SOIL REHABILITATION: CAN DUNG BEETLES IMPROVE POST-MINING LAND-USE OPTIONS?

PROJECT NO: 8.2.8

Final report to

Coaltech

The Coaltech Research Association NPC

May 2018

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

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ACKNOWLEDGEMENTS

We, the authors, would like to acknowledge various contributions made to this work by the following individuals:

- Coaltech for project funding;
- Johann Beukes for his years of support and encouragement;
- The Coaltech Surface and Environment committee for guidance and feedback;
- Staff at mines including Martin Platt, Francois Grove, Annelie Rossouw, & Jaco Fick;
- Staff at the Scarab Research Group at the University of Pretoria including Christian Deschodt, Carmen Jacobs and Catherine Sole. We also thank Johan Van Wyk for field assistance. We *especially* thank Adrian Davis for his time and expertise identifying the beetles.
- Former students and project team members Ms. Alexandra Howard and Ms. Sarah Newman

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EXECUTIVE SUMMARY

By Jackie Dabrowski

PURPOSE OF THIS STUDY

The activities of ants, termites, dung beetles and earthworms are all known to positively impact on a range of soil health parameters. However, to our knowledge, none of these groups have deliberately been applied as part of a land reclamation strategy. The overall aim of this study was to determine whether dung beetles could be used to improve post-mining land-use options through their dung-burial activities. This aim was achieved through a series of experimental and field-scale studies to determine whether dung beetles could maintain their activities along with established ecosystem services in the soils typically found on rehabilitated mines. This information forms the basis of recommendations for the use of dung beetles as a complementary method within the current mix of rehabilitation methods.

SCOPE OF THIS REPORT

This is the final report for the three year research project funded by Coaltech from 2015 – 2018. The report begins with an Executive Summary which summarises the major findings, outputs, and recommendations arising from this project. This report includes all of the chapters submitted for the fulfilment of requirements for the M.Sc. degrees for Jessica Badenhorst (Chapters 1 to 4) and Gustav Venter (Chapters 5 to 8). Work related to these studies occurred between 2015 and 2017 and includes data submitted for both students' B.Sc. Hons degrees at the University of Pretoria. Gustav's M.Sc. built on the project started by Alexandra Howard for her B.Sc. Honours, and her work is therefore included in this report.

OUTPUTS GENERATED BY THIS PROJECT

Apart from the annual progress reports provided to Coaltech in March 2016 and March 2017, a number of additional outputs have been generated by this project and are summarised below:

1) *Tertiary degrees from the University of Pretoria supported by Coaltech bursaries*

Degree	Student	Year graduated
B.Sc. Honours Entomology	Sarah Newman	Autumn 2016
B.Sc. Honours Entomology	Alexandra Howard	Autumn 2016
B.Sc. Honours Entomology	Jessica Badenhorst	Autumn 2016

B.Sc. Honours Entomology	Gustav Venter	Autumn 2016
M.Sc. Entomology	Jessica Badenhorst	Spring 2018
M.Sc. Entomology	Gustav Venter	Spring 2018

2) *Presentations*

Presenter	Conference	Output	Date
Jackie Dabrowski	SACESHA	Oral presentation	Mar. 2015
Jackie Dabrowski	Coaltech colloquium	Oral presentation	Aug. 2015
Jackie Dabrowski	Land Rehabilitation Society of South Africa	Oral presentation	Sep. 2015
Alexandra Howard	Diamond Route Conference	Oral presentation	Oct. 2015
Jackie Dabrowski	SACESHA	Oral presentation	Jan. 2016
Jessica Badenhorst	Land Rehabilitation Society of South Africa	Poster	Sep. 2016
Gustav Venter	Land Rehabilitation Society of South Africa	Poster	Sep. 2016
Gustav Venter	Diamond Route Conference	Oral presentation	Oct. 2016
Jessica Badenhorst	Land Rehabilitation Society of South Africa	Oral presentation	Aug. 2017
Gustav Venter	Land Rehabilitation Society of South Africa	Poster	Aug. 2017

3) *Popular media*

Presenter / author	Platform	Date
Jackie Dabrowski	Classic FM radio interview	Oct. 2015
Jackie Dabrowski / Dylan Slater	Mining Weekly article	May 2017
Gustav Venter	RSG radio interview	Oct. 2016

4) *Research publications*

Authors	Title	Journal	Status
Badenhorst *et al.* 2018	Dung beetle activity improves herbaceous plant growth and soil properties on plots simulating reclaimed mined land in South Africa.	Applied Soil Ecology	Accepted with changes
Venter *et al.* 2018	Dung beetles can tunnel into highly compacted soils from reclaimed mined sites in eMalahleni, South Africa.	Applied Soil Ecology	Accepted with changes

Badenhorst *et al.* 2019	Alleviation of degraded soil conditions by dung beetle activity on reclaimed mined land.	Applied Soil Ecology	In preparation

THE BENEFITS OF DUNG BEETLES FOR POST MINING LAND RECLAMATION

1) Dung beetles can be attracted back to rehabilitated mines

Dung-baited pitfall traps used to determine dung beetle diversity and abundance at 5 rehabilitated mine sites. A total of 9 collections were made over a period of 3 years during summer months. Collectively, 13 921 individual beetles were collected from the 5 mine sites compared to 58 546 from the 3 reference sites. On average there were 54 species at mine sites compared to 76 species at the reference sites. *Although there is an obvious decline in species diversity and abundance at the mine sites, there appear to be sufficient source populations in the vicinity of the mines to re-populate these sites over time with the provision of dung.*

2) Dung beetles can be bred for mass release on mines

Dung beetles have been successfully mass-reared for release in various countries. Attempts were made to breed large numbers of beetles for this project, with the main focus being on 3 species: *Digitonthophagus gazella*; *Onitis alexis*; and *Euoniticellus intermedius*. Our attempts never yielded very high numbers. A visit to a mass-rearing facility in New Zealand in 2016 revealed that in order to breed high numbers of dung beetles for release, a custom-built facility with at least 2 full time staff members is required. The facilities are not expensive and the work is not highly specialised. *Optimising the numbers of beetles requires daily, dedicated attention for the full duration of the breeding season (summer) and less intensive maintenance work during winter. The 3 species identified for breeding naturally occur at the mine sites and therefore remain good prospects for future breeding programmes.*

3) Dung beetles can tunnel into highly compacted soils

Three species of dung beetles applied in field trials on a rehabilitated mine site were able to tunnel into compacted soils with an average penetration resistance of 3 193 kPa, and even at the maximum measurement of 5 000 kPa. Their tunnel depths were slightly shallower than depths reported in other literature. Furthermore, there was evidence of successful breeding with brood balls containing eggs and larvae produced by all three species. The three species investigated occur naturally at all of the mine sites surveyed in this project, which shows that *compacted soils would not prevent tunnelling by a range of dung beetle species naturally present at mine sites*. These results were supported by field trial applications which included a wide range of species applied in enclosures to prevent their escape. They were observed to immediately feed on the dung and were not found at the soil surface at a later stage, thus implying they had managed to tunnel into the soil.

4) Dung beetles improve water infiltration rates

Water infiltration rates were significantly higher on dung beetle treated experimental plots. *Improvements ranged between a 30% and 300% increase in the volume of water infiltrating the soil*. *The latter increase resulted in a difference of approximately 100 mm h^{-1} with dung beetles*. In field trials, the water infiltration rates improved significantly following the application of dung beetles. Improvements were not as drastic as in the experimental plots with a maximum of 35%. The difference in results is likely a reflection of environmental heterogeneity and larger spatial area covered in the field trial. Nevertheless, *this result is highly beneficial for plant growth and results in less surface runoff, which reduces soil loss (erosion) rates.*

5) Dung beetles reduce soil compaction

On experimental plots with dung beetles, soil compaction (penetration resistance) decreased significantly. The greatest increase was seen one month and six months following the 2nd dung beetle application. In the latter measurement, penetration resistance reached a maximum of 430 kPa while the sites with no beetles had maximum values greater than 1100 kPa. This is a *reduction in soil compaction of more than 150%,* and suggests that repeated applications of dung beetles could enhance certain benefits associated with their tunnelling activity. The results of the experimental trial were reinforced in the field trial, where reduction in soil compaction was observed after all dung beetle applications. However, the results were not sustained in the long term. Measurements were taken at random points in the enclosure and therefore included areas where dung beetles had tunnelled and where they had not. These results emphasise that *improvements are localised beneath dung pats, and did not extend beyond this area.*

6) Vegetation biomass is increased where dung beetles are active

Post dung beetle applications on experimental plots, vegetation biomass showed a general pattern of increase. Improvements were significant six months post the 1st dung beetle application, and 1 month after the 2nd dung beetle application, *increasing biomass significantly between 100% and 60%* respectively. In field trials, there was a large increase in biomass (± 50%) following the first dung beetle application. Although biomass was generally higher on the dung beetle treated plots in follow up measurements, the differences were not statistically significant. *Although results were not consistently significant in both the experimental and field trials, there was a general trend of increased vegetation biomass where dung and dung beetles were applied.*

7) *Dung burial leads to increased soil nutrients*

At various stages of measurement post dung application there were significant increases in phosphorus, potassium, calcium, magnesium, and cation exchange capacity measured on experimental and field plots. In particular there were increases in potassium and magnesium, and in the cation exchange capacity. While none of these parameters were maintained at elevated levels throughout the study, *their periodic increase in the dung beetle treated plots indicate a degree of nutrient enrichment not present in the untreated plots*. Fluctuations in nutrients may depend on the nutrient content of the dung buried and the vegetation growth phase.

DO DUNG BEETLES PROVIDE A VIABLE AND PRACTICAL SOLUTION?

1) *The requirement for good quality dung*

Dung beetles feed on, and breed in dung. Without it, they will not be attracted to a site and cannot be sustained. The dung source could be from cattle or game. It could come from animals on site, or it could be collected and spread by humans. The latter would be time intensive but could create jobs. However, it would be more practical to introduce livestock for grazing and to attract beetles. Many endo-parasiticides used to treat livestock contain toxic residues in the dung that are lethal to beetles when ingested. Therefore, if livestock were introduced with one of the aims being to attract dung beetles, then this aspect would need to be carefully managed. This can be achieved through the use of tested 'Dung Beetle Friendly' products and strategically planning treatments to coincide with periods of lower dung beetle abundance.

2) How many beetles are needed to make a difference?

The number of beetles used for most applications in this study was 20 individuals per dung pat. At this rate, a measurable improvement in soil and vegetation parameters was achieved. Most of the dung in a 1 kg dung pat was also buried, reducing effects of pasture fouling and maximising the recycling of nutrients. The positive effects of beetle activity are however, concentrated beneath the dung and don't extend horizontally beyond the dung pat to a great degree. Unless every square inch of ground is covered with dung and worked by beetles (which is impractical), the effects will inevitably be heterogenous in space and time.

3) Would mass rearing and release of beetles be required?

A single cow deposits about 2.5 kg of faeces between 10 and 24 times a day in one large, or several smaller pats. If we consider that 20 beetles were required to bury 1 kg of dung in approximately 48 hrs, then we would need between 500 and 1 200 beetles to process the dung produced by a single cow in one day. On average, 309 individual beetles were collected from each mine site during each sampling trip. Therefore we can safely assume that the numbers of naturally occurring beetles required to process all the dung produced even by a small herd of cattle will initially be insufficient to bury all of their dung on rehabilitated mine sites. Although the population would grow with time, mass-rearing and release would provide an initial boost to the population and would be recommended.

RECOMMENDATIONS FOR FUTURE RESEARCH

The benefits of dung beetle activity are well established for agro-ecosystems and, based on the results of this project, have for the most part been shown to apply to reclaimed mine sites. Future research should therefore focus on methods for the application, management and monitoring of dung beetles on reclaimed mines.

Livestock would need to be introduced to a number of reclaimed mine sites. Ideally sites would include some of those assessed in this project as we have a good understanding of the baseline dung beetle assemblage and environmental conditions. Livestock could be introduced to suitably grassed, fenced areas and rotated after a period of 4-6 weeks. Once livestock have been introduced, we could then attempt to answer the following research questions:

- Given the provision of dung producing livestock, how would naturally occurring versus mass-reared and released dung beetle populations fluctuate and influence their environment?
- How do naturally present dung beetle assemblages respond to the presence of dung-producing livestock on reclaimed mines?
- When applying mass-reared beetles, at what point can we be certain that a sufficiently numerous and self-sustaining population of beetles has been established?
- Is the vegetation growth response to grazing and dung beetle activity sufficient to provide a sustainable grazing system on reclaimed mine sites? What quantities of livestock could be supported?

Chapter 1 BIOLOGICAL INTERACTIONS ON POST-MINING SOIL

By Jessica Badenhorst

1.1 HISTORY OF COAL MINING

Land is valuable, in the literal and figurative sense. Not only is the price of vacant land increasing, humans are dependent on agricultural land to provide food security. With untransformed land in limited supply, it is important to rehabilitate and restore disturbed land to an appropriate land use potential. One of the biggest causes of land degradation is mining, which has transformed more than 22% of Earth's ice-free land area (excluding Africa).

Historical records and radiocarbon dating suggest that the first use of coal may date back as far as 3 490 BC (Dodson et al. 2014). Although coal was not actively mined, the use of near-surface coal to supply heat and light was recognised as a valuable commodity, particularly in areas where wood was scarce (Théry et al. 1996). Large-scale coal mining only commenced at the beginning of the Industrial Revolution in the 19th century, advancing national productivity by association with railroads, metallurgy and steam power (Freese, 2004). Opencast (surface) coal mining needs specialised machinery − to strip overburden and dig out coal in large quantities for it to be financially feasible − which only became available in the 20th century (Coulson, 2012).

Due to the destructive approach of opencast mining, vegetation and soil in the overburden is displaced and so are the biological interactions associated with it. Approximately 500 000 hectares of land are disturbed by mining every year globally (Johnson and Lewis, 1995).

1.2 CONDITIONS ON OPEN-CAST COAL MINES

Mining activities negatively affect the physical, chemical and biological properties of soil. Frequent problems associated with previously mined land include excessive erosion, nutrient leaching and increased mineralisation rates (Kołodziej et al. 2016). The destruction of vegetation cover and disturbance of hydrological cycles lead to a hostile environment with little land use potential. Acidic soil is a common problem associated with coal mines. These soils are deficient in calcium (Ca), magnesium (Mg) and phosphorus (P) and contain an excess of hydrogen ions and aluminium (Krstic et al. 2012). In very acidic soils ($pH < 5$), aluminium toxicity is a prevalent problem for the growth of plants. Aluminium ions are passively taken up by plants by means of osmosis, inhibiting the growth of plant roots and lateral root formation (Krstic et al. 2012).

Before mining starts, topsoil is gathered from the mine by means of heavy equipment (increasing soil compaction), moved to a storage area and stockpiled for some time (Strohmayer 1999). After stockpiling, which affects the quality of the soil depending on the depth and the length of time the stockpile was stored, the topsoil is moved back to the mining area when mining activities have ceased (Strohmayer 1999). Subsoil (the soil layer beneath the topsoil) lacks the microbial communities and organic matter necessary to sustain plants and may mix with stored topsoil, creating problems for the establishment of vegetation when rehabilitation commences (Visser *et al.* 1984). The topsoil can also contain an increased bioavailability of metals due to low pH, elevated sand content, greater compaction, lack of moisture and low organic matter content (Sheoran *et al.* 2010). As soil compaction increases by spreading the soil with heavy equipment, the ability of plant roots to penetrate soil is limited and ceases entirely at a penetration resistance of approximately 2.5 MPa (Taylor, 1971; Mason et al., 1988).

1.3 ENVIRONMENTAL IMPACT OF COAL MINING

Coal mining constituted the second largest segment of income in the South African mining sector in 2012 with a value of R96 097 million – closely following platinum metal ore (R117 150 million; Lehohla, 2014). In 2012, the South African mining industry spent R1715 million on the rehabilitation of coal and lignite mines, more than nine times that of any other type of mining rehabilitation (Lehohla, 2014).

It has long been known that the mining and use of coal has a negative impact on the health of miners as well as the environment. Some of these impacts include spontaneous combustion of coal, air and water pollution and land transformation (Younger, 2004). The negative impacts have been viewed as an unavoidable consequence of contributing to a country's GDP and energy requirements (Toren and Unal, 2001). In developing countries (like South Africa) coal mining is essential for regional development, contributing significantly to the employment of areas with large coal deposits (Koko, 2015). Even though coal mining is still important in providing many developing countries' energy needs, there has been a noticeable shift towards renewable energy in developed countries. In 2016, the global coal consumption dropped by 1.6% as opposed to its average increase of 1.9% per year since

2005 (Katakey, 2017). Renewable energy, such as solar and wind energy, is becoming more available and affordable, and has influenced the decision of multiple countries to shut down many of their coal mines. In South Africa, most coal mines will be decommissioned by 2050 (Moeng, 2018). Following mine closure, most countries require the mined land to be rehabilitated or reclaimed.

1.4 MINE RECLAMATION

Mine reclamation seeks to return land disturbed by mining to pre-mining conditions which is appropriate for surrounding land uses (Bowman and Baker, 1998). Reclamation also aims to create usable land contours that facilitate productivity and protect the environment. Like many large-scale management programs, reclamation of land is most effective and sustainable when it integrates the interactions of multiple disciplines, thereby restoring ecological, hydrological, recreational and other functions of the disturbed land (Kuter, 2013; Pearman, 2009).

When monitoring the success and sustainability of a reclamation plan, physical factors (soil compaction, water quality) and vegetation (plant biomass, richness) are usually measured; whereas fauna are generally excluded (Smyth and Dearden, 1998; Cristescu et al. 2013). This is based on the assumption that fauna will return to the area after the flora has re-established (Block et al., 2001; Thompson and Thompson, 2004). The assumption is possibly flawed in the sense that ecosystem functionality relies on the many services that fauna provide, like nutrient cycling, soil aeration, pollination, pest control and seed dispersal (Nichols et al. 2008; Frouz et al. 2006).

There are many steps involved in reclaiming disturbed land which usually includes topsoil management, managing overburden and soil and landscape design (Krutka and Jingfeng, 2013). Topsoil management is critical because it is a valuable and scarce resource. Revegetation of disturbed land is essential and will not be successful without adequate topsoil of a good quality. Establishing vegetation in mine-disturbed soils will stabilise the soil surface area and reduce the seepage of water through the mine spoils which potentially increases acid mine drainage in groundwater resources (Limpitlaw et al. 1997). Grass is mostly used for revegetation purposes because it has a high turnover of roots and could aid land capability by providing feed for grazing animals (Limpitlaw et al. 1997).

1.5 SOIL FERTILITY AND PLANT GROWTH

To improve soil fertility, organic matter plays a significant role in providing microflora with energy, aiding in the formation of soil structure, and assisting in the water holding capacity of the soil (Frouz et al. 2006). Adding organic matter to soil aids it in resisting soil degradation as well as alleviating soil compaction, therefore decreasing soil strength (Carter, 2002). Organic matter is mostly composed of decomposing plant and animal life, as well as their excretions. Animal manure has been extensively used by farmers to improve soil fertility, focussing on alleviating soil compaction caused by heavy machinery and cattle. It has been reported that the addition of green leaf manure increased water infiltration rates by 0.4 $cm.h^{-1}$ and decreased bulk density by 0.02 $Mg.m^{-3}$ (Reddy, 1991). Plant growth is dependent on nutrient availability in the soil, particularly nitrogen (N) and P. Nutrients in manure, such as P, K, Mg and Na, enhance and regulate important processes within vegetation and increase plant growth (Hutton et al. 1967).

Nitrogen found in manure occurs mostly as organic ammonium which needs to be mineralised to its inorganic form for plant uptake (Pettygrove 2009; Pratt and Castellanos 1981). In order for plants to incorporate N into their tissues, ammonium has to go through two processes namely nitrification and assimilation; these processes may be complicated by the presence of free anions (such as phosphate, sulphate and nitrate) in the soil, binding to positively-charged ammonium cations (Lamb et al. 2014). Ammonium may accumulate in soil and will not be absorbed by plants if soil pH is low (<5.5) or if there is a lack of organic matter by depressing microbial ammonium oxidation (Mengel and Kirby 1987). These are common features of post-mining soils. Soil pH influences the fertility of the soil, where the pH level determines the availability of plant nutrients and affects plant growth (Jones, 2012). Soil pH affects the cation exchange capacity of soil, which could cause soil to be deficient in Ca and Mg (Fertilizer Industry Federation of Australia, 2006).

Cation exchange capacity (CEC) is the ability of soil to retain cations, particularly Ca, Mg, potassium (K) and sodium (Na). When cations are bound by negatively-charged soil or organic matter particles, these cations become available to plants (Ketterings et al., 2007). Soils with a greater clay content tend to have a higher CEC whereas sandy soils rely on organic matter to increase CEC (Brown and Lemon, 2016). Adding organic matter to soil can increase the CEC four to 50 times per given weight than clay, but it requires years to take effect (Ketterings et al., 2007; Brown and Lemon, 2016).

If manure is not incorporated into the soil, most N is lost due to volatilisation and will not be available for plant uptake. Although manure can be mechanically worked into the soil using machines or labour, it is not sustainable and will be an unavoidable reoccurring expense. In natural and agricultural systems, manure is utilised and broken down by various soil macrofauna and microbes. The burrowing activities of soil macrofauna greatly influence decomposition, nutrient cycling and water movement of soil (Bot and Benites, 2005). Dung beetles are among the most important and efficient invertebrates that contribute to the burial and decomposition of dung (Lee and Wall 2006).

1.6 SOIL INVERTEBRATES

Soil fauna contribute greatly to soil structure and have been estimated to represent about 23% of all described organisms (± 360 000 species), 85% of these species being arthropods (Culliney, 2013). Soil invertebrates play significant roles in most important soil functions, especially in water infiltration, soil erosion, plant growth, regulating soil organic matter and nutrient cycling (Lavelle and Spain, 2001). Most soil invertebrate research has focussed on ants, termites, and earthworms. Many species of earthworms, termites, ants and dung beetles have comparable burrowing activities. Creating subterranean tunnels facilitates soil mixing, alleviates soil compaction and modifies soil structure. Earthworms ingest and excrete soil matter, altering soil resources and fertility; however, these animals can only exert radial pressures of approximately 200 kPa (Lavelle et al. 1997).

Mechanical loosening of highly compacted soils by using tines or radial blades may result in the re-compaction of treated soil as this method does not necessarily consider the degree to which soil structure is degraded (Spoor et al. 2003).

Entomoremediation is a novel term used to describe the decontamination of soil using insects, particularly those considered to be ecosystem engineers (Ewuim, 2013). The groups of insects that are most applicable to this term include ants, termites, collembolans and beetles. Many soil invertebrates have been found to accumulate metals; ants that were collected in metal-polluted sites had high concentrations of various metals (Pb, Cd, Cu, Zn, Fe and Mn) in their midgut epithelium (Rabitsch, 1997). Many soil invertebrates can sequester metals at least to the extent that it is no longer hazardous for their survival (Hopkin 1989). With the development of this new term, comes the possibility of "Entomoreclamation" – the use of insects to remediate degraded soil.

It is implicit that the mass rearing of candidate insects will be required for effective treatment of degraded soil. Termites and dung beetles have been mass-reared; however, there is no research available investigating their ability to decontaminate soil (Hayakawa and Kamashita, 1990; Leuthold et al., 2004). Dung beetles are exceptional candidates to improve degraded soil as they have successfully been mass-reared, transported and introduced to various locations around the world (McKay, 1976).

Insects with similar burrowing activities as those exhibited by paracoprid dung beetles have shown similar significant improvements to water infiltration rates. Areas where the old nest materials of a termite species (*Anoplotermes* spp.) were present had an infiltration rate 27 times higher than surrounding, unmodified soil (Martius 1990). Likewise, areas around ant nests increased the infiltration rates threefold compared to those of surrounding farmlands (Majer et al. 1987). Subterranean termite and ant nests contribute greatly to enhancing physical and chemical soil properties, particularly increasing water infiltration and aeration (Martius 1990).

1.7 DUNG BEETLES

Dung beetles, from the subfamily Scarabaeinae, have evolved to specialise their feeding mainly on dung. They are further classified based on the way they process dung into three functional guilds. Rollers (telecoprids) break off a piece of dung from the dung pat, form it into a ball and roll it away to avoid competition; tunnellers (paracoprids) construct broodball and bury them at the bottom of tunnels which are dug directly beneath the dung pat; dwellers (endocoprids) complete their entire lifecycle inside the dung pat where they feed and breed.

Many studies have found that dung beetles are actively involved in the ecological processes of soil, particularly nutrient cycling (Brussaard and Runia, 1984; Halffter and Edmonds, 1982; Nichols et al. 2007; Farias and Hernandez, 2017). In agroecosystems, dung beetles have been observed to enhance plant growth by directly contributing to soil bioturbation, soil aeration and nutrient cycling (Hanafy, 2012; Farias and Hernandez, 2017). Coprophagous beetles do not usually disperse over great distances to locate dung and are therefore sensitive to environmental changes like habitat loss and have been found to function as important bioindicators (Favila and Halffter, 1997; McGeoch et al., 2002; Spector, 2006; Salah, 2014). It is theorised that highly compacted soils may limit the tunnelling activity of dung beetles and, in turn, their effects on soil properties and plant growth.

The importance of the dung burial service provided by dung beetles is illustrated by the Australian Dung beetle Project (Bornemissza 1976). Exotic dung beetles from various countries, including South Africa, were introduced into Australia because these beetles co-evolved alongside large herbivores and bovines, therefore being capable of processing and utilising the abundant cattle dung on Australian pastures (Bornemissza 1976). Great care was taken when selecting species for introduction; the dung beetles had to be compatible with Australian weather and soil types, with a low risk of predation, would not themselves become pests and would remove most of the dung in 48 hours (Bornemissza 1976). The program was highly successful with some dung beetle species establishing sustainable populations in Australia. Of the 23 species that have successfully established, 13 of these species were introduced from South Africa (Edwards 2007).

Certain dung beetles are more efficient at processing dung. Tunnellers, especially species larger in size, may remove more dung as compared to rollers and dwellers (Shahabuddin, 2014). In the absence of nocturnal large-bodied tunnellers, dung removal can decrease with as much as 75% (Slade et al. 2007). Research done by Manning et al. (2016) also suggests that functionally diverse groups of dung beetles may provide a variety of ecosystem services, and that redundancy is an important characteristic of a thriving ecosystem.

The economic value of dung beetles in the United States of America was calculated by Losey and Vaughn (2006) and is summarised in Table 1. About a third of dung produced by cattle in the USA is processed by dung beetles, other dung is either treated or the surface where the cattle are maintained is artificial. This table highlights the contribution of dung beetles as ecosystem engineers to agro-ecosystems. A similar study was done in the United Kingdom where dung beetles were estimated to save the country approximately £367 million each year in the cattle industry (Beynon et al. 2015).

Table 1. Modified table from Losey and Vaughn (2006) showing the economic value of dung beetle activity in the United States of America.

Total economic losses averted annually as a result of accelerated burial of livestock faeces by dung beetles			
Billions of US dollars			
	Estimated losses		
Cause of loss	No dung beetle activity	Current dung beetle activity	Losses averted
Forage fouling	0.65	0.53	0.12
Nitrogen volatilisation	0.31	0.25	0.06
Parasitism	.098	0.91	0.07
Pest flies	1.83	1.70	0.13
Total losses averted			0.38

1.8 RESEARCH QUESTIONS

Many studies have evaluated the effects of soil invertebrates on soil and plant properties, but a research gap remains in assessing the effects and benefits of soil invertebrates for degraded and compacted soil. As the incorporation of manure into soil will improve many aspects of the soil structure (nutrient content, soil fertility, soil pH, moisture etc.), it was determined that dung beetles could provide a good option to accomplish this due to their tunnelling and dung burial activity.

In this study, the tunnelling activities of dung beetles were assessed focussing on their effects on soil and plant properties of reclaimed mined land, both in a controlled environment and in the field. The research questions presented asked:

1) How does dung beetle activity influence soil properties and herbaceous plant growth response on:

- Constructed plots simulating reclaimed mined conditions?
- Reclaimed mined land?

2) To what extent can we rely on naturally-occurring dung beetles on the reclaimed mined land to incorporate dung into the soil?

These questions will be addressed in the following chapters.

Chapter 2 DUNG BEETLE ACTIVITY IMPROVES HERBACEOUS PLANT GROWTH AND SOIL PROPERTIES ON PLOTS SIMULATING RECLAIMED MINED LAND

By Jessica Badenhorst

This chapter has been prepared according to the guidelines for the Journal of Applied Soil Ecology, and has been accepted with changes which have been made and the manuscript has been re-submitted.

2.1 INTRODUCTION

As the need for enhanced agricultural production becomes more important with a growing world population, land degradation may be a threat to the productive capacity of the land. Soil contamination, compaction, erosion and leaching are common features of degraded land and contribute to a decline in plant productivity. Human activities have modified > 50% of the earth's surface, agriculture being the main contributor (Hooke et al., 2012). Unlike agriculture, mining activities permanently alter the land's topography, drastically impairing land capability. Surface coal mining destroys 2-11 times more land than underground mining by removing vegetation and disturbing soil hydrologic regimes (Li, 2006).

In some countries, mine closure necessitates the return of land to viable land use capabilities such as agriculture. The effects of mining activities and wastes include the loss of grazing areas for animals and cultivated land, loss of agricultural production, water and air pollution, soil erosion, loss of biodiversity and geo-environmental disasters (Sheoran et al., 2010).

Degraded soils found on mining sites experience many problems regarding the establishment and maintenance of herbaceous plants related to soil such as loss of soil horizons and structure, poor soil fertility, reduced soil pH, extreme nutrient leaching, decreased nutrients available for plants, decreased cation exchange capacity, increased soil erosion and increased compaction (Mensah, 2015). Topsoil is essential for vegetation establishment. Improving the condition of topsoil by reducing N-losses and increasing soil nutrients and microbes is central to an effective reclamation plan (Sheoran et al., 2010). Vegetation establishment, following top soil improvement, contributes greatly to restoration of hydrological processes as this develops over time in association with the plant community (Clark and Zipper, 2016).

In developing countries, a common post-closure land use is low-intensity grazing (Limpitlaw and Briel, 2014). The nutritional value of plants is determined by their protein content, which is derived from the plants N content. Herbage feed-value becomes increasingly important when cattle are used to graze areas and turns into an expensive practice when fertilisers are needed to improve vegetation quality, as crude protein content is otherwise too low. Cattle manure generally contains five essential nutrients for plant growth (N, P, K, Ca and Mg) and is abundant in organic matter (Onwudike, 2010). Phosphorus is involved in root development and energy storage, K promotes plant metabolism, Ca has a major role in cell integrity and membrane permeability and Mg is actively involved in photosynthesis (Silva and Uchida, 2000). Nitrogen is vital for protein synthesis.

Dung beetles in the subfamily Scarabaeinae are classified by their predominant activity when processing dung. The three major functional guilds are telecoprids (dung beetles create a dung ball from a portion of a dung pat, roll the dung ball away and bury the dung ball at a different location from the dung source), endocoprids (dung beetles complete their entire lifecycle inside a dung pat) and paracoprids (dung beetles construct tunnels directly underneath dung pats, forming a continuous link to the dung source). An estimated 70% of southern Africa’s approximately 780 species of dung beetles are tunnelers (Davis et al., 2008).

The ecosystem services provided by dung beetles have been extensively reviewed by Nichols et al. (2008), stating that dung beetles play an important role in parasite suppression, secondary seed dispersal and nutrient cycling. Dung beetles contribute greatly to ecosystem functionality, particularly in the case of nutrient cycling and plant growth enhancement (Tixier et al., 2015). Nitrogen found in dung is integrated into the soil much faster if an adequate number of dung beetles are present, reducing the amount of N lost due to ammonia volatilisation from 80% to a value between 5-15% (Yamada et al., 2007). Dung beetles enhance air permeability in soil, facilitate the transfer of nutrients in dung to soil, leading to an increase in herbage feed-value, biomass and nutritive value of the vegetation (Mittal, 1993; Bang et al., 2005; Yamada et al., 2007). High-diversity assemblages of dung beetles are likely to improve functionality in a range of ecosystems, thereby contributing to ecosystem services (Manning et al., 2016). Tunnelling by dung beetles can improve various physical and hydrological aspects of soil by increasing water infiltration rates leading to higher soil moisture and reducing soil bulk density. Improved water infiltration rates result in reduced surface water runoff which ultimately reduces rates of soil erosion (Brown et al., 2010). Slade et al. (2015) showed that the presence of dung beetles promoted bacterial transfer across soil-dung interface, subsequently increasing community- and function similarity. Dung beetles have been mass-reared and introduced into Australia, New Zealand, the United States of America and elsewhere for the purpose of dung burial, pest control and to facilitate pasture improvement in agro-ecosystems (Edwards, 2007; Bertone, 2005; Dymock, 1993; Bornemissza, 1976).

Most studies of the activities of dung beetles are undertaken to better understand their role and benefits in agro-ecosystems (Beynon et al., 2012, Farias and Hernández, 2017). Few studies have investigated their ability to maintain their activities and associated benefits in systems with extreme soil degradation, such as on reclaimed coal mines. In particular, soil bulk density rates on rehabilitated coal mines can be in excess of 1.8 g/cm^3 while those in agro-ecosystems are generally in the range of 1.1 to 1.5 g/cm^3 (Sheoran et al., 2010, Haigh and Sansom, 2007). Highly compacted soil may present a physical barrier to the tunnelling abilities of dung beetles. However, if they are able to maintain their activities under these conditions then dung beetles present a potentially valuable resource to be considered for improving reclaimed mined land conditions to further increase the range of viable land-use options. The objectives of this study were to determine whether dung beetles applied to simulated reclaimed mine soils can maintain their beneficial activities by measuring: (1) the properties of soil in terms of penetration resistance (kPa), nutrients (mg/kg), pH, cation exchange capacity (cmol(+)/kg) and water infiltration rate ($mm.h^{-1}$); (2) the growth response of plants in terms of above-ground biomass (g/m^2) and crude protein content (%); (3) the longevity of these effects on experimental plots. It is hypothesised that areas where dung beetles have been active will have:

- Higher water infiltration rates
- Lower soil penetration resistance (reduced bulk density)
- Greater soil nutrient content, pH and cation exchange capacity
- Increased plant biomass and protein content
- Effects lasting at least 6 months

2.2 METHODS

2.2.1 Field collection of dung beetles

During January 2015 *Euoniticellus intermedius* (Reiche, 1849), *Digitonthophagus gazella* (Fabricius, 1787) and *Onitis alexis* (Klug, 1835) were collected from a rural area in Brits, North-West Province (-25.273877, 27.778443) for the purpose of establishing a breeding programme. The dung beetles were collected by locating cattle dung pats and gathering the relevant species. Pitfall traps were also used by placing a 2 L bucket into the ground, covering the top of the bucket with a steel mesh (20 mm x 20 mm) and placing 1.5 kg of cattle dung on top of the mesh. These three species were chosen based on their successful establishment in the Australian dung beetle project, and occur naturally in the Highveld of South Africa, where coal mining is concentrated (Bornemissza 1976).

2.2.2 Breeding dung beetles

Dung beetles of the above-mentioned species were placed in 2 L buckets filled with sand, leaving enough space for a 3 cm layer of cattle dung and a lid with gauze insert for aeration. Beetles of each species were divided into pairs (one male and one female) per bucket, and fed a diet of 1 kg fresh cattle dung twice a week. The sand was sieved once a week in order to locate brood balls. The brood balls were placed in buckets separate to the beetles and were kept moist by applying small amounts of water with a watering can twice per week. The ambient temperature and humidity during the summer was sufficient for beetle survival and brood ball development. During the winter, the breeding environment was kept at 25^{o}C using an air conditioning system and at a relative humidity of 50-60% using a humidifier.

2.2.3 The study site

The experiments were conducted at the University of Pretoria experimental farm, Gauteng, South Africa, at an altitude of 1 308 m.a.m.s.l. (S25.752295, E28.252754). A total of 12 plots constructed of brick were used, each measuring 1 m^3. The soil profile typical of rehabilitated mined land was simulated by layering 60 cm of waste coal, followed by 30 cm of subsoil, and finally a 30 cm layer of topsoil. The soil used in the uppermost layer was a Hutton / Clovelly soil mixture, which is commonly used for rehabilitation purposes (Viljoen & Associates, 2013). The soil was classified as a sandy loam consisting of 77% sand, 6% silt and 17% clay. Preferential water flow along the edges of the plots was reduced by constructing concrete ridges on the interior walls. A grass seed mixture typically used in the mine rehabilitation process was applied to the experimental plots. At the time of the study, the plant community was dominated by *Digitaria eriantha* (Steud.) and *Chloris gayana* (Kunth).

The mean annual temperature was 17.8 °C; mean annual precipitation was 697 mm. All plots were exposed to the same ambient environmental conditions.

2.2.4 Preparation of experimental plots

Each of the 12 plots was covered with a 1m^3 insect gauze (mesh size 1.4 mm x 1.4 mm) enclosure using iron rods (1.5 m in length, 10 mm thick) as support and weighted down with bricks on the walls of the plots (Figure 1). This was to prevent the movement of beetles in and out of the plots. Three treatments were applied to each of four replicated plots: dung + beetle; dung only; control (no dung, no beetles; Figure 2). The first treatment was applied in April, 2015 and the second treatment was applied in March, 2016. All dung used in this study was collected from grass-fed, drench-free cattle.

Figure 1. The constructed experimental plots situated at the University of Pretoria. The plots were covered with insect gauze.

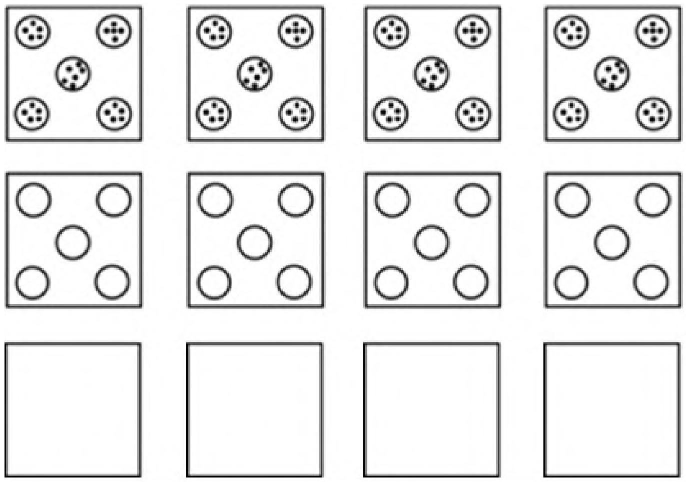


Figure 2. The first treatment contained five 1 kg dung pats and 100 dung beetles per plot. The second treatment contained five 1 kg dung pats on four plots. The third treatment contained no dung and no dung beetles on four plots. Size categories for paracoprid dung beetles: small (6-9 mm), medium (10-12 mm) and large (15-22 mm).

For the dung and beetles (D+B) treatment, each of the dung beetle plots received a total application of 100 dung beetles and five 1 kg fresh cattle dung pats placed on the soil surface (Figure 2). Dung pats were applied in such a manner that all soil surface areas were covered in dung over time, with the first application placed alternatingly and the second application filling the areas that had not been exposed to dung. Dung and beetles were applied during autumn months in two separate applications within a period of 18 months. Three species of paracoprid (tunnelling) dung beetles were used in the treatments: *E. intermedius*, *D. gazella* and *O. alexis*. The variation in dung beetle body size was selected to ensure a range of tunnel widths and to reflect the body size range of dung beetles in the natural environment. All dung beetles were allowed to roam freely within the plots where they were placed.

The dung only (D) treatment consisted of five 1 kg fresh cattle dung pats placed on the soil surface in the manner described above with no dung beetles in order to study the effect of dung placement alone. The no dung + no beetles (X) treatment represented reference conditions from which to compare the results of the other two treatments.

Measurements of effects were repeated one month after each treatment application in May 2015 and May 2016 as well as six months after the treatment applications (in September 2015 and September 2016) to determine the longevity of effects.

2.2.5 Herbaceous plant biomass and protein content

The herbaceous plant biomass ($g.m^{-2}$) was calculated by trimming the herbaceous plant cover (predominantly grasses) down to 5 cm above the soil surface, placing the cuttings into paper bags which were then oven-dried at 65°C for 48 hours, and weighed. The crude content (%) of the dried herbaceous material was measured by Nvirotek (NviroTek Laboratoriums (Pty) Ltd. to determine herbaceous plant quality, and in turn, an important component of herbage feed-value.

2.2.6 Soil properties

A 100 g sample from the top 20 cm of topsoil was collected randomly from each experimental plot and was analysed by Nvirotek (NviroTek Laboratoriums (Pty) Ltd). Soil pH (1 to 2.5 ratio extraction with 1.0 M KCl ; determined with pH meter), and soil nutrient content including phosphorus (P; 1 to 7.5 ratio extraction with BRAY I extractant; determined colourimetrically), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) and sulphur (S; 1 to ten ratio extraction with 1.0 M ammonium acetate; determined by inductively coupled plasma analysis) was measured as well as the cation exchange capacity (CEC; saturation by 1.0 M ammonium acetate, washed by ethanol and extracted with 1.0 M KCl; determined colourimetrically) and soil particle size (% clay, sand, silt; Bouyoucos method). The analyses provided information on the soil's ability to bind essential nutrients and to determine which nutrient levels were more readily improved by paracoprid dung burial.

2.2.7 Soil strength

Penetration resistance (kPa) was measured using a handheld penetrometer (Geotron Hand Penetrometer, serial 100401, model P5) and was recorded for each centimetre up to 20 cm in depth. A total of five measurements were taken randomly per plot. One can infer a level of soil compaction from penetration resistance (soil strength) which may indicate the degree to which paracoprid dung beetles can reduce soil compaction.

2.2.8 Water infiltration rate

Water infiltration rates were measured to determine the influence of dung beetle tunnelling on the infiltration of water into the soil. A double ring infiltrometer was driven into the soil with a hammer for at least 1 cm after which water was added to the outer ring and manually maintained at a constant level. Water was then added to the inner ring. The time that the water level took to decrease by 2 cm was measured and converted to $mm.h^{-1}$ (Gregory et al., 2005). This method was repeated five times per plot.

2.2.9 Data analysis

Using Statistica 13 (StatSoft, Dell Inc., ver. 10), the data were analysed to ensure the assumptions for parametric tests were met. Data which were not normally distributed were transformed using logarithmic transformations. One-way analysis of variance (ANOVA) and *post-hoc* Tukey's HSD tests were used to compare group means and to determine whether herbaceous plant and soil parameters differed significantly between treatments. Statistical significance was assumed at $p < 0.05$. A Principal Components Analysis (PCA) was used to detect groupings in the plots using the measured soil and herbaceous plant parameters to assess possible relationships among the variables.

2.3 RESULTS

2.3.1 Herbaceous plant biomass and protein content

Post-hoc comparisons using the Tukey's HSD test indicated that biomass yield was significantly greater ($p < 0.001$) for plots with dung beetles (D+B) compared to plots with only dung (D) and reference plots (X) for measurements taken six months after the first treatment application; the same trend was not observed six months after the second application (Figure 3). One month after the second application, the average biomass yield for D+B treatments was 150.38 $g.m^{-2}$ ± 12.72, approximately 80 $g.m^{-2}$ more than the D and X treatments. The results showed no significant difference between D treatments and X treatments for measurements taken one month and six months post the first and second treatment applications ($p > 0.05$). Herbaceous plant protein content was not significantly different among any of the treatments for measurements taken (Figure 4).

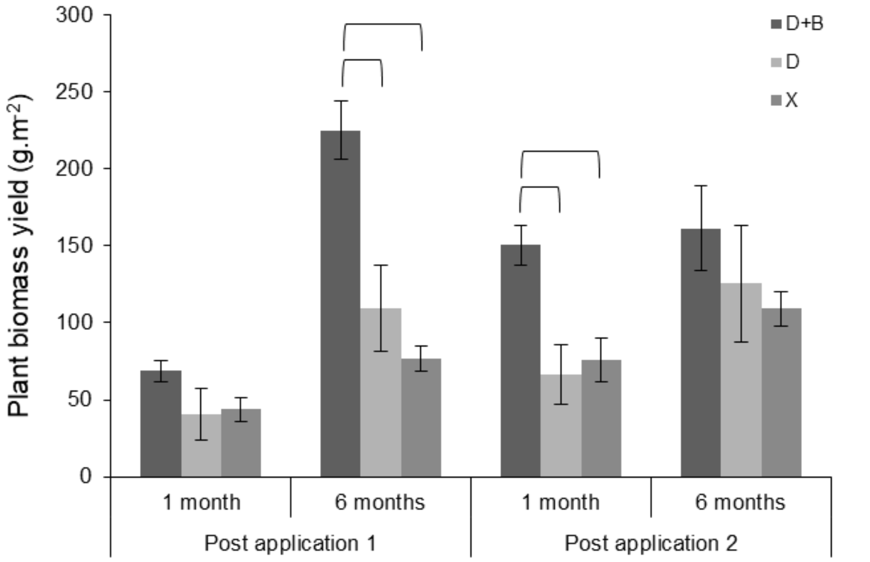


Figure 3. Mean ± SE values for herbaceous plant biomass yield (g.m^{-2}) measurements taken one month and six months post the first treatment application, and one month and six months post the second treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); and no-dung-or-dung beetles (X; n= 4). [* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$].

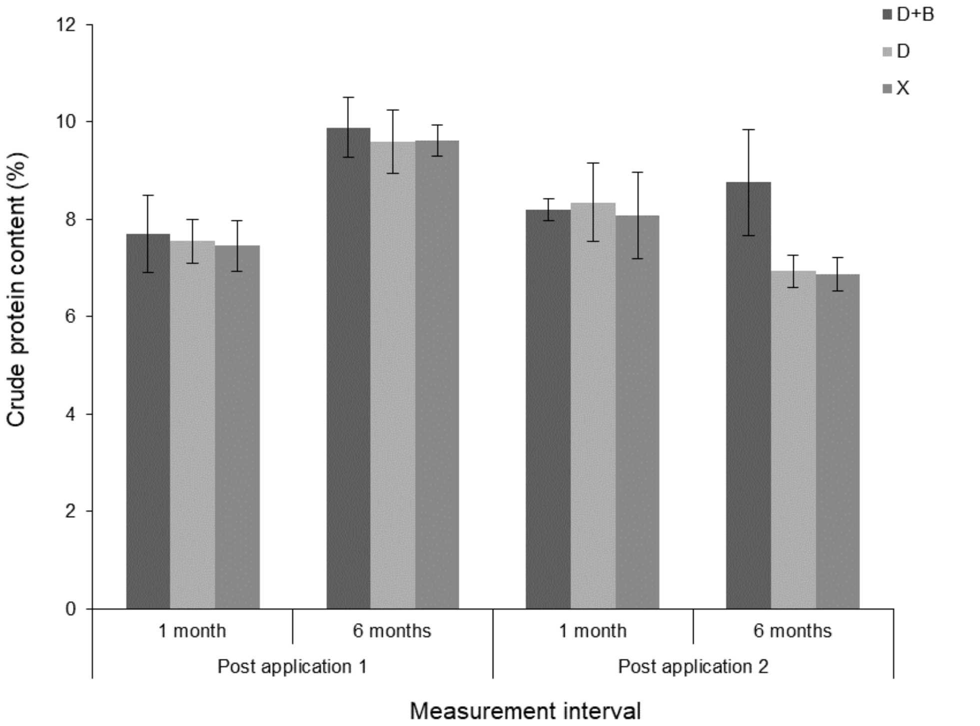


Figure 4. Mean ± SE values for herbaceous plant crude protein content (%) measurements taken one month and six months post the first treatment application, and one month and six months post the second treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); and no-dung-or-dung beetles (X; n= 4). [* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$].

2.3.2 Water infiltration rates

Water infiltration was significantly higher for D+B treatments when measurements were taken immediately after treatment application, as well as six months later ($p < 0.01$). A one-way ANOVA indicated that there was no significant difference between D and X treatments except when measurements were taken six months after the first application (Figure 5). The average water infiltration rate for D+B treatments reduced after the second application was made, decreasing from 152.97 $mm.h^{-1}$ ± 5.67 to 96.83 $mm.h^{-1}$ ± 6.10.

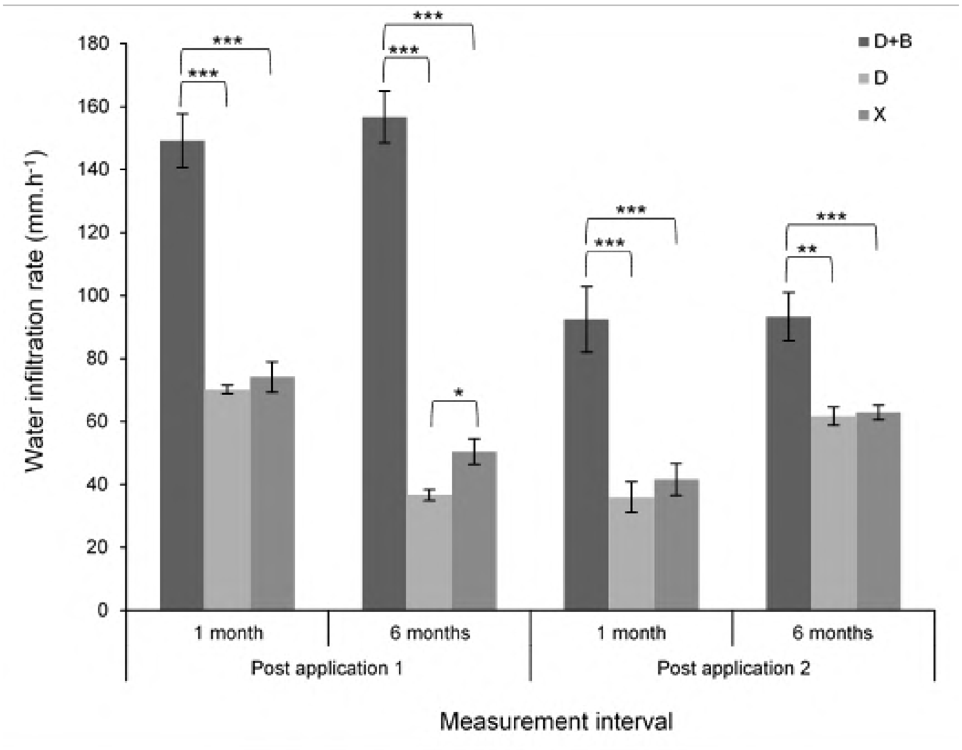


Figure 5. Mean ± SE values for water infiltration rate ($mm.h^{-1}$) measurements taken one month and six months post the first treatment application, and one month and six months post the second treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); and no-dung-or-dung beetles (X; n= 4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

2.3.3 Soil strength

No statistical significance was seen among any of the treatments for measurements taken one month after the first application (Figure 6). Soil penetration resistance was significantly lower at soil depths of 1-2 cm, 4-14 cm and 18-20 cm six months after the first treatment application, with D+B treatments having a lower penetration resistance when compared to D and X treatments (Figure 7). D and X treatments had similar penetration resistance values. Plots with dung beetles had significantly lower penetration resistance between 2-18 cm, and no difference was observed for D and X treatments (Figure 8). In comparison, the measurements taken six months after the second treatment application increased linearly in penetration resistance as a greater depth was reached (Figure 9). D+B treatments had a significantly lower penetration resistance at a depth of 2-19 cm when compared to D and X treatments.

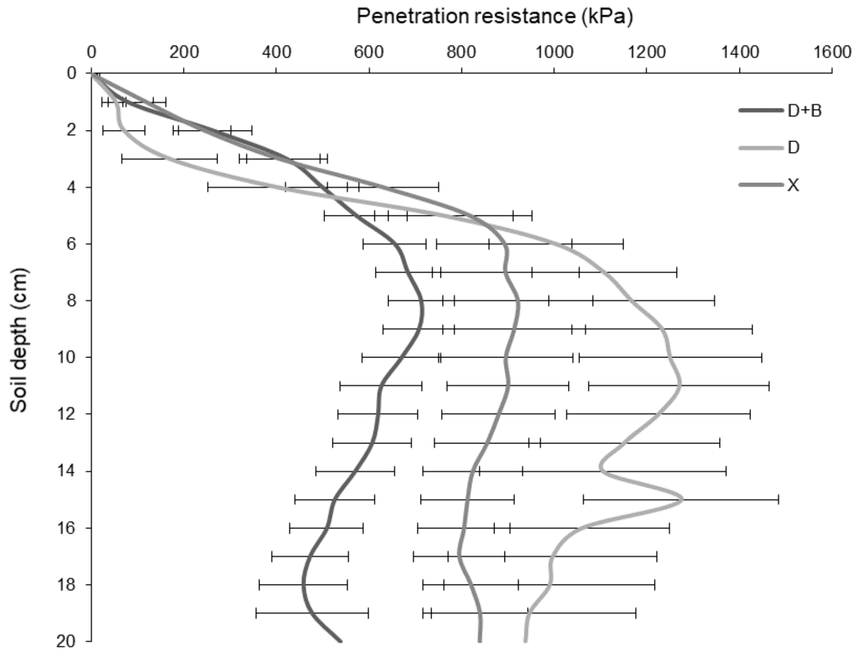


Figure 6. Mean ± SE values for soil penetration resistance (kPa) measurements taken one month post the first treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); and no-dung-or-dung beetles (X; n= 4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

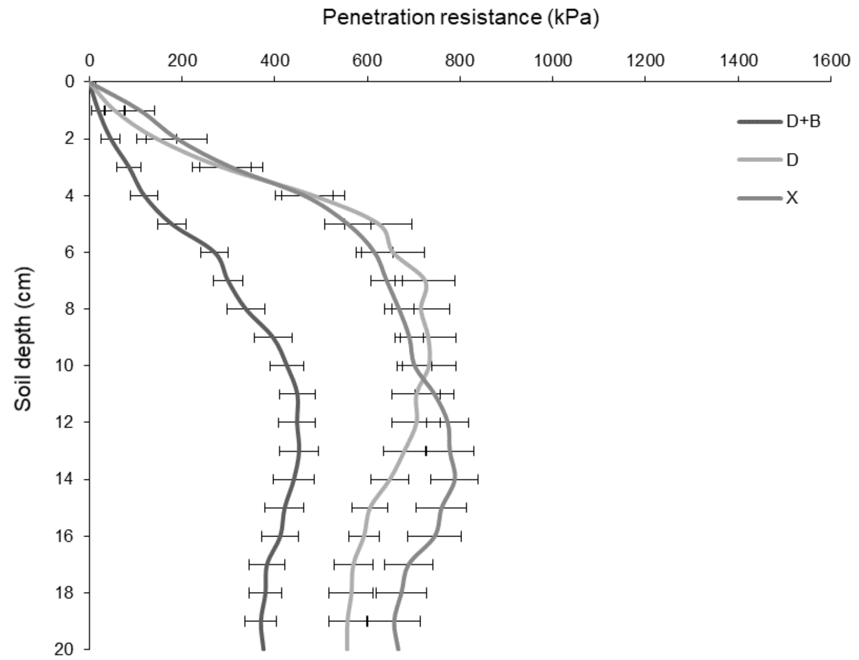


Figure 7. Mean ± SE values for soil penetration resistance (kPa) measurements taken six months post the first treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); and no-dung-or-dung beetles (X; n= 4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

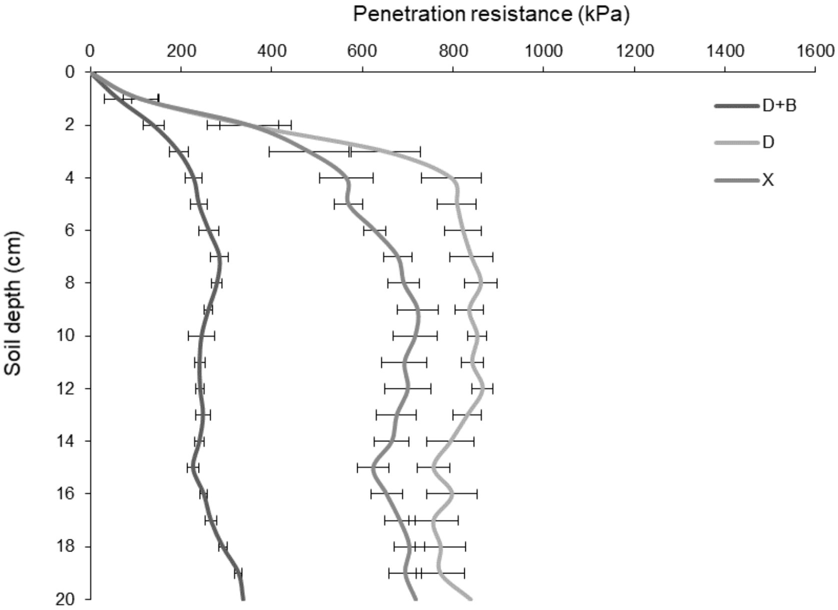


Figure 8. Mean ± SE values for soil penetration resistance (kPa) measurements taken one month post the second treatment application Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); and no-dung-or-dung beetles (X; n= 4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

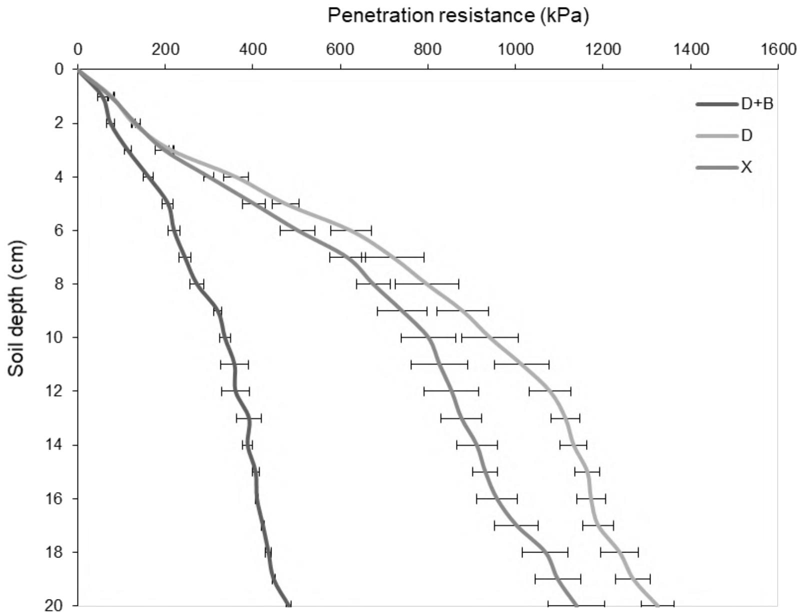


Figure 9. Mean ± SE values for soil penetration resistance (kPa) measurements taken six months post the second treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); and no-dung-or-dung beetles (X; n= 4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

2.3.4 Soil properties

Post-hoc Tukey's HSD tests indicated that there were significant differences for K, Ca and AmAc levels in the soil analysed one month after the first application (**Error! Reference ource not found.**). Only K was significant six months post the first application. No statistical significance was seen for any parameter one month after the second application. The analysis done for measurements taken six months post the second application indicated that P, Mg and CEC were significantly greater for D+B treatments.

The CEC for D+B plots were 4.49 cmol (+)/ kg ± 0.17, averaging on 1.2 cmol (+)/ kg more than D and X treatments (3.19 cmol (+)/ kg ± 0.29 and 3.39 cmol (+)/ kg ± 0.33, respectively). All comparisons between D and X treatments were not significant (p >0.05).

Table 2. Mean ± SE of soil parameters measured from three treatments; dung-and-dung beetles (D+B), dung-only (D) and no-dung-or-dung beetles (X). Measurements were taken after the first application, six months after the first application, after the second application and six months after the second application.

	Post application 1						Post application 2					
	One month			Six months			One month			Six months		
Soil properties	D+B	D	X	D+B	D	X	D+B	D	X	D+B	D	X
pH	5.7 ± 0.2	5.6 ± 0.1	5.9 ± 0.2	6.1 ± 0.2	5.8 ± 0.2	5.7 ± 0.3	5.7 ± 0.2	6.1 ± 0.3	5.4 ± 0.2	6.3 ± 0.2	5.7 ± 0.3	6 ± 0.3
P ($mg.kg^{-1}$)	9.2 ± 2.9	5.2 ± 3.1	3.7 ± 1.4	20.9 ± 8.1	9.2 ± 3.5	10.3 ± 4.5	18.2 ± 3.9	12.6 ± 2.6	12.1 ± 3	**21.7 ± 5.6 ***	12.7 ± 3.3	5.7 ± 1.4
K ($mg.kg^{-1}$)	**335.4 ± 50 ****	93.3 ± 14.6	74.7 ± 27.3	**295.5 ± 59.7 ***	168.1 ± 49.1	111.2 ± 19.5	144.3 ± 19.6	240.5 ± 46.2	115.3 ± 14	164.6 ± 17	108.5 ± 13.2	115.1 ± 23.6
Ca ($mg.kg^{-1}$)	**94.1 ± 5.4 ***	60.6 ± 8.1	57.6 ± 8.8	448.5 ± 72.9	271 ± 40.9	300.2 ± 32.6	445.5 ± 52.3	472.4 ± 74.1	367.7 ± 28	447.3 ± 47.2	321.8 ± 37.3	317.9 ± 30.8
Na ($mg.kg^{-1}$)	22.6 ± 5.9	20.7 ± 5	20.4 ± 2.6	34.5 ± 2.5	34 ± 3.1	37.3 ± 5.9	18.4 ± 0.8	16.6 ± 1.2	13.6 ± 0.7	18.2 ± 4.2	15.5 ± 2.3	14.4 ± 1.3
Mg ($mg.kg^{-1}$)	302.9 ± 31.5	190.3 ± 32.1	194.6 ± 27.5	178.9 ± 23 *	121 ± 20	102.7 ± 10.3	172 ± 29.4	169.7 ± 24.4	91.5 ± 4.6	**165.3 ± 25.4 ***	90.5 ± 13.2	77.3 ± 7
AmAc ($mg.kg^{-1}$)	**19.7 ± 1.4 ***	14.3 ± 1.3	15.2 ± 1.1	27.8 ± 2.9	32 ± 8.3	33.5 ± 5.4	9.4 ± 0.9	14 ± 3.2	9.1 ± 0.6	20.2 ± 1.9	37.2 ± 18.5	21.1 ± 1.8
CEC [cmol(+)/kg]	2.4 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	ND	ND	ND	2.1 ± 0.5	2.2 ± 0.3	1.8 ± 0.5	**4.5 ± 0.2 ***	3.2 ± 0.3	3.4 ± 0.3

* − $p \leq 0.05$

** − $p \leq 0.01$

ND – not determined

2.3.5 Principal Components Analysis

The D+B treatments were clearly separated from the D and X treatments for measurements taken one month after treatment applications as well as six months later. For Principal Component 1 (PC1), Mg had a high factor loading for each analysis with a correlation between 0.90 and 0.98 (Table 3). After the first application, Ca and Mg had the highest factor loading for PC1 with correlations of 0.98 each. Principal Component 1 and 2 combined accounted for 73.91% of the total variation (Figure 10). Similarly, Mg and Ca, as well as K, had the highest factor loadings for measurements taken six months after the first treatment application with a correlation of -0.95, -0.96 and -0.93, respectively. The separation of the three treatments is seen along the PC1 axis, with D+B treatments being distinct from D and X treatments (Figure 11).

Although PC 1 explained most of the variation seen for all PCA results, PC 2 showed a clearer trend in the data with D+B treatments being separated from the other two treatments along the PC2 axis for measurements taken after the second application (Figure 12). Separation in treatments seen for measurements taken after the second application was mainly due to PC 2 where the highest factor loadings were from herbaceous plant biomass and water infiltration rate at a correlation of 0.85 and -0.82, separately. Principal component 1 and 2 combined accounted for 61.87% of the total variation.

Six months after the second application, the highest factor loadings for PC1 were seen for Mg (0.93) and crude protein content of the herbaceous plants (0.85; Figure 13).

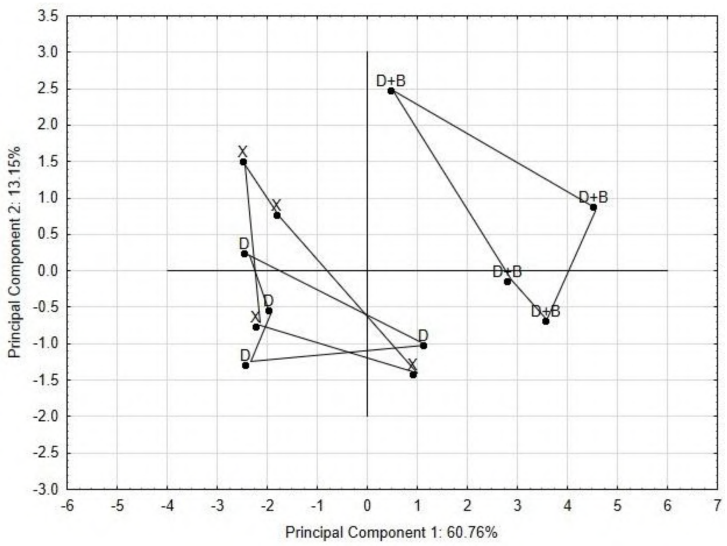


Figure 10. Principal component analysis (PCA) of 11 variables, namely water infiltration rate, herbaceous plant biomass yield, herbaceous plant crude protein content and various soil nutrients (P, K, Ca, Na, Mg and AmAc) and soil properties (CEC and pH) for four dung-and-dung beetles treatments (D+B), four dung-only treatments (D) and four no-dung-or-dung beetles treatments (X). Data represented were measured one month post the first treatment application.

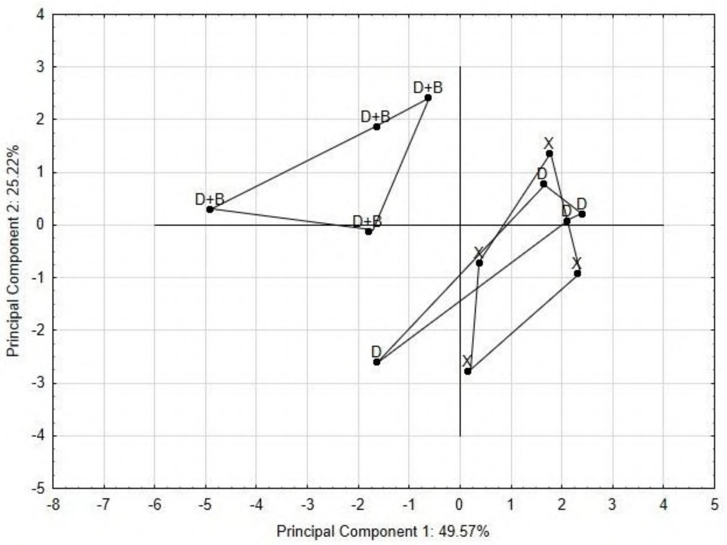


Figure 11. Principal component analysis (PCA) of 10 variables, namely water infiltration rate, herbaceous plant biomass yield, herbaceous plant crude protein content and various soil nutrients (P, K, Ca, Na, Mg and AmAc) and soil properties (pH) for four dung-and-dung beetles treatments (D+B), four dung-only treatments (D) and four no-dung-or-dung beetles treatments (X). Data represented were measured six months post the first treatment application.

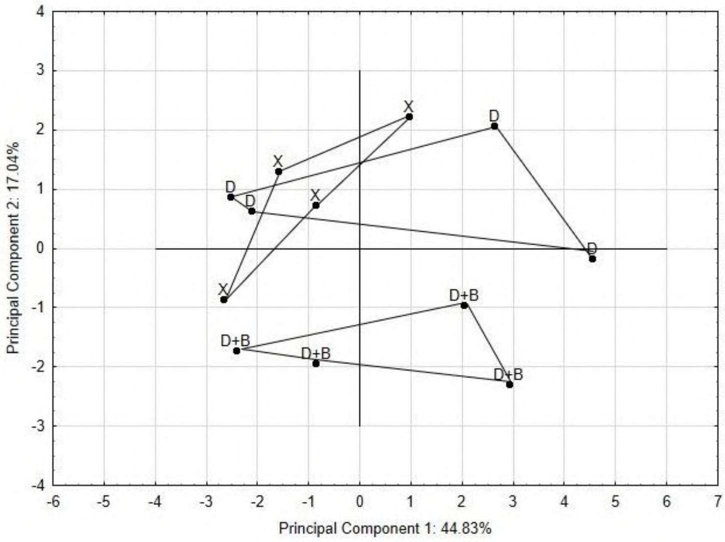


Figure 12. Principal component analysis (PCA) of 11 variables, namely water infiltration rate, herbaceous plant biomass yield, herbaceous plant crude protein content and various soil nutrients (P, K, Ca, Na, Mg and AmAc) and soil properties (CEC and pH) for four dung-and-dung beetles treatments (D+B), four dung-only treatments (D) and four no-dung-or-dung beetles treatments (X). Data represented were measured one month post the second treatment application.

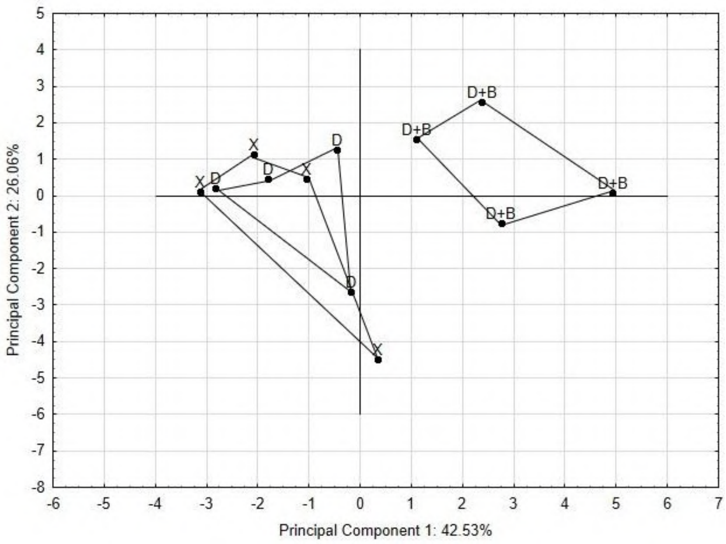


Figure 13. Principal component analysis (PCA) of 11 variables, namely water infiltration rate, herbaceous plant biomass yield, herbaceous plant crude protein content and various soil nutrients (P, K, Ca, Na, Mg and AmAc) and soil properties (CEC and pH) for four dung-and-dung beetles treatments (D+B), four dung-only treatments (D) and four no-dung-or-dung beetles treatments (X). Data represented were measured six months post the second treatment application.

Table 3. Factor loadings of 11 variables for measurements in principal components 1 and 2 (PC 1 and PC 2) from the principal component analyses (Fig.10 – 13).

	Post application 1				Post application 2			
	One month		Six months		One month		Six months	
Variable	PC 1	PC 2	PC 1	PC 2	PC1	PC2	PC1	PC2
Water infiltration rate	0.80	-0.32	-0.69	0.45	-0.12	0.85	0.49	0.33
Biomass	0.71	0.56	-0.63	0.61	0.36	-0.82	0.82	0.40
Crude protein content	0.17	0.55	-0.50	-0.48	0.59	0.13	0.85	0.15
pH	-0.38	0.71	-0.32	0.57	0.26	-0.20	0.19	0.88
P	0.85	-0.26	-0.87	-0.10	0.36	-0.44	0.84	-0.01
K	0.94	0.15	-0.93	-0.03	0.93	0.28	0.83	-0.40
Ca	0.98	-0.09	-0.96	-0.03	0.84	-0.22	0.82	0.27
Na	0.42	-0.33	-0.42	-0.75	0.82	-0.37	0.67	-0.11
Mg	0.98	-0.04	-0.95	0.09	-0.90	0.15	0.93	0.18
AmAc	0.92	-0.08	-0.29	-0.89	0.81	0.10	-0.18	0.02
CEC	0.90	-0.05	ND	ND	0.48	-0.03	0.83	-0.19

ND - not determined

2.4 DISCUSSION

The results of this study confirm that several of the established benefits associated with dung beetle tunnelling are maintained in compacted, degraded soils typically associated with rehabilitated mines. Despite the compacted soil, dung beetles managed to tunnel into the soil resulting in increased water infiltration rates and reduced soil penetration resistance. Following this, herbaceous plant growth was enhanced as more nutrients and water were available for plant uptake.

The most noteworthy finding of this study was the higher rate of water infiltration seen for treatments containing dung beetles. A recent study from New Zealand showed that water infiltration rates were mainly limited to the plant root zone as the tunnels are sealed off by brood balls at the bottom (Forgie, unpublished). The topsoil depth of most South African reclaimed mine soils range from 166 mm to 536.82 mm, which falls considerably short of the recommended minimum topsoil depth of 1 m (Morgenthal, 2003; Harris et al., 1989). Water infiltration and permeability was found to be 129% deeper in presence of the activity of dung beetles, stressing the importance of applying a thicker topsoil layer to post-mining lands as dung beetles are naturally-occurring (Richardson and Richardson, 2000). High water infiltration rates may be problematic in post-mining soil as the water might seep through to the coal layer below the topsoil, increasing acid mine drainage seepage to groundwater.

Similar to what other studies have found (Miranda et al. 2001; Lastro, 2006; Forgie et al. 2013), the above-ground plant biomass yield was significantly higher where dung beetles were active. The increased herbaceous plant biomass for D+B treatments could be attributed to plant roots having direct access to nutrients contained in the dung as well as higher water infiltration rates. Increased soil aeration associated with the dung beetle tunnels improves plant growth (Jones, 2005).

Penetration resistance was observed to be greater for treatments that did not contain dung beetles. The activity of dung beetles occurs mostly within the first 10 cm of the soil, whereby their burrowing-activity loosens the top layer of soil (Bang et al., 2005), as was reflected by the results obtained in this study. The loosening of the top layer of soil may further increase water infiltration rate by creating a more porous soil structure. High soil strength hinders the root growth of plants, resulting in a decrease in nutrient and water uptake as well as poor herbaceous plant cover (Chan and Barchia, 2007).

Percentage N content in vegetation has been found to increase significantly when dung beetles were active on a site (Bertone et al., 2006). In this experiment, the activity of dung beetles did not have a significant effect on the crude protein content of the herbaceous plants for any of the treatments. An increasing trend (but not statistically significant) was seen six months post the second application, with D+B treatments having greater crude protein content than D or X treatments. This could be explained by the relationship between N uptake and soil pH, whereby N needs to be mineralised to inorganic N for plant uptake and will not occur if the pH of the soil is low (<5.5; Mengel and Kirby, 2001).

Although the soil parameters of treatments with dung beetles had few statistically significant differences when compared to D and X treatments, the results may be biologically meaningful. The pH in the soil on plots with dung beetles increased from 5.65 to 6.32 after two treatment applications, possibly improving nutrient uptake (Jones, 2012).

Magnesium ions, abundant in the soil containing dung beetles, play a vital role in photosynthetic organisms. Magnesium in dung is important for photosynthesis and movement of sugars within a plant (Silva and Uchida, 2000; Marschner, 1995). As Mg is one of the exchangeable cations mostly associated with CEC, plots with dung beetles that had high Mg content also had a higher CEC than plots that did not contain dung beetles.

Even though little to no dung beetle activity was observed six months after each treatment application, there appeared to be no correlation between the amount of nutrients in the soil and when the treatment application took place. There was no significant decrease in nutrients for treatments containing dung beetles over the six months where no treatments were applied. This result suggests that the effects of dung beetle activity may be present for an extended time after the treatment application is made.

Chapter 3 Alleviation of degraded soil conditions by dung beetle activity on reclaimed mined land.

By Jessica Badenhorst

3.1 INTRODUCTION

Laboratory or controlled studies are conducted in a controlled environment. The studies, although less variable, do not reflect the 'natural' conditions experienced when doing a field study. Controlled studies evaluate theoretical concepts and exclude many of the complexities seen with field data. Field studies are more accurate in terms of the various interactions expected in a 'natural' environment, but more variables arise that cannot be controlled and may result in an inconstant data set. Although experimental and field studies are usually done on different scales, the results may have different comparative advantages.

Research frequently investigates the difference between controlled environment experiments and field experiments. Most of these studies provided the same conclusions. Laboratory or controlled experiments provide well-defined data with few variables, but may rely on uncertain extrapolations, whereas field experiments give more directly applicable results, but can be less suitable for quantitative analysis due to many variations in the environment (Talling, 1960; Rudich et al. 2007; Poorter et al. 2016). Integrating these two approaches provides opportunities to make a variety of observations which can be used to understand what is strongly generalisable.

The need to replicate a controlled study in the field results from possible influences of confounding factors occurring in the natural environment. Minimising the effects of confounding factors in the field will lead to data that more accurately represent what is found in nature. This highlights the importance of having a well-designed experimental set up and frequently observing the interactions in field experiments.

Many confounding factors may influence experiments on rehabilitated post-mining land. If the mine is still operational in certain areas, dust blown from stockpiles may influence dung beetle activity, as was observed during the study reported in Chapter 6. The abundance of birds in the post-mining area may also reduce the number of insects in the system, as they cannot disperse great distances like birds. In this study, it was important to limit the confounding factors by isolating the experiment from external influences such as predation, other invertebrates and coprophagous organisms.

Organisms are classified into functional groups according to their shared characteristics and how they utilise resources. A diversity of functional groups in an ecosystem has been associated with a greater long-term stability (Cadotte et al. 2011). Arthropods constitute some of the most important functional groups pertaining to ecosystem functionality; many arthropods are directly associated with soil processes, seed dispersal and pollination. The effects of ants, termites and earthworms have been frequently documented, highlighting how the tunnelling activities of these groups have improved soil mixing, drainage, aeration and root penetration (Wali and Kannowski 1975).

Dung beetles, from the subfamily Scarabaeinae, actively contribute to various ecosystem services including nutrient cycling, parasite suppression and soil hydrological processes (Nichols et al. 2008; Brown et al. 2010). Dung beetles have been found to bury up to 78% of dung applied to soil (Fincher et al. 1981). Omaliko (1984) and Miranda et al. (2001) reported that dung decomposition increased concentrations of nitrogen, potassium, phosphorus, magnesium and calcium of soil up to 42-56 days after dung exposure. The application of tunnelling dung beetles could provide a sustainable approach to reclaiming degraded soil; they tend to remain within an area where dung is abundant and generally do not disperse great distances to locate a new dung source (Favila and Halffter, 1997).

The objectives of this study were to determine whether dung beetles applied to reclaimed mine soils can maintain their beneficial activities by measuring (1) the properties of soil in terms of penetration resistance (kPa), nutrients (mg/kg), pH, cation exchange capacity (cmol(+)/kg) and water infiltration rate ($mm.h^{-1}$); (2) the growth response of plants in terms of above-ground biomass (g/m^2) and crude protein content (%); (3) the effects of naturally-occurring dung beetles compared to applied dung beetles and (4) the longevity of these effects on experimental plots. It is hypothesised that confounding factors will lead to more variation in the data, and that areas where applied dung beetles have been active will have:

- Higher water infiltration rates
- Lower soil penetration resistance
- Greater soil nutrient content, pH and cation exchange capacity

- Increased plant biomass and protein content
- Effects lasting at least 6 months

Furthermore, applied dung beetles will be more effective when processing dung pats compared to dung beetles that occur naturally on the reclaimed mined site.

3.2 METHODS

3.2.1 Study site

The experiments were conducted at a reclaimed mine located in eMalahleni (Witbank). Underground mining commenced in the 19^{th} century, however surface coal mining only started in 1979. The reclaimed mine site chosen for the experiments had homogenous properties in terms of vegetation cover and slope, and rehabilitation age was approximately 15-20 years. The soil was classified as a sandy loam consisting of 65% sand, 17% silt and 18% clay.The study area had an average daily temperature of 23 ^{o}C, was at an altitude of 1570 m.a.m.s.l., and had an average summer rainfall of 114 mm and winter rainfall of 14.5 mm.

3.2.2 Preparation of enclosures

Sixteen enclosures of 4 x 4 m in size (and approximately 1 m high) were built on the reclaimed mined site (Figure 14). The enclosures were constructed with white shade netting (SpectraNet 50), cable ties and iron rods (1.5 m long, 10 mm in diameter). The overhanging shade netting was secured to the ground to discourage dung beetles from escaping.

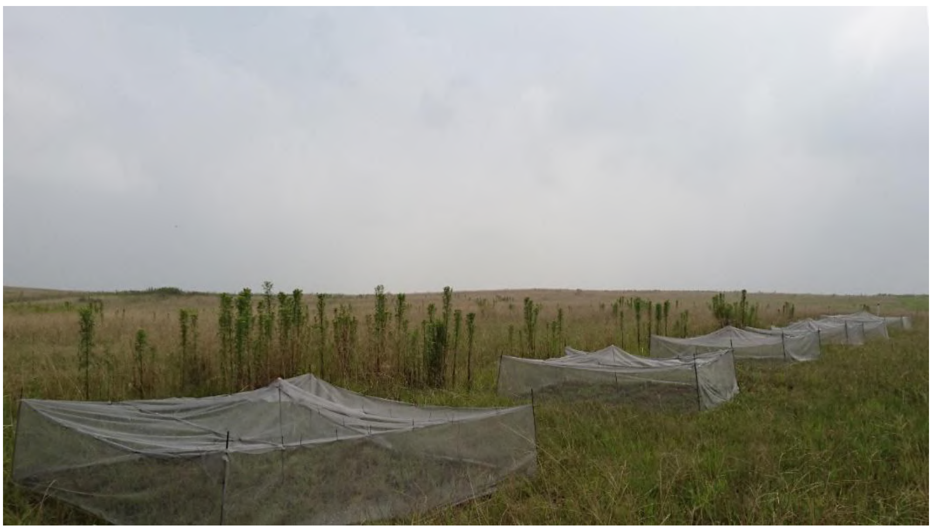


Figure 14. The constructed field plots situated at reclaimed mine site in eMalahleni. The plots were covered with shade netting and secured.

Four treatments with four replicates of each were applied. Each treatment and replicate were spaced 5 m apart from one another and treatments were allocated randomly (Figure 15). Tunnelling dung beetles were collected from neighbouring farms close to the reclaimed mined land and were divided up equally for each treatment application that required the presence of dung beetles. Pit fall traps were used to collect live dung beetles two days before the application took place and the beetles were kept in breadbins with fresh cattle dung. Tunnelling dung beetles were identified and used for the experiment. These species included *Euoniticellus intermedius, Digitonthophagus gazella, Onitis alexis* and *Copris mesacanthus.*

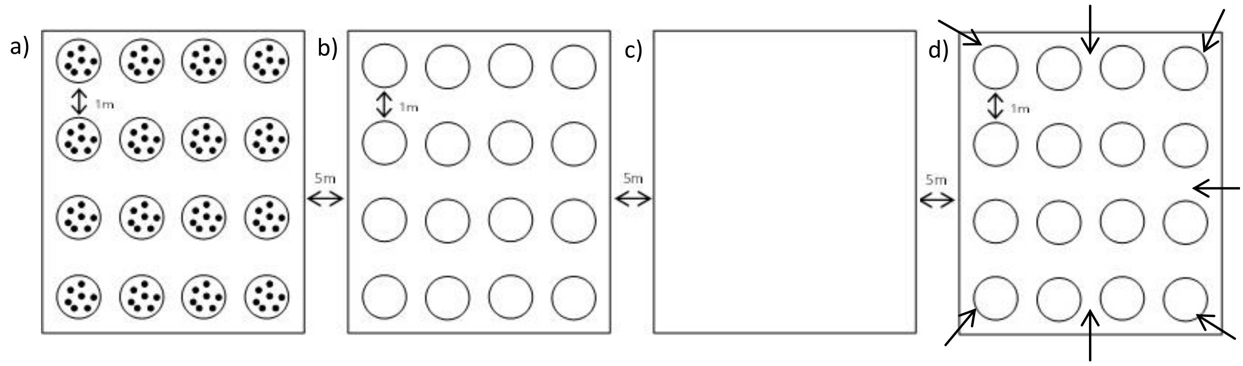


Figure 15. Sample design showing one replicate of each treatment. Four replicates of each treatment were spaced 5 m apart from one another. All treatments were closed off by means of individual 4 x 4 m insect gauze enclosures. The first treatment (a) contained 16 1 kg dung pats. The second treatment (b) contained 16 1 kg dung pats. The third treatment (c) contained no dung and no dung beetles. The fourth treatment (d) allowed for natural colonisation of dung by dung beetles. Dung beetles collected were divided up equally among the enclosures that required applied dung beetles (340 dung beetles per replicate).

The first treatment (D+B) contained a total of 16 dung pats placed 1 m apart with 340 dung beetles applied randomly in the enclosure. The second treatment (D) had 16 dung pats only, placed 1 m apart and the third treatment had no dung or dung beetles (X). The fourth treatment (N) contained 16 dung pats placed 1 m apart and allowed for naturally-occurring dung beetles to colonise the dung for a period of 72 hours, after which time these replicates were closed off with shade netting enclosures.

In total, three treatment applications were made within a 17 month period. Plant productivity and soil property measurements were taken one month after each treatment application and repeated after a six month interval. The first treatment was applied in March, 2016, the second treatment was applied in February, 2017 and the third treatment was applied in April, 2017.

3.2.3 Measurement of plant and soil properties

The herbaceous plant biomass was reported in grams per 4 m^2 plot. Soil samples of approximately 200 g were taken in a diagonal transect up to 10 cm in depth per plot. Penetration resistance and water infiltration rates were measured in a diagonal transect per plot. Please refer to Section 2.2 for the methods.

3.3 RESULTS

3.3.1 General observations

The tunnelling dung beetles immediately started burrowing into the soil when applied to the dung pats. Naturally-occurring dung beetles immediate arrived as dung pats were being placed. *Gymnopleurus pumilus* was the most abundant naturally-occurring roller dung beetle (Figure 16).

Figure 16. Naturally-occurring *Gymnopleurus pumilus* burrowing into highly compacted soil on the reclaimed mined land in eMalahleni.

On the study site, dung beetle broodballs were recorded at various depths with the deepest broodball around a depth of 20 cm (Venter, et al., unpublished). There was a clear difference in dung pat decomposition between treatments, especially when comparing dung-only and applied dung beetle treatments (Figure 17). Six months after the third treatment application, there was evidence of further dung decomposition (Figure 18).

Figure 17. Left: One month after the dung only treatment was applied on reclaimed mined land. Right: One month after the dung and dung beetle treatment was applied on reclaimed mined land.

Figure 18. Six months after the dung and dung beetle treatment was applied on reclaimed mined land.

3.3.2 Herbaceous plant biomass and crude protein content

One month post application 1, D+B treatments yielded significantly more plant biomass when compared to D, X and N treatments (Figure 19). The average biomass yield for D+B treatments was 466.38 g ± 59, almost 200 g more than the D, X and N treatments. There was no difference observed in biomass yield six months post application 1 (September). Although D+B treatments yielded more biomass one month (March) post application 2 (394.13 g ± 12.14), the results were variable for D, X and N treatments (282.13 g ± 52.23, 328.5 g ± 37.91 and 357 g ± 43.48, respectively). Following the second application, the third application measurements were also highly variable for all treatments. No significant difference was observed between treatments one month, and six months after the third application.

Herbaceous plant protein content was higher on average for D+B treatments compared to D, X and N treatments, although the difference was not significant for all treatments (Figure 20; $p > 0.05$). The highest protein content for all treatments was observed six months post application 1.

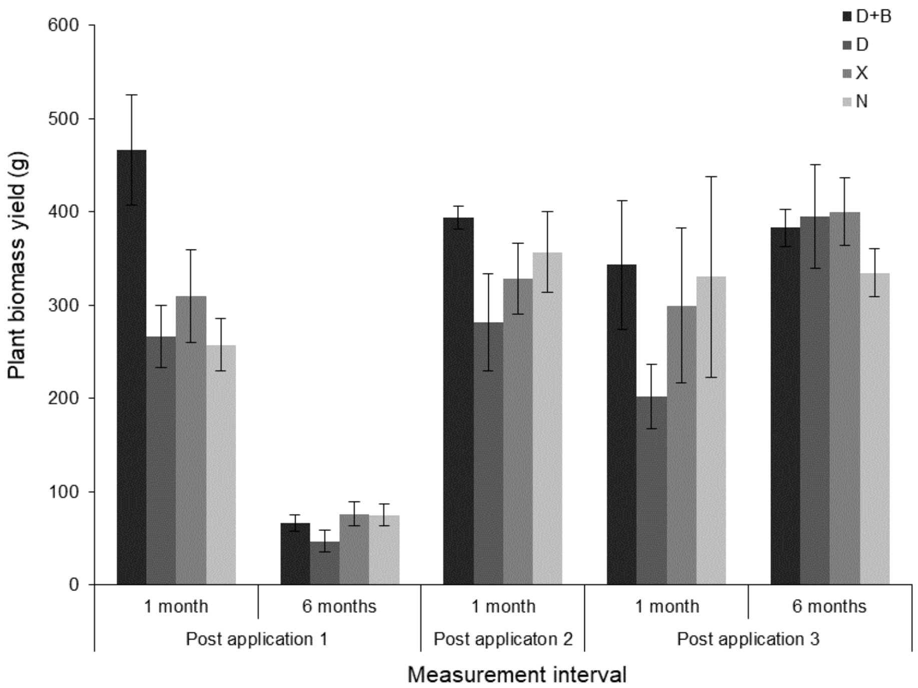


Figure 19. Mean ± SE values for herbaceous plant biomass yield (g) measurements taken one month and six months post the first treatment application, one month post the second treatment application, and one month and six months post the third treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); no-dung-or-dung beetles (X; n= 4); and naturally-occurring dung beetles (N; n=4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

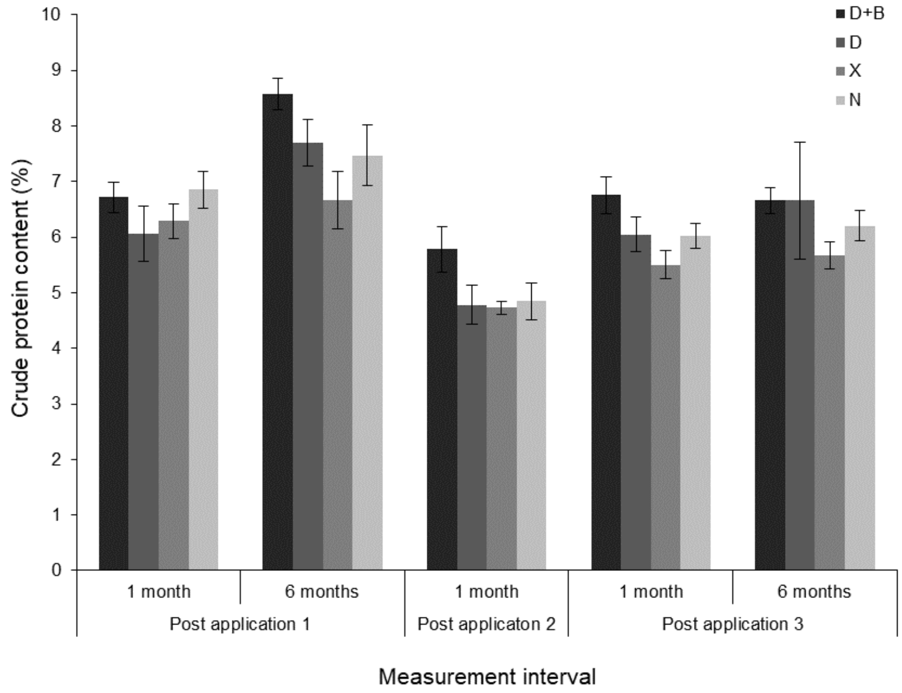


Figure 20. Mean ± SE values for herbaceous plant crude protein content (%) measurements taken one month and six months post the first treatment application, one month post the second treatment application, and one month and six months post the third treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); no-dung-or-dung beetles (X; n= 4); and naturally-occurring dung beetles (N; n=4). [* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$].

3.3.3 Water infiltration rate

Water infiltration rates were higher for most measurement intervals where applied dung beetles were active, except six months post application 1 (Figure 21). *Post-hoc* Tukey HSD tests indicated that water infiltration rates were significantly higher for D+B treatments compared to X treatments one month post application 1. After the second and third applications, D+B treatments continued to have higher water infiltration rates, but this was not significant when compared to D treatments post application 2 and X treatments post application 3 ($p > 0.05$). Water infiltration rates showed a generally increasing trend for all treatments from the first to the final measurements.

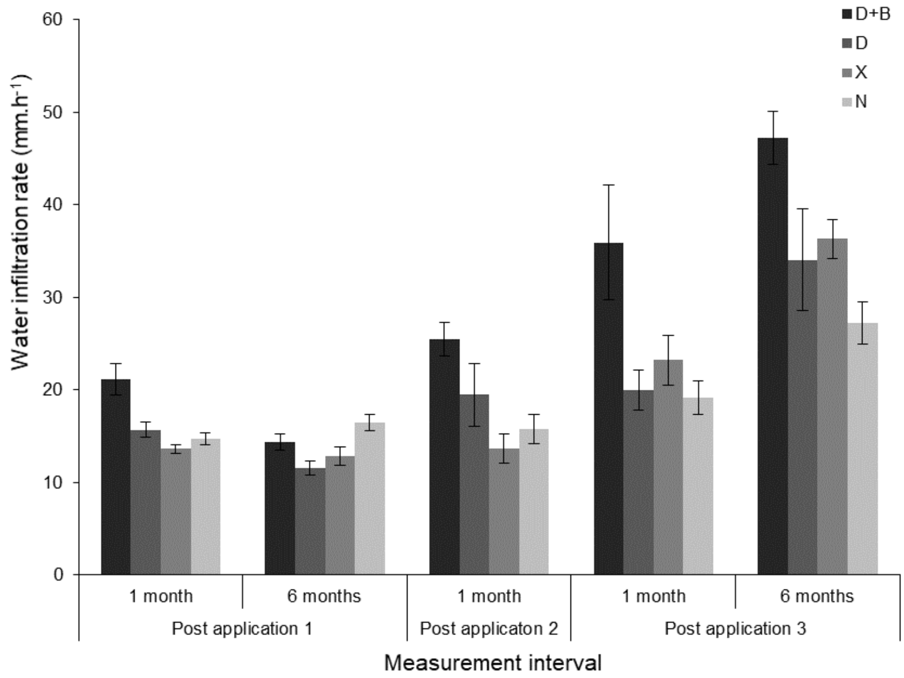


Figure 21. Mean ± SE values for water infiltration rate (mm.h^{-1}) measurements taken one month and six months post the first treatment application, one month post the second treatment application, and one month and six months post the third treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); no-dung-or-dung beetles (X; n= 4); and naturally-occurring dung beetles (N; n=4). [* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$].

3.3.4 Soil strength

Penetration resistance measurements were significantly lower between the soil depths of 2 and 15 cm one month after the first application for D+B treatments ($p < 0.05$; Figure 22). When comparing D, X and N treatments, no difference was observed.

Between 1 and 19 cm, there was no difference when comparing treatments six months after the first application (Figure 23). At 20 cm, penetration resistance was significantly reduced for D+B treatments when compared to X and N treatments, but not D treatments ($p < 0.05$). One month post application 2, D+B treatments had significantly lower penetration resistance at 2 – 10 cm compared to D, X and N treatments (Figure 24).

A similar trend in penetration resistance measurements was seen one month after the third application; however, D+B treatments were only significantly lower at 7 – 9 cm (Figure 25). No significant difference was observed six months post application 3 (Figure 26).

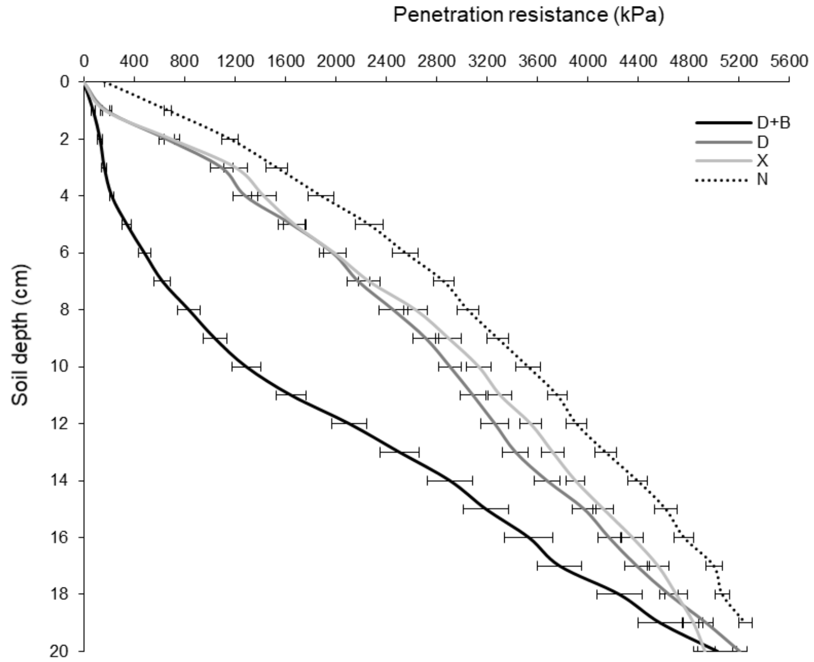


Figure 22. Mean ± SE values for soil penetration resistance (kPa) measurements taken one month post the first treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); no-dung-or-dung beetles (X; n= 4); and naturally-occurring dung beetles (N; n=4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

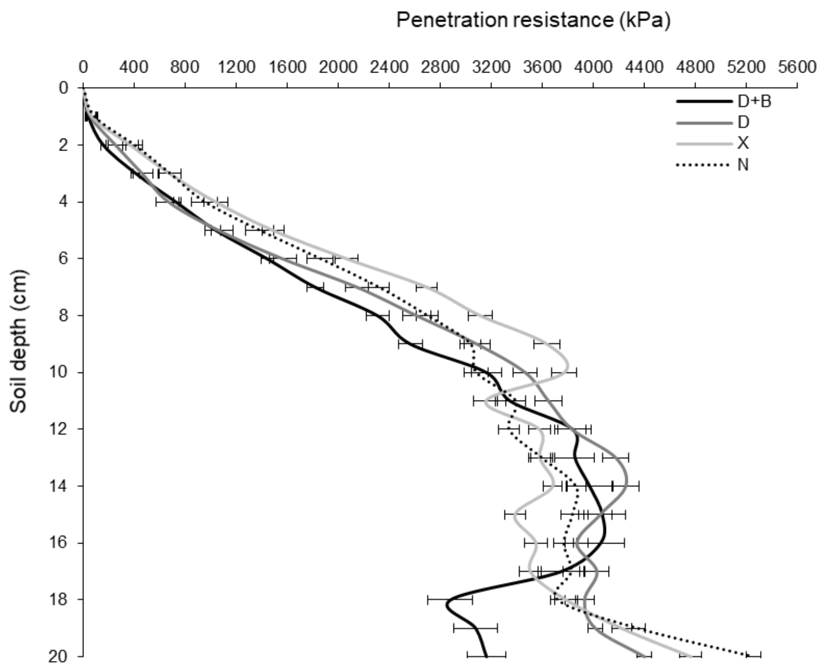


Figure 23. Mean ± SE values for soil penetration resistance (kPa) measurements taken six months post the first treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); no-dung-or-dung beetles (X; n= 4); and naturally-occurring dung beetles (N; n=4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

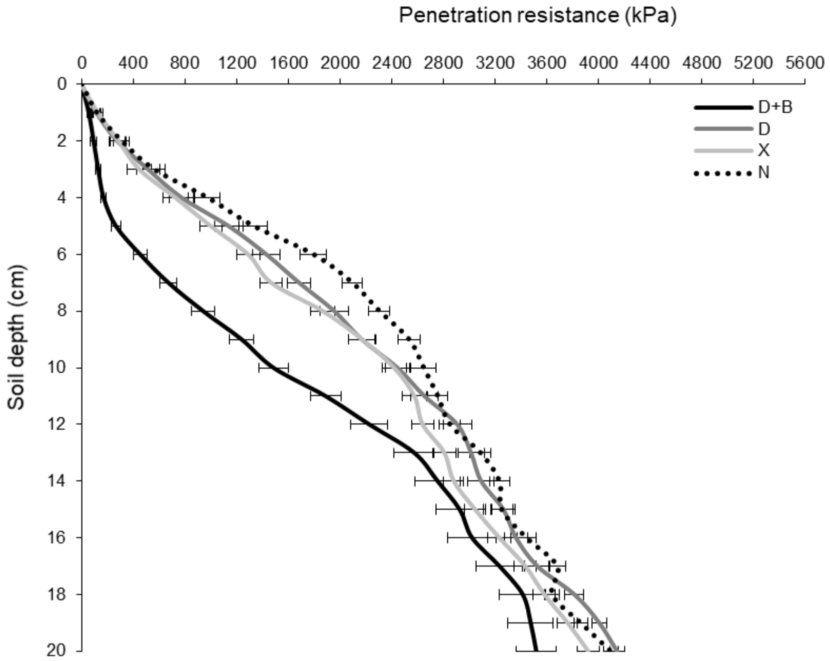


Figure 24. Mean ± SE values for soil penetration resistance (kPa) measurements taken one month post the second treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); no-dung-or-dung beetles (X; n= 4); and naturally-occurring dung beetles (N; n=4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

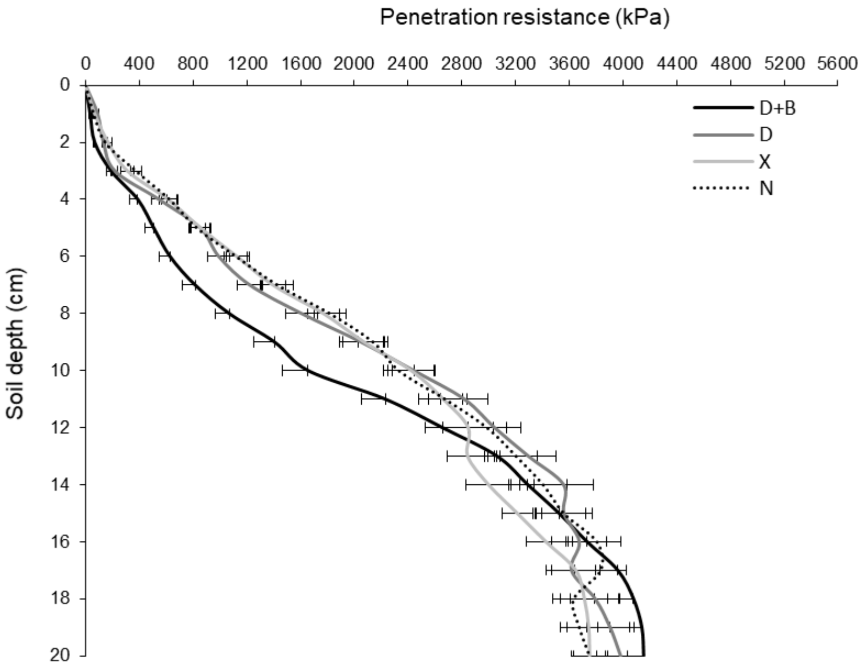


Figure 25. Mean ± SE values for soil penetration resistance (kPa) measurements taken one month post the third treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); no-dung-or-dung beetles (X; n= 4); and naturally-occurring dung beetles (N; n=4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001]

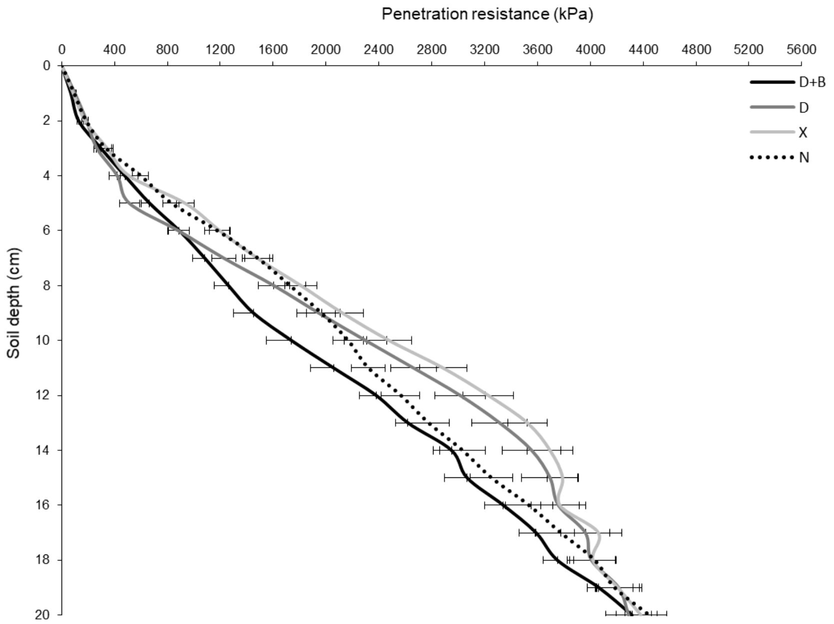


Figure 26. Mean ± SE values for soil penetration resistance (kPa) measurements taken six months post the third treatment application. Treatment applications were: dung-and-dung beetles (D+B; n= 4); dung-only (D; n= 4); no-dung-or-dung beetles (X; n= 4); and naturally-occurring dung beetles (N; n=4). [* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001].

3.3.5 Soil properties

All D+B treatments for all measurement intervals had higher Mg content, but this was only significantly higher one month post application 1 (103.43 $mg.kg^{-1}$ ± 13.57), and one and six months post application 3 (240.1 $mg.kg^{-1}$ ± 14.51 and 180.58 ± 30.62, respectively; Table 4). Ammonium acetate, Ca and P were not significantly different for any treatment during all measurement intervals. Six months post application 1, CEC was significantly higher for X treatments (D+B: 5.17 cmol(+)/kg ± 0.53; D: 4.42 cmol(+)/kg ± 0.23; X: 7.3 cmol(+)/kg ± 0.36; N: 5.45 cmol(+)/kg ± 0.49), after which it was not significantly higher for any other measurement interval. The D+B treatments had higher pH compared to D, X and N treatments for most measurement intervals, except one month post application two (D+B: 4.12 ± 0.1; D: 3.99 ± 0.04; X: 4.11 ± 0.04; N: 4.14 ± 0.12). One month and six months post application 3, D+B treatments had significantly higher pH, K, Na and Mg content.

Table 4. Mean ± SE of soil parameters measured from four treatments; dung-and-dung beetles (D+B), dung-only (D), no-dung-or-dung beetles (X) and naturally-occurring dung beetles. Measurements were taken one- and six months after the first application, one month after the second application, and one- and six months after the third lication.

Soil properties	Post application 1								Post application 2				Post application 3							
	One month				Six months				One month				One month				Six months			
	D+B	D	X	N	D+B	D	X	N	D+B	D	X	N	D+B	D	X	N	D+B	D	X	N
pH	4 ± 0.3	4.4 ±0.1	4.4 ±0.1	4.2 ±0.1	4.2 ±0.1	4.1 ±0.03	4 ±0.04	4.1 ±0.1	4.1 ±0.1	4 ±0.04	4.1 ±0.04	4.1 ±0.1	**5 ±0.2 *** **	4.2 ±0.1	4.2 ±0.02	4.3 ±0.1	**4.9 ±0.2 ***	4.3 ±0.1	4.2 ±0.1	4.2 ±0.1
P ($mg.kg^{-1}$)	67.9 ±22	108.3 ±15.4	68 ±14.1	57.1 ±9	67 ±19.4	99.3 ±17.4	73.2 ±11.9	49.3 ±10.5	41.2 ±20.3	82.6 ±17.7	63.4 ±22.3	64 ±19	51.1 ±7.9	56.5 ±16.2	56.8 ±14.4	63.3 ±15.5	104.2 ±19.4	85.3 ±7.1	67.1 ±10.1	57.1 ±9.6
K ($mg.kg^{-1}$)	154 ±25.6	125.3 ±7.8	146.5 ±19.3	116.8 ±13.6	112.3 ±9.7	152.2 ±7.6	142.8 ±24.8	112.8 ±16.5	103.7 ±23.7	76 ±8	104.6 ±13	93.8 ±13.4	**268.3 ±44.5 ***	113.1 ±20.6	138.4 ±28.5	132.5 ±16.7	**266.2 ±20.7 *****	95.3 ±8.1	111.4 ±12.8	111.6 ±16
Ca ($mg.kg^{-1}$)	417.2 ±52.7	373.3 ±25.3	480.6 ±96.1	397.5 ±70.3	383.2 ±51.1	348.6 ±15.8	429.2 ±57.7	426.9 ±52.6	331.7 ±42.1	325.3 ±49.3	290 ±70.8	418.4 ±133.6	459 ±36.9	334.1 ±85.1	373.2 ±29.6	445.4 ±57.4	392.6 ±42.7	298.5 ±46.3	355.8 ±43	363.8 ±42
Na ($mg.kg^{-1}$)	18.3 ±4.7	19.8 ±4.9	15.6 ±2.3	14.9 ±2.7	15.5 ±1.9	17.1 ±1.5	18.7 ±3.1	15.8 ±1.8	10.7 ±1.8	15.3 ±3.6	9.4 ±0.9	14.4 ±1.7	**36.1 ±7.9 ****	12.4 ±3.2	8.4 ±0.2	15.8 ±2.3	**36.8 ±9.8 ***	11.6 ±1.6	10.1 ±1	12.4 ±2.1
Mg ($mg.kg^{-1}$)	**103.4 ±13.6 ***	61.2 ±3.1	70.5 ±10.5	57.9 ±8.9	67.2 ±11.8	48.9 ±1.9	52.7 ±10.1	55.1 ±6.9	77.2 ±18.3	65.7 ±12.2	51.2 ±11.5	74.1 ±20	**240.1 ±14.5 *****	71.6 ±11.4	73.7 ±8.5	87 ±11.9	**180.6 ±30.6 ****	49 ±4.2	59.4 ±9.6	58.2 ±7
AmAc ($mg.kg^{-1}$)	40.2 ±4	59.4 ±8.5	71.8 ±19.1	51.8 ±6.6	53.9 ±10.3	89.4 ±9.6	77.7 ±17.2	73 ±18	36.7 ±1.8	50.2 ±8.6	46 ±6.1	49.2 ±7	56.2 ±11.4	38.9 ±6.5	35.3 ±7.5	51.8 ±10.8	81.1 ±12.6	66.1 ±5.1	84.6 ±5.2	72.5 ±6.3
CEC [cmol(+)/kg]	2.4 ±0.7	2.8 ±0.2	3.5 ±0.6	2.9 ±0.3	5.2 ±0.5	4.4 ±0.2	**7.3 ±0.4 ***	5.5 ±0.5	1.7 ±0.1	1.8 ±0.2	2.2 ±0.8	2.4 ±0.6	2.9 ±0.4	3.3 ±0.3	3.2 ±0.3	3.5 ±0.4	5.6 ±0.5	5.5 ±0.5	5.8 ±0.4	5.5 ±0.3

* − $p \le 0.05$; ** − $p \le 0.01$; *** − $p \le 0.001$

3.3.6 Principal Components Analysis

One month after the first application, D+B treatments were clearly distinguished from D, X and N treatments, and were separated along the principal component 1 (PC1) axis with the exception of one D+B plot (Figure 27). Magnesium and K had the highest factor loading scores of -0.96 and -0.83, respectively (Table 5). The measurements for D, X and N treatments were observed to be similar, with two X plots and one N plot being outliers. No clear pattern was seen six months after the first application, although D and X treatments were similarly distributed along the PC1 and PC2 axes (Figure 28). Principal Component 1 and 2 combined to account for 54.94% of the total variation. Likewise, there was no noticeable pattern one month post application 2; however the D+B treatments were the only treatments clustered together along the PC2 axis (Figure 29). This could be attributed to the plant biomass, which had a factor loading score of -0.81 for PC2. The high factor loading for plant biomass seen correlates to the increase in plant biomass observed for D+B treatments in Figure 19. Similar patterns were observed one month, and six months post application 3 (Figure 30 and Figure 31). In both cases, D+B treatments were separated from D, X and N treatments along the PC1 axis. Magnesium, pH and Na had the highest factor loading scores for both one month (0.96, 0.93 and 0.87, respectively) and six months post application 3 (0.93, 0.87 and 0.94, respectively).

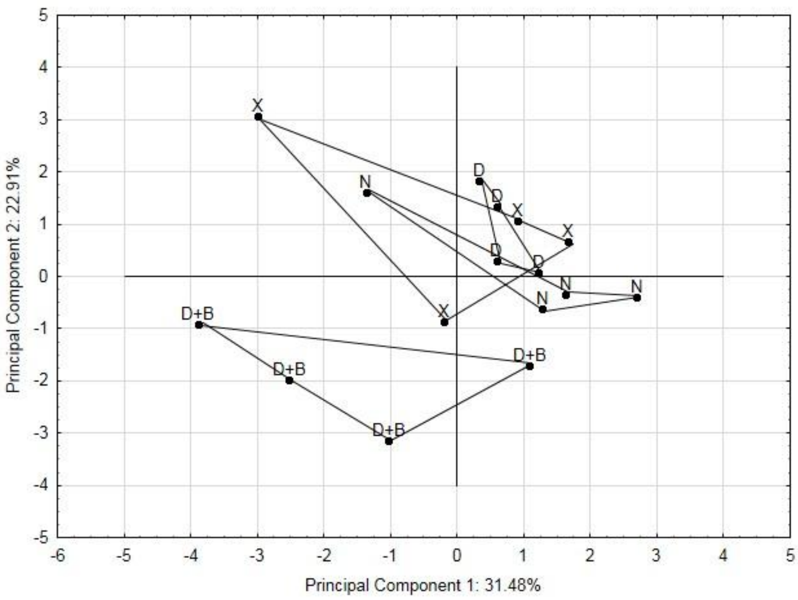


Figure 27. Principal component analysis (PCA) of 11 variables, namely water infiltration rate, herbaceous plant biomass yield, herbaceous plant crude protein content and various soil nutrients (P, K, Ca, Na, Mg and AmAc) and soil properties (pH and CEC) for four dung-and-dung beetles treatments (D+B), four dung-only treatments (D), four no-dung-or-dung beetles treatments (X) and four naturally-occurring dung beetles (N). Data represented were measured one month post the first treatment application.

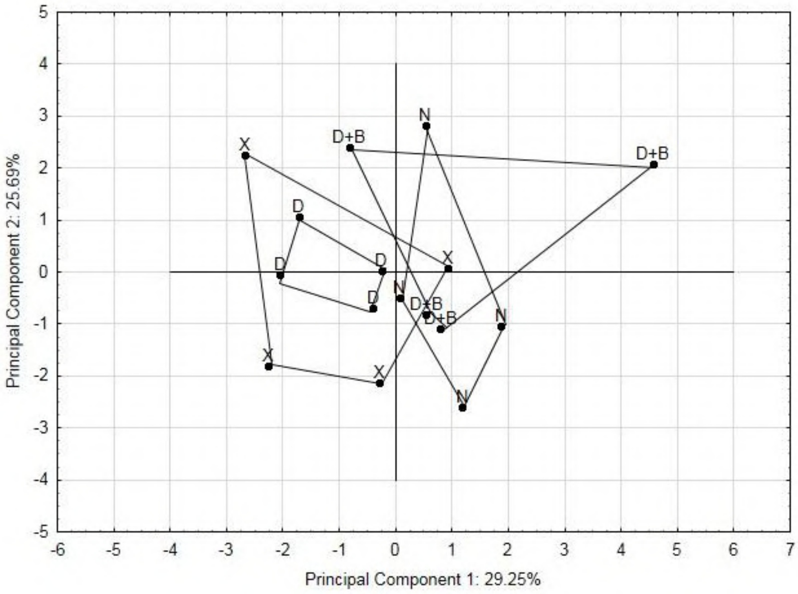


Figure 28. Principal component analysis (PCA) of 11 variables, namely water infiltration rate, herbaceous plant biomass yield, herbaceous plant crude protein content and various soil nutrients (P, K, Ca, Na, Mg and AmAc) and soil properties (pH and CEC) for four dung-and-dung beetles treatments (D+B), four dung-only treatments (D), four no-dung-or-dung beetles treatments (X) and four naturally-occurring dung beetles (N). Data represented were measured six month post the first treatment application.

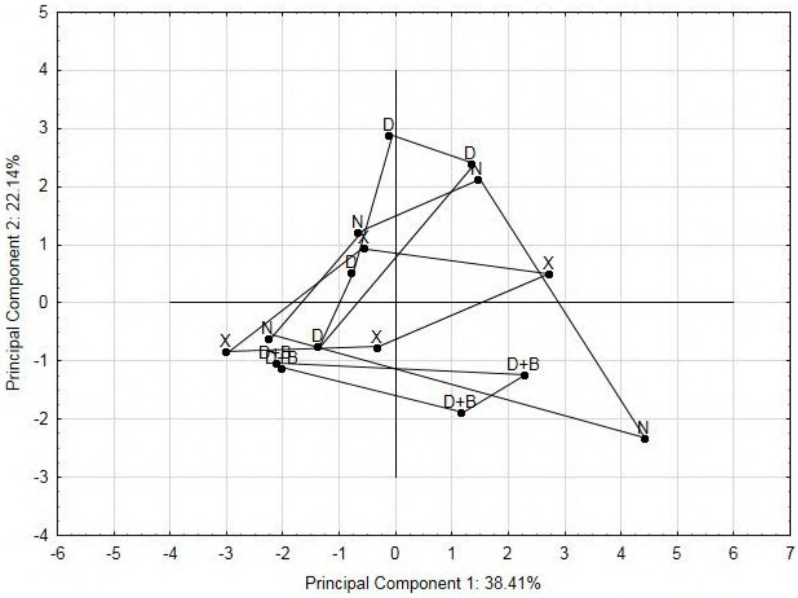


Figure 29. Principal component analysis (PCA) of 11 variables, namely water infiltration rate, herbaceous plant biomass yield, herbaceous plant crude protein content and various soil nutrients (P, K, Ca, Na, Mg and AmAc) and soil properties (pH and CEC) for four dung-and-dung beetles treatments (D+B), four dung-only treatments (D), four no-dung-or-dung beetles treatments (X) and four naturally-occurring dung beetles (N). Data represented were measured one month post the second treatment application.

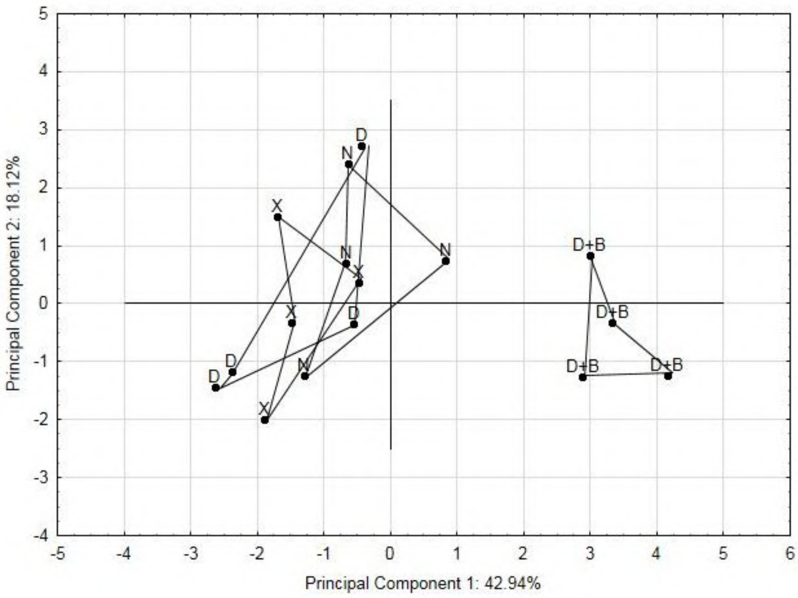


Figure 30. Principal component analysis (PCA) of 11 variables, namely water infiltration rate, herbaceous plant biomass yield, herbaceous plant crude protein content and various soil nutrients (P, K, Ca, Na, Mg and AmAc) and soil properties (pH and CEC) for four dung-and-dung beetles treatments (D+B), four dung-only treatments (D), four no-dung-or-dung beetles treatments (X) and four naturally-occurring dung beetles (N). Data represented were measured one month post the third treatment application.

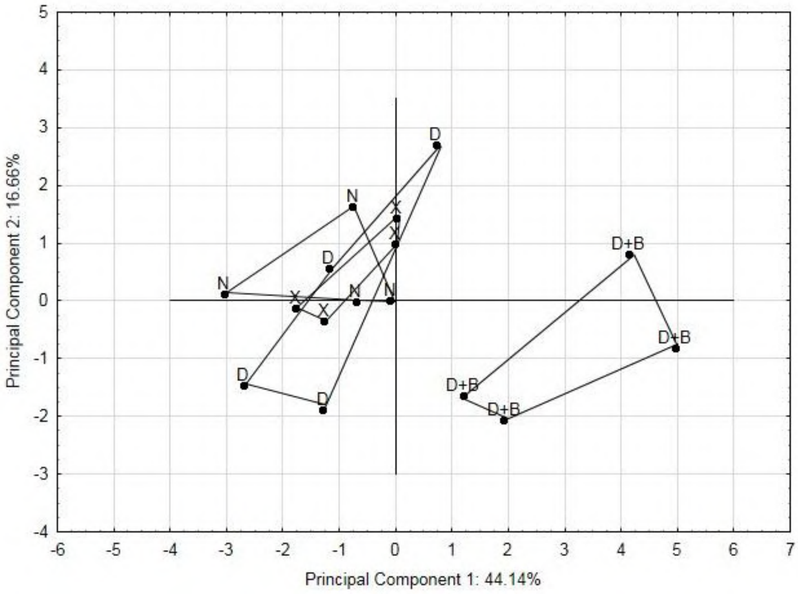


Figure 31. Principal component analysis (PCA) of 11 variables, namely water infiltration rate, herbaceous plant biomass yield, herbaceous plant crude protein content and various soil nutrients (P, K, Ca, Na, Mg and AmAc) and soil properties (pH and CEC) for four dung-and-dung beetles treatments (D+B), four dung-only treatments (D), four no-dung-or-dung beetles treatments (X) and four naturally-occurring dung beetles (N). Data represented were measured six months post the third treatment application.

Table 5. *Factor loadings of 11 variables for measurements in principal components 1 and 2 (PC 1 and PC 2) from the principal component analyses (Fig.27 – 31).*

	Post application 1				Post application 2		Post application 3			
	One month		Six months		One month		One month		Six months	
Variable	PC 1	PC 2	PC 1	PC 2	PC1	PC2	PC1	PC2	PC1	PC2
Water infiltration rate	-0.49	-0.38	0.75	-0.23	-0.003	0.03	0.61	-0.34	0.42	-0.7
Biomass	-0.48	-0.67	0.28	-0.14	0.28	-0.81	0.51	-0.32	0.1	0.4
Crude protein content	0.11	0.48	0.4	0.4	0.02	-0.11	0.59	-0.06	0.29	0.06
pH	-0.37	-0.60	0.54	0.77	0.67	-0.46	0.93	-0.11	0.87	-0.27
P	-0.02	0.5	-0.73	0.46	0.75	0.54	0.11	0.62	0.69	0.01
K	-0.83	0.25	-0.65	0.64	0.76	-0.28	0.84	0.13	0.9	-0.18
Ca	-0.44	0.26	-0.21	0.71	0.94	-0.07	0.49	0.75	0.65	0.64
Na	-0.8	0.4	-0.74	0.25	0.49	0.59	0.87	0.05	0.94	-0.09
Mg	-0.96	0.25	0.37	0.86	0.7	-0.49	0.96	0.12	0.93	-0.11
AmAc	0.21	0.57	-0.59	-0.29	0.51	0.77	0.44	-0.09	0.47	0.19
CEC	-0.61	0.65	-0.32	-0.22	0.83	0.09	-0.17	-0.06	0.4	0.78

3.4 DISCUSSION

This study provided evidence supporting many of the stated hypotheses, which stated that dung beetles could improve various soil properties and herbaceous plant growth on reclaimed mined land. The highly compacted soil did not present a barrier to the dung beetles as dung was mostly buried by dung beetles on D+B plots, whereas dung remained on the soil surface for all other treatments. A month after each treatment application, dung pats were completely broken down for plots containing applied dung beetles, whereas dung pats for dung-only and naturally-occurring dung beetles remained mostly untouched. This suggests that naturally occurring dung beetles on reclaimed mines may not be able to achieve a measurable improvement in soil and plant properties without augmenting their numbers with additional beetle applications.

The most prominent result of this study was the increase in water infiltration rates where applied dung beetles were active, although the effects were only observed after the second treatment application. This could be explained by considering the resistance of the soil to change, where more frequent treatment applications may be required for lasting effects. Doube et al. (2003) and Brown et al. (2010) similarly found that dung beetle tunnels increase water infiltration rate when compared to experiments without dung beetles on natural soils. Water infiltration rate and plant growth is related to soil compaction, therefore alleviating soil compaction is vital to improving soil hydrological properties.

Root growth is affected by soil compaction and low pH. Even though plant biomass was noticeably improved for areas with applied dung beetles, the yield was highly variable after the second treatment application. During the second treatment application, a cyclone moved through the area and caused the collapse of many enclosures built for the experiment. This could have negatively influenced the plant biomass yield for all replicates and may explain the variable biomass measurements, because certain areas were more affected by totally or partially collapsed enclosures. Along with increased access to water, the increase in plant growth may be attributed to a greater root – soil contact as well as a lowering in pH, resulting in greater root growth. Furthermore, dung beetle tunnels may have provided unobstructed corridors for plant roots to penetrate deeper into the soil, gaining access to important nutrients found in the dung buried by the dung beetles (Edwards and Aschenborn, 1987).

The tunnelling activity of dung beetles had a remarkable effect on soil strength, substantially reducing soil compaction. Alleviating soil compaction on post-mining soils is important for the topsoil layer where the majority of plant growth takes place. The results of this study suggested that dung beetles are able to alleviate soil compaction within the top 10 cm, but that the longevity of these effects may initially depend on the frequency of the treatment applications; or until a self-sustaining population of dung beetles have established. Dung beetles were able to tunnel into soil compacted to a penetration resistance of more than 5 000 kPa. As dung beetle broodballs were observed at depths of 20 cm, it is likely that dung beetles are able to complete their lifecycles on post-mining soil. Brown et al. (2010) similarly showed that dung beetle activity decreases bulk density.

Throughout all measurement intervals, plots containing dung beetles had the greatest Mg content. Magnesium and Ca are important nutrients for soil texture and the ideal ratio of Ca:Mg has been reported to be 3:1, yet several studies have shown that agricultural production does not depend on an ideal Ca:Mg ratio but may be influenced by lime applications, which increases soil pH (McLean and Brown, 1984; Simson et al., 1979; Eckert, 1987).

Although pH was not significantly higher for all plots with applied dung beetles, an increase in pH was noted and is biologically relevant for plant root growth. Decreased root size and root branching results in a decrease of nutrient uptake. Aluminium ions become toxic in acidic soil and greatly limits plant growth (Bojórquez-Quintal et al., 2017). Aluminium content in the post-mining soil was not determined. Soil pH will decrease faster with time if the CEC of the soil is low, which was not reflected in the results; however, soil pH was much more variable for treatments with low CEC (Brown and Lemon, 2016).

According to the PCAs, dung beetle activity also resulted in similar trends in the data, especially after the third treatment application. This may suggest that dung beetle activity could improve soil parameters if treatment applications were more frequent. Furthermore, there was no separation of the data when considering treatments that did not contain applied dung beetles, suggesting that the changes seen for treatment with applied dung beetles could be attributed to dung beetle activity. It is also clear that naturally-occurring dung beetles of the mine did not have a significant effect on any plant or soil parameters measured. The natural abundance and body size of dung beetles on the mine was not sufficient to completely remove the dung available to them.

The effects of dung beetle activity were not apparent six months after the first application; however, soil parameters were significantly affected by dung beetle activity six months after the third application. The second and third treatment applications took place a month apart from each other, which could suggest that more frequent treatment applications may be required for highly degraded soil in a variable environment.

Chapter 4 Discussion, conclusions and further research recommendations

By Jessica Badenhorst

4.1 GENERAL DISCUSSION AND CONCLUSIONS

Poor soil conditions and limited plant growth have been persistent problems in restoring land for post-mining land capabilities. There has been a large gap in knowledge concerning alternative or complementary approaches to alleviating severe soil compaction which do not involve disturbing plant growth or require continuous maintenance. In this study, the beneficial effects of dung beetle activity were investigated on reclaimed mined land. The following research questions were asked:

1) How does dung beetle activity influence soil properties and herbaceous plant growth response on:

    - Constructed plots simulating reclaimed mined conditions?
    - Reclaimed mined land?

2) To what extent can we rely on naturally-occurring dung beetles on the reclaimed mined land to incorporate dung into the soil?

The experiments were conducted over a 3-year period in which these research questions were addressed. In conclusion, dung beetle activity represents the opportunity to greatly improve reclaimed mined land conditions with effects having the potential to last for six months if enough dung and dung beetles are present. Presently, naturally-occurring dung beetles on the reclaimed mined land are not capable of processing the amount of dung required to ameliorate degraded soils.

The conclusions were based on the improved soil and plant properties recorded in the results for areas where applied dung beetles were active. The study provided evidence that the activity of dung beetles presents as a non-invasive, sustainable and complementary treatment to the current mine reclamation standards. The results suggest that an additional treatment of dung beetles should be considered for current mine reclamation plans, especially for areas where the post-mining land use was determined to be grazing. Furthermore, there is a potential for job creation where individuals would need to be trained for dung beetle breeding programmes.

The activity of dung beetles greatly improved the water infiltration rates, soil penetration resistance and herbaceous plant biomass yield of simulated reclaimed mined land. These parameters are highly impacted during the reclamation process, emphasising the benefits of incorporating dung beetles as a complementary strategy to improve rehabilitation efforts. Soil pH was similarly improved but was not significant for each measurement interval. Acidic soil (of at least 5) appears to have no significant effect on dung beetle activity, although it is uncertain if low pH affects the life cycle of dung beetles in terms of larva development, adult emergence and the fecundity of the emerged adults. Research conducted by Venter et al. (unpublished) suggests that dung beetles are capable of tunnelling into reclaimed mined land soil, constructing brood balls and depositing eggs. However, establishing whether the dung beetles emerge as fully-functional adults was not part of the study.

The improvements observed in soil and plant properties are attributed to the tunnelling activity of dung beetles. Although variable field results were obtained, this was expected due to the complexity of the environment. The field plots were exposed to extreme weather conditions, resulting in highly variable data due to parts of the enclosures collapsing. Rodent tunnels were also observed on two plots, which could have further influenced the results. Many invertebrate species were present in the field including grasshoppers (Caelifera), ground beetles (Carabidae), ants (Formicidae), and spiders (Araneae). These invertebrates may have affected the outcomes of the results had they been inadvertently enclosed on some of the plots.

The results highlight the importance of applying an appropriately deep topsoil layer to mine spoils as dung beetles create tunnels and increase water infiltration rate. In natural soils, *Onitis alexis* typically constructs tunnels to a maximum depth of 23 cm, and *O. alexis* broodballs were recorded at 20 cm in highly compacted soils exceeding a penetration resistance of 5000 kPa (Edwards and Aschenborn, 1987; Venter, et al. unpublished). This could have many serious implications as dung beetles occur naturally in coal mining areas and cattle grazing is not always monitored or restricted.

Introducing dung beetles and other beneficial invertebrates such as earthworms, ants and termites can increase biodiversity of an area, potentially attracting many beneficial species including birds and rodents. An important finding of the study was the improvement in soil compaction, as all other soil and plant properties are directly or indirectly associated with it. Plant roots are only able to penetrate soil at a resistance of approximately 3 500 kPa, highlighting the importance of loosening soil structure and increasing aeration (Bengough and Mullins, 1990). Water infiltration is drastically reduced by soil compaction, increasing surface water run-off which can cause erosion (Castellano and Valone, 2007). Even though livestock grazing has been linked to an increase in soil compaction and reduced infiltration rate, studies have found that once continuous grazing ceased, these impacts were reversed (Chyba et al, 2014; Sharrow 2007). It is therefore important to rotate cattle grazing periods to allow soil and plants to recover and to avoid overgrazing. Adding dung beetles and dung to compacted soil could decrease tillage costs, especially if dung beetles are able to establish a self-sustaining population.

Using the activities of dung beetles in addition to conventional reclamation methods may accelerate the reclamation of post mining soils, but it may only be practical on a smaller area. It was observed that the effects dung beetles are concentrated directly beneath the dung pats on highly degraded soil. It is important to emphasise that more soil area was covered in dung on the simulated plots as opposed to plots on the reclaimed mined land. Since dung pats were spaced 1 m apart on the reclaimed mined land plots, the measurements taken in transect would have had a lower probability of being taken on an area where dung beetles were active. This could affect the comparability of the two experiments but provides important insights into what could be expected with less coverage of dung and lower abundance of beetles. Due to various climatic conditions during this study, dung beetles available for the experiments were limited and the experimental design had to be altered accordingly.

When comparing controlled- and field experiments, careful consideration should be given to the differences between the experimental designs and the complexity of the environments. The simulated plot measurements were taken in a simpler environment as opposed to the field conditions on the reclaimed mined land, leading to results that had fewer additional interactions that could have affected the outcomes of the data. These additional interactions were observed to be rodent tunnels, extreme weather events, possibly dust fallout from discard stockpiles and variation in topsoil depth. Therefore, results were less variable and a definite pattern in the data could be observed. Many interactions were associated with the field experiment, and the results reflected the complex environment the measurements were taken in. A general trend, but not a pattern, could be seen and more accurately reflected what could be more practical. Both experiments provided crucial insight into the effects of dung beetles on reclaimed mined land.

To maximise the dung processing effects of dung beetles, it is suggested that a body-size range of tunnelling dung beetles be bred in a facility and transferred to relevant areas with a continuous supply of dung preferably from managed livestock, or through dung placement. . Large-bodied dung beetles have been found to be more successful in processing dung; though, the more functionally-diverse dung beetles present in an ecosystem, the more ecological services are provided (Manning, et al. 2016). Furthermore, it should be considered to include nutrient-dense organic matter materials into the diet of cattle, if possible, or to mix cattle dung with these materials. Biochar has been found to significantly increase cattle body size as well as improve soil fertility (Doube, 2015; Joseph, et al. 2015). Experimental studies have also emphasised the importance of combining multiple organic matter materials when treating highly degraded soils in order to significantly improve soil pH, bulk density, and plant biomass (Coetzee et al. 2015).

It is advised to combine dung beetle application with short duration (1 to 15 days), high intensity grazing and rotate the grazing periods every 20 to 60 days for optimal results, especially if cattle are to return to the same paddock (Meehan et al. 2011). Cattle will not graze pastures covered in manure and plant roots need to regrow before the area can be grazed again (Dohi et al. 1991). High intensity grazing allows for the regeneration of plant roots following grazing, leading to sustainable pasture growth.

To stress the importance of dung beetles for economic growth, results from Chapter 3 were extrapolated and are represented in Table 6. While these results are estimates, they provide a good indication of how dung beetles could contribute to crop yield or hay production. A 45.21% increase was seen for D+B treatments in comparison to D treatments. Even though only a 22% increase was observed for X and N treatments in comparison to D+B treatments, it may be a significant increase for a farmer. Miranda et al. (2000) demonstrated that dung beetles and dung (25 mg N per 1 kg dung pat) yielded plant dry matter of 191.8 g/m^2 compared to fertiliser (100 kg/ha N) yielding 126.2 g/m^2 after 110 days. This may indicate that dung beetle activity is comparable to fertiliser application.

Table 6. Mean ± SE of extrapolated biomass yield data measured at a reclaimed mined land. The four treatments were: dung-and-dung beetles (D+B), dung-only (D), no-dung-or-dung beetles (X) and naturally-occurring dung beetles.

Treatment	Plant biomass yield (kg/ha)
D+B	793.83 ± 218.7
D	498.98 ± 134.21
X	634.06 ± 148.36
N	637.73 ± 158.78

4.2 FURTHER RESEARCH AND RECOMMENDATIONS

Due to the variability of climatic parameters during this study, it is recommended that soil measurements be taken over a longer period to obtain a comprehensive body of data. Future studies should investigate the effects of dung beetle activity on post-mining soils on a large scale, using a diverse selection of functional groups. Furthermore, studies should look at combining cattle dung with a grass seed mix to determine if the seeds are able to germinate once buried by dung beetles. It is also worth considering the effects of other flora and fauna and the ecological services they provide, to create a sustainable and natural environment. It is also unknown if the fecundity of dung beetles is affected by unfavourable conditions of post-mining soil, and the feasibility of combining dung beetle applications with high density grazing.

There is further research potential in combining dung and dung beetle application with other organic matter shown to increase plant yield such as woodchips and fly ash.

Chapter 5 The coal mining sector in eMalahleni (South Africa) and how dung beetles can assist in reclamation efforts

M.Sc. introductory chapter by Gustav Venter

5.1 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.

5.2 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 32; Universal Coal 2017).

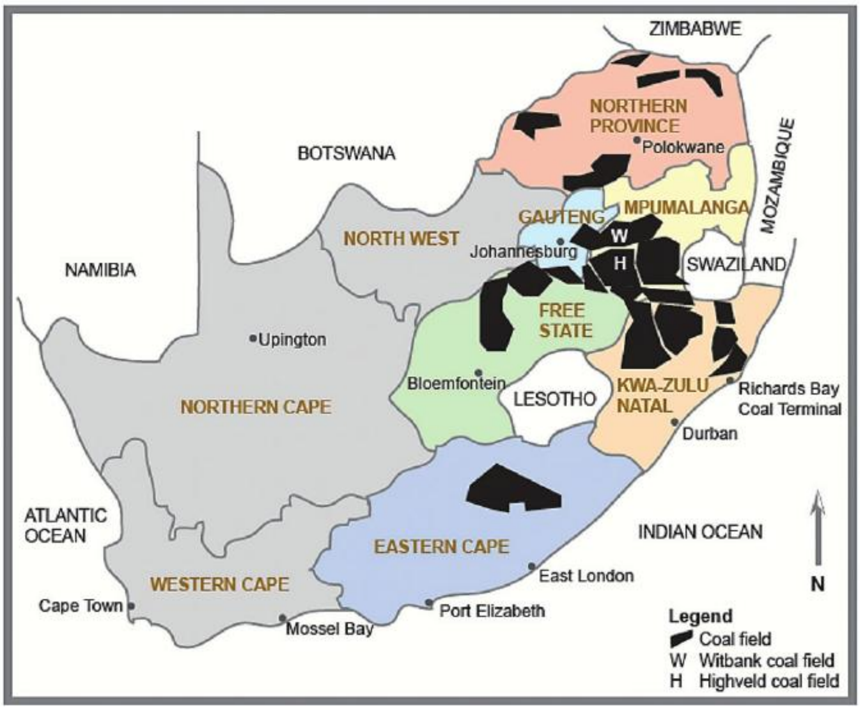


Figure 32. The coalfields of South Africa, highlighting the Highveld and Witbank as primary coal producing areas. Adapted from Pinetown et al. 2007.

5.3 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 Institute, 2005; World coal organisation, 2017). 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.

5.4 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, Bullock, Hälbich, & Lindsay, 2001b; 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).

5.5 IMPACT ON THE ENVIRONMENT

5.5.1 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 33; Ghose *et al.* 1989; Sheoran *et al.* 2010).

Figure 33. Habitat destruction after 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).

5.5.2 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.* 2001a). Acid accumulation can then adversely affect water, soil, vegetation and animals in the region (Ochieng *et al.* 2010).

Figure 34. Reclaimed mined soil 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, Simcock, & Mitchell, 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).

5.6 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, very 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 (Frouz, Pižl, & Tajovský, 2007). The study by Frouz in 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.

5.6.1 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.* 2008a). 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.* 2008a).

5.6.2 Factors that influence their regional distribution

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.* 2008a).

5.6.3 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.* 2008a).

Because the majority of dung beetles tunnel into soil to construct nests, soil type might affect their local distribution (Osberg *et al.* 1994a). 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.* 2008a). Soils that are deep and sandier tend to support the highest diversity, with specialist species occurring at either extreme (Davis *et al.* 2008b).

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.* 2008b). 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.* 2008b; 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.* 2008b).

5.6.4 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.* 2008b).

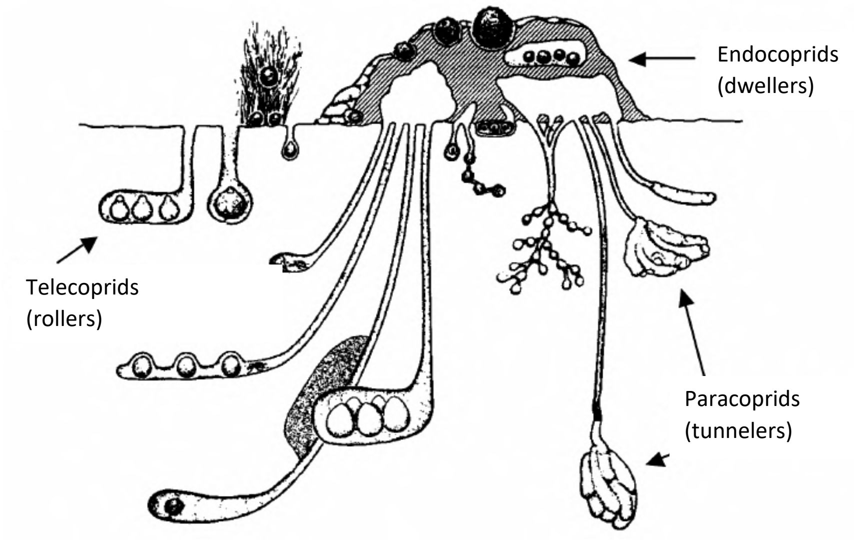


Figure 35. Basic illustration of three nesting behaviours based on dung utilisation.

5.6.5 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.

5.7 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 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 the vicinity; 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 6 How dung beetle assemblages are affected by environmental factors across reclaimed mined sites in eMalahleni.

By Gustav Venter

6.1 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). Because of 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, 1991b; Ridsdill-Smith, 2014). 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.* 1994a). These properties are known to influence nesting properties and have a strong link to offspring survival (Osberg *et al.*1994b). 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 *et al.* 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 handful of 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 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.

Because of their ability 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 a 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 were too low, species of interest needed to be identified for 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 different sites.

6.2 METHODS

6.2.1 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.

6.2.2 Study sites

A list of the selected study sites along with basic descriptors is presented in Table 7.

Table 7. Key characteristics of the reclaimed and reference sites. All sites used during the population assemblage study for the period from 2015 to 2017.

Site	Dominant 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: 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 7; Figure 36). For comparison, three reference areas were selected. Two were commercial cattle farms (Site 7 & 8) and one was a private 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.

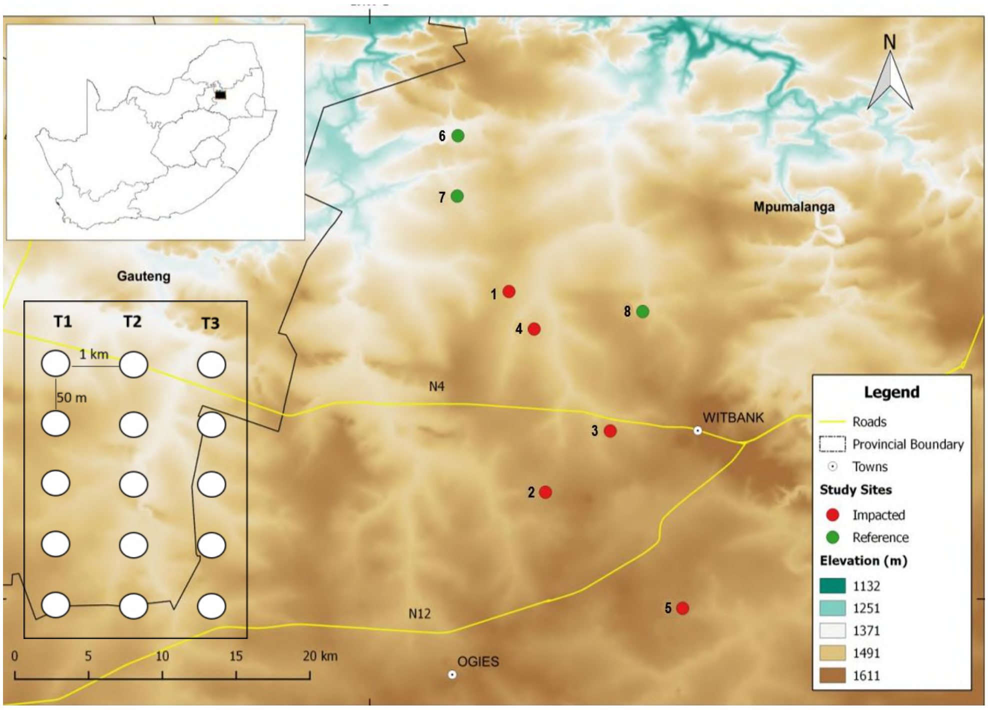


Figure 36. Local map of geographical relationship between sites. Red markers indicate reclaimed mined sites and green markers indicate reference sites. Trap design displayed in lower left corner. T1-T3 refers to Transect 1 to 3 respectively.

6.2.3 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 early 2015 during the first rainfall season, this included collection during February, March and April. Sampling during 2016 took place at the same times 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% soap solution water (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.

6.2.4 Environmental variables

These measurements were made in October and November of 2016, and February and April of 2017. I-buttons were used to measure temperature and humidity for each site every 2 minutes for the duration of each sampling trips. I-buttons (DS1923L-F5/MAXIM) 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 entirety of the project. These data were collected from Witbank weather station 0515320 8 and Kleinkopje weather station 0478391 9 from January 2015 until May 2017.

6.2.5 Data analysis

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 (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 (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 (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 obtained *p*-values 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.

6.3 RESULTS

6.3.1 Dung beetle assemblage

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 37).

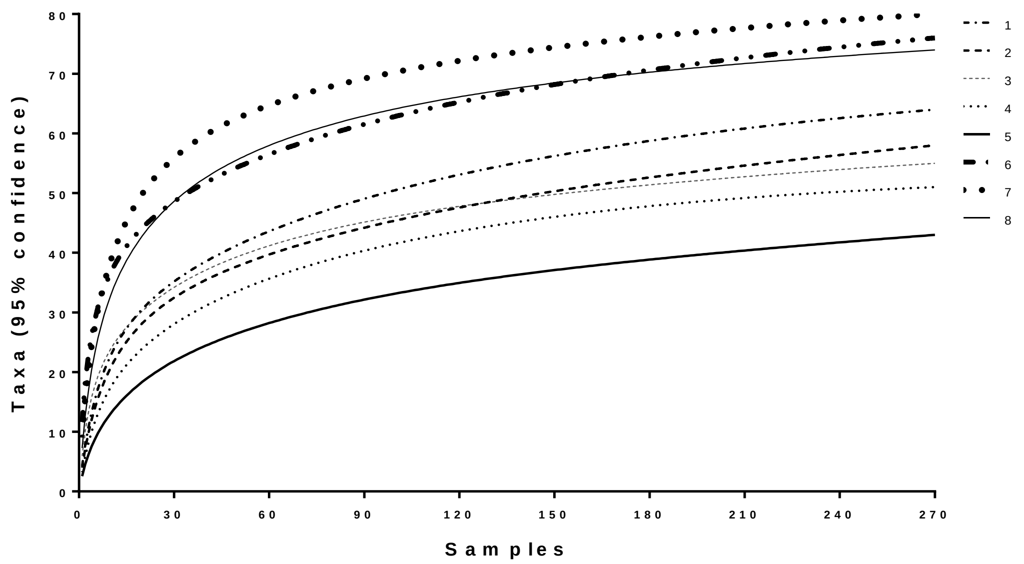


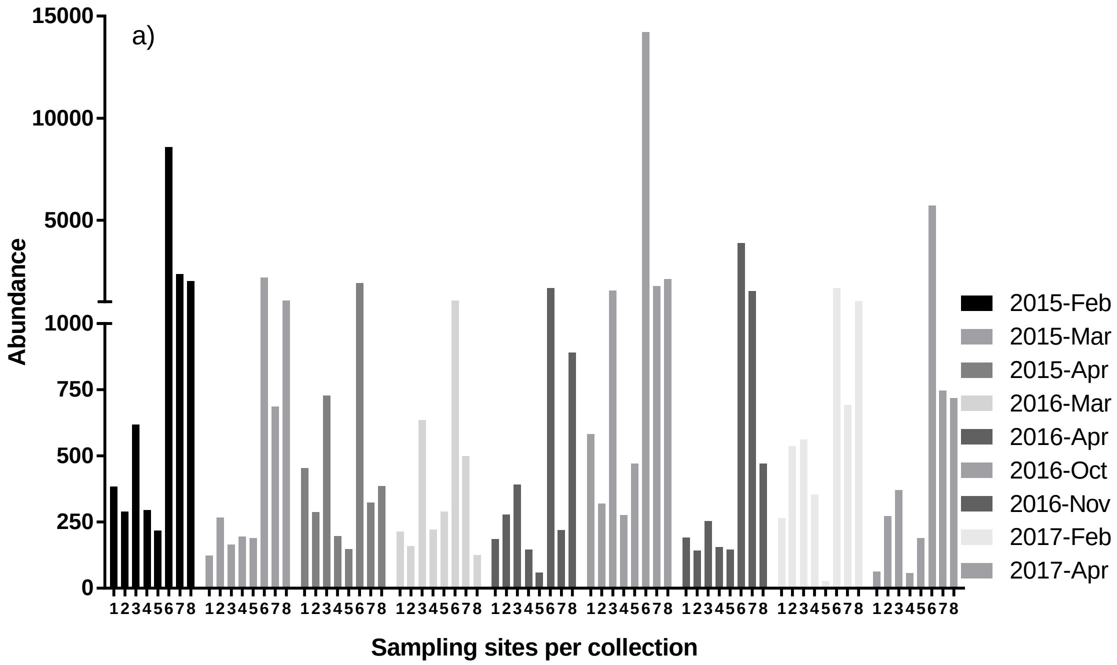
Figure 37. Species accumulation curve (MaoTau) for 270 samples collected during nine sampling periods between March 2015 and April 2017, for 5 reclaimed mined sites (1-5), two cattle farms (7 & 8) and a nature reserve (6).

The 72 467 specimens of Scarabaeinae collected from all sites included members of nine tribes and 96 species (Table 11; Appendix 1). Predictably, the sites with the most abundant beetles were the reference sites, with the highest abundance of 40 914 individual specimens collected at site 6 (nature reserve) (Table 11). Of the reclaimed mine sites, Site 3 had the most 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 from 43 species. The number of species per site, in ranked order from most to least abundant were summarised in Table 12 (Appendix 2).

Scarabaeus ambiguus (Boheman, 1857) was the single most abundant species with 11 534 individuals in total (Table 11). These were collected across all the sites with most of the individuals being collected from the 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.

Dung beetle abundance was significantly higher at the nature reserve (Site 6) than any other site (Figure 38 a; $F_{(7,\ 56)} = 8.61$, $p<0.05$). 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 38 b; $F_{(7,56)} = 17.61$, $p<0.05$). 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 2017 when compared to early 2015 ($F_{(8,56)} = 6.04$, $p < 0.05$).



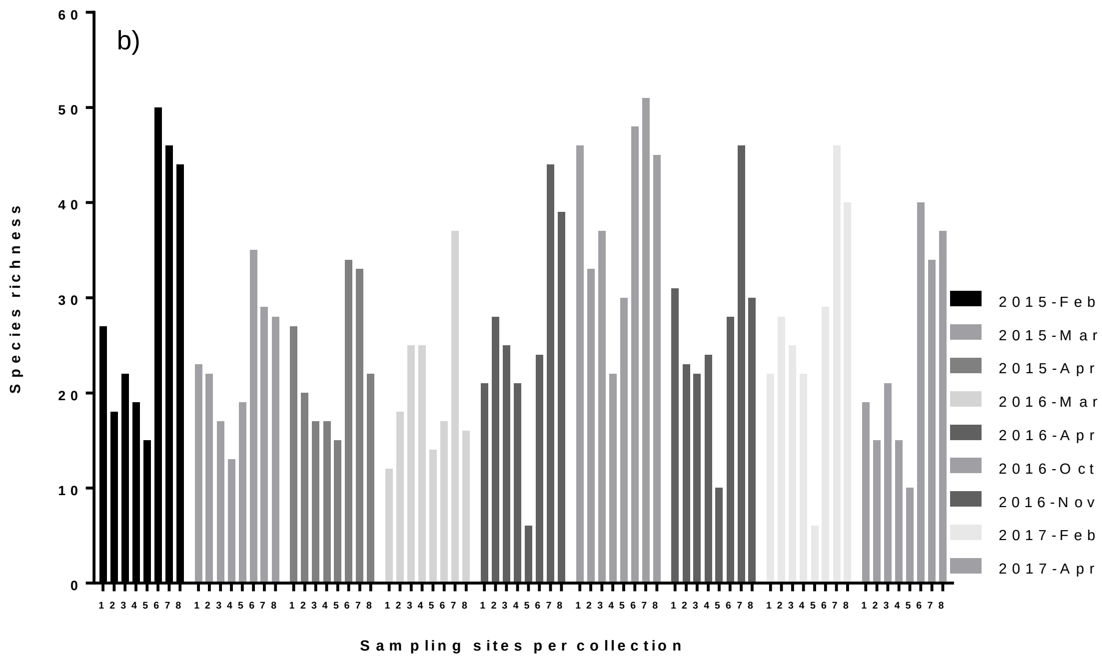


Figure 38. Total dung beetle abundance (a) and species richness (b) for all sites and sampling periods. Sites 1-5 are reclaimed mined sites, Sites 7-8 are cattle farms and Site 6 is the nature reserve.

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 nature reserve is the furthest removed, with cattle farms in close proximity to reclaimed mined sites (Figure 39). Site 5 is the furthest removed from the reference sites and other mined sites. The UPGMA dendrogram (Bray-Curtis) further reiterates the dissimilarity between reference sites and reclaimed mined sites (Figure 40).

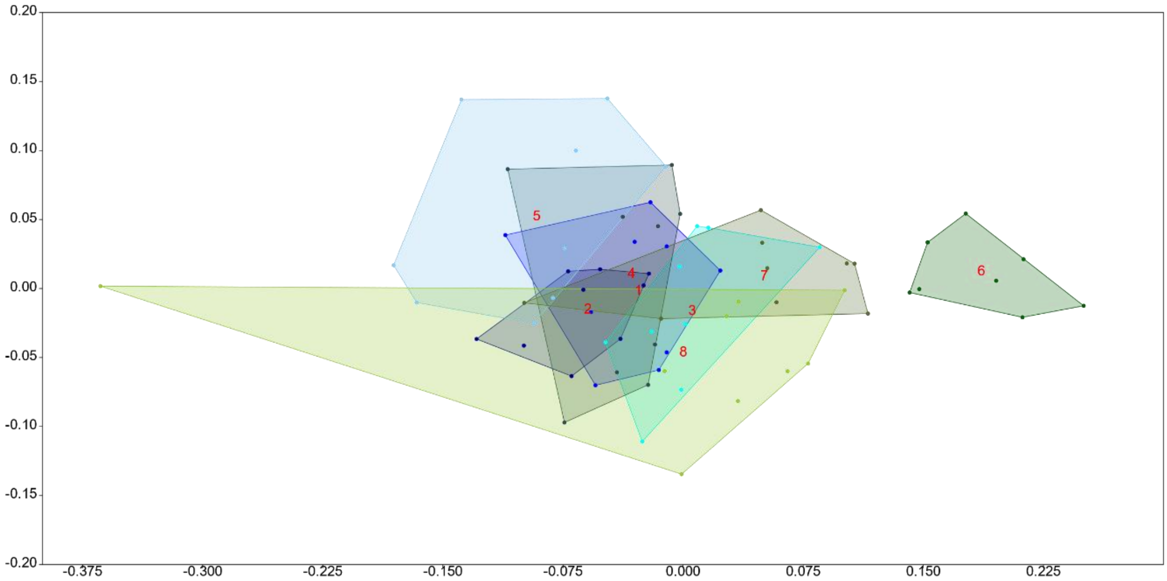


Figure 39. Non-metric multidimensional scaling ordination that shows patterns of distribution for the assemblages between 5 reclaimed mined sites (site 1-5) and three reference sites (Site 6-8) based on the Bray-Curtis similarity index.

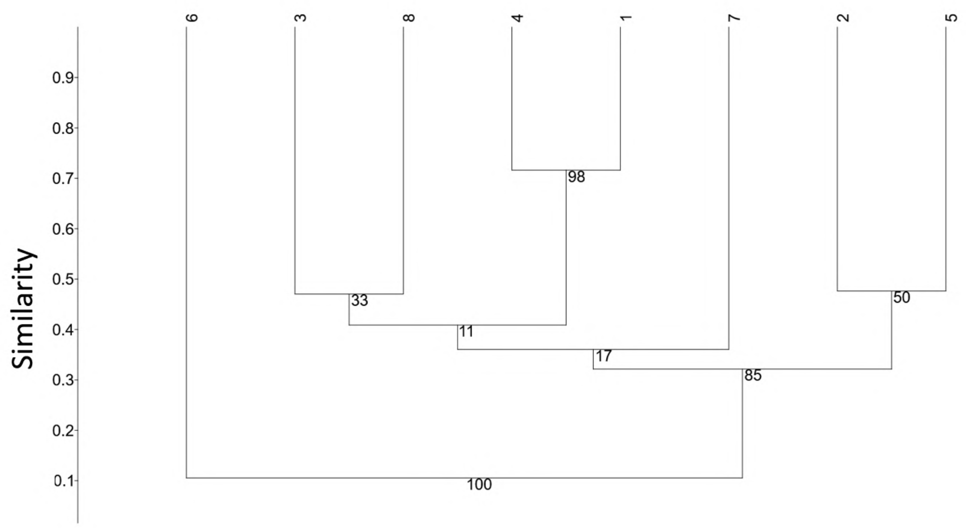


Figure 40. Classical UPGMA dendrogram depicting similarity of assemblages between sites. Bootstrapping at 9999 with Bray- Curtis similarity.

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 8). Lower index values indicate sites that are lower in species richness or have sites that have high numbers of individual species.

Table 8. Diversity indices for reclaimed mined sites (Site 1-5), nature reserve (Site 6) and cattle farms (Site 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

6.3.2 Environmental variables

The reference sites had soil profiles that were less homogenized than that of the mined sites, with higher sand percentages (Table 9). 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 10). Climate (temperature, humidity and rainfall) was similar between sites during each sampling trip (Table 10).

Table 9. Soil composition (Clay, Silt and Sand percentage) for each site.

		Soil composition % (Mean ± SD)								
Study Site	N	Clay			Silt			Sand		
Reclaimed mines										
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
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

Table 10. 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

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 9) were all found to account for higher species richness as determined in the general linear model (Figure 41). 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.

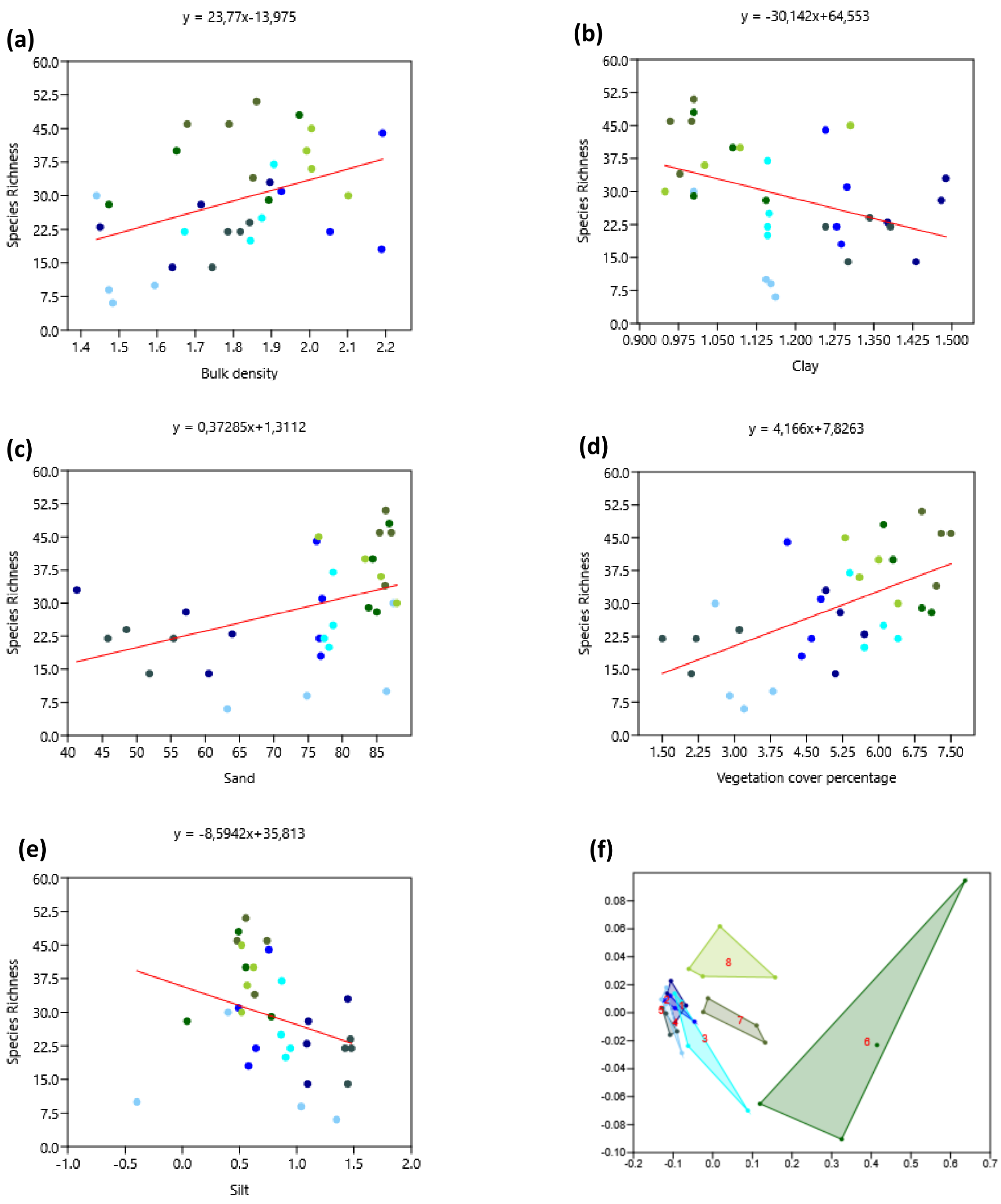


Figure 41. Linear regression for (a) bulk density (R^2= 0.22), (b) clay (R^2=0.16), (c) sand (R^2=0.15), (d) vegetation cover (R^2=0.29) and (e) silt (R^2=0.09) to species richness. (f) nMDS plot of different sites ordinated according to environmental similarities. Blue sites indicate reclaimed mined sites, whilst green indicate reference areas.

The Canonical Correspondence Analyses indicated that the measured environmental variables had a significant influence on the dung beetle assemblages across the difference sites (Figure 42).

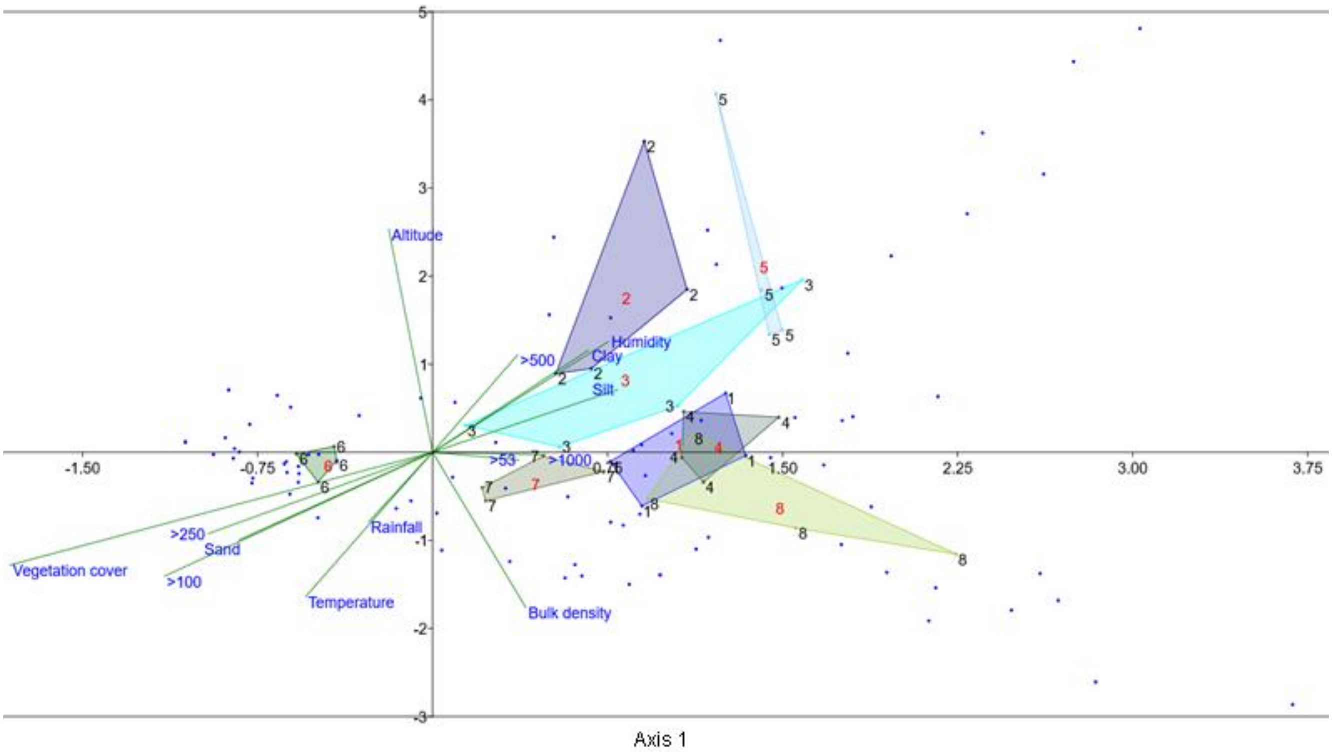


Figure 42. Canonical Correspondence Analysis (CCA) ordination of dung beetle assemblages across reclaimed mined sites (Site 1-5), Nature reserve (Site 6) and Cattle farms (Site 7 & 8). Vector lines indicate influence of the environmental variables on dung beetle assemblage with length indicating relative strength. Convex hulls indicate each study site across nine sampling seasons with blue dots indicating species. Values (>53- >1000 indicate soil particle size). Eigenvalue 0.42.

6.4 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 or absence of dung wasn't determined in this study, the assumption was made that dung provision would be very low as no livestock are maintained or encouraged on the reclaimed mine sites. 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 conditions through their tunnelling abilities.

Dung beetle abundance was much higher on the nature reserve compared to any other site. 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 reclaimed mined 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. 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. 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 nature reserve) and the reclaimed mined sites. 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 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 that indicates their similarity in assemblage structure across the various sampling trips. The nature reserve is not as closely related, as supported by the placement of the reserve as the outgroup in the dendrogram. 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 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, 1991a). 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 documented 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 sites (Site 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 and 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; Oikos, 2016; 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 stark, the plant physiognomy and microclimates are more similar between grasslands (nature reserve) and farms than would be the case in other biomes such as savannahs and forests.

No indicator 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 binodus* and *Caccobius sp* 1 that were recorded in low numbers. 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 to improve pastures, reduce dung breeding pests and provide many other services.

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 such as *E. intermedius* and *D. gazella* are all species that have been successfully used in the past to improve pasture conditions. Many of these species thrive on cow dung and do not require multiple sources and types thereof. This is an ideal situation as the mined sites will most probably have only cattle dung available. Additionally, due to the high numbers 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 beetle numbers 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 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 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 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 nature reserve that might indicate a more specialised and better-established assemblage. Temperature differences seem to affect species more that are affiliated with the nature reserve (Site 6) along with vegetation cover and monthly total rainfall. Climate (rainfall and temperature patterns) is known to influence dung beetles on a more seasonal scale than on diel activity patterns (Davis, 2002).

The 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.* 1994a 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 two daily samplings during dusk and dawn. 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. On farming sites, traps were prone to destruction due to cattle movement and traps in the nature reserve (site 6) were subject to removal by jackals and baboons. During March 2016, construction of a housing complex on one of our sites 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 beetles to 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 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 mined soils.

Chapter 7 Dung beetles can tunnel into highly compacted soils from reclaimed mine sites in eMalahleni, South Africa.

By Gustav Venter

This chapter has been prepared according to the guidelines for the Journal of Applied Soil Ecology, and has been accepted with changes which have been made and the manuscript has been re-submitted.

7.1 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.* 2010b). 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. A Study by 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 in 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 in females during nest construction (Davis *et al.* 2008b). 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.* 2008b). 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.* 2008a).

Three dung beetle species that have been successfully bred for export 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.

7.2 METHODS

7.2.1 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.

7.2.2 Study taxa

Three species of dung beetles (Family: Scarabaeinae) were used in this study: *Onitis alexis* (Klug, 1835), *Digitonthophagus gazella* (Fabricius, 1787) and *Euoniticellus intermedius* (Reiche, 1849). 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. Beetles (150 per species) used in the study were captured from wild populations two days before application, kept in a climate controlled room at 32 ºC with a 12-hour day-night cycle, and starved for one day prior to application.

7.2.3 Experimental design

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 43: 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 dug into the soil with no gaps for the beetles to escape (Figure 43). 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.

7.2.4 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.

7.3 RESULTS

From the 450 applied 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 44a). 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 44b) 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 44c) 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 ($R^2 > 0.7$).

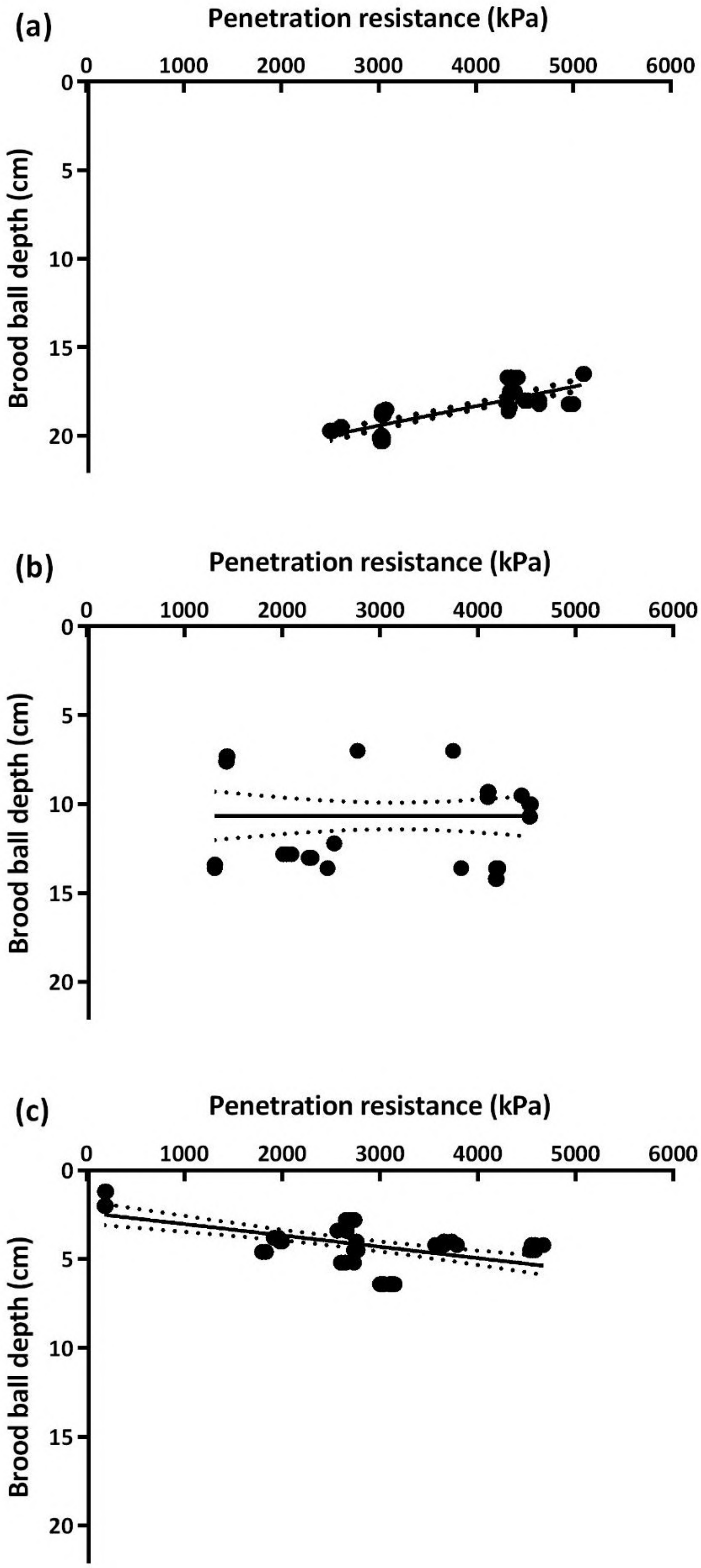


Figure 44: Terminal brood ball depth (cm) plotted against mean penetration resistance (kPa) for three study species. (a) Digitonthophagus gazella, (b) Onitis alexis, (c) Euoniticellus intermedius.

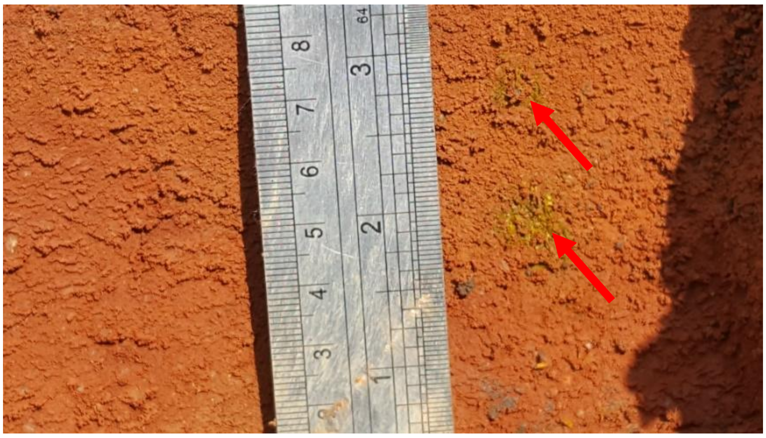


Figure 45: *Euoniticellus intermedius* brood noted close to the soil surface.

7.4 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.

Digitonthophagus gazella was primarily active between 16 cm and 21 cm, while a previous observation had recorded depths up to 35 cm (Samper & 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 interface with the waste coal layer. Another study has shown that species from Onthophagini may limit the depth of tunnels to avoid brood ball placement below the subterranean water level (Sowig, 2017) and may extend to other substrates that are not feasible for broods.

Onitis alexis brood ball depths lacked a distinct relationship to that of increasing penetration resistance. However, there was no definitive influence observed of the compacted soil on *O. alexis* burrowing depth. A previous study has placed the zone of activity for *O. alexis* between 10 cm and 23 cm with an average depth of approximately 17 cm (Edwards & Aschenborn, 1987). The results from this study therefore indicate that although *O. alexis* tunnels 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 previous observations recording depths of up to 20 cm (Figure 15; Ridsdill-Smith, 2014).

Tunnel depths were shallower than what has been recorded for each species in the past. However, the depths were lower 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 (Ridsdill-Smith, 2014). 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 amount and rate of dung burial. The findings of this study suggested 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 could 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. Because the abilities of dung beetles to improve soil conditions are coupled with their activities within the soil, these findings provide a basis for such a project in the future.

Chapter 8 Insights from the assessment of dung beetle assemblages and their tunneling ability for post-mining reclamation.

By Gustav Venter

8.1 CONCLUSIONS

The two studies that were conducted that were constructed 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,* that were highly abundant on reclaimed mined sites were highlighted as possible candidates for breeding and release programs to boost naturally occurring 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.

8.2 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 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, buffalo, zebra etc.) similar to the nature reserve or domesticated cattle, sheep, donkeys and goats similar to that of the farms around 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. 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 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, beetle communities could, in time, increase on their own or a combination of other strategies such as mass breeding. 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 (Ridsdill-Smith, 2014). Dung beetle species that are better adapted on 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 beetles. This could subsequently lead to more effective improvement in the soil and yield greater diversity of vegetation and associated beetles and other fauna.

8.3 DUNG BEETLE TUNNELLING ON RECLAIMED MINE SOILS

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, beetles were not found to be active in the coal layer and seemed to avoid tunnelling into it (in this study, Chapter 7). This provides another level of assurance to the use of dung beetles for rehabilitation.

8.4 FUNCTIONAL DIVERSITY

An important factor in dung beetle assemblages apart from species richness and abundance is that of functional diversity. Dung beetles are currently categorized in one of seven functional groups (FGs) that vary depending on interaction with dung (Doube, 1990). The magnitude of beneficial services provided by dung beetles might change depending on the structure of 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 very beneficial to determine the influence of different dung beetle functional groups as well as the ratio that would be most effective at delivering beneficial results. This knowledge could contribute to more effective rehabilitation of soils with a reduced, but effective, assemblage of beetles. A study by 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 this study, the absence of a single functional group (large nocturnal tunnellers) reduced dung removal by 75% (Slade *et al.* 2007). This suggests that a diverse functional assemblage is required for dung beetles to maximize ecosystem services.

8.5 SECONDARY SEED DISPERSAL

Another topic that needs further exploration is the dispersal of seeds by dung beetles and how it could benefit 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).

8.6 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 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.

8.7 CONCLUDING REMARKS

Despite mining activity, a relatively high diversity of dung beetles were identified on and around reclaimed 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.

Chapter 9 References

Acocks, J. P. H. (1988). Veld types of South Africa. (Third edition). *Mem. Bot. Surv. S. Afr.*, *57*, 1–146.

Aitken, G. A. (1994). Permian palynomorphs from the number 5 seam, Ecca group, Witbank/Highveld Coalfields, South Africa. *Palaeontologia Africana*.

Almeida, S., Louzada, J., Sperber, C., & Barlow, J. (2011). Subtle Land-Use Change and Tropical Biodiversity: Dung Beetle Communities in Cerrado Grasslands and Exotic Pastures. *Biotropica*, *43*(6), 704–710.

Andresen, E. (2002). Dung beetles in a Central Amazonian rainforest and their ecological role as secondary seed dispersers. *Ecological Entomology*, *27*(3), 257–270.

Andresen, E., & Levey, D. J. (2004). Effects of dung and seed size on secondary dispersal, seed predation, and seedling establishment of rain forest trees. *Oecologia*, *139*(1), 45–54.

Bache, B. W. (1984). The role of calcium in buffering soils. *Plant, Cell and Environment , 7*, 391–395.

Bang, H. S., Lee, J., Kwon, O. S., Na, Y. E., Jang, Y. S., & Kim, H. W. (2005). Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. *Applied Soil Ecology , 29*, 165-171.

Bassett, I. E., Simcock, R. C., & Mitchell, N. D. (2005). Consequences of soil compaction for seedling establishment: Implications for natural regeneration and restoration. *Austral Ecology*, *30*(8), 827–833.

Bell, F. G., Bullock, S. E. T., Hälbich, T. F. J., & Lindsay, P. (2001a). Environmental impacts associated with an abandoned mine in the Witbank Coalfield, South Africa. *International Journal of Coal Geology*, *45*(3), 195–216.

Bell, F. G., Bullock, S. E. T., Hälbich, T. F. J., & Lindsay, P. (2001b). Environmental impacts associated with an abandoned mine in the Witbank Coalfield, South Africa. *International Journal of Coal Geology*, *45*(3), 195–216.

Bengough, A., Mullins, C. (1990). Mechanical impedance to root growth: a review of experimental techniques and root growth responses. J. Soil Sci. 41, 341–358.

Bertone, M., Green, J., Washburn, S., Poore, M., Sorenson, C., Watson, D.W. (2005). Seasonal Activity and Species Composition of Dung Beetles (Coleoptera: Scarabaeidae and Geotrupidae) Inhabiting Cattle Pastures in North Carolina. Ann. Entomol. Soc. Am. 98, 309D321

Bertone, M. A., Green, J. T., Washburn, S. P., Poore, M. H., & Watson, D. W. (2006). The Contribution of Tunneling Dung Beetles to Pasture Soil Nutrition. *Forage and Grazinglands , 4*.

Beynon, S.A., Mann, D.J., Slade, E.M, Lewis, O.T. (2012). Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. J. Appl. Ecol. 49, 1365–1372.

Beynon, S.A., Wainwright, W.A., Christie, M. (2015). The application of an ecosystem services framework to estimate the economic value of dung beetles to the UK cattle industry. Ecol. Entomol. 40, 124–135.

Bicknell, J. E., Phelps, S. P., Davies, R. G., Mann, D. J., Struebig, M. J., & Davies, Z. G. (2014). Dung beetles as indicators for rapid impact assessments: Evaluating best practice forestry in the neotropics. *Ecological Indicators*, *43*, 154–161.

Block, W.A., Franklin, A.B., Ward, J.P., Ganey, J.L., White, G.C. (2001). Design and implementation of monitoring studies to evaluate the success of ecological restoration on wildlife. Restor. Ecol. 9, 293-303.

Blume, R. R. (1984). Euoniticellus intermedius (Coleoptera:Scarabaeidae): description of adults and immatures and biology of adults. *Environmental Entomology , 13*, 1064-1068.

Bojórquez-Quintal, E., Escalante-Magaña, C., Echevarría-Machado, I., Martínez-Estévez, M. (2017). Aluminum, a Friend or Foe of Higher Plants in Acid Soils. Front. Plant Sci. 8, 1767.

Bornemissza, G. F. (1960). Could dung eating insects improve our pastures? *Journal of Australian Institute of Agricultural Science , 26*, 54–56.

Bornemissza, G. F. (1976). The Australian dung beetle project 1965-1975. *Australian Meat Research Committee Review , 30*, 1–30.

Bot, A., Benites, J. (2005). The importance of soil organic matter. Retrieved from Food and Agriculture Organization: http://www.fao.org/docrep/009/a0100e/a0100e.pdf

Bowman, B., & Baker, D. (1998). Mine Reclamation Planning in the Canadian North. *Northern Minerals Program, Working Paper No.1. Canadian Arctic Resources Committee* , 82.

Boyer, S., Wratten, S., Pizey, M., & Weber, P. (2011). Impact of soil stockpiling and mining rehabilitation on earthworm communities. *Pedobiologia*, *54*(SUPPL.).

Brändle, M., Durka, W., & Altmoos, M. (2000). Diversity of surface dwelling beetle assemblages in open-cast lignite mines in Central Germany. *Biodiversity and Conservation*, *9*(9), 1297–1311.

Brown, J., Scholtz, C. H., Janeau, J.-L., Grellier, S., & Podwojewski, P. (2010). Dung beetles (Coleoptera: Scarabaeidae) can improve soil hydrological properties. *Applied Soil Ecology , 46*, 9-16.

Brown, K., Lemon, J. (2017). Cations and Cation Exchange Capacity, Soil Quality Pty Ltd. http://www.soilquality.org.au/factsheets/cation-exchange-capacity (accessed 17.08.21).

Brussaard, L. (1983). Reproductive behaviour and development of the dung beetle Typhaeus typhoeus (Coleoptera, Geotrupidae). *Tijdschr Entomol*, *126*(10), 203–231.

Brussaard, L., Runia, L.T. (1984). Recent and ancient traces of scarab beetles activity in sandy soils of Netherlands. Geoderma 34, 229-50.

Cadotte, M.W., Carscadden, K., Mirotchnick, N. (2011). Beyond species: functional diversity and the maintenance of ecological processes and services. J. Appl. Ecol. 48, 1079–1087.

Carter, M.R. (2002). Soil quality for sustainable land management: organic matter and aggregation interactions that maintain soil functions. Agron. J. 94, 38–47.

Castellano, M.J., Valone, T. (2007). Livestock, soil compaction and water infiltration rate: Evaluating a potential desertification recovery mechanism. J. Arid Environ. 71, 97-108.

Chamber of Mines of South Africa. (2016). *Integrated annual review*. Johannesburg.

Chan, K.Y., Barchia, I. (2007). Soil compaction controls the abundance, biomass and distribution of earthworms in a single dairy farm in south-eastern Australia. Soil Tillage Res. 94, 75-82.

Chown, S. L. (2010). Temporal biodiversity change in transformed landscapes: a southern African perspective. *Philosophical Transactions on the Royal Society B , 365*, 3729–3742.

Chyba, J., Kroulik, M., Krištof, K., Misiewicz, P.A., Chaney, K. (2014). Influence of soil compaction by farm machinery and livestock on water infiltration rate on grassland. Agron. Res. 12, 59-64.

Clark, E. V., & Zipper, C. E. (2016). Vegetation influences near-surface hydrological characteristics on a surface coal mine in eastern USA. *Catena , 139*, 241-249.

Coetzee, M., Truter, W., Du Toit, C.J.L. (2015). Organic matter contribution of ameliorants to surface mined soil reclamation and carbon storage. Masters dissertation in Environmental Management, University of Pretoria, Pretoria.

Cook, A. P., & Lloyd, P. J. D. (2012). The estimation of greenhouse gas emissions from South African surface and abandoned coal mines. *Journal of the Southern African Institute of Mining and Metallurgy*, *112*(12), 1087–1090.

Cowan, A. K., Lodewijks, H. M., Sekhohola, L. M., & Edeki, O. G. (2016). *In situ bioremediation of South African coal discard dumps*.

Coulson, M. (2012). The History of Mining. Harriman House Ltd., Hampshire.

Cristescu, R.H., Rhodes, J., Frére, C., Banks, P.B. (2013). Is restoring flora the same as restoring fauna? Lessons learned from koalas and mining rehabilitation. J. Appl. Ecol. 50, 423–431.

Davis, A. L. V. (1996). Habitat associations in a South African, summer rainfall, dung beetle community (Coleoptera: Scarabaeidae, Aphodiidae, Staphylinidae, Histeridae, Hydrophilidae). *Pedobiologia*, *40*(3), 260–280.

Davis, A. L. V. (1997). Climatic and biogeographical associations of southern African dung beetles (Coleoptera : Scarabaeidae). *African Journal of Ecology*, *35*(1), 10–38.

Davis, A. L. V. (2002). Dung beetle diversity in South Africa: influential factors, conservation status, data inadequacies and survey design. *African Entomology*, *10*(1), 53–65.

Davis, A.L.V., Frolov, A.V., Scholtz, C.H. (2008). The African Dung Beetle Genera. Protea Book House, Pretoria, South Africa.

Davis, A. L. V., & Philips, T. K. (2009). Regional fragmentation of rain forest in West Africa and its effect on local dung beetle assemblage structure. *Biotropica*, *41*(2), 215–220.

Davis, A. L. V., & Scholtz, C. H. (2001). Historical vs. ecological factors influencing global patterns of scarabaeine dung beetle diversity. *Diversity and Distributions*, *7*(4), 161–174.

Davis, A. L. V., & Scholtz, C. H. (2004). Local and regional species ranges of a dung beetle assemblage from the semi-arid Karoo/Kalahari margins, South Africa. *Journal of Arid Environments*, *57*(1), 61–85.

Davis, A. L. V., Swemmer, A. M., Scholtz, C. H., Deschodt, C. M., & Tshikae, B. P. (2014). Roles of environmental variables and land usage as drivers of dung beetle assemblage structure in mopane woodland. *Austral Ecology*, *39*(3), 313–327.

Davis, A. L. V., van Aarde, R. J., Scholtz, C. H., & Delport, J. H. (2003). Convergence between dung beetle assemblages of a post-mining vegetational chronosequence and unmined dune forest. *Restoration Ecology*, *11*(1), 29–42.

Davis, A. L. V., van Aarde, R. J., Scholtz, C. H., Guldemond, R. A. R., Fourie, J., & Deschodt, C. M. (2013). Is microclimate-driven turnover of dung beetle assemblage structure in regenerating coastal vegetation a precursor to re-establishment of a forest fauna? *Journal of Insect Conservation*, *17*(3), 565–576.

Dirzo, R., & Raven, P. H. (2003). Global state of biodiversity and loss. *Annual Review of Environment and Resources*, *28*, 137–167.

Dodson, J., Li, X., Sun, N., Atahan, P., Xinying, Z., Liu, H., Yang, Z. (2014). Use of coal in the Bronze Age in China. The Holocene 24, 525 –530.

Dohi, H., Yamada, A., Entsu, S. (1991). Cattle feeding deterrents emitted from cattle faeces. J. Chem. Ecol. 17, 1197 – 1203.

Doube, B. M. (1983). The habitat preference of some bovine dung beetles (Coleoptera : Scarabaeidae) in Hluhluwe Game Reserve , South Africa. *Bulletin of Entomological Research*, *73*, 357–371.

Doube, B. M. (1990). A functional classification for analysis of the structure of dung beetle assemblages. *Ecological Entomology*, *15*, 371–383.

Doube, B.M., Dalton, G., Ford, B. (2003). Dung beetles to improve the quality of catchment water in Australia. National Conference on Integrated Catchment Management, University of Western Sydney, Parramatta.

Doube, B. (2015). Dung beetles, biochar and improved water quality and pasture growth: interim report. Dung Beetle Solutions Australia, Adelaide.

Dymock, J. J. (1993). A case for he introduction of additional dung-burying beetles (Coleoptera: Scarabaeidae) into New Zealand. *New Zealand Journal of Agricultural Research , 36*, 163-171.

Eckert, D.J. (1987). Soil test interpretations: Basic cation saturation ratios and sufficiency levels. In. J.R. Brown (ed.) Soil Testing: Sampling, Correlation, Calibration, and Interpretation. Special Publication No. 21. Soil Science Society of America.

Edwards, P. B., & Aschenborn, H. H. (1987). Patterns of Nesting and Dung Burial in Onitis Dung Beetles: Implications for Pasture Productivity and Fly Control. *Journal of Applied Ecology , 24*, 837-851.

Edwards, P. (2007). *Introduced Dung Beetles in Australia 1967-2007: Current status and future directions.* Queensland: Landcare Australia.

Ehrlich, P. R. (1988). The Loss of Diversity: Causes and Consequences. In *Biodiversity* (pp. 21–28).

Estrada, A., Coates-Estrada, R., Dadda, A. A., & Cammarano, P. (1998). Dung and carrion beetles in tropical rain forest fragments and agricultural habitats at Los Tuxtlas, Mexico. *Journal of Tropical Ecology*, *14*(5), 577–593.

Evans, A. V., & Hogue, J. N. (2004). *Introduction to California Beetles.* Los Angeles: University of California Press.

Ewuim, S.C. (2013). Entomoremediation – A novel *in situ* bioremediation approach. Anim. Res. Int. 10, 1681 – 1684.

Farias, P.M., Hernández, M.I.M. (2017). Dung Beetles Associated with Agroecosystems of Southern Brazil: Relationship with Soil Properties. Rev. Bras. Ciênc. Solo, 41, e0160248.

Favila, M., Halffter, G. (1997). The use of indicator groups for measuring biodiversity as related to community structure and function. Acta Zool. Mex. 72, 1-25.

Federico, G. (2005). *Feeding the World: An Economic History of Agriculture, 1800-2000* (1st ed.). New Jersey: Princeton University Press.

Feer, F., & Hingrat, Y. (2005). Effects of forest fragmentation on a dung beetle community in French Guiana. *Conservation Biology*, *19*(4), 1103–1112.

Ferreira, M. C. (1969). Os escarabideos de Africa (Sul do Saara). *Revista de Entomologia* *de Mozambique*, (11), 5–1088.

Fertilizer Industry Federation of Australia, I. (2006). Australian Soil Fertility Manual. CSIRO, Australia, Collingwood.

Fertilizer Society of South Africa, (2007). Fertilizer handbook, 7th revised edition. FSSA, Pretoria.

Fiene, J. G. (2014). The benefits of dung beetles to cattle producers. *Boehringer Ingelheim* , 1-3.

Fincher, G. T., Stewart, T. B., & Davis, R. (1970). Attraction of Coprophagous Beetles to Feces of Various Animals. *The Journal of Parasitology*, *56*(2), 378.

Fincher, G. T., Monson, W. G., & Burton, G. W. (1981). The potential value of dung beetles in pastureecosystems. *Agronomy Journal* , 775–779

Forgie, S. A., Paynter, Q., Zhao, Z., Flowers, C., & Fowler, S. V. (2013). The impact of tunnelling and dung burial by new exotic dung beetles (Coleoptera: Scarabaeinae) on surface run-off, survivorship of a cattle helminth, and pasture foliage biomass in New Zealand pastures. *Landcare Research* , 1-18.

Freese, B. (2004). Coal: A Human History. Penguin Books. New York, p. 137.

Frouz, J., Elhottová, D., Kuráz, V., Sourková, M. (2006). Effects of soil macrofauna on other soil biota and soil formation in reclaimed and unreclaimed post mining sites: Results of a field microcosm experiment. Appl. Soil Ecol. 33, 308-320.

Frouz, J., Pižl, V., & Tajovský, K. (2007). The effect of earthworms and other saprophagous macrofauna on soil microstructure in reclaimed and un-reclaimed post-mining sites in Central Europe. *European Journal of Soil Biology*, *43*(SUPPL. 1).

Galatowitsch , S. M. (2012). *Ecological Restoration.* Sunderland: Sinauer Associates Inc.

Ghose, M. K. (2004). Effect of opencast mining on soil fertility. *Journal of Scientific and Industrial Research*, *63*(12), 1006–1009.

Ghose, M. K., Tiwary, R. K., Harris, J. A., Birch, P., & Short, K. C. (1989). Coal Mine Restorations. *Soil Use and Management*, *5*(4), 1006–1009.

Gillard, P. (1967). Coprophagous beetles in pasture ecosystems. *Journal of the Australian Institute of Agricultural Science , 33*, 30–34.

Giller, P. S., & Doube, B. M. (1989). Experimental analysis of inter- and intraspecific competition in dung beetle communities. *Journal of Animal Ecology*, *58*(1), 129–142.

Gregory, J.H., Dukes, M.D., Miller, G.L., Jones, P.H. (2005). Analysis of double-ring infiltration techniques and development of a simple automatic water delivery system. Online. Appl. Turfgrass Sci. doi:10.1094/ATS-2005-0531-01-MG.

Guo, L., Wu, G., Li, Y., Li, C., Liu, W., Meng, J., Liu, H., Yu, X., Jiang, G. (2016). Effects of cattle manure compost combined with chemical fertilizer on topsoil organic matter, bulk density and earthworm activity in a wheat–maize rotation system in Eastern China. Soil and Tillage Research, 156, 140 – 147.

Haigh, M.J., Sansom, B. (2007). Soil compaction, runoff and erosion on reclaimed coal-lands (UK). Int. J. Min. Reclam. Environ. 13, 135-146.

Halffter, G., Edmonds, W.D. (1982). The Nesting Behavior of Dung Beetles (Scarabaeinae): An Ecological and evolutive approach. Instituto de Ecologia, México.

Hamza, M.A., Anderson, W.K. (2005). Soil compaction in cropping systems. A review of the nature, causes and possible solutions. Soil Tillage Res. 82, 121–145.

Hanski, I., & Cambefort, Y. (1991). *Dung Beetle ecology*. (I. Hanski & Y. Cambefort, Eds.). New Jersey: Prinston University Press.

Hanafy, H.E.M. (2012). Effect of dung beetles, *Scarabaeus sacer* (Scarabaeidae: Scarabaeinae) on certain biochemical contents of leaves and fruits of tomato and squash plants. J. Appl. Sci. Res. 8, 4927-4936.

Harris, J.P., Birch, P., Short, K.C. (1989). Changes in the microbial community and physio-chemical characteristics of top soils stockpiled during opencast mining. Soil Use Manage. 5, 161-168.

Hayakawa, H., Hamashita, N. (1990). Studies in mass rearing methods of the dung battle *Onthophagus gazella* 4. A preliminary test on artificial diets for rearing the larvae. Annual Report of the Society of Plant Protection of North Japan 41, 182 – 183.

Hernanz, J. L., Peixoto, H., Cerisola, C., & Sanchez-Giron, V. (2000). An empirical model to predict soil bulk density profiles in field conditions using penetration resistance, moisture content and soil depth. *Journal of Terramechanics , 37*, 67-184.

Hopkin, S.P. (1989). Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier Applied Science, Barking, UK.

Horgan, F. G. (2005). Effects of deforestation on diversity, biomass and function of dung beetles on the eastern slopes of the Peruvian Andes. *Forest Ecology and Management*, *216*(1–3), 117–133.

Hughes, R. D., Tyndale Biscoe, M., Walker, J., & Biscoe, M. T. (1978). Effects of introduced dung beetles (Coleoptera: Scarabaeidae) on the breeding and abundance of the Australian bushfly, Musca vetustissima Walker (Diptera: Muscidae). *Bulletin of Entomological Research*, *68*(3), 361–372.

Hurlbert, S.H. (1984). Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54, 187–211.

Hutton, J.B., Jury, K.E., Davies, E.B. (1967). The intake and utilisation of potassium, sodium, calcium, phosphorus, and nitrogen in pasture herbage by lactating dairy cattle. N.Z. J. Agric. Res. 10, 367-388.

Hutton, S. A., & Giller, P. S. (2003). The effects of the intensification of agriculture on northern temperate dung beetle communities. *Journal of Applied Ecology*, *40*(6), 994–1007.

Johnson, D.L., Lewis, L.A. (1995). Land Degradation: Creation and Destruction, Blackwell, Oxford.

Jones, J.B. (2005). Hydroponics: A Practical Guide for the Soilless Grower. CRC Press, Florida, United States of America.

Jones, J.B. (2012). Plant Nutrition and Soil Fertility Manual, Second Edition. CRC Press, Florida, United States of America.

Jouquet, P., Huchet, G., Bottinelli, N., Thu, T. D., & Duc, T. T. (2012). Does the influence of earthworms on water infiltration, nitrogen leaching and soil respiration depend on the initial soil bulk density? A mesocosm experiment with the endogeic species Metaphire posthuma. *Biology and Fertility of Soils*, *48*(5), 561–567.

Joseph, S.D., Pow, D., Dawson, K., Mitchell, D.R.G., Rawal, A., Hook, J., Taherymoosavi, S., Van Zweiten, L., Rust, J., Donne, S.Munroe, P., Pace, B., Graber, E., Thomas, T., Nielsen, S., Ye, J., Lin, Y. Genxing, P., Lianqing, L., Solaiman, Z.M. (2015). Feeding Biochar to Cows: An Innovative Solution for Improving Soil Fertility and Farm Productivity. Pedosphere 25, 666–679.

Katakey, R. (2017). World Coal Production Just Had Its Biggest Drop on Record. Retrieved from Bloomberg Markets: https://www.bloomberg.com/news/articles/2017-06-13/coal-s-era-starts-to-wane-as-world-shifts-to-cleaner-energy

Kensa, M. V. (2011). Bioremediation - An overview. *Journal of Industrial Pollution Control*.

Klein, B. C. (1989). Effects of Forest Fragmentation on Dung and Carrion Beetle Communities in Central Amazonia. *Ecology*, *70*(6), 1715–1725.

Koko, M. (2015). Coal is the lifeblood of the South African economy. Retrieved from Eskom: http://www.eskom.co.za/Pages/CoaL.aspx

Kołodziej, B., Bryk, M., Słowińska-Jurkiewicz, A, Otremba, K., Gilewska, M. (2016). Soil physical properties of agriculturally reclaimed area after lignite mine: A case study from central Poland. Soil and Tillage Res. 163, 54–63.

Krstic, D., Djalovic, I., Nikezic, D. Bjelic, D. (2012). Aluminium in Acid Soils: Chemistry, Toxicity and Impact on Maize Plants, Food Production - Approaches, Challenges and Tasks. InTech, DOI: 10.5772/33077.

Krutka, H., Jingfeng, L. (2013). Case Studies of Successfully Reclaimed Mining Sites. Retrieved from Cornerstone: http://cornerstonemag.net/case-studies-of-successfully-reclaimed-mining-sites/

Kundu, N. K., & Ghose, M. K. (1997). Soil profile Characteristic in Rajmahal Coalfield area. *Indian Journal of Soil and Water Conservation , 25*, 28-32.

Kuter, N. (2013). Reclamation of Degraded Landscapes due to Opencast Mining, Advances in Landscape Architecture, DOI: 10.5772/55796.

Lamb, J.A., Fernandez F.G., Kaiser D.E. (2014). Extension Specialists in Nutrient Management. University of Minnesota, Minneapolis.

Lang, O. (2010). The dangers of mining around the world. Retrieved from BBC News: http://www.bbc.com/news/world-latin-america-11533349

Larsen, T. H., & Forsyth, A. (2005). Trap spacing and transect design for dung beetle biodiversity studies. *Biotropica*, *37*(2), 322–325.

Lastro, E. (2006). Dung beetles (Coleoptera: Scarabaeidae and Geotrupidae) in North Carolina Pasture Ecosystems. North Carolina State University, Raleigh.

Lavelle, P. & Spain, A.V. (2001). Soil ecology. Kluwer Scientific, Amsterdam.

Lavelle, P. (1997). Faunal activities and soil processes: adaptive strategies that determine ecosystem function. Adv. Ecol. Res. 27, 93 – 132.

Lee, C.M., Wall, R. (2006). Cow-dung colonization and decomposition following insect exclusion. Bull. Entomol. Res. 96, 315–322.

Lehohla, P. (2014). *Mining Industry, 2012.* Pretoria: Statistics South Africa.

Leuthold, RH., Triet, H., Schildger, B. (2004). Husbandry and breeding of African giant termites (*Macrotermes jeanneli*) at Berne Animal Park. Zoologische Garten 74, 26e37.

Li, M.S. (2006). Ecological restoration of mineland with particular reference to the metalliferous mine wasteland in China: a review of research and practice. Sci. Total Environ. 357, 38e53.

Limpitlaw, D., Aken, M., Lodewijks, H., & Viljoen, J. (2005). Post-mining rehabilitation, land use and pollution at collieries in South Africa, (April), 1–10.

Limpitlaw, D., & Briel, A. (2014). Post-mining land use opportunities in developing countries. *The Southern African Institute of Mining and Metallurgy , 114*, 137-142.

Limpitlaw, D., Aken, M., Kilani, J., Mentis, M., Nell, J.P., Tanner, P.D. (1997). Rehabilitation & soil characterization, Proceedings of the 11th International Conference on Coal Research, Calgary, Canada, 9-12 September, pp 297 – 309.

Losey, J.E. & Vaughan, M. (2006). The Economic Value of Ecological Services Provided by Insects. BioScience 56, 311 – 323.

Macqueen, A., & Beirne, B. P. (1975). Effects of cattle dung and dung beetle activity on growth of beardless wheatgrass in British Columbia. *Canadian Journal of Plant Science , 55*, 961–967.

Maiti, S. K., & Ghose, M. K. (2005). Ecological restoration of acidic coal mine overburden dumps- an Indian case study. *Land Contamination and Reclamation , 13*, 361-369.

Majer, J.D., Walker, T.C., Berlandier, F. (1987). The role of ants in degraded soils within Dryandra State Forest. Mulga Res. Cent. J. 9, 15 – 16.

Manning, P., Slade, E. M., Beynon, S. A., & Lewis, O. T. (2016). Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agriculture, Ecosystems and Environment , 218*, 87-94.

Marschner, H. (1995). Mineral Nutrition in Higher Plants. Academic Press, San Diego.

Martius, C. (1990). The Influence of Geophagous Termites on Soils of Inundation Forests in Amazonia – First Results, in Veeresh, G.K., Mallik, B., Viraktamath, C.A., (Eds), Social Insects and the Environment. Leiden, Netherlands, pp. 209 – 210.

Martin-Piera, F., & Lobo, J. M. (1996). A comparative discussion of trophic preferences in dung beetle communities. *Miscellania Zoologica Barcelona*, *19*, 13–31.

Marvier, M., Kareiva, P., & Neubert, M. G. (2004). Habitat destruction, fragmentation, and disturbance promote invasion by habitat generalists in a multispecies metapopulation. *Risk Analysis*.

Materechera, S. A., Dexter, A. R., & Alston, A. M. (1991). Penetration of very strong soils by seedling roots of different plant species. *Plant and Soil*, *135*(1), 31–41.

McAndrews, G.M., Liebman. M., Cambardella, C. A., Richard, T.L. (2002). Residual effects of composted and fresh solid swine (*Sus scrofa* L.) manure on soybean [*Glycine max* (L.) Merr.] growth and yield. Agronomy Journal, *98*, 873 – 882.

McCarthy, T. S. (2011). The impact of acid mine drainage in South Africa. *South African Journal of Science*, *107*(5/6), 1–7.

McGeoch, M.A., Rensburg, B.J.V., Botes, A. (2002). The verification and application of bioindicators: a case study of dung beetles in a savanna ecosystem. J. Appl. Ecol. 39, 661-672.

McKay, A. (1976). The industrious dung beetle, surprise and enterprise. Fifty Years of Science for Australia's CSIRO, CSIRO Publishing, Australia.

McLean, E.O., Brown, J.R. (1984). Crop response to lime in the Midwestern United States. In Soil Acidity and Liming.

Meehan, M.A., Sedivec, K.K., DeKeyser, E.S. (2011). Grazing Riparian Ecosystems: Grazing Systems Facts sheet. Retrieved from North Dakota State University: https://www.ag.ndsu.edu/pubs/ansci/range/r1540.pdf

Mengel, K., Kirkby, E.A., 2001. Principles of Plant Nutrition. Kluwer Academic Publishers, Dordrecht.

Mensah, A. K. (2015). Role of revegetation in restoring fertility of degraded mined soils in Ghana: A review. *International Journal of Biodiversity and Conservation , 7*, 57-80.

Miranda, C. H., Santos, J. C., & Bianchin, I. (2000). The role of Digitonthophagus gazella in pasture cleaning and production as a result of burial of cattle dung. *Pasturas Tropicales , 22*, 14-18.

Mittal, I.C. (1993). Natural manuring and soil conditioning by dung beetles. Tropical Ecology 34, 150-159.

Morgenthal, T. L. (2003). *The assessment of topsoil degradation on rehabilitated coal discard dumps.* Potchefstroom: North-West University Publishing.

Morgenthal, T. L., & Van Rensburg, L. (2004). Ecosystem development on seven rehabilitated discard dumps. *African Journal of Range and Forage Science , 21*, 57-66.

Mosaddeghi, M.R., Hajabbasi, M.A., Hemmat, A., Afyuni, M. (2000). Soil compactibility as affected by soil moisture content and farmyard manure in central Iran. Soil and Tillage Research, *55*, 87 – 97.

Mrzljak, J., & Wiegleb, G. (2000). Spider colonization of former brown coal mining areas - Time or structure dependent? *Landscape and Urban Planning*, *51*(2–4), 131–146.

Mucina, L., & Rutherford, M. C. (2006). *The vegetation of South Africa, Lesotho and Swaziland.Strelitzia 19. Strelitzia* (Vol. 19).

Mueller, K. E., Tilman, D., Fornara, D. A., & Hobbie, S. E. (2013). Root depth distribution and the diversity-productivity relationship in a long-term grassland experiment. *Ecology*, *94*(4), 787–793.

Naeem, S., Thompson, L. J., Lawler, S. P., Lawton, J. H., & Woodfin, R. M. (1995). Empirical evidence that declining species diversity may alter the performance of terrestrial ecosystems. *Philosophical Transactions: Biological Sciences , 347*, 259-262.

Nealis, V. G. (1977). Habitat associations and community analysis of south Texas dung beetles (Coleoptera: Scarabaeinae). *Canadian Journal of Zoology*, *55*(1), 138–147.

Nel, L., Truter, W. F., Kellner, K., & van Deventer, P. (2014). *The value of coated Rhodes grass seed in rehabilitation.* Free State: 50th Annual Congress of the Grassland Society of Southern Africa.

Nervo, B., Tocco, C., Caprio, E., Palestrini, C., & Ronaldo, A. (2014). The effects of body mass on dung removal efficiency in dung beetles. *PLoS ONE , 9*, 1-9.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., & Favila, M. E. (2008). Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological Conservation , 141*, 1461-1474.

Nichols, E., & Gardner, T. A. (2011). Dung Beetles as a Candidate Study Taxon in Applied Biodiversity Conservation Research. In L. W. Simmons, & T. J. Ridsdill-Smith, *Ecology and Evolution of Dung Beetles* (pp. 267-291). New Jersey: Blackwell Publishing Ltd.

Ochieng, G., Seanego, E. S., & Nkwonta, O. I. (2010). Impacts of mining on water resources in South Africa: A review. *Scientific Research and Essays*, *5*(22), 3351–3357.

Ohsowski, B. M., Klironomos, J. N., Dunfield, K. E., & Hart, M. M. (2012). The potential of soil amendments for restoring severely disturbed grasslands. *Applied Soil Ecology , 60*, 77–83.

Omaliko, C.P.E. (1984). Dung decomposition and its effects on soil component of a tropical grassland ecosystem. Trop. Ecol. 25, 214-220.

Onwudike, S.U. (2010). Effectiveness of Cow Dung and Mineral Fertilizer on Soil Properties, Nutrient Uptake and Yield of Sweet Potato (Ipomoea batatas) in Southeastern Nigeria. Asian J. Agric. Res. 4, 148-154.

Osberg, D. C., Doube, B. M., & Hanrahan, S. A. (1994). Habitat specificity in African dung beetles: the effect of soil type on the survival of dung beetle immatures (Coleoptera Scarabaeidae). *Tropical Zoology*, *7*(1), 1–10.

Pettygrove, G.S., (2009). Nitrogen Mineralisation. Regents of the University of California, Los Angeles.

Pinetown, K. L., Ward, C. R., & van der Westhuizen, W. A. (2007). Quantitative evaluation of minerals in coal deposits in the Witbank and Highveld Coalfields, and the potential impact on acid mine drainage. *International Journal of Coal Geology*, *70*(1–3 SPEC. ISS.), 166–183.

Platt, M. (2006). *Rehabilitation of Mined Land.* Witbank: Coaltech.

Pomfret, J. C., & Knell, R. J. (2006). Sexual selection and horn allometry in the dung beetle Euoniticellus intermedius. *Animal Behaviour , 71*, 567–576.

Poorter, H., Fiorani, F., Pieruschka, R., Wojciechowski, T., van der Putten, W.H., Kleyer, M., Schurr, U., Postma, J. Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. New Phytol. 212, 838 – 855.

Pratt, P.F., Castellanos, J.Z., (1981). Available nitrogen from animal manures. California Agriculture.

Quintero, I., & Roslin, T. (2005). Rapid recovery of dung beetle communities following habitat fragmentation in central Amazonia. *Ecology*, *86*(12), 3303–3311.

Rabitsch, W.B., 1997. Seasonal metal accumulation patterns in the red wood ant Formica pratensis (Hymenoptera) at contaminated and reference sites. J. Appl. Ecol. 34, 1455–1461.

Raghuvanshi, S. P., Chandra, A., & Raghav, A. K. (2006). Carbon dioxide emissions from coal based power generation in India. *Energy Conversion and Management*, *47*(4), 427–441. https://doi.org/10.1016/j.enconman.2005.05.007

Reid, W. V., Mooney, H. A., Cropper, A., Capistrano, D., Carpenter, S. R., Chopra, K., … Zurek, M. B. (2005). *Millenium Ecosystem Assessment - Ecosystems and human well-being: Synthesis*. *Island Press, Washington, DC*.

Reddy, M.S., (1991). Effects of soil amendments on the hardening of red sandy loams (chalka soils) of Andhra Pradesh. Ann. Agric. Res. 12, 174–176.

Richardson, P., Richardson, R., (2000). Dung beetles and their effects on soil. Ecol. Restor. 18, 116-117.

Ridsdill-Smith, T. J. (2014). Effect of soil moisture on brood ball production by Onthophagus binodis Thunberg and Euoniticellus intermedius . *Australian Journal of Entomology*, *25*, 75–78.

Rousk, J, Brookes, P.C., Baath, E., (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. App.l Environ. Microb. 75, 1589–1596.

Rudich, Y., Donahue, N.M., Mental, T.F., (2007). Aging of Organic Aerosol: Bridging the Gap between Laboratory and Field Studies. Annu. Rev. Phys. Chem. 58, 321–352.

Ruiz-jaen, M. C., & Aide, T. M. (2005). Restoration success: How is it being measured? *Restoration Ecology , 13*, 569–577.

Salah, S., Hasanah, U., Elijonnahdi, E., (2014). Effectiveness of dung beetles as bioindicators of environmental changes in land-use gradient in Sulawesi, Indonesia. Biotropia 21, 53-63.

Salt, D. E., Smith, R. D., & Raskin, I. (1998). Phytoremediation. Annual review of plant physiology. *Annual Review of Plant Physiology and Plant Molecular Biology*, *49*, 643–668.

Samper, J. R., & Piera, F. M. (1995). Onthophagine nesting bahaviour.pdf. *European Journal of Entomology*, *92*, 667–679.

Scholtz, Clarke H. , Davis, Adrian L.V. and Kryger, U. (2009). *Evolutionary biology and conservation of dung beetles*.

Scott, B., Ranjith, P. G., Choi, S. K., & Khandelwal, M. (2010). A review on existing opencast coal mining methods within Australia. *Journal of Mining Science*, *46*(3), 280–297.

Sharrow, S.H., (2007). Soil compaction by grazing livestock in silvopastures as evidenced by changes in soil physical properties. Agrofor. Syst. 71, 215-223.

Sheoran, A. S., Sheoran, V., & Poonia, P. (2008). Rehabilitation of mine degraded land by metallophytes. *Mining Engineers Journal , 10*, 11-16.

Sheoran, V., Sheoran , A. S., & Poonia, P. (2010). Soil Reclamation of Abandoned Mine Land by Revegetation: A Review. *International Journal of Soil, Sediment and Water , 3*, 1-20.

Sheoran, V., Sheoran, A. S., & Poonia, P. (2010). Soil Reclamation of Abandoned Mine Land by Revegetation: A Review. *International Journal of Soil, Sediment and Water , 3*, 1-20.

Shepherd, V. E., & Chapman, C. A. (1998). Dung beetles as secondary seed dispersers: impact on seed predation and germination. *Journal of Tropical Ecology*, *14* (September 2000), 199–215.

Silva, J.A, Uchida, R., (2000). Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture. College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa.

Simson. C.R., R.B. Corey, and M.E. Sumner. (1979). Effect of varying Ca:Mg ratios on yield and composition of corn (*Zea mays*) and alfalfa (*Medicago sativa*). Commun. Soil Sci. Plant Anal. 10:153-162.

Slade, E.M., Mann, D.J., Villanueva, J.F. and Lewis, O.T., (2007). Experimental evidence for the effects of dung beetle functional group richness and composition on ecosystem function in a tropical forest. J. Anim. Ecol. 76, 1094–1104.

Slade, E. M., Riutta, T., Roslin, T., & Tuomisto, H. L. (2015). The role of dung beetles in reducing greenhouse gas emissions from cattle farming. *Scientific Reports*, *6*. https://doi.org/10.1038/srep18140

Slade, E. M., Roslin, T., Santalahti, M., & Bell, T. (2015). Disentangling the 'brown world' faecal-detritus interaction web: dung beetle effects on soil. *Oikos* .

Smyth, C., Dearden, P., (1998). Performance standards and monitoring requirements of surface coal mine reclamation success in mountainous jurisdictions of western North America: a review. J. Environ. Manage. 53, 209- 229.

Sorensen, P. (2009). Mine closure in South Africa. In *Proceedings - European Metallurgical Conference, EMC 2009* (Vol. 1, pp. 189–204).

Spoor, G., Tijink, F.G.J. and Weisskopf, P. (2003). Subsoil compaction: risk, avoidance, identification and alleviation. Soil Till. Res. 73, 175 – 182.

Sowig, P. (2017). Nordic Society Oikos Brood Care in the Dung Beetle Onthophagus vacca (Coleoptera : Scarabaeidae): The Effect of Soil Moisture on Time Budget , Nest Structure , and Reproductive Success Author (s): Peter Sowig Published by : Wiley on behalf of Nordic S. *Ecography*, *19*(3), 254–258.

Sparling, G. (1992). Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. *Australian Journal of Soil Research , 30*, 195.

Spector, S., (2006). Scarabaeine dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae): an invertebrate focal taxon for biodiversity research and conservation. Coleopt. Bull. 5, 71-83.

Strohmayer, P. (1999). Soil Stockpiling for Reclamation and Restoration activities after Mining and Construction. *Student On-Line Journal , 4*, 1-6.

Swart, E. (2003). The South African Legislative Framework for Mine Closure. *The Journal of The South African Institute of Mining and Metallurgy* , 489-492.

Talling, J.F., (1960). Comparative laboratory and field studies of photosynthesis by a marine planktonic diatom. Limnol. Oceanogr. 5, 62–77.

Tanner, P., & Mohr-Swart, M. (2007). Guidelines for the rehabilitation of mined land.

Taylor, H.M., (1971). Effects of soil strength on seedling emergence, root growth and crop yields. Compaction of Agricultural Soils, St. Joseph, MI.

Théry, I, Gril, J, Vernet, J.L., Meignen, L., Maury, J., (1996). Coal used for fuel at two prehistoric sites in southern France: Les Canalettes (Mousterian) and Les Usclades (Mesolithic). J. Archaeolog. Sci. 23, 509–512.

Thompson, S., Thompson, G., (2004). Adequacy of rehabilitation monitoring practices in the Western Australian mining industry. Ecol. Manage. Restor. 5, 30 - 33.

Thurow, T. L., Blackburn, W. H., & Taylor, C. A. (1986). Hydrologic characteristics of vegetation types as affected by livestock grazing systems. *Journal of Range Management , 39*, 505-509.

Tilman, D. (2001). Forecasting Agriculturally Driven Global Environmental Change. *Science*, *292*(5515), 281–284.

Tixier, T., Bloor, J. M., & Lumaret, J. P. (2015). Species-specific effects of dung beetle abundance on dung removal and leaf litter decomposition. *Acta Oecologica , 69*, 31-34.

Toren, T., Ünal, E., (2001). Assessment of Open Pit Coal Mining Impacts Using Remote Sensing: A Case Study from Turkey. 17th International Mining Congress and Exhibition of Turkey- IMCET2001, 2001, ISBN 975-395-417-4. General Directorate of Turkish Coal Enterprises, Ankara, Turkey.

Truter, W. F., Bredell, T., Olivier, J., Heerden, D. van, & Mosebi, P. (2013). The impact of surface coal mining on the pre- and post-mining agricultural potential of the grassland biome of Southern Africa. *Revitalising Grasslands to Sustain Our Communities: Proceedings, 22nd International Grassland Congress, 15-19 September, 2013, Sydney, Australia*.

Tscharntke, T., Steffan-Dewenter, I., Kruess, A., & Thies, C. (2002). Characteristics of insect populations on habitat fragments: A mini review. In *Ecological Research* (Vol. 17, pp. 229–239).

Tyndale-Biscoe, M. (1990). *Common Dung Beetles in Pastures of South-eastern Australia.* Brisbane: CSIRO Australia.

Unger, P. W., & Kaspar, T. C. (1993). Soil Compaction and Root Growth: A Review. *Agronomy Journal Abstract*, *86*(5), 759–766.

Viljoen. (2013). *Arnot coal mine specialist soil assessment as part of the consolidated EMPR.* Johannesburg: Golder Associates.

Visser, S., Fujikawa, J., Griffiths, C.L., Parkinson, D., (1984). Effect of topsoil storage on microbial activity, primary production and decomposition potential. Plant and Soil 82, 41-50.

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (2008). Human domination of Earth's ecosystems. In *Urban Ecology: An International Perspective on the Interaction Between Humans and Nature* (pp. 3–13).

Vulinec, K., Lambert, J. E., & Mellow, D. J. (2006). Primate and dung beetle communities in secondary growth rain forests: Implications for conservation of seed dispersal systems. *International Journal of Primatology*, *27*(3), 855–879.

Wali, M.K., Kannowski, P.B., (1975). Prairie Ant Mound Ecology: Interrelationships of Microclimate, Soils and Vegetation. In Prairie: A Multiple View; Wali, M.K., Ed.; University of North Dakota Press. USA, North Dakota.

Waterhouse, D. F. (1974). The biological control of dung. *Scientific American*, *230*(4), 100–109.

Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere , 50*, 775–780.

World Coal association. (2012). *Coal matters*.

World Coal Institute. (2005). *the Coal Resource*. *World Coal Institute*.

World coal orginisation. (2017). Coal and electricity.

Yamada, D., Imura, O., Shi, K., & Shibuya, T. (2007). Effect of tunnelerdung beetles on cattle dung decomposition, soil nutrients and herbage growth Grassland. *Science , 53*, 121–129.

Younger, P.L., 2004. Environmental impacts of coal mining and associated wastes: a geochemical perspective. Geological Society, London, Special Publications 236, 169-209.

Zhao, Y., Wang, P., Li, J., Chen, Y., Ying, X., & Liu, S. (2009). The effects of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat–maize cropping system. *European Journal of Agronomy , 31* (1), 36–42.

Zhao, Z., Shahrour, I., Bai, Z., Fan, W., Feng, L., & Li, H. (2013). Soils development in opencast coal mine spoils reclaimed for 1-13 years in the West-Northern Loess Plateau of China. *European Journal of Soil Biology , 55*, 40-46.

Bache, B. W. (1984). The role of calcium in buffering soils. *Plant, Cell and Environment , 7*, 391–395.

Bang, H. S., Lee, J., Kwon, O. S., Na, Y. E., Jang, Y. S., & Kim, H. W. (2004). Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. *Applied Soil Ecology , 29*, 165–171.

Bang, H. S., Lee, J., Kwon, O. S., Na, Y. E., Jang, Y. S., & Kim, H. W. (2005). Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. *Applied Soil Ecology , 29*, 165-171.

Bertone, M. A., Green, J. T., Washburn, S. P., Poore, M. H., & Watson, D. W. (2006). The Contribution of Tunneling Dung Beetles to Pasture Soil Nutrition. *Forage and Grazinglands , 4*.

Blume, R. R. (1984). Euoniticellus intermedius (Coleoptera:Scarabaeidae): description of adults and immatures and biology of adults. *Environmental Entomology , 13*, 1064-1068.

Bornemissza, G. F. (1960). Could dung eating insects improve our pastures? *Journal of Australian Institute of Agricultural Science , 26*, 54–56.

Bornemissza, G. F. (1976). The Australian dung beetle project 1965-1975. *Australian Meat Research Committee Review , 30*, 1–30.

Bowman, B., & Baker, D. (1998). Mine Reclamation Planning in the Canadian North. *Northern Minerals Program, Working Paper No.1. Canadian Arctic Resources Committee* , 82.

Brown, J., Scholtz, C. H., Janeau, J.-L., Grellier, S., & Podwojewski, P. (2010). Dung beetles (Coleoptera: Scarabaeidae) can improve soil hydrological properties. *Applied Soil Ecology , 46*, 9-16.

Chown, S. L. (2010). Temporal biodiversity change in transformed landscapes: a southern African perspective. *Philosophical Transactions on the Royal Society B , 365*, 3729–3742.

Clark, E. V., & Zipper, C. E. (2016). Vegetation influences near-surface hydrological characteristics on a surface coal mine in eastern USA. *Catena , 139*, 241-249.

Dymock, J. J. (1993). A case for he introduction of additional dung-burying beetles (Coleoptera: Scarabaeidae) into New Zealand. *New Zealand Journal of Agricultural Research , 36*, 163-171.

Edwards, P. B., & Aschenborn, H. H. (1987). Patterns of Nesting and Dung Burial in Onitis Dung Beetles: Implications for Pasture Productivity and Fly Control. *Journal of Applied Ecology , 24*, 837-851.

Edwards, P. (2007). *Introduced Dung Beetles in Australia 1967-2007: Current status and future directions.* Queensland: Landcare Australia.

Evans, A. V., & Hogue, J. N. (2004). *Introduction to California Beetles.* Los Angeles: University of California Press.

Federico, G. (2005). *Feeding the World: An Economic History of Agriculture, 1800-2000* (1st ed.). New Jersey: Princeton University Press.

Fiene, J. G. (2014). The benefits of dung beetles to cattle producers. *Boehringer Ingelheim* , 1-3.

Fincher, G. T., Monson, W. G., & Burton, G. W. (1981). The potential value of dung beetles in pastureecosystems. *Agronomy Journal* , 775–779.

Forgie, S. A., Paynter, Q., Zhao, Z., Flowers, C., & Fowler, S. V. (2013). The impact of tunnelling and dung burial by new exotic dung beetles (Coleoptera: Scarabaeinae) on surface run-off, survivorship of a cattle helminth, and pasture foliage biomass in New Zealand pastures. *Landcare Research* , 1-18.

Galatowitsch , S. M. (2012). *Ecological Restoration.* Sunderland: Sinauer Associates Inc.

Gillard, P. (1967). Coprophagous beetles in pasture ecosystems. *Journal of the Australian Institute of Agricultural Science , 33*, 30–34.

Hernanz, J. L., Peixoto, H., Cerisola, C., & Sanchez-Giron, V. (2000). An empirical model to predict soil bulk density profiles in field conditions using penetration resistance, moisture content and soil depth. *Journal of Terramechanics , 37*, 67-184.

Kundu, N. K., & Ghose, M. K. (1997). Soil profile Characteristic in Rajmahal Coalfield area. *Indian Journal of Soil and Water Conservation , 25*, 28-32.

Lehohla, P. (2014). *Mining Industry, 2012.* Pretoria: Statistics South Africa.

Limpitlaw, D., & Briel, A. (2014). Post-mining land use opportunities in developing countries. *The Southern African Institute of Mining and Metallurgy , 114*, 137-142.

Limpitlaw, D., & Briel, A. (2014). Post-mining land use opportunities in developing countries. *Mining, Environment and Society Conference* (pp. 137-142). Witbank: The Southern African Institute of Mining and Metallurgy.

Macqueen, A., & Beirne, B. P. (1975). Effects of cattle dung and dung beetle activity on growth of beardless wheatgrass in British Columbia. *Canadian Journal of Plant Science , 55*, 961–967.

Maiti, S. K., & Ghose, M. K. (2005). Ecological restoration of acidic coal mine overburden dumps- an Indian case study. *Land Contamination and Reclamation , 13*, 361-369.

Manning, P., Slade, E. M., Beynon, S. A., & Lewis, O. T. (2016). Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agriculture, Ecosystems and Environment , 218*, 87-94.

Mensah, A. K. (2015). Role of revegetation in restoring fertility of degraded mined soils in Ghana: A review. *International Journal of Biodiversity and Conservation , 7*, 57-80.

Mensah, A. K. (2015). Role of revegetation in restoring fertility of degraded mined soils in Ghana: A review. *International Journal of Biodiversity and Conservation , 7*, 57-80.

Miranda, C. H., Santos, J. C., & Bianchin, I. (2000). The role of Digitonthophagus gazella in pasture cleaning and production as a result of burial of cattle dung. *Pasturas Tropicales , 22*, 14-18.

Morgenthal, T. L. (2003). *The assessment of topsoil degradation on rehabilitated coal discard dumps.* Potchefstroom: North-West University Publishing.

Morgenthal, T. L., & Van Rensburg, L. (2004). Ecosystem development on seven rehabilitated discard dumps. *African Journal of Range and Forage Science , 21*, 57-66.

Naeem, S., Thompson, L. J., Lawler, S. P., Lawton, J. H., & Woodfin, R. M. (1995). Empirical evidence that declining species diversity may alter the performance of terrestrial ecosystems. *Philosophical Transactions: Biological Sciences , 347*, 259-262.

Nel, L., Truter, W. F., Kellner, K., & van Deventer, P. (2014). *The value of coated Rhodes grass seed in rehabilitation.* Free State: 50th Annual Congress of the Grassland Society of Southern Africa.

Nervo, B., Tocco, C., Caprio, E., Palestrini, C., & Ronaldo, A. (2014). The effects of body mass on dung removal efficiency in dung beetles. *PLoS ONE , 9*, 1-9.

Nichols, E., & Gardner, T. A. (2011). Dung Beetles as a Candidate Study Taxon in Applied Biodiversity Conservation Research. In L. W. Simmons, & T. J. Ridsdill-Smith, *Ecology and Evolution of Dung Beetles* (pp. 267-291). New Jersey: Blackwell Publishing Ltd.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., & Favila, M. E. (2008). Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological Conservation , 141*, 1461-1474.

Ohsowski, B. M., Klironomos, J. N., Dunfield, K. E., & Hart, M. M. (2012). The potential of soil amendments for restoring severely disturbed grasslands. *Applied Soil Ecology , 60*, 77–83.

Platt, M. (2006). *Rehabilitation of Mined Land.* Witbank: Coaltech.

Pomfret, J. C., & Knell, R. J. (2006). Sexual selection and horn allometry in the dung beetle Euoniticellus intermedius. *Animal Behaviour , 71*, 567–576.

Rabitsch, W.B. (1997). Seasonal metal accumulation patterns in the red wood ant Formica pratensis (Hymenoptera) at contaminated and reference sites. *Journal of Applied Ecology, 34,* 1455–1461.

Reddy, M.S. (1991). Effects of soil amendments on the hardening of red sandy loams (chalka soils) of Andhra Pradesh. *Annual Agricultural Research, 12,* 174–176.

Ruiz-jaen, M. C., & Aide, T. M. (2005). Restoration success: How is it being measured? *Restoration Ecology , 13*, 569–577.

Sheoran, A. S., Sheoran, V., & Poonia, P. (2008). Rehabilitation of mine degraded land by metallophytes. *Mining Engineers Journal , 10*, 11-16.

Sheoran, V., Sheoran , A. S., & Poonia, P. (2010). Soil Reclamation of Abandoned Mine Land by Revegetation: A Review. *International Journal of Soil, Sediment and Water , 3*, 1-20.

Sheoran, V., Sheoran, A. S., & Poonia, P. (2010). Soil Reclamation of Abandoned Mine Land by Revegetation: A Review. *International Journal of Soil, Sediment and Water , 3*, 1-20.

Slade, E. M., Roslin, T., Santalahti, M., & Bell, T. (2015). Disentangling the 'brown world' faecal-detritus interaction web: dung beetle effects on soil. *Oikos* .

Sparling, G. (1992). Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. *Australian Journal of Soil Research , 30*, 195.

Strohmayer, P. (1999). Soil Stockpiling for Reclamation and Restoration activities after Mining and Construction. *Student On-line Journal , 4*, 1-6.

Strohmayer, P. (1999). Soil Stockpiling for Reclamation and Restoration activities after Mining and Construction. *Student On-Line Journal , 4*, 1-6.

Swart, E. (2003). The South African Legislative Framework for Mine Closure. *The Journal of The South African Institute of Mining and Metallurgy* , 489-492.

Thurow, T. L., Blackburn, W. H., & Taylor, C. A. (1986). Hydrologic characteristics of vegetation types as affected by livestock grazing systems. *Journal of Range Management , 39*, 505-509.

Tixier, T., Bloor, J. M., & Lumaret, J. P. (2015). Species-specific effects of dung beetle abundance on dung removal and leaf litter decomposition. *Acta Oecologica , 69*, 31-34.

Tyndale-Biscoe, M. (1990). *Common Dung Beetles in Pastures of South-eastern Australia.* Brisbane: CSIRO Australia.

Viljoen. (2013). *Arnot coal mine specialist soil assessment as part of the consolidated EMPR.* Johannesburg: Golder Associates.

Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere , 50*, 775–780.

Yamada, D., Imura, O., Shi, K., & Shibuya, T. (2007). Effect of tunnelerdung beetles on cattle dung decomposition, soil nutrients and herbage growth Grassland. *Science , 53*, 121–129.

Zhao, Y., Wang, P., Li, J., Chen, Y., Ying, X., & Liu, S. (2009). The effects of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat–maize cropping system. *European Journal of Agronomy , 31* (1), 36–42.

Zhao, Z., Shahrour, I., Bai, Z., Fan, W., Feng, L., & Li, H. (2013). Soils development in opencast coal mine spoils reclaimed for 1-13 years in the West-Northern Loess Plateau of China. *European Journal of Soil Biology , 55*, 40-46.

Chapter 10 Appendices

10.1 DUNG BEETLE SPECIES LIST

Table 11. 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 (7 & 8) and a nature reserve (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
	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
	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
	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
	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
	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
Sisyphini												
	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

10.2 DUNG BEETLE SPECIES ABUNDANCE PER SITE

Table 12. 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 cafferBoheman, 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 cafferBoheman, 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 deceptorPéringuey, 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 cafferBoheman, 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 cafferBoheman, 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 deceptorPéringuey, 1901	1
Catharsius sesostris Waterhouse, 1888	1	*Caccobius obtusus* (Fahraeus, 1857)	1
Onitis deceptorPéringuey, 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	*Scarabaeus goryi* (Castelnau, 1840)	433

Catharsius tricornutus (DeGeer, 1778)	7	*Pachylomera opaca* van Lansberge, 1874	432
Onthophagus asperulus d'Orbigny, 1905	7	*Allogymnopleurus splendidus* (Bertolini, 1849)	396
Onthophagus cinctipennis Quedenfeldt, 1884	7	*Proagoderus chalcostolus* (d'Orbigny, 1902)	382
Proagoderus sapphirinus (Fahraeus, 1857)	7	*Onthophagus pallidipennis* Fahraeus, 1857	332
Onitis pecuarius van Lansberge, 1875	6	*Caccobius ferrugineus* (Fahraeus, 1857)	225
Onthophagus deterrens Péringuey, 1901	6	*Onthophagus aeruginosus* Roth, 1851	177
Onthophagus pauxillus d'Orbigny, 1902	6	*Onthophagus fimetarius* Roth, 1851	159
Scarabaeus heqvisti zur Strassen, 1962	6	*Euoniticellus intermedius* (Reiche, 1848)	148
Epidrepanus caelatus (Gerstaecker, 1871)	5	*Onthophagus vinctus* Erichson, 1843	134
Oniticellus planatus Castelnau, 1840	5	*Neosisyphus rubrus* (Paschalidis, 1974)	134
Onthophagus binodis Thunberg, 1818	5	*Liatongus militaris* (Castelnau, 1840)	110
Drepanocerus kirbyi Kirby, 1828	4	*Onthophagus sp. nr granilifer*	108
Neosisyphus rubrus (Paschalidis, 1974)	4	*Epidrepanus caelatus* (Gerstaecker, 1871)	84
Eodrepanus fastiditus (Péringuey, 1901)	3	*Catharsius sesostris* Waterhouse, 1888	63
Onitis tortuosus Houston, 1983	3	*Metacatharsius troglodytes* Boheman, 1857	63
Cleptocaccobius convexifrons (Raffray, 1877)	3	*Onthophagus rasipennis* d'Orbigny, 1908	48
Catharsius aegus Génier	2	Cheironitis sp. *nr scabrosus*	38
Onitis cafferBoheman, 1857	2	*Kheper nigroaeneus* (Boheman, 1857)	35
Onthophagus ebenus Péringuey, 1888	2	*Hamonthophagus depressus* (Harold, 1871)	28
Pachylomera femoralis (Kirby, 1828)	2	*Pedaria picea Fahraeus*, 1857	25
Pedaria picea Fahraeus, 1857	2	*Tiniocellus eurypygus eurypygus* Branco, 2010	25
Metacatharsius troglodytes Boheman, 1857	1	*Euonthophagus sp.*	25
Cyptochirus ambiguus (Kirby, 1828)	1	*Onthophagus cribripennis* d'Orbigny, 1902	25
Cheironitis sp. *nr scabrosus*	1	*Euoniticellus triangulatus* (Harold, 1873)	24
Caccobius obtusus (Fahraeus, 1857)	1	*Kheper lamarcki* (Macleay, 1821)	23
Onthophagus sp. (small, short, shiny)	1	*Eodrepanus fastiditus* (Péringuey, 1901)	21
Onthophagus vinctus Erichson, 1843	1	*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 cafferBoheman, 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		