiliation to soils of varying hardness, composition and particle size and the combined water retention abilities thereof (Davis, 1996; Nealis, 1977; Osberg *et al.* 1994). These properties are known to influence nesting properties and have a strong link to offspring survival (Osberg *et al.*1994). Homogenised topsoil on reclaimed mined soils is known to be extremely compacted, nutrient deprived and have fluctuating extremes of water retention abilities (Bell, *et al.* 2001b; Boyer *et al.* 2011; Truter *et al.* 2013). Because of the above-mentioned factors, soil type also influences dung beetle assemblages through the preferences of some species (Hanski & Cambefort, 1991).

Soil conditions also influence vegetation cover that in turn has an impact on the local dung beetle population (Davis *et al.* 2014). Dung beetle association are not primarily dependent on plant heterogeneity but rather on the shade and microclimate related components produces by vegetation known as physiognomy (Davis *et al.* 2013; Doube, 1983). Mine altered shade availability has significant impacts on beetle assemblages particularly when the historical land cover had been predominantly forest (highly shaded) (Davis *et al.* 2013). Although eMalahleni is predominantly covered with grassland (lowered availability of shade), alteration in the vegetation structure may still have an influence on dung beetle fauna.

Both species richness and abundance of dung beetles in an area is also closely linked to the availability of a range of dung types and its abundance (Davis & Scholtz, 2001; Martin-Piera & Lobo, 1996). Unfortunately, large dung producing animals were mostly excluded from reclaimed mined sites to prevent harm to the miners, animals and herdsman. The lowered or absent availability of dung may further reduce the affiliation of dung beetles with these sites, that may require a dung establishment regime for beetle assemblages to increase.

A few studies have been conducted to investigate the effects of mining on dung beetle assemblages. These studies were primarily focussed on forests or woodland biomes (Davis *et al.* 2014; Davis *et al.* 2003). Even though eMalahleni (Mpumalanga) is the primary coal producer in South Africa, no study has yet assessed the impact on local dung beetle communities. Based on previous studies, we could expect that both dung beetle abundance and species richness will decline (Davis *et al.* 2014; Horgan, 2005). Although both variables tend to decline on disturbed / agricultural / mined areas, species richness seems most affected, possibly due to the reduction of a variety of dung sources.

The ability of Dung Beetles to improve soil physicochemical and hydrological properties through bioturbation and active incorporation of nutrient-rich dung, their presence is highly valuable on reclaimed mined soil (Nichols *et al.* 2008). For this reason, this study was undertaken to establish the dung beetle assemblages on coal mines of eMalahleni and compare them to reference sites that include Telperion Nature Reserve that is more representative of the vegetation and habitat before alteration and farms that have a high density of dung producing cattle. If the local abundances are too low, species of interest need to be identified for future breeding and release programs.

It was hypothesised that the assemblage of dung beetles will be higher on reference sites when compared to mined sites, with cattle farms having a similar high abundance but lowered species composition. Secondly, these differences will most likely be due to the absence of a diverse / abundant availability of dung (not investigated) and environmental differences in soil, vegetation, microclimate and soil bulk density between the sites.

2.2 Methods

2.2.1 Study taxa

For this study, only true dung beetles from the subfamily Scarabaeinae (Order Coleoptera, Family Scarabaeidae) were taken into consideration for identification and subsequent data analyses.

2.2.2 Study sites

Table 1: Key characteristics of the reclaimed and reference sites. All sites used during the population assemblage study for the period from February 2015 to April 2017.

Site	Predominant land use	GPS Co-ordinates	Altitude (m)	Annual Rainfall (mm)	Time since rehabilitation (years)
1	Reclaimed mined site	25°47'44.1"S 29°05'39.8"E	1 479	671	4
2	Reclaimed mined site	25°55'44.3"S 29°07'08.7"E	1 550	690	3
3	Reclaimed mined site	25°53'17.7"S 29°09'46.7"E	1 510	649	7
4	Reclaimed mined site	25°49'13.8"S 29°06'41.1"E	1 471	659	3
5	Reclaimed mined site	26°00'22.0"S 29°12'43.2"E	1 570	624	16
6	Reference site: Telperion Nature Reserve	25°48'32.0"S 29°11'05.9"E	1 521	684	N/A
7	Reference site: Cattle farm	25°43'55.7"S 29°03'33.5"E	1 430	662	N/A
8	Reference site: Cattle farm	25°41'31.2"S 29°03'35.2"E	1 440	673	N/A

Five surface coal mines with reclaimed areas were selected from the eMalahleni (Witbank) area in Mpumalanga, South Africa (Table 1; Figure 5). For comparison, three reference areas were selected. Two were commercial cattle farms (Site 7 & 8) and one was a private Telperion

Nature Reserve (Site 6). All sites were at least 6 km apart from one another. The area was classified as Mesic Highveld Grassland by Mucina & Rutherford (2006), that receives approximately 700 mm of rain per year, mainly in the summer months (Mucina & Rutherford, 2006). The vegetation type in the area is primarily Bankenveld that consists of Mesic grasslands, forested ravines, woodlands and wetlands (Acocks, 1988). Both cattle farms were chosen based on the information that no intensive, historic cultivation has taken place there. These cattle farms were primarily for pasture-fed beef production and included regular treatment of animals with anti-parasitics.

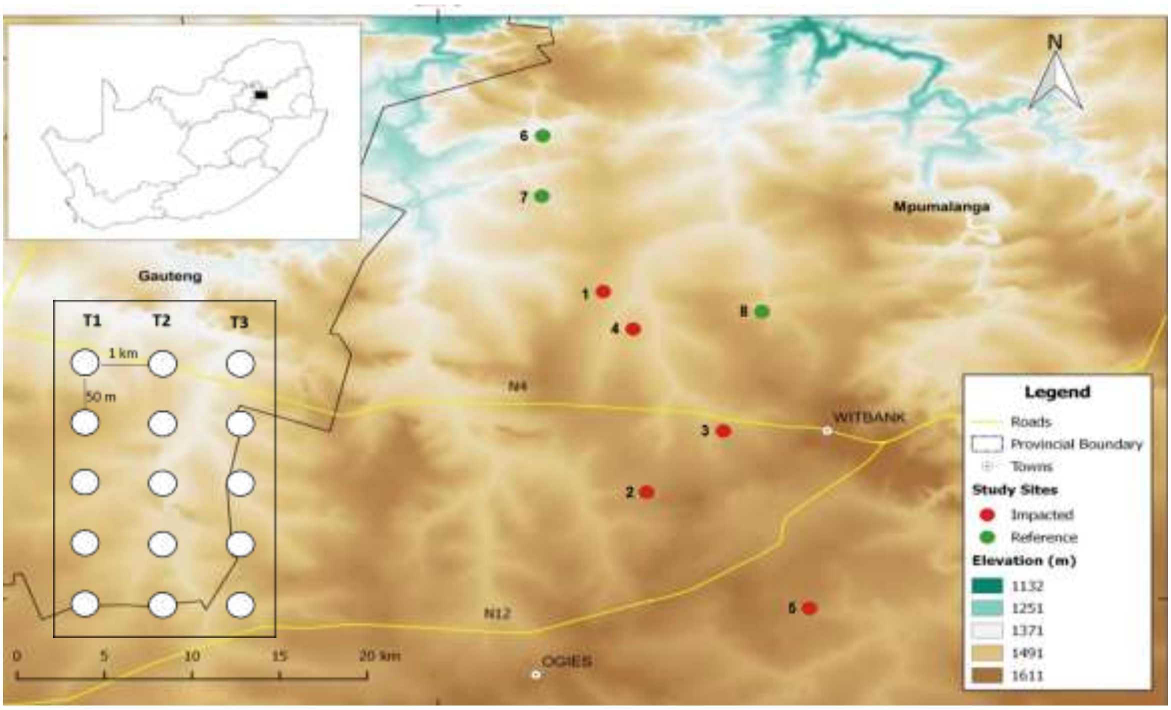

Figure 5: Local map of geographical relationship between sites. Red markers indicate reclaimed mined sites and green markers indicate reference sites. Trap design displayed in lower left corner. T1-T3 refers to transect 1 to 3 respectively.

2.2.3 Dung beetle sampling

Over a period of three years, dung beetles were sampled on nine occasions during the summer months. The first collection took place in 2015 during the first rainfall season, this included collection during February, March and April. Sampling during 2016 again took place February, March and April in with additional collections in October and November. Samples were collected in 2017 during February and April. This sampling protocol encompassed both inter-seasonal and inter-annual variation.

The sampling protocol consisted of three linear transects at each study site, each consisting of five traps, separated by 50 m (Figure 5). The traps comprised of a 2 L bucket filled with 250 ml of 5% water soap solution (to decrease surface tension). The soil was dug out to place the bucket into the soil making sure it was flush with the soil surface. A 250 ml dung bait was wrapped in curtain netting and suspended over the middle of the open bucket using wire. The bait consisted of a cattle-pig manure mixture in some three-part cattle to one-part pig manure ratio. This composite 3:1 ratio is known to attract more than half the species present in each locality (Davis, 2002).

A 48-hour sampling protocol was followed for each sampling trip, each trap was baited every 12 hours, and specimens were collected every 24 hours. This sampling schedule is known to account for the majority of the local diversity (Larsen & Forsyth, 2005). During collection of the samples, the specimens were removed from the soap mixture using a small sieve. The specimens were stored in 95% ethanol until identification. The beetles were categorised into morpho-species for species-level identification by Dr Adrian Davis of the Scarab Research Group at the University of Pretoria.

2.2.4 Environmental variables

These measurements were made in October and November of 2016, and February and April of 2017. iButtons® (DS1923L-F5/MAXIM) were used to measure temperature and humidity for each site every two minutes for the duration of each sampling trips. iButtons® were placed one meter above the soil surface and covered with a 1 L white bucket to shield them from wind and rain. I-button data was recorded on ColdChain Thermodynamics Microdevice CTMD software. A rain gauge was placed one meter above the soil surface at each site and rainfall was recorded every day during the sampling trips. Vegetation cover was assessed for each site by means of a Point Bridge meter. Four measurements (at least five meters apart) were taken at each transect of each site to obtain an average vegetation coverage. The Point Bridge meter consists of ten metal pins that are evenly spaced, each contact point with vegetation would represent 10% of the vegetation cover. The sampler was blindfolded and allowed to randomly select an area to place down the meter. A soil bulk density cylinder (250 ml) was used to take three samples along each transect at each site. Additional data were obtained from the South African Weather Services that included temperature, windspeed, rainfall and humidity for the duration of the project. These data were collected from Witbank Weather Station (0515320 8) and Kleinkopje Weather Station (0478391 9) from 01 January 2015 until 31 May 2017.

2.2.5 Data analyses

Beetles collected during each sampling period were identified and compiled into a list for analyses of assemblage per site and season. With this list, total species abundance and species richness could be determined (using Microsoft Excel 2013). To determine if sampling was sufficient, a species accumulation curve (Mao Tau's Rarefaction) was constructed for each site using P.A.S.T. 3.1.7. Where applicable, Bray-Curtis dissimilarity was used as it is a well-known and robust measurement to determine relationships in biological fields. A multiple comparison two-way ANOVA, coupled with Tukey's post-hoc test was used to determine if there was a significant difference in species richness and abundance between any of the sites and sampling seasons (using Graph-pad Prism 6). To determine if reclaimed mined sites differed more in terms of dung beetle assemblage between sites than within sites for nine sampling periods, an analysis of similarity (ANOSIM) was used in Graph-pad Prism 6. This was strengthened by using a permutational multivariate analysis of variance (perMANOVA) to compare sites based on beta-diversity. The p-values for both aforementioned tests were corrected using Bonferroni's Criteria. Furthermore, to visualise the similarity or dissimilarity between sites in terms of species richness and abundance, non-metric Multidimensional Scaling (nMDS) ordination was constructed using P.A.S.T. 3.1.7. An Unweighted Pair Group Method with Arithmetic mean (UPGMA) was used in addition to the nMDS to determine similarity between sites, based on dung beetle assemblage with Bray-Curtis dissimilarity measures (bootstrapping at 9999). Various diversity indices were calculated for all sites with a focus on both Shannon-Wiener and Simpson's Diversity Indices using Rstudio 2012 and tested for significance using Two-way ANOVA. The IndVal package in Rstudio was used to determine if any indicator species were present at the reclaimed mined sites. All analyses were considered significant if the *p*-values obtained were less than 0.05.

The relationship between the measured environmental variables and the beetle assemblage across a spatial and temporal gradient was assessed using a Canonical Correspondence Analyses (CCA) in P.A.S.T. 3.1.7. Environmental data was not collected during 2015 and the first two collection periods of 2016, with the first collection that included this data being October 2016. Using Linear regression, every variable was tested against abundance and species richness to determine if they had a significant influence (Graph-pad Prism 6). When applicable data were log-transformed and had to comply with a Shapiro-Wilks test for normal distribution of the data.

2.3 Results

2.3.1 Assemblage

The 72 467 specimens of Scarabaeinae collected from all sites included members of nine tribes and 96 species (Table 6). Predictably, the sites with the most abundant beetles were the reference sites, with the highest abundance of 40 914 individual specimens collected at Telperion Nature Reserve (site 6) (Table 6). Of the reclaimed mine sites, Site 3 had the highest abundance with 5 272 individual specimens collected. Site 5 had the least abundance and species richness of all the sites with 1 735 specimens collected for 43 species. The number of species per site, in ranked order from most to least abundant is summarised in Table 7.

Scarabaeus ambiguus (Boheman, 1857) was the single most abundant species with 11 534 individuals in total. These were collected across all the sites with most of the individuals being collected from the Telperion Nature Reserve (Site 6). *Proagoderus sapphirinus* (Fahraeus, 1857), *Onthophagus sp. 1* (*nr sugillatus* NW), *Onthophagus pauxillus* d'Orbigny (1902), *Pachylomera femoralis* (Kirby, 1828), *Onthophagus cyaneoniger* d'Orbigny (1902), *Kurtops signatus* and *Scarabaeus heqvisti* zur Strassen (1962) were the nine most abundant species after *S. ambiguus* and comprised 60% of all the specimens collected during the three years of the study.

Species rarefaction curves for each site (Mao Tau) approached an asymptote, indicating that sampling was sufficient for the methodology followed, with taxa accumulation increasing by a negligible amount if sampling were to continue (Figure 6).

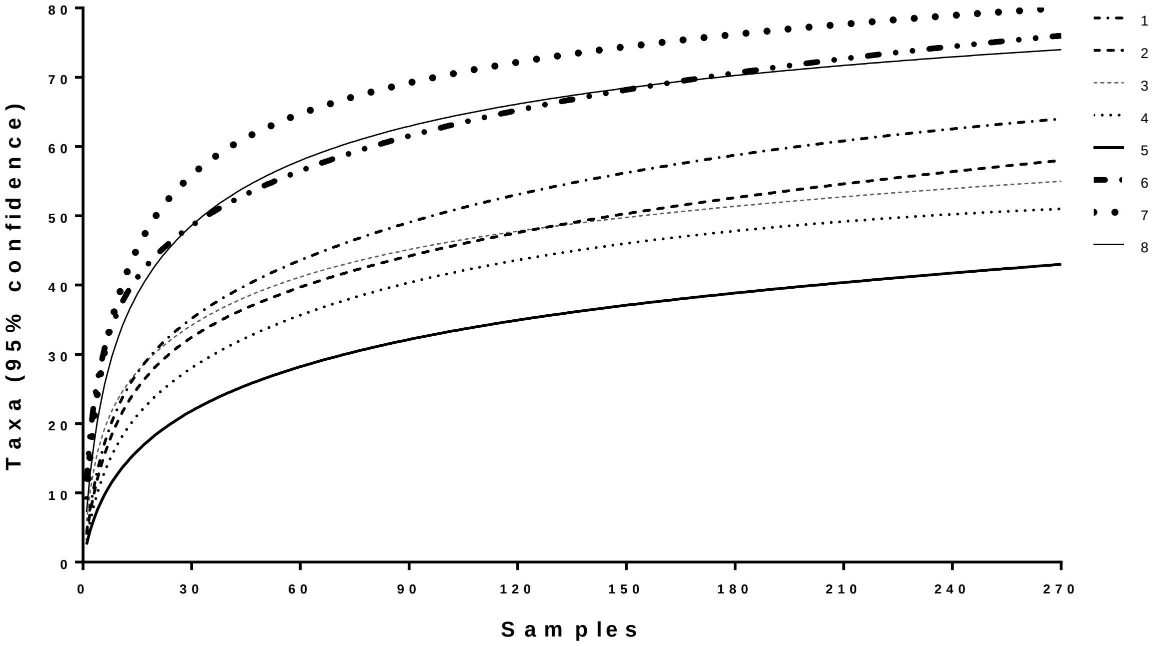

Figure 6: Species accumulation curve (Mao Tau) for 270 samples collected during nine sampling periods between March 2015 and April 2017, for 5 reclaimed mined sites (1-5), two cattle farms (7 & 8) and Telperion Nature Reserve (6).

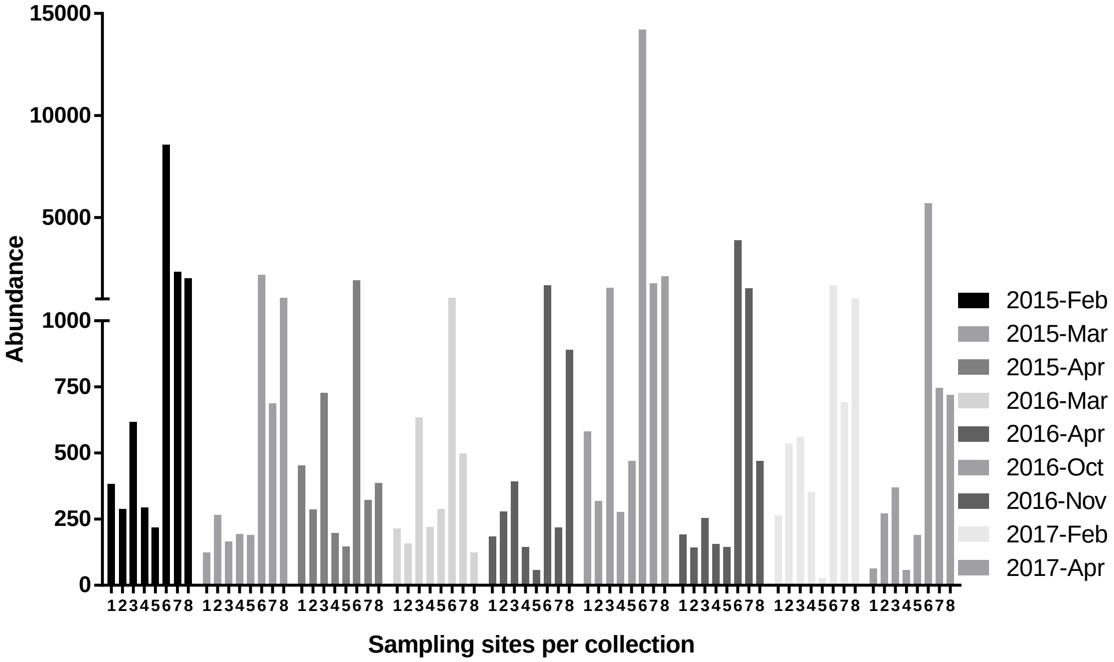

Figure 7: Total dung beetle abundance for all sites and sampling periods. Sites 1-5 are reclaimed mined sites, Sites 7-8 are cattle farms and the Telperion Nature Reserve (Site 6).

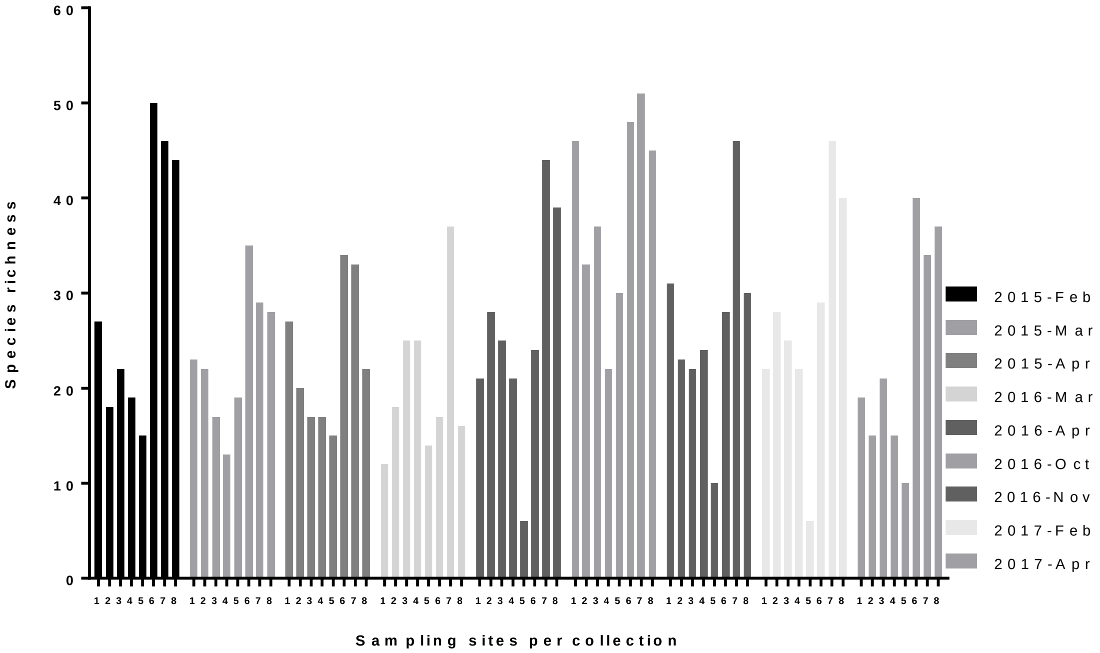

Figure 8: Total species richness for all sites and sampling periods. Site 1-5 indicate reclaimed mined sites, Site 7-8 indicate cattle farms and site 6 indicates the Telperion Nature Reserve.

Dung beetle abundance was significantly higher at the Telperion Nature Reserve (Site 6) than any other site (Figure 7; $F_{(7,\ 56)} = 8.61$, $p<0.05$). Dung beetle abundance at the two farm reference sites was not significantly higher than any of the reclaimed mined sites.

Species richness differed significantly between sampling sites (Figure 8; $F_{(7,56)} = 17.61$, $p<0.05$). Site 1 (reclaimed mine) was only significantly different when compared to site 5 (reclaimed mine) and site 7 (cattle farm). Sampling season also significantly influenced species richness, with higher values after October 2016 when compared to early 2015 ($F_{(8,56)} = 6.04$, $p < 0.05$).

A one-way ANOSIM determined that reclaimed mined sites were significantly dissimilar from reference sites in terms of dung beetle assemblages ($R=0.55$, $p<0.05$) (higher similarity within mined sites and reference sites than between mined sites and reference sites).

Reclaimed mine sites 1, 3 and 4 were also significantly different from site 2 and 5. An nMDS ordination showed a cluster that comprised all the reference sites, of which the Telperion Nature Reserve is the furthest removed, with cattle farms in close proximity to reclaimed mined sites (Figure 9). Site 5 is the furthest removed from the reference sites and other mined sites. The UPGMA dendrogram (Bray-Curtis) also shows the dissimilarity between reference sites and reclaimed mined sites (Figure 10).

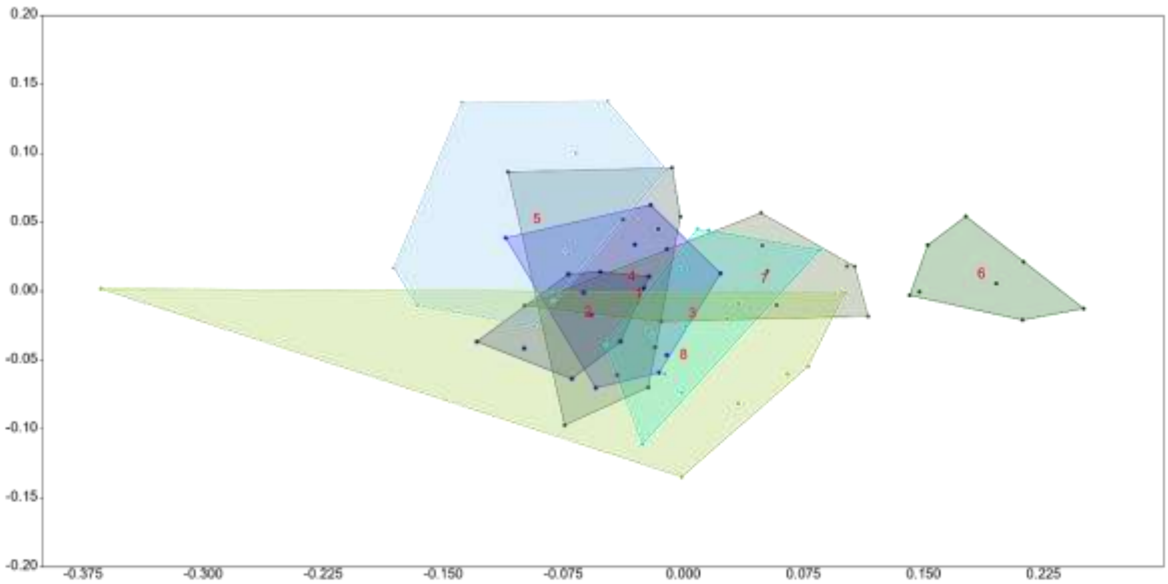

Figure 9: Non-metric multidimensional scaling ordination that shows patterns of distribution for the assemblages between 5 reclaimed mined sites (Sites 1-5) and three reference sites (Sites 6-8) based on the Bray-Curtis similarity index.

Species diversity indices, including Shannon Wiener and Simpson's indices, were relatively low with no significant difference between sites ($F_{(7,\ 48)} = 1.00$, $p > 0.05$; Table 2). Lower index values indicate sites that are lower in species richness or have sites that have high numbers of individual species.

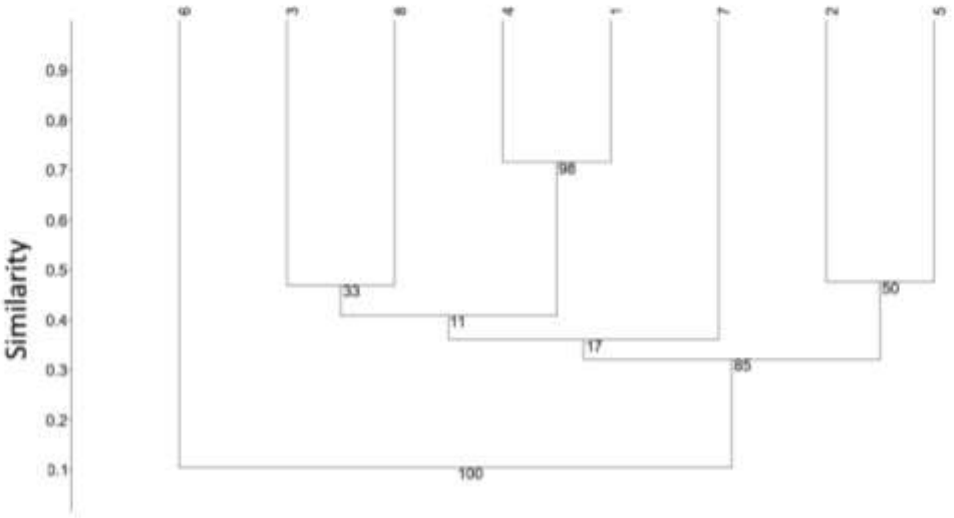

Figure 10: Classical UPGMA dendrogram depicting similarity of assemblages between sites. Bootstrapping at 9999 with Bray- Curtis similarity.

Table 2: Diversity indices for reclaimed coal mined sites (Sites 1-5), Telperion Nature Reserve (Site 6) and cattle farms (Sites 7-8).

Site	1	2	3	4	5	6	7	8
Species Richness	64	58	55	51	43	76	80	74
Abundance	2466	2552	5272	1896	1735	40914	8804	8828
Dominance	0,07	0,12	0,07	0,08	0,18	0,10	0,08	0,09
Simpson	0,93	0,88	0,93	0,92	0,82	0,89	0,92	0,91
Shannon	3,11	2,79	2,90	2,92	2,35	2,74	3,14	2,99
Evennes	0,35	0,28	0,33	0,36	0,24	0,20	0,29	0,27
Brillouin	3,06	2,74	2,88	2,86	2,31	2,74	3,12	2,97
Menhinick	1,29	1,15	0,76	1,17	1,03	0,38	0,85	0,79
Margalef	8,07	7,27	6,30	6,63	5,63	7,06	8,70	8,04
Equitability	0,75	0,69	0,72	0,74	0,63	0,63	0,72	0,70
Fisher alpha	12,01	10,56	8,56	9,65	7,98	9,03	12,14	11,07
Berger-Parker	0,14	0,30	0,13	0,14	0,36	0,23	0,21	0,20
Chao-1	71,33	76,20	59,00	52,67	45,50	85,17	84,67	76,50

2.3.2 Environment

Increasing bulk density ($F_{(1, 30)} = 8.61$, $p < 0.05$; $R^2 = 0.22$) vegetation cover ($F_{(1, 30)} = 12.07$, $p < 0.05$; $R^2 = 0.29$) and sand percentage ($F_{(1, 30)} = 5.46$, $p<0.05$; $R^2 = 0.15$) (Table 3) were all found to account for higher species richness as determined in the general linear model (Figure 11). An increase in clay ($F_{(1, 30)} = 5.58$, $p<0.05$; $R^2 = 0.16$) and silt ($F_{(1, 30)} = 3.09$, $p>0.05$; $R^2 = 0.09$) percentage was found to be associated with a decrease in species richness with only clay being highly significant. No other variable was found to influence abundance or species richness. The reference sites had soil profiles that were less homogenized than that of the mined sites, with higher sand percentages (Table 4). Although bulk densities were comparable between all sites, mined sites had highly compacted clay dominant soils. Reference sites also had higher vegetation cover than the reclaimed mined sites (Table 3). Climate (temperature, humidity and rainfall) was similar between sites during each sampling trip (Table 3). Canonical Correspondence Analyses indicated that the measured environmental variables had a significant influence on the dung beetle assemblages across the difference sites (Figure 12).

Table 3: Environmental variables collected for four sampling periods between October 2016 and April 2017.

Site	Date (M-Y)	Temperature average (°C) (Mean ± SD)	Humidity average (%) (Mean ± SD)	Monthly Rainfall (mm) (Mean ± SD)	Bulk density (g/cm^3) (Mean ± SD)	Vegetation cover (%)
1	10-2016	23,56 ± 0,78	51,89 ± 1,05	84 ± 1,33	2,19 ± 1,06	4,8 ± 1,32
1	11-2016	26,6 ± 0,90	49,06 ± 0,61	224,6 ± 0,5	1,93 ± 1,02	4,1 ± 0,32
1	02-2017	26,69 ± 2,66	76,03 ± 1,75	127,8 ± 4,8	2,05 ± 1,02	4,6 ± 0,84
1	04-2017	20,2 ± 1,89	71,65 ± 1,30	113,6 ± 2,23	2,19 ± 1,62	4,4 ± 0,70
2	10-2016	23,18 ± 1,17	53 ± 1,26	94 ± 0,88	1,9 ± 1,55	5,7 ± 1,34
2	11-2016	26,18 ± 0,81	58,83 ± 0,84	250,33 ± 1,78	1,45 ± 1,07	4,9 ± 0,88
2	02-2017	26,32 ± 2,27	71,56 ± 1,53	138,38 ± 2,03	1,72 ± 1,05	5,2 ± 0,79
2	04-2017	18,74 ± 1,05	97,04 ± 1,07	125,25 ± 1,79	1,64 ± 1,33	5,1 ± 1,10
3	10-2016	23,18 ± 0,57	53 ± 0,71	105 ± 1,09	1,91 ± 0,84	6,6 ± 0,84
3	11-2016	25,63 ± 0,26	59,27 ± 0,48	288,35 ± 0,64	1,67 ± 0,89	5,4 ± 0,70
3	02-2017	26,23 ± 0,96	18,1 ± 0,76	145,21 ± 1,32	1,88 ± 1,53	6,1 ± 0,57
3	04-2017	18,74 ± 1,26	97,04 ± 1,04	136,25 ± 0,41	1,85 ± 0,89	5,7 ± 0,82
4	10-2016	23,56 ± 0,59	51,89 ± 1,05	81,5 ± 2,82	1,82 ± 0,83	2,5 ± 1,51
4	11-2016	26,2 ± 1,06	49,78 ± 0,88	204,4 ± 1,46	1,84 ± 0,68	1,5 ± 0,71
4	02-2017	25,16 ± 1,01	66,53 ± 1,02	123,82 ± 1,11	1,79 ± 1,03	2,2 ± 1,03
4	04-2017	20,2 ± 0,96	71,65 ± 0,87	120,59 ± 1,33	1,74 ± 1,16	2,2 ± 0,79
5	10-2016	24,39 ± 1,53	49,17 ± 1,02	79 ± 1,41	1,44 ± 0,78	3,6 ± 0,52

5	11-2016	25,69	±	1,40	57,65	±	1,18	184,2	±	1,45	1,59	±	1,11	2,6	±	0,97
5	02-2017	25,04	±	0,53	73,48	±	0,66	119,84	±	0,97	1,48	±	1,07	3,2	±	0,79
5	04-2017	18,8	±	1,37	82,06	±	1,06	127,57	±	0,67	1,47	±	1,11	2,9	±	0,74
6	10-2016	24,65	±	1,10	50,72	±	1,10	94,33	±	0,95	1,97	±	1,18	7,1	±	1,10
6	11-2016	27,39	±	2,63	55,08	±	1,86	254,43	±	0,62	1,47	±	1,23	6,1	±	1,10
6	02-2017	28,47	±	0,94	77,7	±	1,02	137,13	±	2,17	1,89	±	0,93	6,9	±	1,10
6	04-2017	22,25	±	1,05	78,36	±	1,06	125,03	±	1,09	1,65	±	1,61	6,3	±	1,06
7	10-2016	22,77	±	1,03	52,44	±	1,00	89,17	±	1,07	1,86	±	1,10	7,4	±	0,97
7	11-2016	26,18	±	1,60	59,24	±	1,38	239,51	±	1,06	1,68	±	2,63	7,3	±	1,16
7	02-2017	23,3	±	1,36	71,68	±	1,25	132,47	±	0,96	1,79	±	0,94	7,2	±	1,14
7	04-2017	20,2	±	2,22	71,65	±	1,74	119,32	±	1,21	1,85	±	1,05	7,3	±	1,25
8	10-2016	24,25	±	1,02	57,55	±	0,92	99,67	±	0,88	2	±	1,03	6,3	±	0,82
8	11-2016	26,5	±	1,98	64,57	±	1,52	271,39	±	0,70	2,1	±	1,60	5,3	±	1,06
8	02-2017	25,53	±	1,85	84,51	±	1,59	141,17	±	1,02	1,99	±	1,36	6	±	1,33
8	04-2017	19,5	±	2,24	76,85	±	2,04	130,64	±	0,86	2,01	±	2,22	5,6	±	1,84

Table 4: Soil composition (clay, silt and sand percentage) for each site.

		Soil composition % (Mean ± SD)								
Study Site	N	Clay			Silt			Sand		
Reclaimed mined sites										
1	4	19,1	±	0,76	4,25	±	1,1	76,65	±	0,34
2	4	27,95	±	3,23	16,3	±	7,8	55,73	±	10
3	4	14,02	±	0,05	7,88	±	0,69	78,13	±	0,62
4	4	21,05	±	2,58	28,53	±	1,61	50,4	±	4,16
5	4	13,17	±	2,06	8,8	±	10,14	77,95	±	11,38
Telperion Nature Reserve										
6	4	11,52	±	1,82	3,45	±	2,01	85	±	1,3
Cattle farms										
7	4	9,675	±	0,46	4,1	±	1,07	86,25	±	0,7
8	4	13,02	±	4,99	3,63	±	0,43	83,33	±	4,92

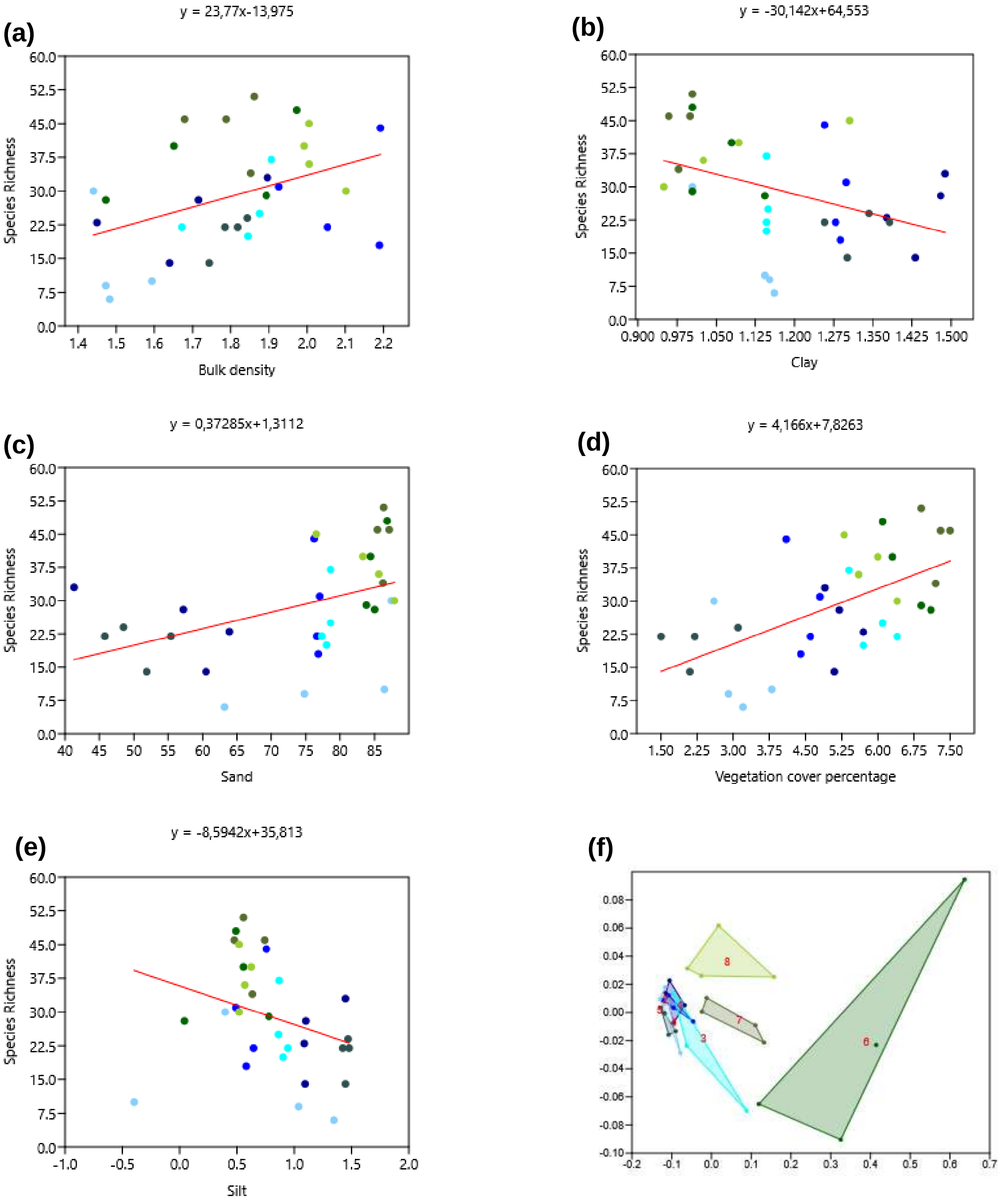

Figure 11: Linear regression for (a) bulk density (R^2= 0.22), (b) clay (R^2=0.16), (c) sand (R^2=0.15), (d) vegetation cover (R^2=0.29) and (e) silt (R^2=0.09) to species richness. (f) nMDS plot of different sites ordinated according to environmental similarities. Blue sites indicate reclaimed mined sites, whilst green indicate reference areas.

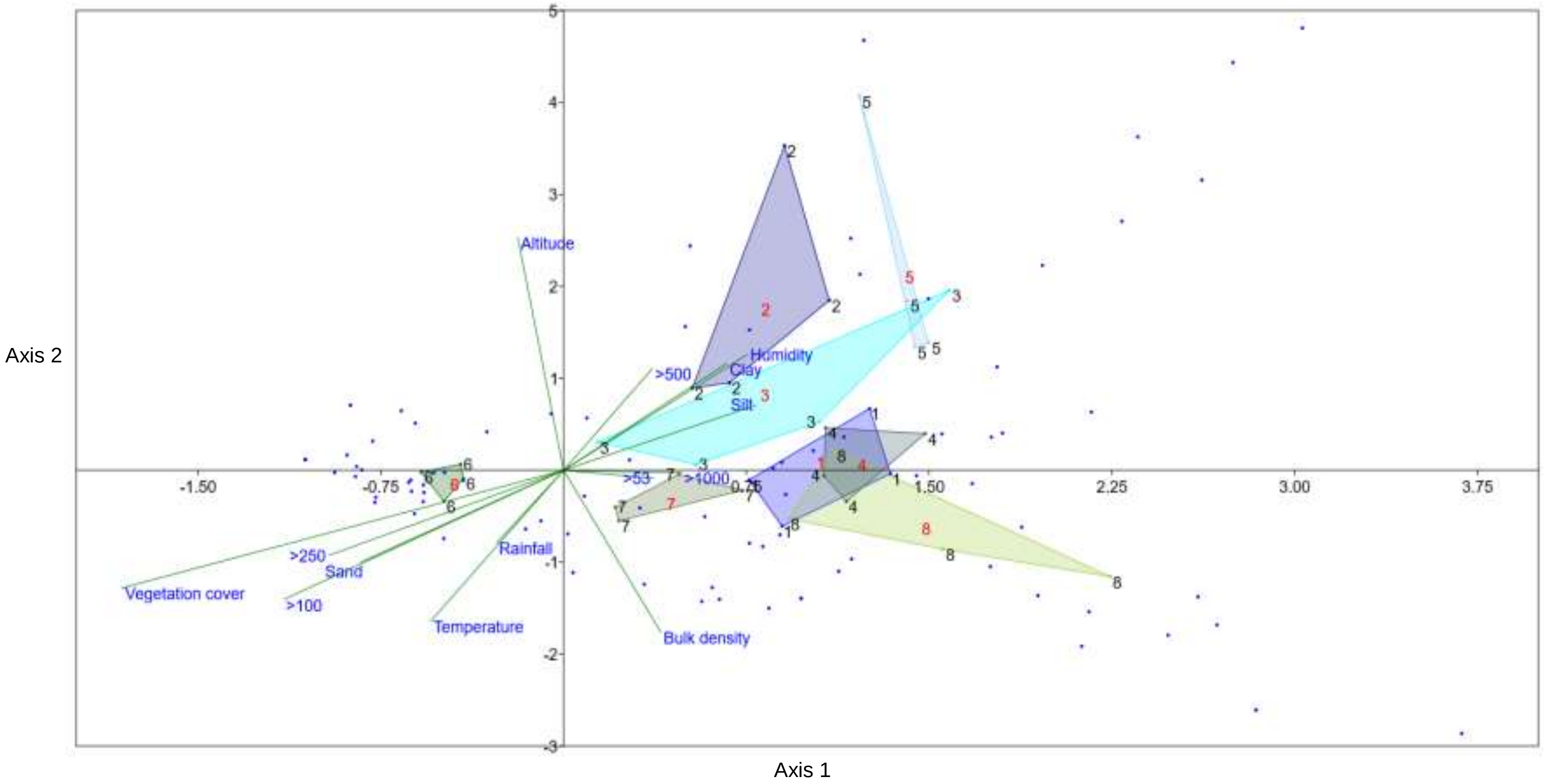

Figure 12: Canonical Correspondence Analysis (CCA) ordination of dung beetle assemblages across reclaimed mined sites (Sites 1-5), Telperion Nature Reserve (Site 6) and Cattle farms (Sites 7-8). Vector lines indicate influence of the environmental variables on dung beetle assemblage with length indicating relative strength. Convex hulls indicate each study site across nine sampling seasons with blue dots indicating species (abundance and richness). Values (>53- >1000 indicate soil particle size). Eigenvalue 0.42.

2.4 Discussion

This study has demonstrated that with the provision of dung, dung beetles are still active on reclaimed mined sites, most likely mediated by farms that act as source populations in the vicinity. Although assemblage structure differed between land use types with significantly lowered abundance on reclaimed mined sites, diversity of dung beetles was higher than anticipated. These differences were most likely due to the absence of a diverse group of dung producing mammals and abiotic variables related to soil condition and vegetation cover. Although the presence / absence of dung wasn't measured in this study, the assumption was made that dung provision would be low as no livestock are maintained or encouraged on the rehabilitated coal mine sites. Otherwise it sounds like it's an unknown that could be problematic. Despite having a lower diversity of beetles and dung sources, mined sites have a relatively high abundance of some species that could vastly improve mining rehabilitation conditions through their tunnelling abilities.

Dung beetle abundance was much higher on the Telperion Nature Reserve compared to any other site (Table 5). Reference farms were found to be comparable to reclaimed mined sites in terms of abundance even though the total abundance of beetles was slightly higher for farms. Although the diversity of species was lower on reclaimed sites than reference sites, the cumulative species richness was relatively high for disturbed conditions, with the season-specific numbers varying more for mine sites than that of the reference sites (Table 5). Two studies by Davis *et al.* (2014) and Almeida *et al.* (2011) had similar findings where comparable dung beetle abundances were found between disturbed and "natural" sites with disturbed sites showing a lowered diversity of dung beetles. Species diversity indices yielded no difference between sites with most values being extremely low (Table 5). This indicated that most sites were dominated by a few species that were captured in higher abundance with many species only represented by a few collected individuals for the site. The similarity in indices between sites may also be due to either the large sample size or due to the fine scale of sampling.

Although farms and mined sites were similar in dung beetle abundance and in some cases beetle diversity, the assemblage structure (distribution of numbers between species) was found to be significantly different between the reference sites (farms and Telperion Nature Reserve) and the reclaimed mined sites (Figure 7, R=0.55, $p<0.05$). Site 1 was found to be only slightly different from the closely situated cattle farm (Site 7) approximately 6.20 km away. Additionally, site 8 and 3 were more related in terms of assemblage structure as the farm site 8 was only 8.57 km from the mined site 3. Despite being separated by a similar distance (9.10 km), Site 7 and 4 were not closely related and this may be due to the frequently used large dirt road separating the sites. Site 5, on the other hand, was the furthest from the two sampled farm sites and shows the lowest degree of similarity of all the mined sites.

The clusters of the farms and mined sites overlap extensively in the nMDS ordination (Figure 9) that indicates their similarity in assemblage structure across the various sampling trips. The Telperion Nature Reserve is not as closely related, as supported by the placement of the reserve as the outgroup in the dendrogram (Figure 10). The overlap in community structure between cattle farms and reclaimed mined sites could suggest that farms adjacent to mined sites act as source populations that could colonise the mined sites when dung becomes available. This also indicates that dung beetle species associated with pastures or farms would be well adapted to utilise mined sites. There are however still some dissimilarities between the reference sites and the mined sites that may be due to some species that prefer disturbed areas or are colonising the mined sites from other farms that were not sampled.

Although the direct loss of habitat and other environmental and anthropogenic factors might have led to the dissimilarity between mined sites and the reference sites, the decline of dung beetle numbers and diversity on reclaimed sites are most likely related to the absence of a diverse set of dung producing mammals (Hanski & Cambefort, 1991). Many studies have shown a positive correlation between abundance and species richness of mammals and that of dung beetles (Estrada *et al.* 1998; Feer & Hingrat, 2005; Klein, 1989). Dung availability, freshness and type will have an influence on the dung beetle community structure (Fincher *et* *al.* 1970). Goats, sheep and even cattle were occasionally observed at Sites 1-3 despite all the reclaimed mined sites (in this study) actively discouraging domestic herbivores, during the span of this study, to ensure the safety of the miners, herdsman and animals. Notably, no medium / large herbivorous mammals were observed on either of the two least abundant dung beetle sites (Sites 4 & 5). Given that many rehabilitation programs aim to utilise post-mining lands for cattle grazing, the notion that the local dung beetle abundance can increase is possible, at least to resemble the structure of the current reference farms. Quintero & Roslin (2005), found that dung beetle assemblages of forest fragments in Central Amazonia had returned to a natural state in a decade with the regeneration of secondary vegetation. This effect might also be seen with species more adapted to pastures and mined lands when dung becomes available in the future. These findings are supported by several other studies that have investigated dung beetle communities across fragmented landscapes (Davis & Philips, 2009; Estrada *et al.* 1998; Tscharntke *et al.* 2002). More valuably, the results of this study are supported by a study conducted by Davis *et al.* (2014), in which dung beetle responses were compared to environmental and land use changes in the Phalaborwa-Timbavati Mopaneveld, South Africa. Davis *et al.* (2014) found a higher dissimilarity in dung beetle assemblage between natural areas and mined land, than between natural areas and farming lands. This similarity could be less pronounced in our study as it occurs in a grassland biome. Although the difference in vegetation cover and dung diversity is noticeable, the plant physiognomy and microclimates are more similar between grasslands (Telperion Nature Reserve) and cattle farms than would be the case in other biomes such as savannas and forests.

No indicator dung beetle species were identified for any of the mined sites, despite some species occurring at high numbers only on mined sites. Species that only occurred on mined sites include *Onthophagus binodis* (n=6) and *Caccobius sp.* 1 *that* were recorded in low numbers (n=1). Additionally, some species have been identified that occurred in higher numbers on some reclaimed mined sites than on reference sites like *Euoniticellus intermedius* and *Digitonthophagus gazella*. These two generalist species have been mass-reared and used extensively in the past (Bornemisszza, 1970) to improve pastures, reduce dung breeding pests and provide many other services (Bertone *et al.* 2006; Miranda *et al.* 2000). Their presence on mined sites could prove to be beneficial in the future.

It was clear that although dung beetle assemblage structure was different between sites and species richness was lower on mined sites, that many species can colonise these sites once dung becomes available. The beetle species that do occur in high numbers on reclaimed mined sites (Site 1-5) such as *E. intermedius* and *D. gazella* are both species that have been successfully used in the past to improve pasture conditions (Bornemissza, 1970). These species thrive on cow dung and do not require multiple dung sources and types thereof (Miranda *et al.* 2000). This is an ideal situation as the mined sites will most probably only have cattle dung available. Additionally, due to the high numbers (Table 5) in which these beetles were observed to colonise the mined sites with limited application of dung, it seems unnecessary that breeding and release of dung beetles will be required. If a stable source of dung is present, applied regularly by workers or by grazing cattle, a beneficial population of dung beetles could be maintained for the purposes of improving soil quality.

The increased number of beetles sampled between October and February indicated a previously described seasonal pattern of beetles that emerge after the winter diapause that correlates with an increase of rainfall and temperature (Davis, 1996). The impact of rain on recorded numbers for this study might have been skewed due to the large storms during our November 2016 trip. During this collection period, rainstorms flooded many of the traps on various sites. Dung beetles tend to abstain from flying and feeding on colder, overcast wet days and only emerge (in possibly higher numbers) immediately after significant rains. This might also have resulted in lowered abundance and diversity on days that rain was recorded. The influence of environmental variation justifies the requirement for inter and intra seasonal sampling frequency.

The Canonical Correspondence Analysis (Figure 12) showed that at least 40% of the variation between sites can be explained by the measured environmental variables. The farm sites (Site

7 & 8) were again grouped closely with the reclaimed mine sites. Both these land-use types show a higher percentage of clay and silt soils that the beetles are affiliated with as opposed to the Telperion Nature Reserve that has soils of higher sand content. In general, it has been documented that deeper sandier soils will support a higher number of beetles as is present in the Telperion Nature Reserve (Site 6) (Davis, 2002; Nealis, 1977). The scattering of species around the farms and reclaimed mine sites that show a reduced affinity to the relevant environmental vectors, may indicate that mined areas and farms have a higher percentage of generalist species. This is in contrast to the closely grouped species around the Telperion Nature Reserve that might indicate a more specialised and better-established dung beetle assemblage. Temperature differences seem to affect species more that are affiliated with the Telperion Nature Reserve (Site 6) along with vegetation cover and total monthly rainfall (Figure 12). Climate (rainfall and temperature patterns) is known to influence dung beetles on a seasonal scale more so than on diel activity patterns (Davis, 2002).

The Telperion Nature Reserve (Site 6) was the most variable in soil, vegetation and rainfall profile that may have contributed to a higher species richness. It seems that species richness is positively correlated with bulk density if the soil has a higher sand percentage. Hanski & Cambefort (1991) outline that fast-burying dung beetles prefer easily penetrable soils while smaller slow-burying species prefer harder soils. Osberg *et al.* (1994) found that preference to soil type is most likely related to the tendency of soils becoming waterlogged that is more prevalent in soils higher in clay and silt.

A variety of limiting factors affected the design and implementation of this study. Strict mining regulations related to site access impeded efforts to sample according to the schedule known to account for the majority of local diversity (Larsen & Forsyth, 2005). Baiting was only done once in the morning as opposed to the recommended twice daily samplings at dawn and dusk. Labour strikes and blasting were two additional factors that interrupted the sampling efforts on mined sites. Blasting that restricted our access and was especially prevalent on Site 4 may explain the lowered diversity of recorded dung beetles there. On cattle farming sites, traps were prone to destruction due to cattle movement and traps in the Telperion Nature Reserve (Site 6) were subject to removal by jackals and baboons. During March 2016, construction of a housing complex at Site 8 forced us to move a transect.

Species accumulation curves (Mao Tau's Rarefaction) that reached an asymptote for all eight sites showed that sampling at each site was sufficient for the methodology followed. It was suspected that species richness could have been increased to include nocturnal and crepuscular species if an additional baiting could take place in the late afternoon. Additionally, using a variety of dung types might have increased the recorded diversity, as some dung beetles do feed exclusively on certain dung types (Fincher *et al.* 1970). Trapping was primarily done in summer rainfall periods and may exclude some winter occurring dung beetle species. Nonetheless, collected abundance and species richness should provide a good approximation of the beetle assemblage of each site assemblage in the area.

Future studies should consider other environmental variables such as dust (that is a frequent occurrence in the area), light intensity and vegetation height and diversity. These factors could provide further insights along with increased sampling of local farms in the area. Dung beetle functional classification could also increase the current understanding of beetle assemblage structure and could be included in additional studies. This would include describing each species in terms of nesting and dung utilization behaviour, seasonal and daily activity, soil preference and size. Additionally, it is recommended that a variety of reclaimed mined sites should be assessed, as all our sites were managed by a single mining operation that follows a predetermined rehabilitation procedure on all sites. Including sites from other companies may yield different results. Further studies are needed to determine if beetle assemblages can return once sites are completely open to domestic or wild dung producing animals.

The findings of this study thus provide a comprehensive account of the local dung beetle community that was obtained by outlining the beetle assemblage in the area and comparing it to adjacent land use types. Species such as *E. intermedius*, *D. gazelle* and *O. alexis* are both present and abundant on reclaimed coal mine sites and have frequently been mass-reared for dung burial in pastures in Australia and other countries. This provides the foundation for the use of dung beetles to improve soil physicochemical properties on degraded coal mined soils in South Africa and elsewhere in the World (Bett *et al*., 2014).

Chapter 3

Dung beetles can tunnel into highly compacted soils from reclaimed mined sites in eMalahleni, South Africa

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3.1 Introduction

Dung beetles provide numerous ecosystem services through their activities in soil (Nichols *et al.* 2008). They improve soil hydrological properties such as increasing water infiltration rates and reducing soil bulk density due to their bioturbation of soil (Brown *et al.* 2010; Mittal, 1993). Dung beetles improve nutrient cycling by incorporating organic matter into the soil, a process that also promotes secondary dispersal of seeds present in dung (Nichols *et al.* 2008; Shepherd & Chapman, 1998). The aforementioned benefits derived from dung beetle activities collectively work to increase plant biomass yield that may rival that of chemical fertilizers (Bang *et al.* 2005; Miranda *et al.* 2000).

For these reasons, the utilization of dung beetles to improve soil properties and subsequently crop/ plant production on degraded land such as reclaimed mine sites could potentially improve post-mining land use options. Compaction is a major problem associated with reclaimed mine areas that creates significant challenges for establishment and plant root penetration (Bassett *et al.* 2005; Sheoran *et al.* 2010). Agro-ecosystems generally have a soil strength below 2 000 kPa whereas reclaimed mined sites are much more variable, but frequently have values exceeding 3 000 kPa (Materechera *et al.* 1991). Soil compaction not only limits plant growth but may also restrict the abilities of dung beetles to tunnel into the soil and bury dung.

The tunnelling abilities of dung beetles in compacted soils have not been extensively studied. Osberg *et al.* (1994) investigated offspring survival in a range of soil types and moisture content, none of which had any influence on tunnelling depth. Additionally, Brussaard (1983) investigated the influence of soil penetration resistance on the tunnelling ability of a single species. This showed no discernible connection between the depth of the terminal brood ball and the penetration resistance of the soil.

The way in which beetles construct nests is complex and diverse and is summarised in a book by Halffter & Edmonds (1982). Most species construct dung broods (brood balls) that contain single or multiple eggs. There are seven nesting types described, with paracoprids (tunnelers) having three types (Type 1-3), while telecoprids (rollers) and endocoprids each have two types (Types 4-5 and 6-7 respectively) (Halffter & Edmonds, 1982).

Type 1 is characteristic of many slow-burying Onitini, Coprini, Onthophagini, Oniticellini and Dichotomini that usually only require the male for reproduction (Marvier *et al.* 2004). In contrast, Type 2 nests are found in fast-burying members of Dichotomini and Coprini that might show co-operation between males and females during nest construction (Davis *et al.* 2008). Type 3 is a variation in which dung is placed in a shallow tunnel below the soil surface before retrieval and subdivided within the branched nests (Davis *et al.* 2008). Type 4 and 5 nests are characteristic of small beetles from Canthonini, Scarabaeni, Gymnopleurini, Sisiphini, larger beetles from Scarabaeini and some individuals from Canthonini respectively. While Type 6 is not exhibited in any South African taxa, Type 7 is demonstrated in endocoprid Oniticellini (Davis *et al.* 2008).

Three dung beetle species that have been successfully bred for export (Waterhouse, 1974) occur naturally in the coal mining area of eMalahleni, South Africa (Chapter 1). As tunnelling (paracoprid) beetles, *Onitis alexis* (Klug, 1835), *Digitonthophagus gazella* (Fabricius, 1787) and *Euoniticellus intermedius* (Reiche, 1849) would be ideal candidates for mass rearing and application as part of the mine reclamation process, provided they can tunnel into compacted soils. The aim of this study is to determine if the level of soil compaction (measured as penetration resistance) could limit dung beetle tunnelling and if any of the three species of interest are more affected by high compaction rates than others.

3.2 Methods

3.2.1 Study site

The study was conducted on a reclaimed mined section of an open-cast coal mine in eMalahleni, Mpumalanga Province, South Africa (26°0'33.87"S, 29°12'55.32"E). Rehabilitation commenced approximately 16 years prior to the study. Soil penetration resistance at the site ranged from 100 kPa to 5 000 kPa (equipment maximum), with an average of 3 193 kPa that was measured within the first 22 cm of the soil surface. The study took place in an area considered representative of the soil strength on the site and measured approximately 100m^2. The soil in the study area was classified as a sandy clay loam. This soil type (mixed soil with a higher clay content) was common in the area. The typical soil profile consisted of a waste coal layer covered by topsoil that was as shallow as 10 cm in places.

3.2.2 Study taxa

Three species of dung beetles (Subfamily Scarabaeinae) were used in this study: *Onitis alexis*, *Digitonthophagus gazella* and *Euoniticellus intermedius*. All three species are paracoprid in nesting behaviour in that they dig tunnels directly beneath the dung. All three species also construct compound type 1 nests (Halffter & Edmonds, 1982). These nests are variable in construction and are found in slow-burying Oniticellini, Onitini and Ontophagini dung beetles (Davis *et al.* 2008). These nests can contain single or compound broods that are constructed in a linear or branched fashion (Halffter & Edmonds, 1982)

Digitonthophagus gazella is a medium-sized beetle (± 1.1 cm in length) that produces multiple brood balls that are distinctly oval. Brood balls are approximately 2.5 cm by 1 cm.

Onitis alexis is a large beetle (± 2.0 mm in length) and constructs nests that may either be branched or clumped together with brood balls that are larger than that of *D. gazella* and *E. intermedius* and are characteristically sausage-like.

Euoniticellus intermedius is a small beetle (± 0.7 mm in length) that constructs brood balls with well-defined soil plugs separating broods, with spherical brood balls that are smaller than that of the other two species at about 1 cm by 1 cm.

Dung beetles (150 per species) used in the study were captured from wild populations two days before application, kept in a climate-controlled room at 32ºC with a 12-hour day-night cycle, and starved for one day prior to application.

3.2.3 Materials and methods

The study was conducted in the late summer of 2017 (March/April) towards the end of the rainfall period. Soil penetration resistance was measured before beetle application by means of a hand-held penetrometer (Geotron: model LT400). Penetration resistance measurements were taken from the surface every two centimetres to a depth of 30 cm, or until the penetrometer's maximum reading (5 000 KPa) was reached. The penetrometer was used to determine a representative range of penetration resistance (kPa) readings for placement of each of the 30 replicates.

Figure 13: Plastic container covering applied beetles and dung. Ventilated at the sides with mesh (not visible in picture). Numbering on the front facing side of the container indicate penetration resistance measurements in kPa.

A 1 kg fresh cattle dung pat was placed on each replicate. Five individuals (2 female and 3 male) of each species were placed on each dung pat. Dung and beetles were enclosed using an overturned 5 L white plastic container with ventilated mesh siding (with approximate diameter of 10 cm) dug into the soil with no gaps for the beetles to escape (Figure 13). After 14 days, the plastic containers were removed, and every tunnel was individually excavated with a small spade, by carefully following separate tunnel diameters beneath the dung pat. The terminal brood balls were located, and the depth was measured (cm) at this point. Tunnel diameters were examined and related to the shape and size of the brood balls at their terminal ends to determine which species they belonged to, based on the criteria listed above.

3.2.4 Data analysis

Linear regression analysis was used to determine the relationship between penetration resistance (kPa) and tunnel depth (cm) in Rstudio (Version:1.1.383) for all three species.

3.3 Results

From the 450 applied dung beetles, 176 brood balls were recovered across all the replicates.

A total of 64 brood balls were collected in *D. gazella* burrows. *Digitonthophagus gazella* buried dung to the greatest depth of all three species (Figure 14a). Terminal brood ball depth had a marginally inverse relationship with penetration resistance (p<0.05; R^2=0.65). Average brood ball depth was recorded as 18.67 cm with maximum depth measuring 20.30 cm.

For *O. alexis*, 48 brood balls were collected. The tunnel depths of *O. alexis* (Figure 14b) had no significant relationship with penetration resistance (p>0.05). The average brood ball depth was shallower than *D. gazella* at 10.68 cm with a maximum depth of 14.2 cm.

Euoniticellus intermedius also yielded 64 brood balls*. Euoniticellus intermedius* (Figure 14c) had a slightly positive relationship with penetration resistance (p<0.05; R^2=0.35). Tunnels were the shallowest of the three beetle species with an average brood ball depth of 4.08 cm and a maximum of 5.2 cm.

All three species had multiple brood balls recorded beyond the average penetration resistance range of 3 193 kPa and even beyond the equipment maximum of 5 000 kPa. None of the species showed a strong relationship between brood ball depth and penetration resistance ($R^2 > 0.7$).

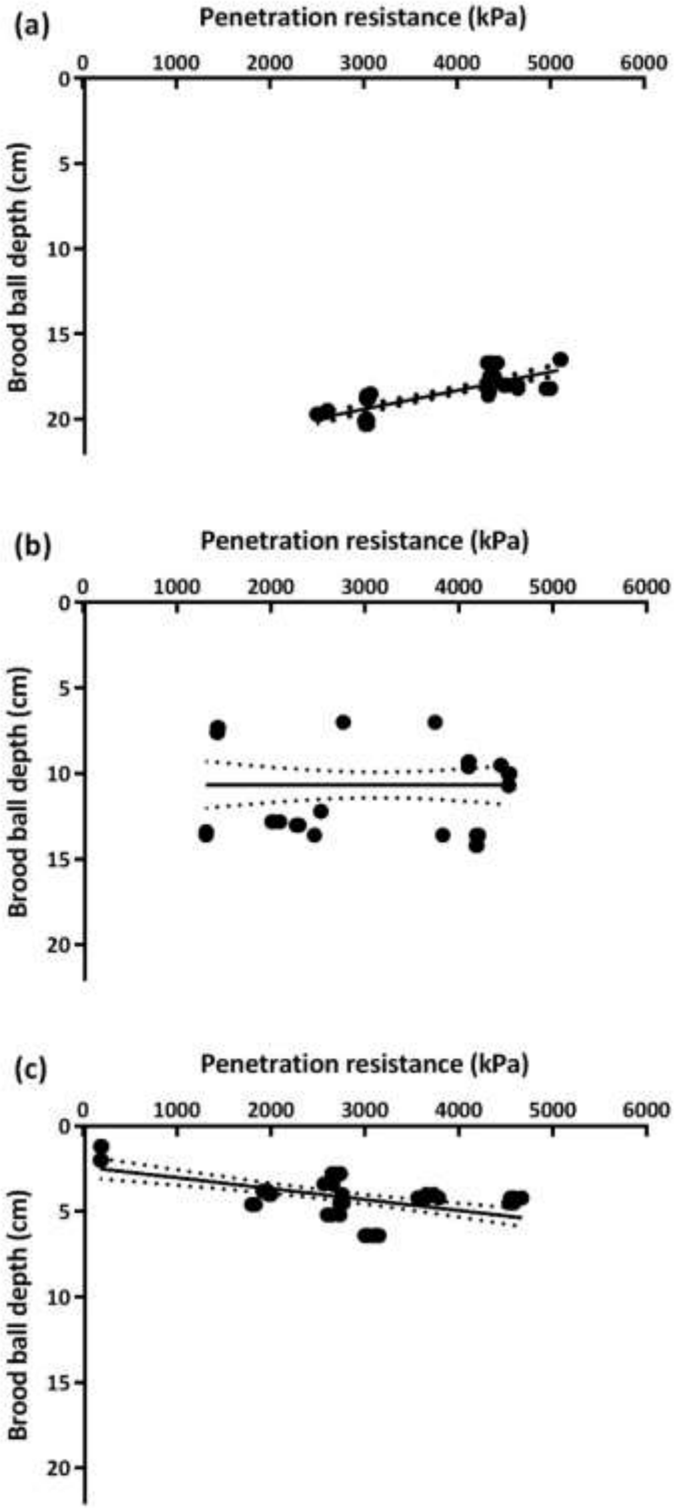

Figure 14: Terminal dung beetle brood ball depth (cm) plotted against mean penetration resistance (kPa) for three species. (a) *Digitonthophagus gazella*, (b) *Onitis alexis*, (c) and *Euoniticellus intermedius*.

Figure 15: *Euoniticellus intermedius* brood noted close to the soil surface.

3.4 Discussion

The three dung beetle species in this study could tunnel into the soil at well past the average penetration resistance of the site at 3 193 kPa and even at the maximum measurement of 5 000 kPa. Brood balls were produced by all three species with eggs and larvae found in many of them.

Penetration resistance had an inconsistent relationship with beetle tunnel depth as brood balls depth was influenced differently for each species (Figure 14).

Digitonthophagus gazella was primarily active between 16 cm and 21 cm, while a previous observation had recorded depths of up to 35 cm (Romero-Samper & Martín-Piera, 1995). The inverse relationship with penetration resistance indicates that their tunnelling may be limited to shallower depths at more extreme levels of penetration resistance. With a single exception, all tunnels were terminated before the interface with the waste coal layer.

Onitis alexis brood ball depths lacked a distinct relationship to that of increasing penetration resistance (Figure 14). However, there was no definitive influence observed of the compacted soil on *O. alexis* burrowing depth. Edwards & Aschenborn (1987) placed the zone of activity for *O. alexis* between 10 cm and 23 cm with an average depth of approximately 17 cm. The results from this study therefore indicate that although *O. alexis* tunnel depths were within the established range for the species, they were however shallower on average at 10.68 cm.

Euoniticellus intermedius, the smallest of the three species, appeared to increase tunnel depths as soil strength increased. They were primarily found within the first 10 cm with Barkhouse & Ridsdill-Smith (1986) recording depths of up to 20 cm for this species (Figure 14;).Tunnel depths were shallower than what has been recorded for each species in the past (albeit in dissimilar soil conditions). However, the depths were less regardless of soil penetration resistance. This might have either been due to the time period of the application or unmeasured properties in the soil such as percentages of sand, silt and clay or soil moisture. Reduced water content would have likely caused structural changes in the nest, such as altered distances between individual broods, and not affected terminal brood ball depth (Barkhouse & Ridsdill-Smith 1986). The shallower tunnel depth might have been influenced by other factors such as low soil moisture, nutrient availability and clay percentage, as topsoil on reclaimed sites is in many cases mixed and degraded (Ghose, 2004).

Intra and interspecific competition may have influenced dung beetle tunnelling behaviour. Giller & Doube (1989) conducted field and laboratory experiments on *O. alexis* and two coprine species to determine the influence of intra and interspecific competition on the amount and rate of dung burial. Their findings suggest that the slower burying *O. alexis* reduced volume of dung buried when two or more pairs were present on the same dung pad. Additionally, the presence of the two coprine species also lowered the amount of dung buried by *O. alexis* without affecting the performance of either coprine species. There has however not been a study to determine the influence on tunnelling depth itself and may prove a valuable parameter to consider. The large number of beetles present on each pat from three different species may have influenced the measured depths of the tunnels. This may explain the reduced depth of tunnels, observed in all three species.

An important observation was that multiple eggs within brood balls for all three species were found with some larvae even being observed. Although testing the ability of beetles to complete an entire life cycle in reclaimed mine soils was not the primary aim of this study, this suggests that dung beetles might be capable of breeding in these soils.

Dung beetle activity was confined to the upper 23 cm of the soil that plays an important role in plant root establishment, as the majority of grassland root biomass occurs within the first 30 cm (Mueller *et al.* 2013). The backfilled tunnels that were produced by the beetles could potentially serve as preferential pathways for root establishment due to the lower compaction, increased water infiltration and aeration when compared to the adjacent soil, which in turn could lead to improved nutrient uptake and plant growth (Unger & Kaspar, 1993).

It was shown that the three selected dung beetle species were able to tunnel into highly compacted mined soils. The abilities of dung beetles to improve soil conditions (Brown *et al.* 2010) are coupled with their activities within the soil, these findings provide a basis for such a project in the future.

Chapter 4

Dung beetle assemblages (Coleoptera: Scarabaeinae) and the implications thereof for soil reclamation during coal mine rehabilitation.

4.1 Conclusions

The two studies (Chapters 2-3) that were conducted to answer four main research questions were addressed with the following conclusions being drawn:

(i) Dung beetles are still active on reclaimed mined sites in eMalahleni, South Africa and could actively colonise these sites given the availability of dung.

(ii) Site-specific environmental variables (soil texture, vegetation and bulk density) did account for differences in species richness.

(iii) Although no indicator species were identified, dung beetle species such as: *Onitis alexis, Digitonthophagus gazella* and *Euoniticellus intermedius,* were abundant (Figure 5) on reclaimed mined sites and are highlighted as possible candidates for breeding and release programs to boost naturally occurring dung beetle numbers.

(iv) The three dung beetles investigated (*Euoniticellus intermedius*, *Onitis alexis* and *Digitontophagus gazella*) were able to tunnel into and construct brood balls in highly compacted mined soils.

4.2 The potential of naturally occurring dung beetles for rehabilitation

The findings of this study provide useful insights into the use of dung beetles as a complementary method for soil improvement in coal mine rehabilitation practices. Although the assemblage of dung beetles on reclaimed mine sites differed from that expected of a "natural" community in this specific region, the relatively high diversity of species is most likely adequate for the purposes of improving soil quality. Of more concern is the lowered abundance of beetles that could drastically reduce the effectiveness of beetle mediated bioturbation in time and space. This becomes important when the effectiveness or the rate of the provided ecosystem services are dependent on dung beetle abundance (Tixier *et al.* 2015).

Fortunately, it is possible that dung beetle abundance could increase substantially through a few hypothetical means. An increased presence of dung producing vertebrates (mammals specifically) that produce a continuous source of nutrition for dung beetles on mined sites, could increase beetle abundance. This could either be wild game (such as eland, black wilderbeest, zebra, etc.) similar to the Telperion Nature Reserve or domesticated cattle, sheep, donkeys and goats similar to that of the farms around the mined sites. The best-case scenario would be to include a variety of dung types that support both dung generalists and specialists and in turn support not only higher abundance, but also a higher diversity of dung beetles. Many mine rehabilitation practices aim to develop mined sites to support independent cattle farms that will help in establishing a higher abundance and diversity of dung beetles. However, it was concluded that dung beetle species that are present are found in high enough numbers to facilitate change without the diversity that is present on natural sites.

Alternatively, communities in and around mine sites could be involved in dung beetle breeding and release programs that could benefit the mines, community, beetles and the soils through a combined job creation and rehabilitation strategy. This strategy would involve recruiting a workforce from local settlements to work as breeders and field workers. Work opportunities exist in the mass breeding and release of identified beetle species, dung collection and field application and monitoring of beetles along with soil improvements.

Lastly, dung beetle communities could, in time, increase on their own or a combination of other strategies such as mass rearing. It has been established that dung beetles improve soil compaction, water permeability and other chemical and physical properties (Bang *et al.* 2005; Brown *et al.* 2010). Many of the issues that are remedied by dung beetle activity, serve to reinforce and increase their presence on a site. As shown in this study and others, the soil type (texture and particle size) had an influence on the beetles affiliated with sites that contained specific soil types (Davis, 2002; Davis *et al.* 2014; Osberg *et al.* 1994). Soil type and texture in return has an influence on the water retention abilities of soil has been documented to affect nest construction and survival of immatures in dung beetles (Barkhouse & Ridsdill-Smith, 1986). Dung beetle species that are better adapted to mined soils could begin soil improvement through their dung burial and bioturbation. The improved soil quality could initiate a positive feedback loop that in turn supports a greater diversity and abundance of dung beetles. This could subsequently lead to more effective improvement in the soil and yield greater diversity of vegetation and associated beetles and other fauna.

4.3 Dung beetle tunnelling on mined soil

The ability for dung beetles to penetrate highly compacted (penetration resistant soils) has been demonstrated by the three tested species (*O. alexis, D. gazella* and *E. intermedius*). This is a good indication that at least some proportion of dung beetles that are present in the area will be able to do the same. One concern regarding the depths to which beetles burrow involved the waste coal layer. Initially it was feared that beetles may extend their activity into the coal layer and subsequently increase acid mine drainage through creating channels of water directly into the pyrite laden spoil. Fortunately, beetle activity generally promotes increased water permeability throughout the soil profile and tunnels are generally backfilled. Additionally, dung beetles were not found to be active in the coal layer and seemed to avoid tunnelling into it (in this study, chapter 3). This provides another level of assurance to the use of dung beetles for coal mine rehabilitation.

4.4 Functional diversity

An important factor in dung beetle assemblages apart from species richness and abundance is that of functional diversity. Dung beetles are categorized into one of seven functional groups (FGs) that vary depending on their interaction with dung (Doube, 1990). The magnitude of beneficial services provided by dung beetles might change depending on the structure of the dung beetle functional diversity. For example, areas that support tunnellers and rollers that bury dung at different rates, might see improved soil conditions over a larger area that is active across many days as opposed to being limited to an area directly around a dung pat.

Therefore, it would be beneficial to determine the influence of different dung beetle functional groups as well as the ratio of these that would be most effective at delivering beneficial soil rehabilitation results. This knowledge could contribute to more effective rehabilitation of soils with a reduced, but effective, assemblage of beetles. Slade *et al.* (2007) found that dung removal rates along with seed dispersal rates were drastically reduced with a decrease in functional dung beetle diversity in tropical forests. In Slade *et al.* (2007) study, the absence of a single functional group (large nocturnal tunnellers) reduced dung removal by 75%. This suggests that a diverse functional assemblage is required for dung beetles to maximize ecosystem services.

4.5 Secondary seed dispersal

Another topic that needs further exploration is the dispersal of seeds by dung beetles and how it could benefit coal mine topsoil rehabilitation practices. It is well established that dung beetles aid in secondary seed dispersal via seeds that are present in translocated dung (Shepherd & Chapman, 1998). Multiple studies have examined the seed dispersal abilities of dung beetles (Andresen, 2002; Shepherd & Chapman, 1998; Vulinec *et al.* 2006). Application of seed-laden dung with subsequent transport and burial of dung, might improve rehabilitation efforts by establishing vegetation at a reduced effort. Additionally seeds that are transported by dung beetles are exposed to nutrient-rich dung, established in the soil by burial and have a reduced risk of predation and infection (Andresen & Levey, 2004; Nichols *et al.* 2008).

4.6 Reducing Greenhouse gases

Coal mining operations are key contributors to global CO_2 emissions through the collection, processing and eventual use of coal for the generation of electricity (Cook & Lloyd, 2012; Raghuvanshi *et al.* 2006). Another major contributor to greenhouse gas (GHG) levels is the dairy and beef industry. Dung beetles have recently been shown to reduce GHG emissions by between 7% and 12% (mainly methane) through their removal of available dung (Slade *et al.* 2015). Although this reduction is only seen at the first two emission levels, if more dung becomes available to dung beetles instead of being removed for other purposes, this amount could be substantially increased (Slade *et al.* 2015). This further increases the usefulness of dung beetles on reclaimed sites.

4.7 Benefits for soil and vegetation on reclaimed mines

In conjunction with this study, Jessica Badenhorst (M.Sc. student) undertook a study to determine specific improvements that dung beetles had on reclaimed mined soils (Unpublished Badenhorst *et al.* 2017). This project involved testing the beneficial influence of dung beetles on soil physico-chemical properties on simulated mined soils as well as larger plots on a reclaimed mined site. During this project, it was concluded that dung beetle activity had significantly lowered soil penetration resistance coupled with an increased plant biomass and water infiltration rates on soils where beetles were active.

4.8 Concluding remarks

Despite mining activity in the eMalahleni area, a relatively high diversity of dung beetles were identified on and around reclaimed coal mined sites with varying abundances. Their ability to penetrate highly compacted soil and improve the quality thereof, make dung beetles prime candidates for use in improving post-mining land use options. Although the dung beetle assemblage identified only applied to the specific region indicated in the study, the application of dung beetles for rehabilitation on post-mining soils, or other areas where soil degradation has occurred, could theoretically be achieved anywhere with a suitable climate.

Appendix

Table 5: Scarabaeinae collected from baited pitfall traps (cow/pig manure mixture) over a three-year period, March 2015- April 2017 from reclaimed mined sites (1-5), cattle farms (Sites 7 & 8) and a Telperion Nature Reserve (Site 6).

						Sites				Abundance per species		
Tribe	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mine totals**	**Ref totals**	**Totals**
Ateuchini												
	Pedaria picea Fahraeus, 1857	6	1	1	2	2	25	72	9	12	106	118
Canthonini												
	Chalconotus convexus (Boheman, 1857)	0	0	0	0	0	7	0	12	0	19	19
	Odontoloma sp.	0	0	0	0	0	2	1	58	0	61	61
Coprini												
	Catharsius aegus Génier	1	0	0	0	2	2	8	0	3	10	13
	Catharsius sesostris Waterhouse, 1888	3	2	1	2	0	63	42	5	8	110	118
	Catharsius tricornutus (DeGeer, 1778)	122	16	27	123	7	456	738	431	295	1625	1920
	Copris elphenor Klug, 1855	0	0	0	0	0	1	1	1	0	3	3
	Copris fidius (Olivier, 1789)	0	0	0	0	0	1	0	0	0	1	1
	Copris inhalatus Quedenfeldt, 1884, ssp. *perturbator* Péringuey, 1901	0	0	0	0	0	2	0	0	0	2	2
	Copris mesacanthus Harold, 1878	0	0	0	0	0	10	0	0	0	10	10
	Copris obesus Boheman, 1857	1	0	10	0	0	6	78	2	11	86	97
	Copris ritsemae Harold, 1875	0	1	0	0	0	1	0	0	1	1	2
	Heliocopris hamadryas (Fabricius, 1775)	3	0	0	2	0	2	7	4	5	13	18

						Sites				Abundance per species		
Tribe	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mine totals**	**Ref totals**	**Totals**
	Metacatharsius sp. (small)	0	0	0	0	0	12	4	5	0	21	21
	Metacatharsius troglodytes Boheman, 1857	2	0	2	1	1	63	36	3	6	102	108
Gymnopleurini												
	Allogymnopleurus splendidus (Bertolini, 1849)	9	4	67	3	0	396	27	2	83	425	508
	Gymnopleurus virens Erichson, 1843	36	157	419	212	622	0	50	39	1446	89	1535
Oniticellini												
	Cyptochirus ambiguus (Kirby, 1828)	0	4	0	0	1	0	1	2	5	3	8
	Drepanocerus kirbyi Kirby, 1828	8	16	5	5	4	6	12	83	38	101	139
	Drepanocerus patrizzii (Boucomont, 1923)	2	3	2	0	0	3	2	7	7	12	19
	Eodrepanus fastiditus (Péringuey, 1901)	8	8	39	5	3	21	12	64	63	97	160
	Epidrepanus caelatus (Gerstaecker, 1871)	41	102	140	72	5	84	31	324	360	439	799
	Euoniticellus intermedius (Reiche, 1848)	273	138	370	272	30	148	241	331	1083	720	1803
	Euoniticellus triangulatus (Harold, 1873)	257	117	455	186	128	24	97	443	1143	564	1707
	Liatongus militaris (Castelnau, 1840)	57	139	160	36	57	110	87	1758	449	1955	2404
	Oniticellus egregius Klug, 1855	0	0	0	0	0	1	1	0	0	2	2
	Chevrolat, 1830	0	0	0	0	0	1	0	0	0	1	1
	Oniticellus planatus Castelnau, 1840	4	6	2	0	5	7	9	7	17	23	40
	Tiniocellus eurypygus Branco, 2010	2	0	3	0	0	25	4	0	5	29	34
Onitini												
	Cheironitis hoplosternus (Harold, 1868)	0	0	0	0	0	0	0	1	0	1	1

						Sites				Abundance per species		
Tribe	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mine totals**	**Ref totals**	**Totals**
	Cheironitis sp. nr scabrosus	157	38	314	69	1	38	40	528	579	606	1185
	Onitis alexis Klug, 1835	2	10	6	9	33	0	6	4	60	10	70
	Onitis caffer Boheman, 1857	27	14	12	16	2	3	22	19	71	44	115
	Onitis deceptor Péringuey, 1901	1	6	1	1	0	3	1	0	9	4	13
	Onitis pecuarius van Lansberge, 1875	1	0	0	0	6	0	1	0	7	1	8
	Onitis tortuosus Houston, 1983	3	1	2	2	3	0	4	2	11	6	17
	Onitis viridulus Boheman, 1857	0	0	0	0	0	0	0	5	0	5	5
Onthophagini												
	Caccobius ferrugineus (Fahraeus, 1857)	0	1	2	0	0	225	14	11	3	250	253
	Caccobius obtusus (Fahraeus, 1857)	2	1	1	1	1	1	2	1	6	4	10
	Caccobius sp. 1	0	1	0	0	0	0	0	0	1	0	1
	Cleptocaccobius convexifrons (Raffray, 1877)	0	0	0	0	3	4	0	0	3	4	7
	Cleptocaccobius viridicollis (Fahraeus, 1857)	16	42	136	6	0	539	57	42	200	638	838
	Digitonthophagus gazella (Fabricius, 1787)	105	15	107	179	71	7	40	43	477	90	567
	Euonthophagus sp.	20	27	31	3	21	25	12	6	102	43	145
	Hyalonthophagus alcyonides (d'Orbigny, 1913)	1	1	0	2	0	8	0	0	4	8	12
	Onthophagus aeruginosus Roth, 1851	49	64	6	35	20	177	314	80	174	571	745
	Onthophagus asperulus d'Orbigny, 1905	0	0	8	0	7	0	0	3	15	3	18
	Onthophagus binodis Thunberg, 1818	0	1	0	0	5	0	0	0	6	0	6
	Onthophagus cinctipennis Quedenfeldt, 1884	39	66	12	16	7	0	31	51	140	82	222

		Sites								Abundance per species		
Tribe	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mine totals**	**Ref totals**	**Totals**
	Onthophagus convexus d'Orbigny, 1908	0	0	0	0	0	3	0	0	0	3	3
	Onthophagus cribripennis d'Orbigny, 1902	27	762	110	9	319	25	116	172	1227	313	1540
	Onthophagus cyaneoniger d'Orbigny, 1902	7	31	672	13	0	2468	79	95	723	2642	3365
	Kheper subaeneus (Harold, 1869)	0	0	0	0	31	0	2	3	31	5	36
	Onthophagus deterrens Péringuey, 1901	0	1	0	0	6	0	5	0	7	5	12
	Onthophagus ebenicolor d'Orbigny, 1902	0	4	0	0	0	0	3	1	4	4	8
	Onthophagus ebenus Péringuey, 1888	4	3	10	2	2	1	10	45	21	56	77
	Onthophagus fimetarius Roth, 1851	353	67	261	200	83	159	438	311	964	908	1872
	Onthophagus fugitivus Péringuey, 1901	0	0	0	0	0	1	0	24	0	25	25
	Onthophagus obtusicornis Fahraeus, 1857	41	62	75	7	49	10	662	124	234	796	1030
	Onthophagus optutus	0	0	0	0	0	0	1	1	0	2	2
	Onthophagus pallidipennis Fahraeus, 1857	23	4	25	22	0	332	30	20	74	382	456
	Onthophagus parumnotatus Fahraeus, 1857	41	38	57	1	113	5	67	27	250	99	349
	Onthophagus pauxillus d'Orbigny, 1902	141	44	270	58	6	2853	268	119	519	3240	3759
	Onthophagus pilosus Fahraeus, 1857	0	2	0	0	0	0	21	24	2	45	47
	Onthophagus pugionatus Fahraeus, 1857	2	1	0	5	0	0	5	2	8	7	15
	Onthophagus pullus Roth, 1851	0	5	2	8	0	3	6	3	15	12	27
	Onthophagus quadrinodosus Fahraeus, 1857	2	0	0	0	0	2	1	0	2	3	5
	Onthophagus rasipennis d'Orbigny, 1908	0	0	0	0	0	48	2	0	0	50	50
	Hamonthophagus depressus (Harold, 1871)	1	3	0	2	0	28	242	26	6	296	302

		Sites								Abundance per species		
Tribe	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mine totals**	**Ref totals**	**Totals**
	Kurtops signatus (Fahraeus, 1857)	14	1	1	1	0	3189	32	32	17	3253	3270
	Onthophagus sp. (?sp. e)	3	2	14	1	0	1	23	0	20	24	44
	Onthophagus sp. (Carrion)	0	0	0	0	0	1	0	0	0	1	1
	Onthophagus sp. (small, short, shiny)	3	11	8	0	1	1	9	11	23	21	44
	Onthophagus sp. nr granilifer	6	0	0	0	0	108	15	0	6	123	129
	Onthophagus sp. nr sugillatus (E. Scarp)	0	0	0	0	0	0	5	3	0	8	8
	Onthophagus sp. nr sugillatus (NW)-undescribed	95	309	631	84	11	2341	173	151	1130	2665	3795
	Onthophagus venustulus Erichson, 1843	4	1	0	0	0	541	24	192	5	757	762
	Onthophagus vinctus Erichson, 1843	64	19	4	30	1	134	224	427	118	785	903
	Onthophahus sp. (black *hildebtandti*)	1	1	0	0	0	14	6	18	2	38	40
	Phalops dregei (Harold, 1867)	2	6	17	3	0	950	15	21	28	986	1014
	Proagoderus chalcostolus (d'Orbigny, 1902)	117	62	16	5	26	382	207	377	226	966	1192
	Proagoderus sapphirinus (Fahraeus, 1857)	57	25	25	36	7	4938	1863	62	150	6863	7013
Scarabaeini												
	Kheper lamarcki (Macleay, 1821)	6	1	8	6	0	23	31	13	21	67	88
	Kheper nigroaeneus (Boheman, 1857)	3	0	1	5	0	35	9	6	9	50	59
	Pachylomera femoralis (Kirby, 1828)	55	2	5	13	2	2980	615	80	77	3675	3752
	Pachylomera opaca van Lansberge, 1874	1	0	4	4	0	432	57	7	9	496	505
	Scarabaeolus flavicornis (Boheman, 1857)	0	0	1	0	0	0	41	4	1	45	46
	Scarabaeus ambiguus (Boheman, 1857)	7	43	569	25	21	9514	741	614	665	10869	11534

						Sites				Abundance per species		
Tribe	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mine totals**	**Ref totals**	**Totals**
Sisyphini	*Scarabaeus goryi* (Castelnau, 1840)	1	0	0	0	0	433	19	1	1	453	454
	Scarabaeus heqvisti zur Strassen, 1962	1	0	0	0	6	2936	114	25	7	3075	3082
	Scarabaeus karae Davis & Deschodt, 2017	0	0	1	0	0	0	2	0	1	2	3
	Scarabaeus rusticus (Boheman, 1857)	4	0	0	3	0	1392	87	20	7	1499	1506
	Neosisyphus fortuitus (Péringuey, 1901)	1	0	0	0	0	9	3	3	1	15	16
	Neosisyphus rubrus (Paschalidis, 1974)	120	40	138	91	4	134	165	1326	393	1625	2018
	Sisyphus caffer Boheman, 1857	0	0	0	2	0	1175	82	33	2	1290	1292
	Sisyphus manni Montreuil, 2015	1	0	6	0	0	804	179	19	7	1002	1009
	Site	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mine totals**	**Ref totals**	**Totals**
	Abundance	2466	2552	5272	1896	1735	40914	8804	8828	13921	58546	72467
	Species Richness	64	58	55	51	43	76	80	74			97

Table 6: Site specific dung beetle assemblages arranged according to highest abundance for each site.

Species	**Site 1**	**Species**	**Site 2**
Onthophagus fimetarius Roth, 1851	353	*Onthophagus cribripennis* d'Orbigny, 1902	762
Euoniticellus intermedius (Reiche, 1848)	273	*Onthophagus sp. nr sugillatus* (NW)-undescribed	309
Euoniticellus triangulatus (Harold, 1873)	257	*Gymnopleurus virens* Erichson, 1843	157
Cheironitis sp. *nr scabrosus*	157	*Liatongus militaris* (Castelnau, 1840)	139
Onthophagus pauxillus d'Orbigny, 1902	141	*Euoniticellus intermedius* (Reiche, 1848)	138
Catharsius tricornutus (DeGeer, 1778)	122	*Euoniticellus triangulatus* (Harold, 1873)	117
Neosisyphus rubrus (Paschalidis, 1974)	120	*Epidrepanus caelatus* (Gerstaecker, 1871)	102
Proagoderus chalcostolus (d'Orbigny, 1902)	117	*Onthophagus fimetarius* Roth, 1851	67
Digitonthophagus gazella (Fabricius, 1787)	105	*Onthophagus cinctipennis* Quedenfeldt, 1884	66
Onthophagus sp. nr sugillatus (NW)-undescribed	95	*Onthophagus aeruginosus* Roth, 1851	64
Onthophagus vinctus Erichson, 1843	64	*Onthophagus obtusicornis* Fahraeus, 1857	62
Liatongus militaris (Castelnau, 1840)	57	*Proagoderus chalcostolus* (d'Orbigny, 1902)	62
Proagoderus sapphirinus (Fahraeus, 1857)	57	*Onthophagus pauxillus* d'Orbigny, 1902	44
Pachylomera femoralis (Kirby, 1828)	55	*Scarabaeus ambiguus* (Boheman, 1857)	43
Onthophagus aeruginosus Roth, 1851	49	*Cleptocaccobius viridicollis* (Fahraeus, 1857)	42
Epidrepanus caelatus (Gerstaecker, 1871)	41	*Neosisyphus rubrus* (Paschalidis, 1974)	40
Onthophagus obtusicornis Fahraeus, 1857	41	*Cheironitis sp. nr scabrosus*	38
Onthophagus parumnotatus Fahraeus, 1857	41	*Onthophagus parumnotatus* Fahraeus, 1857	38
Onthophagus cinctipennis Quedenfeldt, 1884	39	*Onthophagus cyaneoniger* d'Orbigny, 1902	31
Gymnopleurus virens Erichson, 1843	36	*Euonthophagus sp.*	27
Onitis caffer Boheman, 1857	27	*Proagoderus sapphirinus* (Fahraeus, 1857)	25
Onthophagus cribripennis d'Orbigny, 1902	27	*Onthophagus vinctus* Erichson, 1843	19
Onthophagus pallidipennis Fahraeus, 1857	23	*Catharsius tricornutus* (DeGeer, 1778)	16
Euonthophagus sp.	20	*Drepanocerus kirbyi* Kirby, 1828	16
Cleptocaccobius viridicollis (Fahraeus, 1857)	16	*Digitonthophagus gazella* (Fabricius, 1787)	15
Kurtops signatus (Fahraeus, 1857)	14	*Onitis caffer* Boheman, 1857	14
Allogymnopleurus splendidus (Bertolini, 1849)	9	*Onthophagus sp.* (small, short, shiny)	11
Drepanocerus kirbyi Kirby, 1828	8	*Onitis alexis* Klug, 1835	10
Eodrepanus fastiditus (Péringuey, 1901)	8	*Eodrepanus fastiditus* (Péringuey, 1901)	8
Onthophagus cyaneoniger d'Orbigny, 1902	7	*Oniticellus planatus* Castelnau, 1840	6
Scarabaeus ambiguus (Boheman, 1857)	7	Onitis deceptorPéringuey, 1901	6
Onthophagus sp. nr granilifer	6	*Phalops dregei* (Harold, 1867)	6
Kheper lamarcki (Macleay, 1821)	6	*Onthophagus pullus* Roth, 1851	5
Pedaria picea Fahraeus, 1857	6	*Allogymnopleurus splendidus* (Bertolini, 1849)	4
Oniticellus planatus Castelnau, 1840	4	*Cyptochirus ambiguus* (Kirby, 1828)	4
Onthophagus ebenus Péringuey, 1888	4	*Onthophagus ebenicolor* d'Orbigny, 1902	4
Onthophagus venustulus Erichson, 1843	4	*Onthophagus pallidipennis* Fahraeus, 1857	4
Scarabaeus rusticus (Boheman, 1857)	4	*Drepanocerus patrizzii (Boucomont, 1923)*	3
Catharsius sesostris Waterhouse, 1888	3	*Onthophagus ebenus* Péringuey, 1888	3
Heliocopris hamadryas (Fabricius, 1775)	3	*Hamonthophagus depressus* (Harold, 1871)	3
Onitis tortuosus Houston, 1983	3	*Catharsius sesostris* Waterhouse, 1888	2
Onthophagus sp. (?sp. e)	3	*Onthophagus pilosus* Fahraeus, 1857	2

Onthophagus sp. (small, short, shiny)	3	*Onthophagus sp.* (?sp. e)	2
Kheper nigroaeneus (Boheman, 1857)	3	*Pachylomera femoralis* (Kirby, 1828)	2
Metacatharsius troglodytes Boheman, 1857	2	*Copris ritsemae* Harold, 1875	1
Drepanocerus patrizzii (Boucomont, 1923)	2	Onitis tortuosus Houston, 1983	1
Tiniocellus eurypygus eurypygus Branco, 2010	2	*Caccobius ferrugineus* (Fahraeus, 1857)	1
Onitis alexis Klug, 1835	2	*Caccobius obtusus* (Fahraeus, 1857)	1
Caccobius obtusus (Fahraeus, 1857)	2	*Caccobius sp. 1*	1
Onthophagus pugionatus Fahraeus, 1857	2	*Hyalonthophagus alcyonides* (d'Orbigny, 1913)	1
Onthophagus quadrinodosus Fahraeus, 1857	2	*Onthophagus binodis* Thunberg, 1818	1
Phalops dregei (Harold, 1867)	2	*Onthophagus deterrens* Péringuey, 1901	1
Catharsius aegus Génier	1	*Onthophagus pugionatus* Fahraeus, 1857	1
Copris obesus Boheman, 1857	1	*Kurtops signatus* (Fahraeus, 1857)	1
Onitis deceptor Péringuey, 1901	1	*Onthophagus venustulus* Erichson, 1843	1
Onitis pecuarius van Lansberge, 1875	1	*Onthophahus sp.* (black hildebtandti)	1
Hyalonthophagus alcyonides (d'Orbigny, 1913)	1	*Kheper lamarcki* (Macleay, 1821)	1
Hamonthophagus depressus (Harold, 1871)	1	*Pedaria picea Fahraeus*, 1857	1
Onthophahus sp. (black hildebtandti)	1		
Pachylomera opaca van Lansberge, 1874	1		
Scarabaeus goryi (Castelnau, 1840)	1		
Scarabaeus heqvisti zur Strassen, 1962	1		
Neosisyphus fortuitus (Péringuey, 1901)	1		
Sisyphus manni Montreuil, 2015	1		

Species	**Site 3**	**Species**	**Site 4**
Onthophagus cyaneoniger d'Orbigny, 1902	672	*Euoniticellus intermedius* (Reiche, 1848)	272
Onthophagus sp. nr sugillatus (NW)-undescribed	631	*Gymnopleurus virens* Erichson, 1843	212
Scarabaeus ambiguus (Boheman, 1857)	569	*Onthophagus fimetarius* Roth, 1851	200
Euoniticellus triangulatus (Harold, 1873)	455	*Euoniticellus triangulatus* (Harold, 1873)	186
Gymnopleurus virens Erichson, 1843	419	*Digitonthophagus gazella* (Fabricius, 1787)	179
Euoniticellus intermedius (Reiche, 1848)	370	*Catharsius tricornutus* (DeGeer, 1778)	123
Cheironitis sp. *nr scabrosus*	314	*Neosisyphus rubrus* (Paschalidis, 1974)	91
Onthophagus pauxillus d'Orbigny, 1902	270	*Onthophagus sp. nr sugillatus* (NW)-undescribed	84
Onthophagus fimetarius Roth, 1851	261	*Epidrepanus caelatus* (Gerstaecker, 1871)	72
Liatongus militaris (Castelnau, 1840)	160	Cheironitis sp. *nr scabrosus*	69
Epidrepanus caelatus (Gerstaecker, 1871)	140	*Onthophagus pauxillus* d'Orbigny, 1902	58
Neosisyphus rubrus (Paschalidis, 1974)	138	*Liatongus militaris* (Castelnau, 1840)	36
Cleptocaccobius viridicollis (Fahraeus, 1857)	136	*Proagoderus sapphirinus* (Fahraeus, 1857)	36
Onthophagus cribripennis d'Orbigny, 1902	110	*Onthophagus aeruginosus* Roth, 1851	35
Digitonthophagus gazella (Fabricius, 1787)	107	*Onthophagus vinctus* Erichson, 1843	30
Onthophagus obtusicornis Fahraeus, 1857	75	*Scarabaeus ambiguus* (Boheman, 1857)	25
Allogymnopleurus splendidus (Bertolini, 1849)	67	*Onthophagus pallidipennis* Fahraeus, 1857	22
Onthophagus parumnotatus Fahraeus, 1857	57	*Onitis caffer* Boheman, 1857	16
Eodrepanus fastiditus (Péringuey, 1901)	39	*Onthophagus cinctipennis* Quedenfeldt, 1884	16
Euonthophagus sp.	31	*Onthophagus cyaneoniger* d'Orbigny, 1902	13
Catharsius tricornutus (DeGeer, 1778)	27	*Pachylomera femoralis* (Kirby, 1828)	13
Onthophagus pallidipennis Fahraeus, 1857	25	Onitis alexis Klug, 1835	9
Proagoderus sapphirinus (Fahraeus, 1857)	25	*Onthophagus cribripennis* d'Orbigny, 1902	9
Phalops dregei (Harold, 1867)	17	*Onthophagus pullus* Roth, 1851	8

Proagoderus chalcostolus (d'Orbigny, 1902)	16	*Onthophagus obtusicornis* Fahraeus, 1857	7
Onthophagus sp. (?sp. e)	14	*Cleptocaccobius viridicollis* (Fahraeus, 1857)	6
Onitis caffer Boheman, 1857	12	*Kheper lamarcki* (Macleay, 1821)	6
Onthophagus cinctipennis Quedenfeldt, 1884	12	*Drepanocerus kirbyi* Kirby, 1828	5
Copris obesus Boheman, 1857	10	*Eodrepanus fastiditus* (Péringuey, 1901)	5
Onthophagus ebenus Péringuey, 1888	10	*Onthophagus pugionatus* Fahraeus, 1857	5
Onthophagus asperulus d'Orbigny, 1905	8	*Proagoderus chalcostolus* (d'Orbigny, 1902)	5
Onthophagus sp. (small, short, shiny)	8	*Kheper nigroaeneus* (Boheman, 1857)	5
Kheper lamarcki (Macleay, 1821)	8	*Pachylomera opaca* van Lansberge, 1874	4
Onitis alexis Klug, 1835	6	*Allogymnopleurus splendidus* (Bertolini, 1849)	3
Onthophagus aeruginosus Roth, 1851	6	*Euonthophagus sp.*	3
Sisyphus manni Montreuil, 2015	6	*Phalops dregei* (Harold, 1867)	3
Drepanocerus kirbyi Kirby, 1828	5	*Scarabaeus rusticus* (Boheman, 1857)	3
Pachylomera femoralis (Kirby, 1828)	5	*Catharsius sesostris* Waterhouse, 1888	2
Onthophagus vinctus Erichson, 1843	4	*Heliocopris hamadryas* (Fabricius, 1775)	2
Pachylomera opaca van Lansberge, 1874	4	Onitis tortuosus Houston, 1983	2
Tiniocellus eurypygus eurypygus Branco, 2010	3	*Hyalonthophagus alcyonides* (d'Orbigny, 1913)	2
Metacatharsius troglodytes Boheman, 1857	2	*Onthophagus ebenus* Péringuey, 1888	2
Drepanocerus patrizzii (Boucomont, 1923)	2	*Hamonthophagus depressus* (Harold, 1871)	2
Oniticellus planatus Castelnau, 1840	2	*Pedaria picea Fahraeus*, 1857	2
Onitis tortuosus Houston, 1983	2	*Sisyphus caffer* Boheman, 1857	2
Caccobius ferrugineus (Fahraeus, 1857)	2	*Metacatharsius troglodytes* Boheman, 1857	1
Onthophagus pullus Roth, 1851	2	*Onitis deceptor* Péringuey, 1901	1
Catharsius sesostris Waterhouse, 1888	1	*Caccobius obtusus* (Fahraeus, 1857)	1
Onitis deceptor Péringuey, 1901	1	*Onthophagus parumnotatus* Fahraeus, 1857	1
Caccobius obtusus (Fahraeus, 1857)	1	*Kurtops signatus* (Fahraeus, 1857)	1
Kurtops signatus (Fahraeus, 1857)	1	*Onthophagus sp.* (?sp. e)	1
Kheper nigroaeneus (Boheman, 1857)	1		
Scarabaeolus flavicornis (Boheman, 1857)	1		
Scarabaeus karae Davis & Deschodt, 2017	1		
Pedaria picea Fahraeus, 1857	1		

Species	**Site 5**	**Species**	**Site 6**
Gymnopleurus virens Erichson, 1843	622	*Scarabaeus ambiguus* (Boheman, 1857)	9514
Onthophagus cribripennis d'Orbigny, 1902	319	*Proagoderus sapphirinus* (Fahraeus, 1857)	4938
Euoniticellus triangulatus (Harold, 1873)	128	*Kurtops signatus* (Fahraeus, 1857)	3189
Onthophagus parumnotatus Fahraeus, 1857	113	*Pachylomera femoralis* (Kirby, 1828)	2980
Onthophagus fimetarius Roth, 1851	83	*Scarabaeus heqvisti* zur Strassen, 1962	2936
Digitonthophagus gazella (Fabricius, 1787)	71	*Onthophagus pauxillus* d'Orbigny, 1902	2853
Liatongus militaris (Castelnau, 1840)	57	*Onthophagus cyaneoniger* d'Orbigny, 1902	2468
Onthophagus obtusicornis Fahraeus, 1857	49	*Onthophagus sp. nr sugillatus* (NW)-undescribed	2341
Onitis alexis Klug, 1835	33	*Scarabaeus rusticus* (Boheman, 1857)	1392
Kheper subaeneus (Harold, 1869)	31	*Sisyphus caffer* Boheman, 1857	1175
Euoniticellus intermedius (Reiche, 1848)	30	*Phalops dregei* (Harold, 1867)	950
Proagoderus chalcostolus (d'Orbigny, 1902)	26	*Sisyphus manni* Montreuil, 2015	804
Euonthophagus sp.	21	*Onthophagus venustulus* Erichson, 1843	541
Scarabaeus ambiguus (Boheman, 1857)	21	*Cleptocaccobius viridicollis* (Fahraeus, 1857)	539
Onthophagus aeruginosus Roth, 1851	20	*Catharsius tricornutus* (DeGeer, 1778)	456

Onthophagus sp. nr sugillatus (NW)-undescribed	11
Catharsius tricornutus (DeGeer, 1778)	7
Onthophagus asperulus d'Orbigny, 1905	7
Onthophagus cinctipennis Quedenfeldt, 1884	7
Proagoderus sapphirinus (Fahraeus, 1857)	7
Onitis pecuarius van Lansberge, 1875	6
Onthophagus deterrens Péringuey, 1901	6
Onthophagus pauxillus d'Orbigny, 1902	6
Scarabaeus heqvisti zur Strassen, 1962	6
Epidrepanus caelatus (Gerstaecker, 1871)	5
Oniticellus planatus Castelnau, 1840	5
Onthophagus binodis Thunberg, 1818	5
Drepanocerus kirbyi Kirby, 1828	4
Neosisyphus rubrus (Paschalidis, 1974)	4
Eodrepanus fastiditus (Péringuey, 1901)	3
Onitis tortuosus Houston, 1983	3
Cleptocaccobius convexifrons (Raffray, 1877)	3
Catharsius aegus Génier	2
Onitis caffer Boheman, 1857	2
Onthophagus ebenus Péringuey, 1888	2
Pachylomera femoralis (Kirby, 1828)	2
Pedaria picea Fahraeus, 1857	2
Metacatharsius troglodytes Boheman, 1857	1
Cyptochirus ambiguus (Kirby, 1828)	1
Cheironitis sp. *nr scabrosus*	1
Caccobius obtusus (Fahraeus, 1857)	1
Onthophagus sp. (small, short, shiny)	1
Onthophagus vinctus Erichson, 1843	1

Scarabaeus goryi (Castelnau, 1840)	433
Pachylomera opaca van Lansberge, 1874	432
Allogymnopleurus splendidus (Bertolini, 1849)	396
Proagoderus chalcostolus (d'Orbigny, 1902)	382
Onthophagus pallidipennis Fahraeus, 1857	332
Caccobius ferrugineus (Fahraeus, 1857)	225
Onthophagus aeruginosus Roth, 1851	177
Onthophagus fimetarius Roth, 1851	159
Euoniticellus intermedius (Reiche, 1848)	148
Onthophagus vinctus Erichson, 1843	134
Neosisyphus rubrus (Paschalidis, 1974)	134
Liatongus militaris (Castelnau, 1840)	110
Onthophagus sp. nr granilifer	108
Epidrepanus caelatus (Gerstaecker, 1871)	84
Catharsius sesostris Waterhouse, 1888	63
Metacatharsius troglodytes Boheman, 1857	63
Onthophagus rasipennis d'Orbigny, 1908	48
Cheironitis sp. *nr scabrosus*	38
Kheper nigroaeneus (Boheman, 1857)	35
Hamonthophagus depressus (Harold, 1871)	28
Pedaria picea Fahraeus, 1857	25
Tiniocellus eurypygus eurypygus Branco, 2010	25
Euonthophagus sp.	25
Onthophagus cribripennis d'Orbigny, 1902	25
Euoniticellus triangulatus (Harold, 1873)	24
Kheper lamarcki (Macleay, 1821)	23
Eodrepanus fastiditus (Péringuey, 1901)	21
Onthophahus sp. (black hildebtandti)	14
Metacatharsius sp. (small)	12
Copris mesacanthus Harold, 1878	10
Onthophagus obtusicornis Fahraeus, 1857	10
Neosisyphus fortuitus (Péringuey, 1901)	9
Hyalonthophagus alcyonides (d'Orbigny, 1913)	8
Chalconotus convexus (Boheman, 1857)	7
Oniticellus planatus Castelnau, 1840	7
Digitonthophagus gazella (Fabricius, 1787)	7
Copris obesus Boheman, 1857	6
Drepanocerus kirbyi Kirby, 1828	6
Onthophagus parumnotatus Fahraeus, 1857	5
Cleptocaccobius convexifrons (Raffray, 1877)	4
Drepanocerus patrizzii (Boucomont, 1923)	3
Onitis caffer Boheman, 1857	3
Onitis deceptorPéringuey, 1901	3
Onthophagus convexus d'Orbigny, 1908	3
Onthophagus pullus Roth, 1851	3
Odontoloma sp.	2
Catharsius aegus Génier	2

		Copris inhalatus Quedenfeldt, 1884, ssp. *perturbator* Péringuey, 1901	2
		Heliocopris hamadryas (Fabricius, 1775)	2
		Onthophagus quadrinodosus Fahraeus, 1857	2
		Copris elphenor Klug, 1855	1
		Copris fidius (Olivier, 1789)	1
		Copris ritsemae Harold, 1875	1
		Oniticellus egregius klug, 1855	1
		Chevrolat, 1830	1
		Caccobius obtusus (Fahraeus, 1857)	1
		Onthophagus ebenus Péringuey, 1888	1
		Onthophagus fugitivus Péringuey, 1901	1
		Onthophagus sp. (?sp. e)	1
		Onthophagus sp. (Carrion)	1
		Onthophagus sp. (small, short, shiny)	1

Species	**Site 7**	**Species**	**Site 8**
Proagoderus sapphirinus (Fahraeus, 1857)	1863	*Liatongus militaris* (Castelnau, 1840)	1758
Scarabaeus ambiguus (Boheman, 1857)	741	*Neosisyphus rubrus* (Paschalidis, 1974)	1326
Catharsius tricornutus (DeGeer, 1778)	738	*Scarabaeus ambiguus* (Boheman, 1857)	614
Onthophagus obtusicornis Fahraeus, 1857	662	Cheironitis sp. *nr scabrosus*	528
Pachylomera femoralis (Kirby, 1828)	615	*Euoniticellus triangulatus* (Harold, 1873)	443
Onthophagus fimetarius Roth, 1851	438	*Catharsius tricornutus* (DeGeer, 1778)	431
Onthophagus aeruginosus Roth, 1851	314	*Onthophagus vinctus* Erichson, 1843	427
Onthophagus pauxillus d'Orbigny, 1902	268	*Proagoderus chalcostolus* (d'Orbigny, 1902)	377
Hamonthophagus depressus (Harold, 1871)	242	*Euoniticellus intermedius* (Reiche, 1848)	331
Euoniticellus intermedius (Reiche, 1848)	241	*Epidrepanus caelatus* (Gerstaecker, 1871)	324
Onthophagus vinctus Erichson, 1843	224	*Onthophagus fimetarius* Roth, 1851	311
Proagoderus chalcostolus (d'Orbigny, 1902)	207	*Onthophagus venustulus* Erichson, 1843	192
Sisyphus manni Montreuil, 2015	179	*Onthophagus cribripennis* d'Orbigny, 1902	172
Onthophagus sp. nr sugillatus (NW)-undescribed	173	*Onthophagus sp. nr sugillatus* (NW)-undescribed	151
Neosisyphus rubrus (Paschalidis, 1974)	165	*Onthophagus obtusicornis* Fahraeus, 1857	124
Onthophagus cribripennis d'Orbigny, 1902	116	*Onthophagus pauxillus* d'Orbigny, 1902	119
Scarabaeus heqvisti zur Strassen, 1962	114	*Onthophagus cyaneoniger* d'Orbigny, 1902	95
Euoniticellus triangulatus (Harold, 1873)	97	*Drepanocerus kirbyi* Kirby, 1828	83
Liatongus militaris (Castelnau, 1840)	87	*Onthophagus aeruginosus* Roth, 1851	80
Scarabaeus rusticus (Boheman, 1857)	87	*Pachylomera femoralis* (Kirby, 1828)	80
Sisyphus caffer Boheman, 1857	82	*Eodrepanus fastiditus* (Péringuey, 1901)	64
Onthophagus cyaneoniger d'Orbigny, 1902	79	*Proagoderus sapphirinus* (Fahraeus, 1857)	62
Copris obesus Boheman, 1857	78	*Odontoloma sp.*	58
Pedaria picea Fahraeus, 1857	72	*Onthophagus cinctipennis* Quedenfeldt, 1884	51
Onthophagus parumnotatus Fahraeus, 1857	67	*Onthophagus ebenus* Péringuey, 1888	45
Cleptocaccobius viridicollis (Fahraeus, 1857)	57	*Digitonthophagus gazella* (Fabricius, 1787)	43
Pachylomera opaca van Lansberge, 1874	57	*Cleptocaccobius viridicollis* (Fahraeus, 1857)	42
Gymnopleurus virens Erichson, 1843	50	*Gymnopleurus virens* Erichson, 1843	39
Catharsius sesostris Waterhouse, 1888	42	*Sisyphus caffer* Boheman, 1857	33
Scarabaeolus flavicornis (Boheman, 1857)	41	*Kurtops signatus* (Fahraeus, 1857)	32
Cheironitis sp. *nr scabrosus*	40	*Onthophagus parumnotatus* Fahraeus, 1857	27
Digitonthophagus gazella (Fabricius, 1787)	40	*Hamonthophagus depressus* (Harold, 1871)	26

Metacatharsius troglodytes Boheman, 1857	36	*Scarabaeus heqvisti* zur Strassen, 1962	25
Kurtops signatus (Fahraeus, 1857)	32	*Onthophagus fugitivus* Péringuey, 1901	24
Epidrepanus caelatus (Gerstaecker, 1871)	31	*Onthophagus pilosus* Fahraeus, 1857	24
Onthophagus cinctipennis Quedenfeldt, 1884	31	*Phalops dregei* (Harold, 1867)	21
Kheper lamarcki (Macleay, 1821)	31	*Onthophagus pallidipennis* Fahraeus, 1857	20
Onthophagus pallidipennis Fahraeus, 1857	30	*Scarabaeus rusticus* (Boheman, 1857)	20
Allogymnopleurus splendidus (Bertolini, 1849)	27	*Onitis caffer* Boheman, 1857	19
Onthophagus venustulus Erichson, 1843	24	*Sisyphus manni* Montreuil, 2015	19
Onthophagus sp. (?sp. e)	23	*Onthophahus sp.* (black hildebtandti)	18
Onitis caffer Boheman, 1857	22	*Kheper lamarcki* (Macleay, 1821)	13
Onthophagus pilosus Fahraeus, 1857	21	*Chalconotus convexus* (Boheman, 1857)	12
Scarabaeus goryi (Castelnau, 1840)	19	*Caccobius ferrugineus* (Fahraeus, 1857)	11
Onthophagus sp. nr granilifer	15	*Onthophagus sp.* (small, short, shiny)	11
Phalops dregei (Harold, 1867)	15	*Pedaria picea Fahraeus*, 1857	9
Caccobius ferrugineus (Fahraeus, 1857)	14	*Drepanocerus patrizzii (Boucomont, 1923)*	7
Drepanocerus kirbyi Kirby, 1828	12	*Oniticellus planatus* Castelnau, 1840	7
Eodrepanus fastiditus (Péringuey, 1901)	12	*Pachylomera opaca* van Lansberge, 1874	7
Euonthophagus sp.	12	*Euonthophagus sp.*	6
Onthophagus ebenus Péringuey, 1888	10	*Kheper nigroaeneus* (Boheman, 1857)	6
Oniticellus planatus Castelnau, 1840	9	*Catharsius sesostris* Waterhouse, 1888	5
Onthophagus sp. (small, short, shiny)	9	*Metacatharsius sp.* (small)	5
Kheper nigroaeneus (Boheman, 1857)	9	Onitis viridulus Boheman, 1857	5
Catharsius aegus Génier	8	*Heliocopris hamadryas* (Fabricius, 1775)	4
Heliocopris hamadryas (Fabricius, 1775)	7	Onitis alexis Klug, 1835	4
Onitis alexis Klug, 1835	6	*Scarabaeolus flavicornis* (Boheman, 1857)	4
Onthophagus pullus Roth, 1851	6	*Metacatharsius troglodytes* Boheman, 1857	3
Onthophahus sp. (black hildebtandti)	6	*Onthophagus asperulus* d'Orbigny, 1905	3
Onthophagus deterrens Péringuey, 1901	5	*Kheper subaeneus* (Harold, 1869)	3
Onthophagus pugionatus Fahraeus, 1857	5	*Onthophagus pullus* Roth, 1851	3
Onthophagus sp. nr sugillatus (E. Scarp)	5	*Onthophagus sp. nr sugillatus* (E. Scarp)	3
Metacatharsius sp. (small)	4	*Neosisyphus fortuitus* (Péringuey, 1901)	3
Tiniocellus eurypygus eurypygus Branco, 2010	4	*Copris obesus* Boheman, 1857	2
Onitis tortuosus Houston, 1983	4	*Allogymnopleurus splendidus* (Bertolini, 1849)	2
Onthophagus ebenicolor d'Orbigny, 1902	3	*Cyptochirus ambiguus* (Kirby, 1828)	2
Neosisyphus fortuitus (Péringuey, 1901)	3	Onitis tortuosus Houston, 1983	2
Drepanocerus patrizzii (Boucomont, 1923)	2	*Onthophagus pugionatus* Fahraeus, 1857	2
Caccobius obtusus (Fahraeus, 1857)	2	*Copris elphenor Klug*, 1855	1
Kheper subaeneus (Harold, 1869)	2	Cheironitis hoplosternus (Harold, 1868)	1
Onthophagus rasipennis d'Orbigny, 1908	2	*Caccobius obtusus* (Fahraeus, 1857)	1
Scarabaeus karae Davis & Deschodt, 2017	2	*Onthophagus ebenicolor* d'Orbigny, 1902	1
Odontoloma sp.	1	*Onthophagus optutus*	1
Copris elphenor Klug, 1855	1	*Scarabaeus goryi* (Castelnau, 1840)	1
Cyptochirus ambiguus (Kirby, 1828)	1		
Oniticellus egregius klug, 1855	1		
Onitis deceptorPéringuey, 1901	1		
Onitis pecuarius van Lansberge, 1875	1		
Onthophagus optutus	1		
Onthophagus quadrinodosus Fahraeus, 1857	1		

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