DUNG BEETLE ACTIVITY IMPROVES SOIL PROPERTIES AND PLANT GROWTH OF RECLAIMED MINED LAND

By

JESSICA BADENHORST

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Supervisors: Dr. J. Dabrowski
Prof. C. H. Scholtz
Co-supervisor: Dr. W. F. Truter

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DECLARATION

I, Jessica Badenhorst, declare that the dissertation, which I hereby submit for the degree MSc Entomology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: ……………………………………………………….
DATE: 2018/05/01…………………………………………………

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ABSTRACT

Mining activities greatly contribute to economic growth and development in South Africa. However, post-mining soils have limited land-use potential due to low fertility, deficiency in organic matter content and poor physical, chemical, and microbiological properties. Mechanical methods to improve soil conditions, such as ripping, are expensive and provide temporary improvements. Alternatively, exploring biological methods could aid in creating arable land from degraded soils, where the placed soil is sufficiently deep. Dung beetles could potentially improve several aspects of soil degradation, complementing current rehabilitation efforts. Studies in relatively undisturbed soils of agro-ecosystems have found that dung burial introduces essential nutrients to the plant root zone. This study aimed to determine whether these benefits could be maintained in soils on reclaimed mined land, where very high rates of compaction may inhibit tunnelling by dung beetles. Two experiments were conducted. The first experiment investigated the effects of tunnelling dung beetle activity on plots simulating reclaimed mine soil, while the second experiment examined the effects of dung beetle activity on reclaimed mined land near Emalahleni, Mpumalanga. Treatments consisting of dung and dung beetles (D+B), dung only (D) and no dung or dung beetles (X - control) were applied to both the plot and field experiments, with an added treatment of naturally-occurring dung beetles and dung (N) being included for the field-based experiment on reclaimed mined land. In both experiments, soil and plant measurements were taken one month after beetle and dung applications and repeated six months later to determine the longevity of effects. Soil and plant measurements included water infiltration rate, herbaceous plant biomass, plant protein content, soil penetration resistance, and various soil properties and elements. Results obtained from both experiments were similar in most respects. The D+B treatments significantly increased water infiltration rates and magnesium content in the soil. Water infiltration rates for D+B treatments increased by an average of 60% compared to D and X treatments on simulated plots and increased by an average of 38% for reclaimed mined land. Soil strength (penetration resistance) was significantly reduced on the simulated plots and the reclaimed mined land where dung beetles were active. Soil pH was increased by dung beetle activity for both experiments but was variable between sampling intervals. Although plant protein content was not significantly altered for any treatment, it was consistently higher for D+B treatments. Based on these results, the activity of applied dung beetles greatly improved reclaimed mined land soil and in some instances the effects persisted for at least six months with no further dung beetle or dung applications. Where effects were not sustained for six months, it is possible that the random sampling method may have missed effects of dung beetle activity that were concentrated beneath dung pats. A high degree of variability in plant biomass and soil strength was observed on the reclaimed mined land, reflecting the more complex environmental conditions and interactions in the field. Naturally-occurring dung beetles were not as effective as applied dung beetles, because their abundance was much lower and consisted of small-bodied rollers. In conclusion, dung beetle application to reclaimed post-mining soil may alleviate degraded soil conditions but may require frequent applications to augment naturally-occurring populations.

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1. History of coal mining

Land is valuable, in the literal and figurative sense. Not only is the price of land increasing, but 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.

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 nature of opencast mining, vegetation and soil above the overburden is displaced, and so are the biological interactions associated with the stripped soils. Globally, approximately 500 000 hectares of land are disturbed by mining every year (Johnson and Lewis, 1995).

2. Conditions on opencast coal mines

Mining activities negatively affect the physical, chemical and biological properties of soil. Frequent problems associated with soils on previously mined land include excessive erosion, nutrient leaching and increased mineralisation rates (Kołodziej et al., 2016). Rehabilitated mined land often has low land use potential because the replaced soils have low fertility and low permeability due to compaction, resulting in poor plant production. Acidic soil on rehabilitated land is a common problem associated with coal mines. These soils are typically 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 common problem affecting plant growth. 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, usable soil (A-horizon layer – known as topsoil, and non-plinthic B1 horizon material) is stripped ahead of mining by means of heavy equipment, increasing soil compaction (Chamber of Mines of South Africa, 2007). Thereafter, the stripped soil is transported to available mined areas for direct placement (which is preferred), or it is moved to a storage area where it is stockpiled for some time (Strohmayer, 1999). Soil quality will degenerate the longer it is stockpiled, and its availability post-mining will depend on the height and slope of the stockpile. Following mining cessation, soil that was stockpiled is moved back to the mined area where it is placed over reshaped spoils (Strohmayer, 1999). Subsoil (the soil layer beneath the topsoil) lacks the microbial communities and organic matter and fertility necessary to sustain plants, and if mixed with topsoil by overstripping, creates problems for the establishment of vegetation when rehabilitation commences (Visser et al., 1984). These problems include an increase in harmful metals, greater compaction, and low organic matter content (Sheoran et al., 2010). As soil compaction increases by spreading the soil with unsuitable heavy equipment, the ability of plant roots to penetrate soil is limited and ceases entirely at a penetration resistance of approximately 2.5 megapascals (MPa; Taylor, 1971).

3. Environmental impact of coal mining

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 the environmental 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 rate 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 decisions of multiple countries to shut down many of their coal mines, yet South Africa's energy is still largely derived from coal (approximately 86%; Carter and Gulati, 2014). Following the cessation of mining operations, most countries require the mined land to be rehabilitated or reclaimed according to their legislation. In South Africa, the Mineral and Petroleum Resources Development Act (MPRDA), the National Water Act (NWA), and the National Environmental Management Act (NEMA) are key pieces of legislation regulating mine rehabilitation and closure.

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 forms that facilitate agricultural productivity. 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).

When monitoring the success and sustainability of a reclamation plan, physical factors (soil compaction, water quality and quantity) and vegetation (plant biomass and 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). This 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 include topsoil / usable soil management, managing overburden and soil, and landscape design (Krutka and Jingfeng, 2013). Topsoil / usable soil management is critical because it is a valuable and scarce resource. Revegetation of disturbed land is essential and will not be successful without adequate usable soil 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 provides feed for grazing animals (Limpitlaw et al., 1997).

5. Soil fertility and plant growth

To improve soil fertility, organic matter plays an important 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 in resisting soil degradation as well as alleviating soil compaction, therefore decreasing soil strength (Carter, 2002). Organic matter is mostly composed of decomposing plants and animals and includes animal excretions. Animal manure has been extensively used by farmers to improve soil fertility (McAndrews et al., 2006), with an added effect of alleviating soil compaction caused by heavy machinery and cattle (Mosaddeghi et al., 2000; Guo et al., 2016). It has been reported that the addition of green leaf manure increased water infiltration rate 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, Mg, potassium (K) and sodium (Na), enhance and regulate important metabolic processes within vegetation and increase plant growth (Hutton et al., 1967).

Nitrogen found in manure occurs mostly as organic ammonium (NH_4+) that needs to be mineralised to its inorganic form (nitrate – NO_3) for plant uptake (Pettygrove 2009; Pratt and Castellanos 1981). For plants to incorporate N into their tissues, ammonium must 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 which would depress microbial ammonium oxidation (Mengel and Kirby 1987). Low soil pH and deficient organic matter are common features of post-mining soils. Soil pH influences the fertility of the soil, where it determines the availability of plant nutrients and affects plant growth (Jones, 2012). Soil pH determines the cation exchange capacity (CEC) of soil, which could cause soil to be deficient in Ca and Mg (Fertilizer Industry Federation of Australia, 2006).

Cation exchange capacity is the ability of soil to retain cations, particularly Ca, Mg, K and 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 require 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 takes years to build up and have a beneficial effect (Ketterings et al., 2007; Brown and Lemon, 2016).

If manure is not incorporated into the soil, most N found in ammonia 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 within 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).

6. Soil invertebrates

Soil fauna greatly affect 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 relating to soil remediation has focussed on ants, termites, and earthworms. Many species of earthworms, termites, ants and dung beetles have comparable burrowing activities, creating subterranean tunnels that facilitate soil mixing, alleviate soil compaction and modify 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 later 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, and 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 they are lowered to levels that are 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 reclaim 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 (Hayakawa and Kamashita, 1990; Leuthold et al., 2004); however, there is no known research available investigating their ability to decontaminate soil. 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 soils of surrounding farmlands (Majer et al., 1987). Subterranean termite and ant nests greatly enhance the physical and chemical soil properties, particularly by increasing water infiltration rate and soil aeration (Martius, 1990).

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 brood balls 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., 2008; Farias and Hernandez, 2017). In agro-ecosystems, 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 (50-850 m, with large rollers travelling the least distance and large diurnal tunnellers dispersing the furthest) and are therefore sensitive to environmental changes like habitat loss, and have been found to function as important bioindicators of environmental degradation (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, because the dung beetles had to be compatible with Australian weather and soil types, had to have a low risk of predation, would not themselves become pests, and would remove most of the dung in 48 hours (Bornemissza, 1976). The programme 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 larger species, may remove more dung compared to rollers and dwellers (Salah, 2014). In the absence of nocturnal large-bodied tunnellers, dung removal can decrease by 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 a high functional redundancy – the theory that species performing the same functional role in an ecosystem may substitute for another – is an important characteristic of a thriving ecosystem. Furthermore, dung beetle abundance influences dung removal rates, where a 29% reduction in dung removal was seen when dung beetle abundance decreased by 33% (Manning and Cutler, 2018).

Table 1 shows the economic value of dung beetles in the United States of America as calculated by Losey and Vaughn (2006). About one-third of the dung produced by cattle in the USA is processed by dung beetles, with the remaining dung being either treated or deposited onto artificial surfaces where cattle are kept. Table 1 highlights the contribution of dung beetles as important 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 because of accelerated burial of livestock faeces by dung beetles

Billions of US dollars

Cause of loss	Estimated losses: No dung beetle activity	Estimated losses: Current dung beetle activity	Losses averted
Forage fouling	0.65	0.53	0.12
Nitrogen volatilisation	0.31	0.25	0.06
Parasitism	0.98	0.91	0.07
Pest flies	1.83	1.70	0.13
Total losses averted			0.38

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 (including nutrient content, soil fertility, soil pH, moisture content, etc.), it was determined that dung beetles could accomplish this due to their tunnelling and dung burial activities.

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

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

This chapter has been prepared according to the guidelines for the Journal of Applied Soil Ecology and has been accepted with changes which are currently being addressed.

Jessica Badenhorst[a], Jackie Dabrowski[a,b], Clarke H. Scholtz[a], Wayne F. Truter[a]

[a] *Department of Zoology and Entomology, University of Pretoria, Hatfield, Pretoria, Gauteng, 0002, South Africa*

[b] *Sustainability Research Unit, Nelson Mandela University, Private Bag x6531, George 6530, South Africa*

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. Poor soil conditions contribute to the decline in plant productivity. Mining activities permanently alter the land's topography and can drastically impair land capability.

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

Degraded soils found on mining sites experience many problems relating to the establishment and maintenance of herbaceous plants. These include the loss of soil horizons and soil structure, poor soil fertility, reduced soil pH, extreme leaching, decreased nutrient availability for plants, decreased cation exchange capacity, increased soil erosion, and increased compaction (Mensah, 2015). Usable soil placed on reshaped areas after mining is used to provide better conditions for vegetation establishment. Improving the condition of replaced soil by reducing nitrogen-losses, and increasing soil nutrients and microbial populations, is central to an effective reclamation plan (Sheoran et al., 2010). Vegetation establishment, following soil improvement, contributes greatly to restoration of soil hydraulic properties as these develop 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 its protein quantity, which is derived from the plants nitrogen content. Herbage feed-value becomes increasingly important when cattle are used to graze rehabilitated pastures. The application of fertilisers to improve vegetation quality, especially the application of nitrogen to soils to improve the crude protein content of pasture grasses, is an expensive practice. Cattle manure generally contains five essential nutrients for plant growth and is abundant in organic matter (Onwudike, 2010). Phosphorus is involved in root development and energy storage; potassium promotes plant metabolism; calcium has a major role in cell integrity and membrane permeability, and magnesium 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 that 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 that complete their entire lifecycle inside a dung pat), and paracoprids (dung beetles that 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 tunnellers (Davis et al., 2008).

The ecosystem services provided by dung beetles have been extensively reviewed by Nichols et al. (2008), who state that dung beetles play an important role in parasite suppression, secondary seed dispersal, nutrient cycling and plant growth enhancement.

Dung beetles enhance air permeability in soil, facilitating the transfer of nutrients in dung to the soil, leading to an increase in biomass and nutritive value of the vegetation (Mittal, 1993; Bang et al., 2005). High-diversity assemblages of dung beetles are likely to improve functionality in a range of ecosystems, by contributing to ecosystem services (Manning et al., 2016). Tunnelling by dung beetles can improve various physical and hydraulic aspects of soil by increasing water infiltration rates, and leading to higher soil moisture content and reducing soil bulk density. Improved water infiltration rates result in reduced surface water runoff (Brown et al., 2010), which ultimately reduces rates of soil erosion. Slade et al. (2015) showed that the presence of dung beetles promoted bacterial transfer across the soil-dung interface, suggesting that dung beetles may accelerate the dung decomposition processes. Dung beetles have been mass-reared and introduced into Australia, New Zealand, the United States of America and elsewhere, to assist with dung burial, the control of pests, and to facilitate pasture improvement in agro-ecosystems (Edwards, 2007; Bertone, 2005; Dymock, 1993; Bornemissza, 1976).

Most studies of the activities of dung beetles were undertaken to better understand their role and benefits in agro-ecosystems (Beynon et al., 2012, Farias and Hernández, 2017). No studies could be found that investigated the ability of dung beetles to maintain their activities, and associated benefits, in systems with extreme soil degradation, such as on reclaimed land on coal mines. Soil bulk density measurements on rehabilitated land on 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 can maintain their activities under these conditions, then dung beetles present a potentially valuable resource to be considered for improving the condition of soils on reclaimed mined land, to further increase the range of viable post-mining land-use options on these areas. 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), nutrient content (mg.kg^{-1}), 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 (%) and (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;
- Greater soil nutrient content, pH and cation exchange capacity;
- Increased plant biomass and protein content; and
- Effects lasting at least 6 months.

2. Materials and methods

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. Breeding of 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 inserted 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 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.

2.3 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.s.l. (S25.752295, E28.252754). A total of 12 plots were constructed with a brick retaining wall, each plot measuring 1 x 1 x 1 m built upon the soil surface. 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, and the mean annual precipitation was 697 mm. All plots were exposed to the same ambient environmental conditions.

2.4 Preparation of experimental plots

Each of the 12 plots was covered with a 5 m^2 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 (Fig. 1). This was to prevent the movement of beetles in and out of the plots. The experiment comprised of three treatments, each with four replicate plots: dung + beetles (D+B); dung only (D); and control (X - no dung, no beetles). The first application of dung and beetles was made in April 2015 and the second 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.

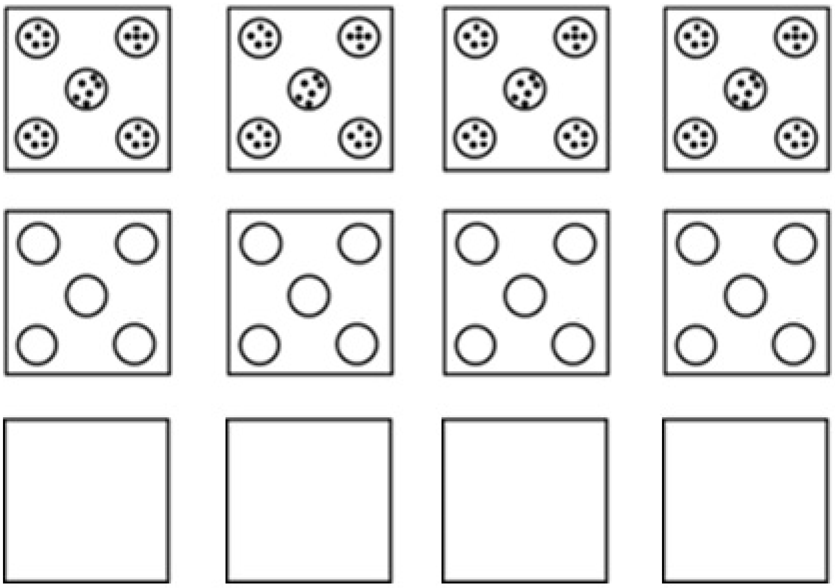

Figure 2. The first treatment contained five 1 kg dung pats and 100 dung beetles per plot. The second treatment contained five 1 kg dung pats on four plots. The third treatment contained no dung and no dung beetles on four plots. Size categories for paracoprid dung beetles were: small (6-9 mm), medium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

For the dung-and-dung beetles (D+B) treatment, each replicate plot received a total of 100 dung beetles and five fresh cattle dung pats (1 kg each) placed on the soil surface (Fig. 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 as shown in Fig. 1 and the second application being placed to fill the areas that had not been exposed to dung. Dung and dung 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 could roam freely within the plots where they were placed.

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

Measurements of effects were made one month after the addition of beetles and dung to the respective plots in May 2015, and six months later (September 2015). Beetles and dung were applied for a second time to the respective treatment plots in May 2016, and measurements again taken one month and six months later (in September 2016). The six-month sampling interval was selected to determine the longevity of effects.

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 that were then oven-dried at 65^{o}C for 48 hours, and weighed. The crude protein content (%) of the dried herbaceous material was analysed by Nvirotek (NviroTek Laboratoriums (Pty) Ltd) to determine herbage feed-value of the grasses.

2.6 Soil properties

A 100 g sample, comprising five randomly-collected subsamples, was collected from the top 20 cm of soil in each experimental plot, and was analysed by Nvirotek (NviroTek Laboratoriums (Pty) Ltd). The following analyses were conducted:

- Soil pH (1 to 2.5 ratio extraction with 1.0 M KCl - determined with pH meter);
- 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);
    - Sulphur (S - 1 to 10 ratio extraction with 1.0 M ammonium acetate, determined by inductively coupled plasma analysis); and
- Cation exchange capacity (CEC - saturation by 1.0 M ammonium acetate, washed by ethanol and extracted with 1.0 M KCl – determined colourimetrically).

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.

2.7 Soil strength

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

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.

2.9 Data analysis

Using Statistica 13 (StatSoft, Dell Inc., ver. 10), the data were analysed to ensure the assumptions for parametric tests were met. Data that were not normally distributed were transformed using logarithmic transformations. One-way analysis of variance (ANOVA) and *post-hoc* Tukey's HSD (honest significant difference) tests were used to compare treatment means and to determine whether herbaceous plant and soil parameters differed significantly between treatments at each independent sampling period. To determine temporal trends in the data and within treatment differences, repeated measures ANOVA was undertaken. Statistical significance was assumed at p <0.05. A Principal Components Analysis (PCA) was used to detect groupings using the measured soil and herbaceous plant parameters to assess possible relationships among the variables.

3. Results

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 control plots (X) for measurements taken six months after the first application of beetles and dung. The same trend was not observed six months after the second application (Fig. 3a). 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 applications ($p > 0.05$).

After the data were consolidated, plots containing dung beetles had a significantly higher plant biomass yield for both sampling intervals (Fig. 4a). Six months after the dung beetle and dung applications, plant biomass yield was somewhat higher compared to one month after the applications.

Herbaceous plant protein content was not significantly different among any of the treatments for measurements taken, although certain trends in the data were seen (Fig. 3b). Six months after the first application of dung and beetles, all treatments increased in protein content.

A clearer influence on the protein content in the plants can be seen after the data were consolidated and analysed with a repeated measures ANOVA (Fig.4b). The activity of dung beetles increased the protein content in the plants, but it did not increase significantly. Furthermore, there is no difference between the D and X treatments when comparing the sampling intervals.

3.2 Water infiltration

Water infiltration was significantly higher for D+B treatments when measurements were taken immediately after the application of beetles and dung, 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 (Fig. 3c). The average water infiltration rate for D+B treatment plots reduced after the second application was made, decreasing from 152.97 mm.h^{-1} ± 5.67 to 96.83 mm.h^{-1} ± 6.10.

Following a repeated measures ANOVA, water infiltration rate was also significantly greater for plots containing dung beetles as opposed to plots with only dung and plots with no dung or beetles (Fig. 4c). Water infiltration rates were similar between the two sampling intervals. Detailed one-way ANOVA and repeated measured ANOVA results for Section 3.1 and 3.2 are indicated in the Appendix, Table A and Table B, respectively.

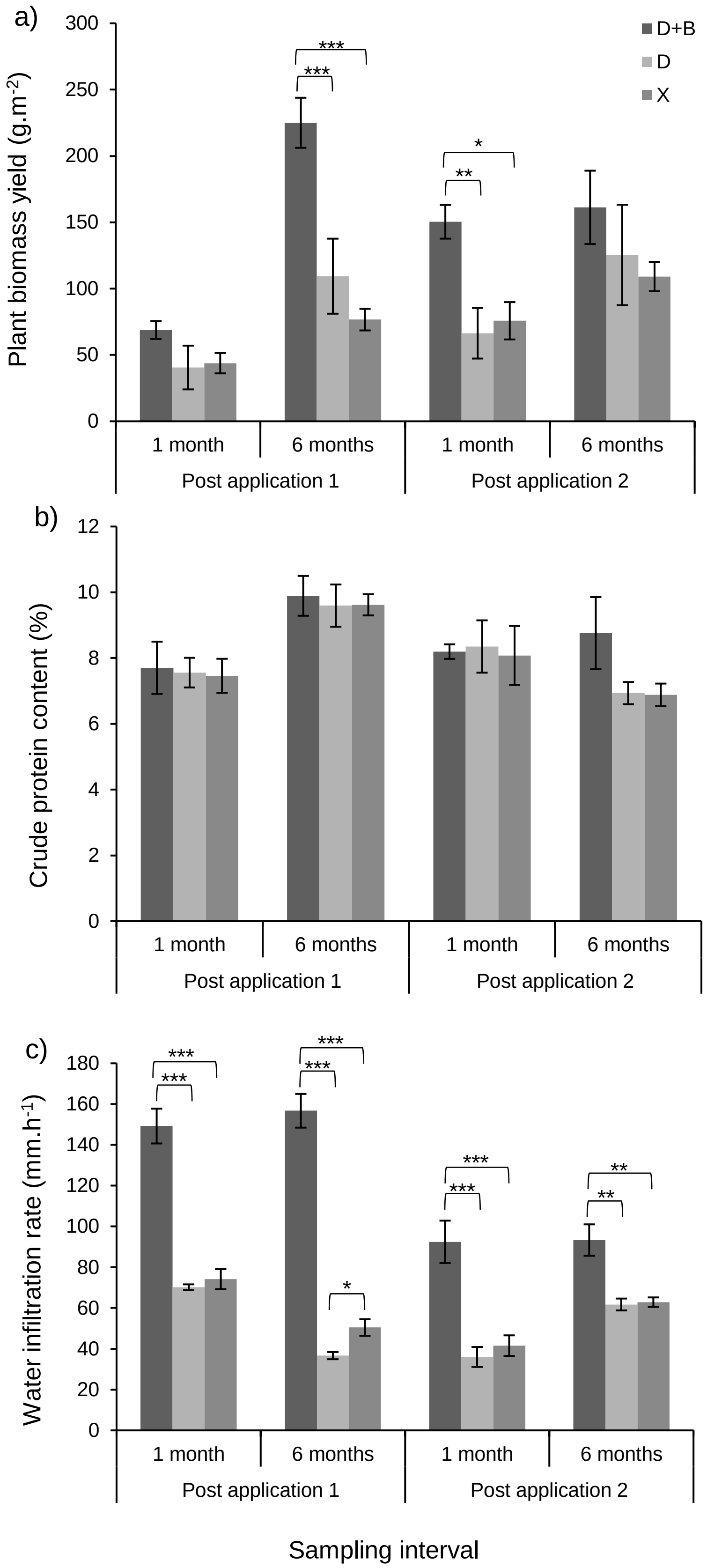

Figure 3. Mean ± SE values for (a) herbaceous plant biomass yield (g.m^{-2}), (b) plant crude protein content (%), and (c) water infiltration rate (mm.h^{-1}) measurements taken one month and six months post the first application of dung and beetles, and one month and six months post the second application. Treatments were: dung-and-dung beetles (D+B; n= 4); dung only (D; n= 4); and control / no dung, no beetles (X; n= 4). [* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$].

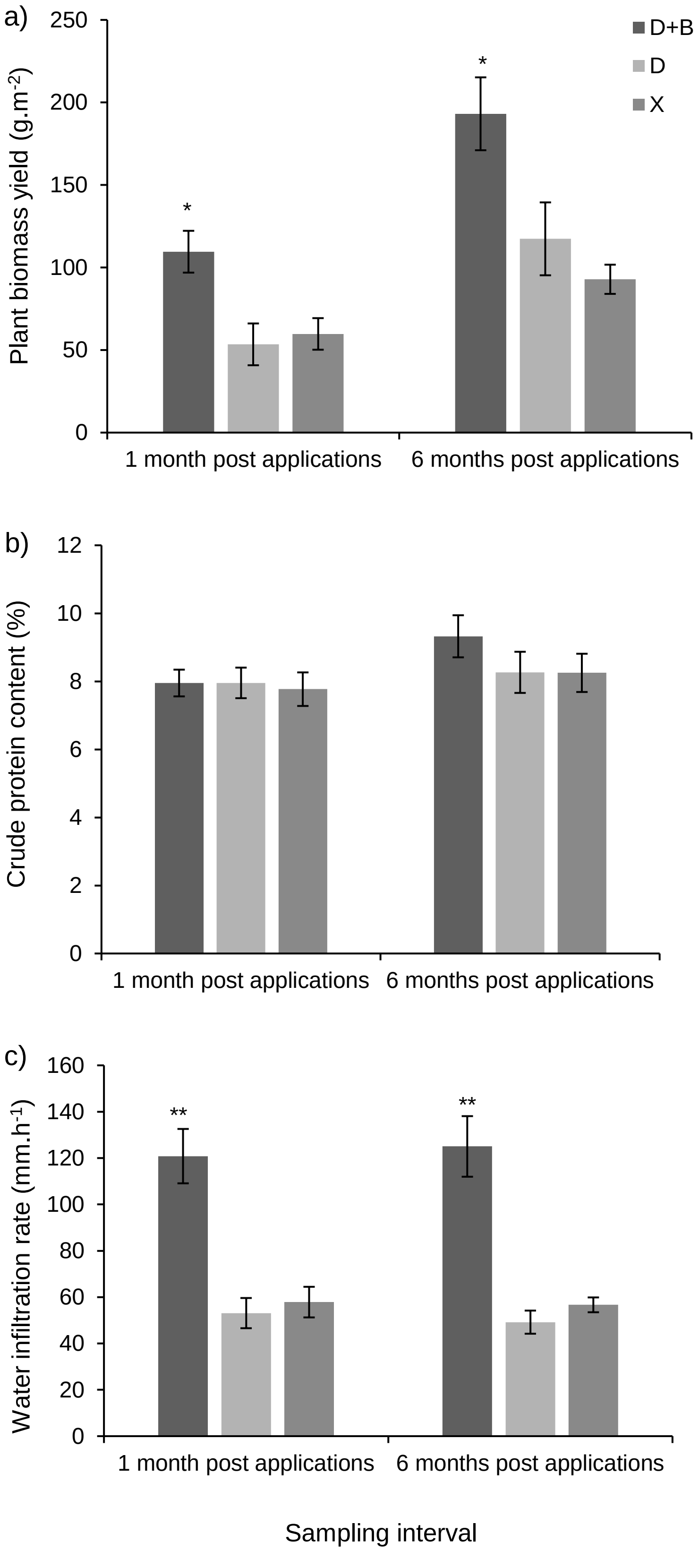

Figure 4. Consolidated mean ± SE values for (a) herbaceous plant biomass yield (g.m^{-2}), (b) plant crude protein content (%), and (c) water infiltration rate (mm.h^{-1}) measurements taken one month and six months post the applications of dung and beetles. Treatments were: dung-and-dung beetles (D+B; n= 4); dung only (D; n= 4); and control / no dung, no beetles (X; n= 4). [* $p \leq 0.05$; ** $p \leq 0.01$].

3.3 Soil strength

No statistical significance was seen among any of the treatments for measurements taken one month after the first application (Fig. 5a). 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 application, with D+B treatments having a lower penetration resistance when compared to D and X treatments (Fig. 5b). 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 (Fig. 5c). In comparison, the measurements taken six months after the second application increased linearly in penetration resistance as a greater depth was reached (Fig. 5d). D+B treatments had a significantly lower penetration resistance at a depth of 2-19 cm when compared to D and X treatments.

After the data were consolidated, plots with dung beetles had significantly lower penetration resistance between 6 and 20 cm one month after the applications took place (Fig. 6a). Six months post beetle and dung applications, penetration resistance was significantly reduced for measurements taken at all depths for consolidated data (Fig. 6b). One-way ANOVA and repeated measures ANOVA F- and *p* values are indicated in the Appendix, Table C and Table D, respectively.

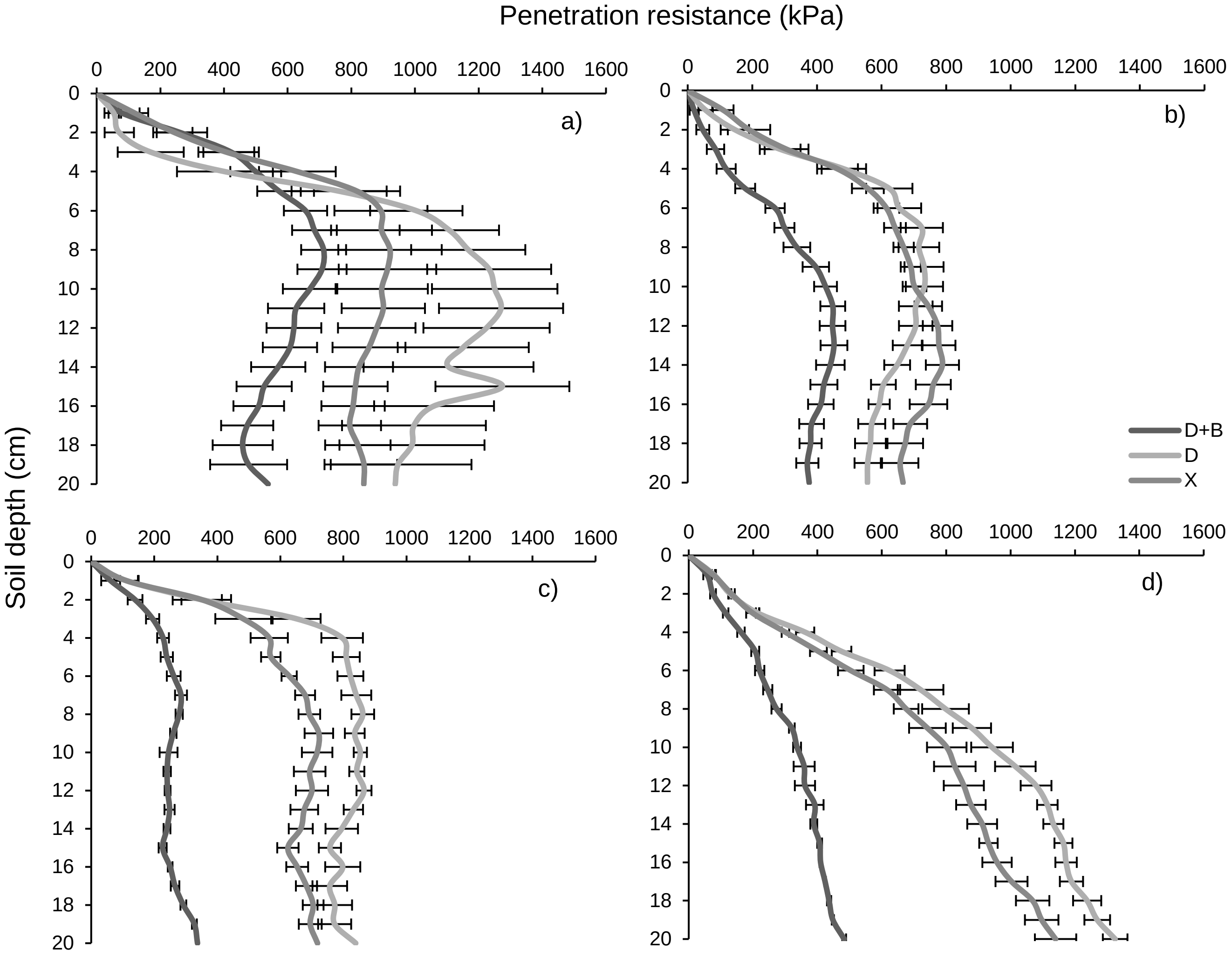

Figure 5. Mean ± SE values for soil penetration resistance (kPa) measurements taken (a) one month and (b) six months post the first application of dung and beetles, and (c) one month and (d) six months post the second application. Treatments were: dung-and-dung beetles (D+B; n= 4); dung only (D; n= 4); and control / no dung, no beetles (X; n= 4).

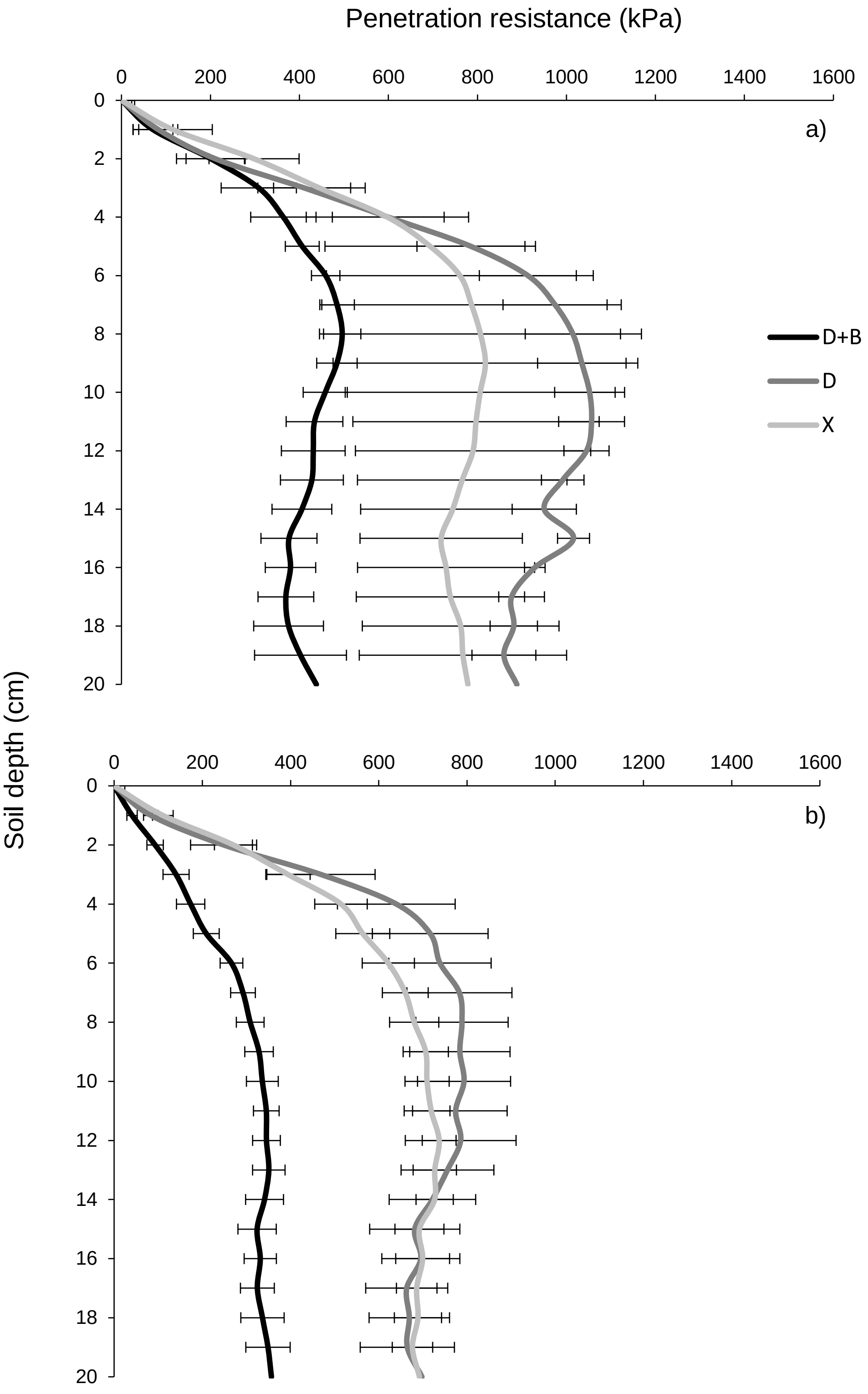

Figure 6. Consolidated mean ± SE values for soil penetration resistance (kPa) measurements taken (a) one month and (b) six months post the applications of dung and beetles. Treatments were: dung-and-dung beetles (D+B; n= 4); dung only (D; n= 4); and control / no dung, no beetles (X; n= 4).

3.4 Soil properties

Post-hoc Tukey's HSD tests indicated that there were significant differences for K, Ca and S levels in the soil analysed one month after the first application (Table 2). 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).

According to repeated measures ANOVA results, no significant differences were observed one month after the treatment applications (Table 3). Six months after the treatment applications, K, Ca, Mg and CEC were determined to be significantly greater for plots where dung beetles were active (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
S (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

* − $p \le 0.05$

** − $p \le 0.01$

ND – not determined

Table 3. Consolidated mean ± SE of soil parameters measured from three treatments: dung-and-dung beetles (D+B), dung only (D) and control / no dung, no beetles (X). Measurements were taken one month and six months after the applications.

Soil properties	Post applications					
	One month			Six months		
	D+B	D	X	D+B	D	X
pH	5.68 ± 0.04	5.84 ± 0.18	5.64 ± 0.08	6.19 ± 0.15	5.75 ± 0.15	5.85 ± 0.18
P (mg.kg^{-1})	13.90 ± 3.07	8.90 ± 3.64	7.89 ± 4.03	21.32 ± 4.59	10.95 ± 2.32	8.03 ± 2.36
K (mg.kg^{-1})	239.87 ± 26.36	166.89 ± 51.99	94.99 ± 27.33	**230.05 ± 38.01***	138.26 ± 14.18	113.13 ± 14.18
Ca (mg.kg^{-1})	374.17 ± 38.55	331.36 ± 71.98	281.13 ± 29.11	**447.90 ± 40.20***	296.43 ± 27.37	309.02 ± 21.03
Na (mg.kg^{-1})	20.51 ± 3.03	18.65 ± 2.39	16.99 ± 1.60	26.33 ± 3.86	24.78 ± 3.92	25.85 ± 5.15
Mg (mg.kg^{-1})	133.54 ± 26.75	115.12 ± 25.23	74.54± 8.03	**172.06 ± 16.36***	106.26 ± 12.58	89.90 ± 7.49
S (mg.kg^{-1})	17.34 ± 3.42	11.84 ± 1.53	12.13 ± 1.08	23.97 ± 2.14	34.59 ± 9.44	27.28 ± 3.52
CEC [cmol(+)/kg]	2.34 ± 0.29	1.79 ± 0.42	1.67 ± 0.26	**4.49 ± 0.17***	3.19 ± 0.29	3.39 ± 0.33

* − $p \leq 0.05$

3.5 Principal component analysis

The D+B treatments were clearly separated from the D and X treatments for measurements taken one month after applications, as well as six months later. For Principal Component 1 (PC1), measurements of Mg had a high factor loading for each analysis with a correlation between 0.90 and 0.98 (Appendix, Table E). After the first application, measurements of 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 (Fig. 7a). Similarly, measurements of Mg and Ca, as well as K, had the highest factor loadings for measurements taken six months after the first 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 (Fig. 7b).

Although PC1 explained most of the variation seen for all PCA results, PC2 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 (Fig. 7c). Separation in treatments seen for measurements taken after the second application was mainly due to PC2 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 61.89% of the total variation.

Six months after the second application (Fig. 7d), the highest factor loadings for PC1 were seen for measurements of Mg (0.93) and crude protein content of the herbaceous plants (0.85).

Following the consolidation of the data, the D+B treatments measured one month after the applications indicate that the data were mainly distributed along PC1. For PC1, measurements of K had a high factor loading one month- and six months after applications with a correlation between 0.92 and 0.94, respectively. One month after application, measurements of Ca and Mg had the same factor loading for PC1 with correlations of 0.79 each. Principal Component 1 and 2 combined accounted for 62.04% of the total variation (Fig. 8a).

Similarly, measurements of Mg, Ca, and K had the highest factor loadings for measurements taken six months after the applications with a correlation of 0.97, 0.94 and 0.92 respectively. Unlike the one month sampling interval, water infiltration rate had a high factor loading of 0.86. The separation of the three treatments is seen along the PC1 axis, with D+B treatments being distinct from D and X treatments (Fig. 8b). Principal component 1 and 2 combined accounted for 70.75% of the total variation. Factor loadings for principal components 1 and 2 for the separate sampling intervals and the consolidated sampling intervals are indicated in the Appendix, Table E and Table F, respectively.

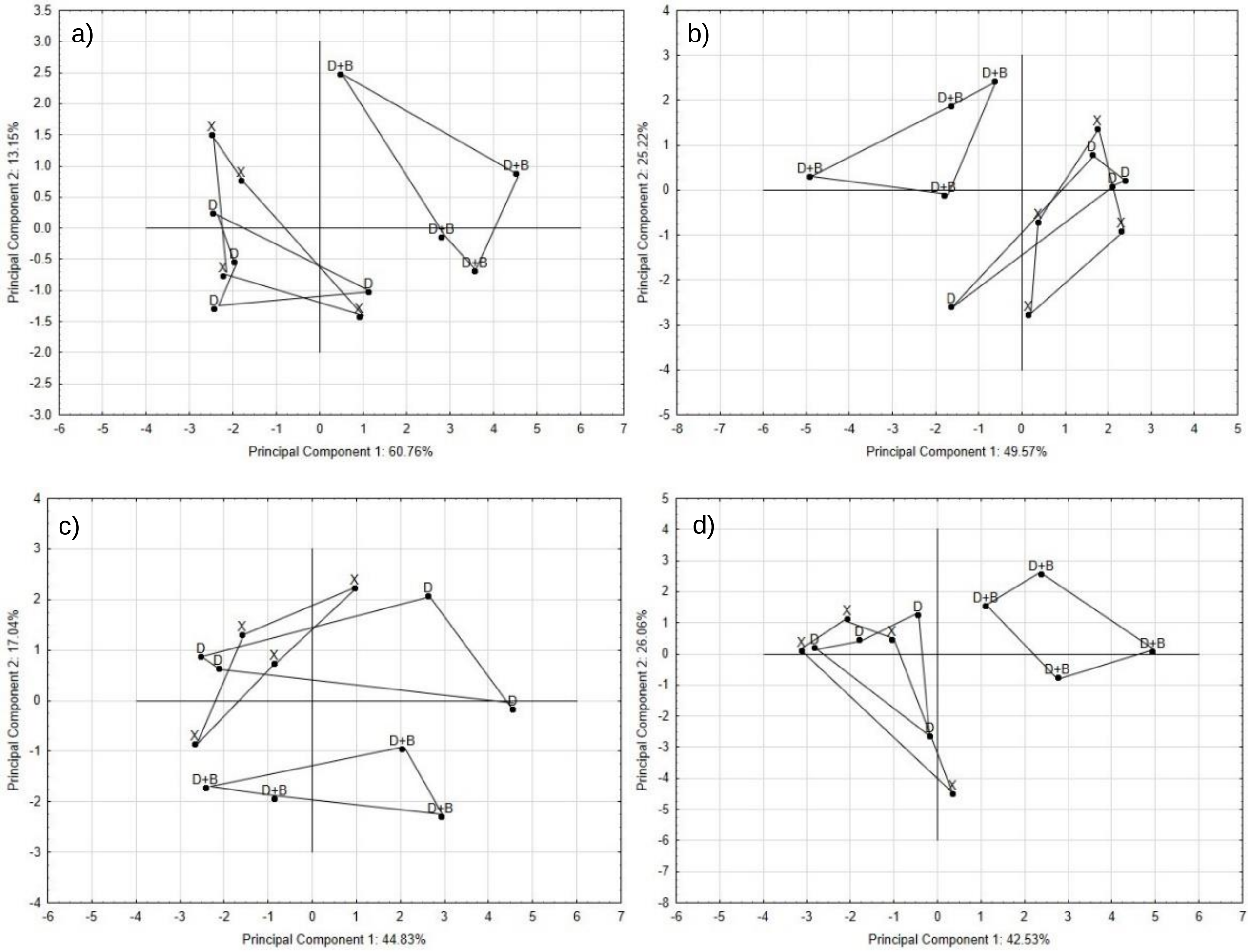

Figure 7. Principal component analyses (PCAs) of 11 variables for: dung-and-dung beetle (D+B); dung only (D); and control / no dung, no beetles (X) treatments. Measurements were taken (a) one month and (b) six months post the first application of dung and beetles, and (c) one month and (d) six months post the second application.

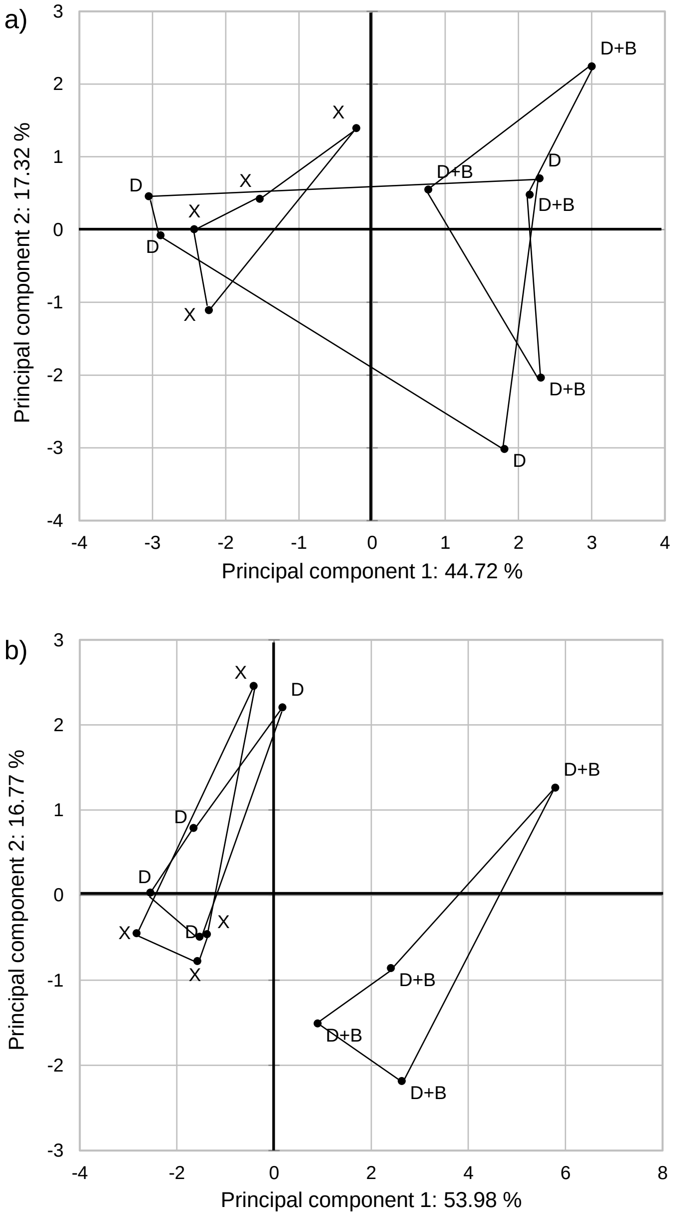

Figure 8. Consolidated principal component analyses (PCAs) of 11 variables for: dung-and-dung beetle (D+B); dung only (D); and control / no dung, no beetles (X) treatments. Measurements were taken (a) one month and (b) six months post the applications of dung and beetles.

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 reclaimed 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 Chamber of Mines of South Africa (2007) recommend a minimum topsoil depth of 150 – 250 mm for a wilderness land use, 250 – 600 mm for a grazing land use and 600+ mm for an arable land capability. Water infiltration rate and permeability was found to be 129% deeper in treatments with active dung beetles, stressing the importance of applying a thicker soil layer of soil to post-mining lands where dung beetles are naturally-occurring (Richardson and Richardson, 2000). High water infiltration rates may be problematic in post-mining soils as the water will seep through to the underlying backfilled spoils, increasing recharge and the volume of excess mine water to be managed at closure.

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 also 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, where 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).

In the current 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, where 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). The percentage N content in vegetation has been found to increase significantly when dung beetles were active on a site (Bertone et al., 2006). Six months after the first application of beetles and dung, all plots had an increase in crude protein, most likely due to various environmental conditions such as temperature and humidity. Temperature and humidity are known to affect a plant’s respiration, assimilation, photosynthesis and metabolism.

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 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). Magnesium is the coordinating metal ion in the chlorophyll molecule, which controls the efficiency of photosynthesis by stabilising thylakoid stacking – the locus for photosynthetic light-reactions (Fork, 1986). 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.

Following the consolidation of the data, clearer patterns emerged as opposed to analysing the data per sampling interval. Water infiltration rate and plant biomass yield was still significantly increased where dung beetles were active, but soil nutrient content differed. According to the repeated measure ANOVA, no soil nutrient content was significantly altered one month after the applications of beetles and dung, yet K, Ca, Mg, and CEC were significantly greater for D+B treatments six months after the applications took place. It is suspected that microbes assisting in nutrient fixation were mostly inactive when the first measurements took place. Microorganisms are known to be abundant in spring (six months after the applications took place) and tend to be lower in winter (one month after the applications took place), and are also less active in acidic soil (Díaz-Raviña et al., 1993). The results of the current study show that the effects of dung beetle application on post-mining soils may be present for an extended time after the application is made. It is suggested that if the experiment were to be replicated, that the applications of dung and dung beetles be made in the spring, where microorganisms in the soil would be able to optimise the use of nutrients in the dung.

CHAPTER 3: ALLEVIATION OF DEGRADED SOIL CONDITIONS ON RECLAIMED MINED LAND BY DUNG BEETLE ACTIVITY

Jessica Badenhorst[a], Jackie Dabrowski[a,b], Clarke H. Scholtz[a], Wayne F. Truter[a]

[a] *Department of Zoology and Entomology, University of Pretoria, Hatfield, Pretoria, Gauteng, 0002, South Africa*

[b] *Sustainability Research Unit, Nelson Mandela University, Private Bag x6531, George 6530, South Africa*

1. Introduction

Laboratory studies are conducted in a controlled environment. The results of these 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 offer different comparative advantages.

Research frequently investigates the difference between controlled environment experiments and field experiments. Most of these studies obtained 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 fallout from stockpiles may influence dung beetle activity, as was observed by Venter et al. (unpublished). 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 avian species. 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, by enclosing the experimental area.

Organisms are classified into functional groups according to their shared characteristics and how they utilise resources. Diverse functional groups in an ecosystem have been associated with greater long-term stability (Cadotte et al., 2011). Arthropods constitute some of the most important functional groups pertaining to ecosystem functionality, with many arthropods being 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 hydraulic properties (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 as 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), nutrient concentrations (mg.kg^{-1}), 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) 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, but 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, because naturally-occurring dung beetles on rehabilitated mines are theorised to be less abundant.

2. Materials and methods

2.1 Study site

The experiments were conducted at a reclaimed mine located in Emalahleni (Witbank). Underground mining in the area commenced in the 19th 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 closest farms are within a 6-km radius of the mine.

The soil was classified as a sandy loam consisting of 65% sand, 17% silt and 18% clay, according to soil texture analyses done by Nvirotek Laboratoriums (Pty) Ltd.

The study area's climate data were provided by the mine. The area had an average daily temperature of 23 ^{o}C, was at an altitude of 1570 m.a.s.l., and had an average summer rainfall of 114 mm and winter rainfall of 14.5 mm. The experiments took place during extreme drought conditions, affecting plant growth on all plots due to lack of water (Baudoin et al. 2017).

2.2 Preparation of enclosures

Sixteen enclosures of 4 m^2 in size (and approximately 1 m high) were built on the reclaimed mined site (Fig. 9). 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.

Four treatments, each with four replicate plots, were installed on site. The replicate plots were spaced 5 m apart in a randomised-plot experimental design (Fig. 10).

Tunnelling dung beetles were collected from neighbouring farms close to the reclaimed mined land and were divided up equally for each treatment requiring dung beetles. Pit fall traps were used to collect live dung beetles two days before they were introduced to the correct plots. Beetles were kept in breadbins with fresh cattle dung before being added to the designated treatment plots. Tunnelling dung beetles were identified and used for the experiment. These species included *Euoniticellus intermedius, Digitonthophagus gazella, Onitis alexis* and *Copris mesacanthus* (Harold, 1878).

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 only 16 dung pats placed 1 m apart, and the third treatment had no dung or dung beetles, representing the control (X). The fourth treatment (N) contained 16 dung pats placed 1 m apart and was uncovered, allowing for naturally-occurring dung beetles to colonise the dung for a period of 72 hours, after which these replicates were closed off with shade netting enclosures.

Beetles and dung were applied to the respective treatment plots on three separate occasions over a 17-month period, viz. March 2016, February 2017 and April 2017. Plant productivity and soil property measurements were taken one month after each application and repeated after a six-month interval.

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 Chapter 2 section 2.5 – 2.9 for the methods.

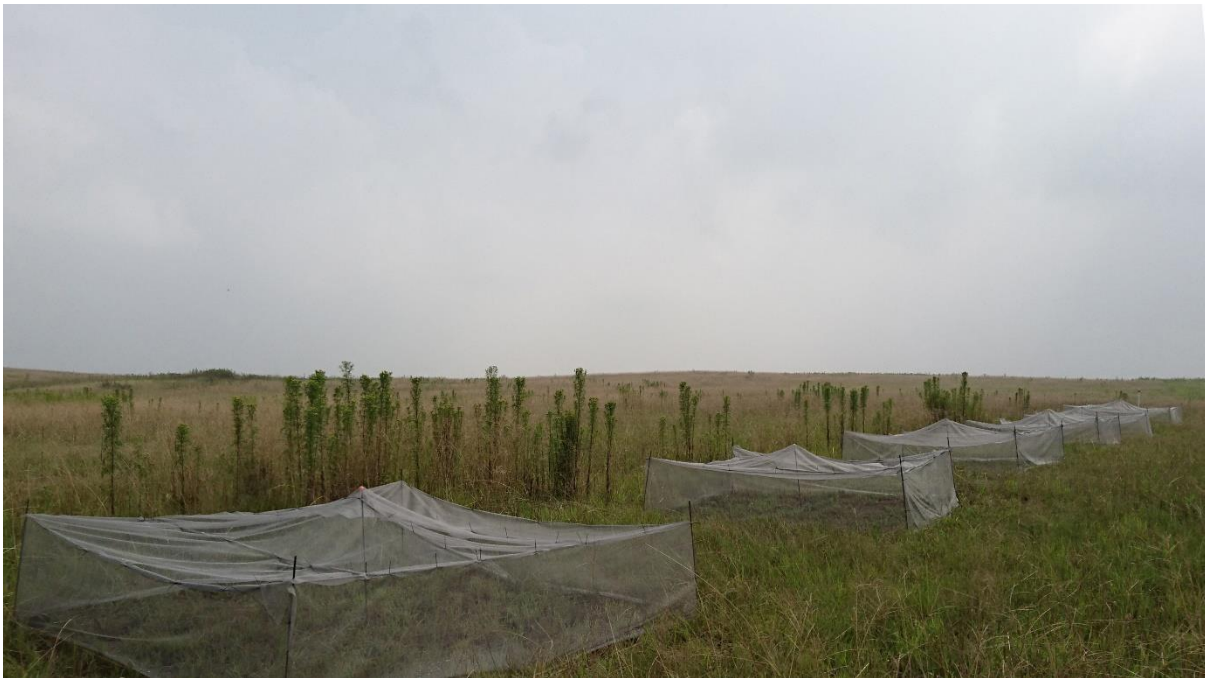

Figure 9. The constructed field plots situated at the reclaimed mined land in Emalahleni. The plots were covered with shade netting and secured.

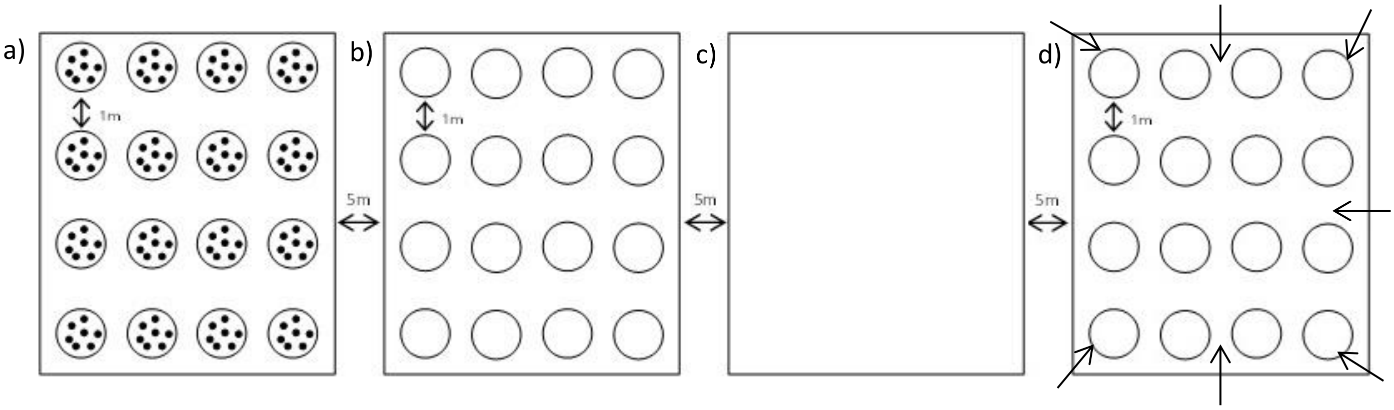

Figure 10. Sample design showing one replicate of each treatment. Four replicates of each treatment were spaced 5 m apart from one another. All treatments were closed off by means of individual 4 x 4 m insect gauze enclosures. The first treatment (a) contained 16 x 1 kg dung pats and dung beetles. The second treatment (b) contained 16 x 1 kg dung pats. The third treatment (c) contained no dung or 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).

3. Results

3.1 General observations

The applied tunnelling dung beetles immediately started burrowing into the soil when they reached the dung pats. Naturally-occurring dung beetles immediately arrived as dung pats were being placed. *Gymnopleurus pumilus* (Reiche, 1847) was the most abundant naturally-occurring roller dung beetle and was seen on the farms where the selected species occurred, but was excluded from the experiment because it is not a tunneller (Fig. 11). No other naturally-occurring dung beetles were observed during the applications of beetles and dung.

Figure 11. Naturally-occurring *Gymnopleurus pumilus* (Reiche, 1847) burrowing into highly compacted soil on the reclaimed mined land in Emalahleni.

On the study site, dung beetle brood balls were recorded at various depths with the deepest brood ball being found at a depth of approximately 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 (Fig. 12). Six months after the beetle and dung applications, there was evidence of further dung decomposition, suggesting that the presence of dung beetles facilitated further dung decomposition without being active (Fig. 13).

Figure 12. 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 13. Six months after the dung-and-dung beetle treatment was applied on reclaimed mined land.

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 (Fig. 14a). 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). 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. After consolidating the data for 1 month after the applications and 6 months after the applications, interesting trends were revealed. Plant biomass yield was significantly greater for D+B treatments (Fig.15a), but was not significant 6 months after the placement of applications.

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 (Fig. 14b; $p > 0.05$). The highest protein content for all treatments was observed six months post application 1. Following the repeated measures ANOVA, one month- and six months after beetle and dung applications, plant protein content was significantly greater for D+B treatments compared to D, X, and N treatments (Fig. 15b), and was not significantly different within treatments.

3.3 Water infiltration rate

Water infiltration rate was higher for most sampling intervals where applied dung beetles were active, except six months post application 1 (Fig. 14c). *Post-hoc* Tukey’s 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. Results from the repeated measures ANOVA are comparable to the one-way ANOVA, with D+B treatments having significantly higher water infiltration rates (Fig.

15a). The difference between the treatments are also more evident when examining the repeated measures ANOVA results. One-way ANOVA and repeated measures ANOVA F- and *p* values for Section 3.2 and 3.3 are indicated in the Appendix, Table G and Table H, respectively.

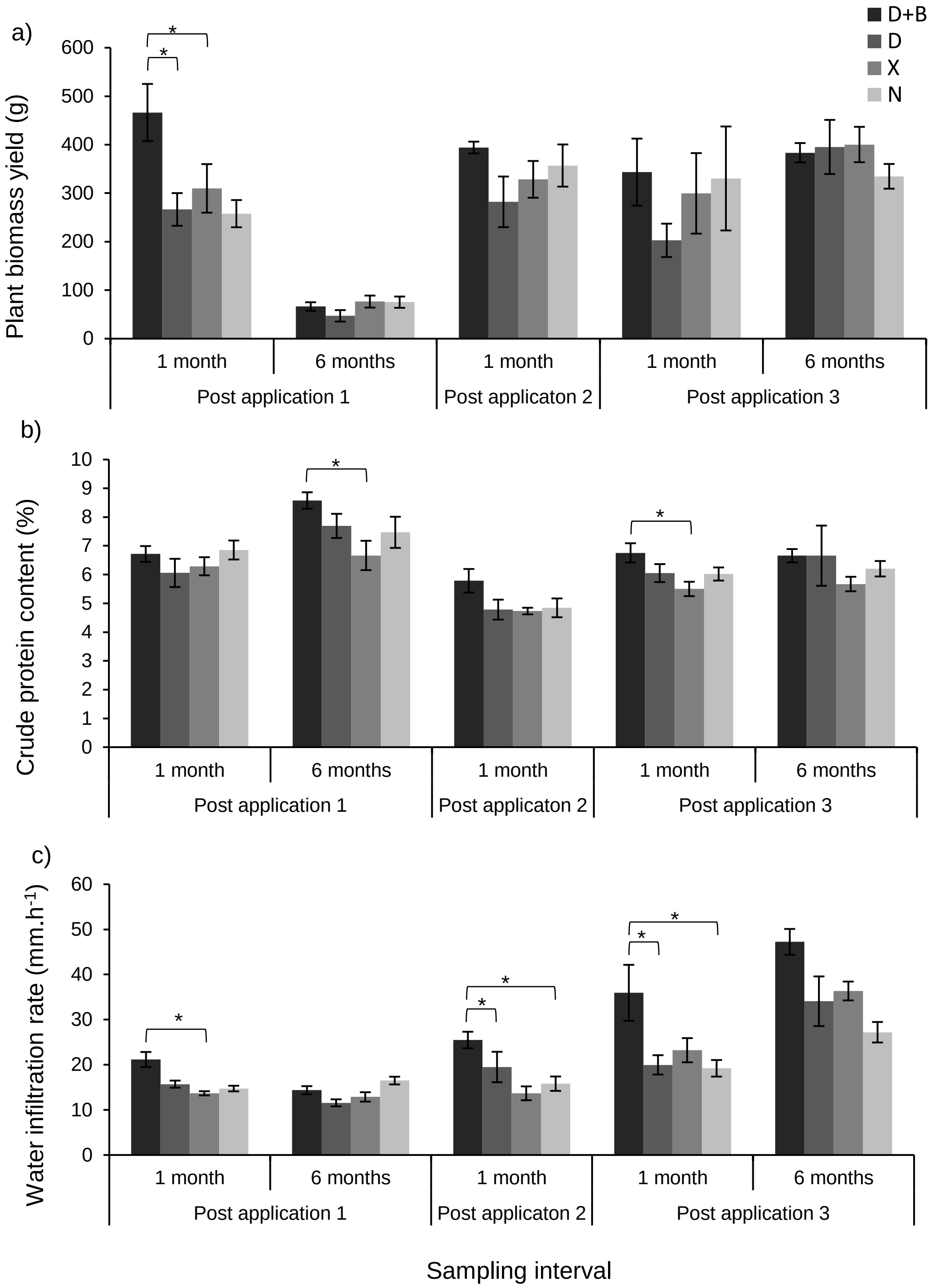

Figure 14. Mean ± SE values for (a) herbaceous plant biomass yield (g), (b) plant crude protein content (%), and (c) water infiltration rate (mm.h^{-1}) measurements taken one month and six months post the first application of dung and beetles, one month post the second application, and one month and six months post the third application. Treatments were: dung-and-dung beetles (D+B; n= 4); dung only (D; n= 4); control / no dung, no beetles (X; n= 4); and naturally-occurring beetles (N; n=4). [* $p \leq 0.05$].

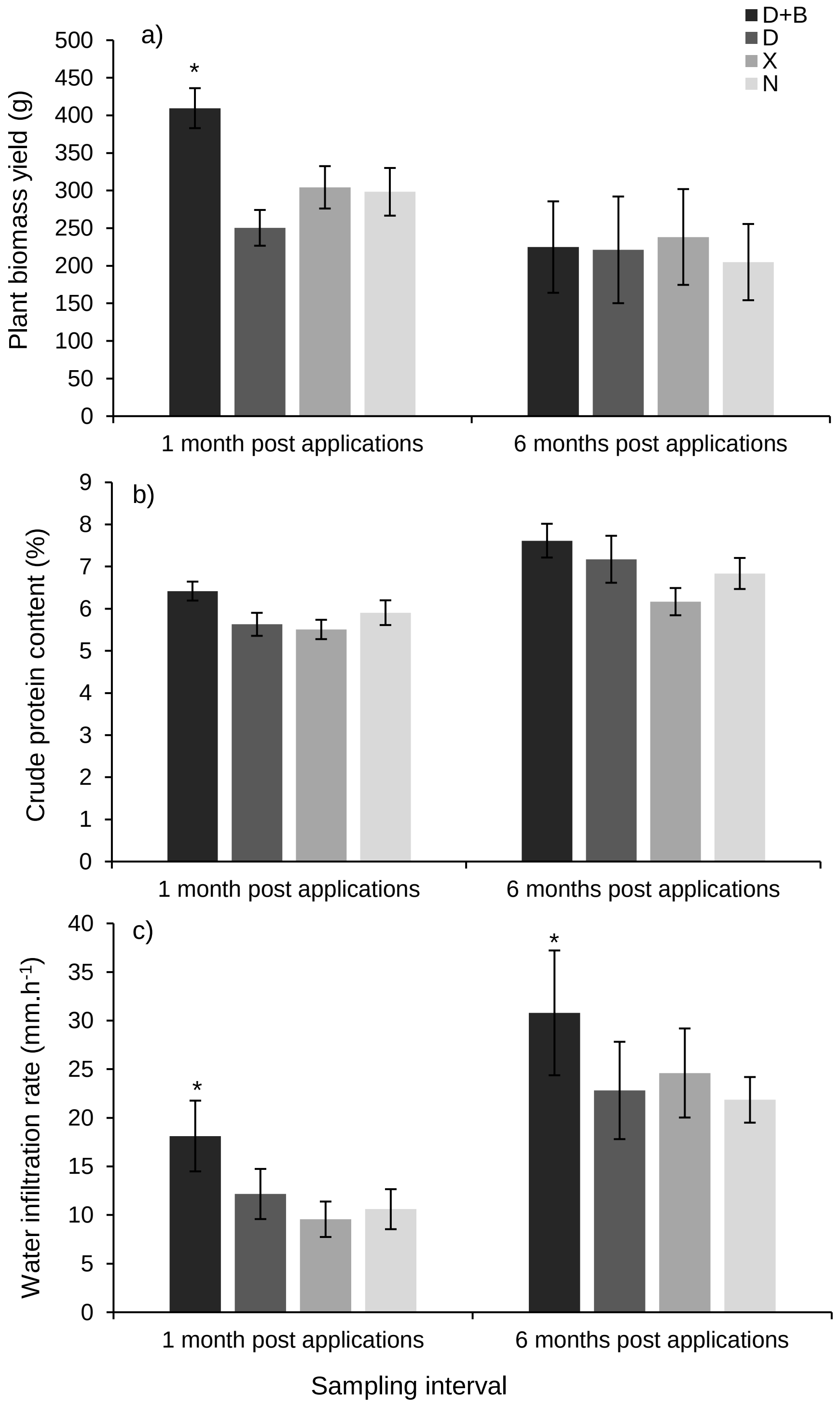

Figure 15. Consolidated mean ± SE values for (a) herbaceous plant biomass yield (g), (b) plant crude protein content (%), and (c) water infiltration rate (mm.h^{-1}) measurements taken one month and six months post the applications of dung and beetles. Treatments were: dung-and-dung beetles (D+B; n= 4); dung only (D; n= 4); control / no dung, no beetles (X; n= 4); and naturally-occurring beetles (N; n=4). [* $p \leq 0.05$].

3.4 Soil strength

Penetration resistance measurements were significantly lower between the soil depths of 3 and 20 cm one month after the first application for D+B treatments ($p < 0.05$; Fig. 16a). 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 (Fig. 16b). At 20 cm, penetration resistance was significantly reduced for D+B treatments when compared to X and N treatments ($p < 0.05$), but not D treatments.

One month post application 2, D+B treatments had significantly lower penetration resistance at 2 – 10 cm compared to D, X and N treatments (Fig. 16c).

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 (Fig. 16d).

A significantly lower penetration resistance was observed at 5 cm for D-treatments six months post application 3 (Fig. 16e). Following repeated measures ANOVA, soil penetration resistance was still significantly different between 1 and 16 cm one month after dung and beetle applications, and at 2-, 3-, 5-, 7-, 9-, and 20 cm for measurements taken six months after applications took place (Fig. 17a and 17b).

One-way ANOVA and repeated measures ANOVA F- and *p* values are indicated in the Appendix, Table I and Table J, respectively.

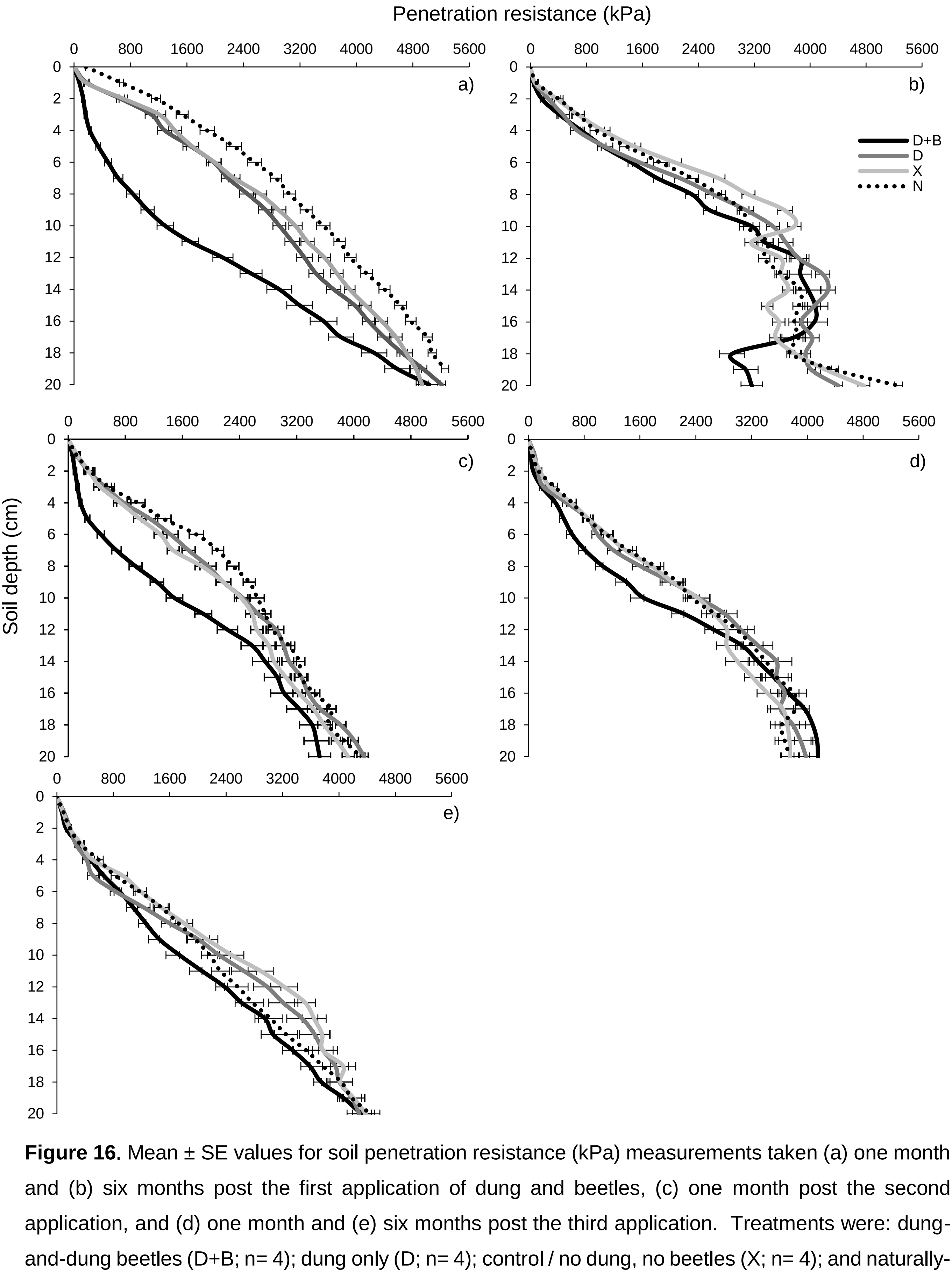

Figure 16. Mean ± SE values for soil penetration resistance (kPa) measurements taken (a) one month and (b) six months post the first application of dung and beetles, (c) one month post the second application, and (d) one month and (e) six months post the third application. Treatments were: dung-and-dung beetles (D+B; n= 4); dung only (D; n= 4); control / no dung, no beetles (X; n= 4); and naturally-occurring beetles (N; n=4).

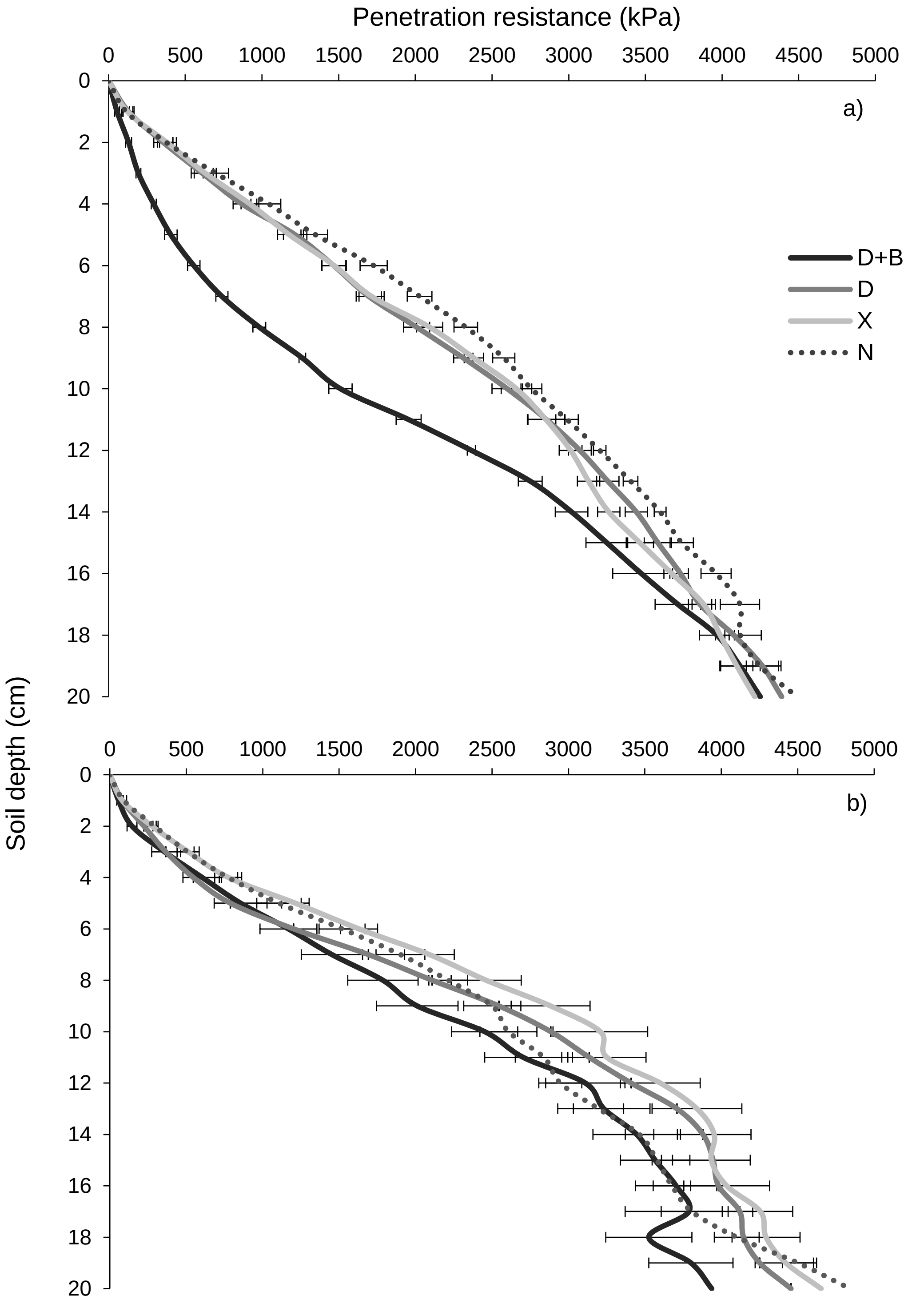

Figure 17. Consolidated mean ± SE values for soil penetration resistance (kPa) measurements taken (a) one month and (b) six months post the applications of dung and beetles. Treatments were: dung-and-dung beetles (D+B; n= 4); dung only (D; n= 4); control / no dung, no beetles (X; n= 4); and naturally-occurring beetles (N; n=4).

3.5 Soil properties

All D+B treatments for all sampling intervals had higher Mg content, but this was only significantly higher one month post application 1, and one and six months post application 3 (Table 4). Sulphur, Ca and P were not significantly different for any treatment during all sampling intervals. Six months post application 1, CEC was significantly higher for X treatments, after which it was not significantly higher for any other sampling interval. The D+B treatments had higher pH compared to D, X and N treatments for most sampling intervals, except one month post application 2. One month and six months post application 3, D+B treatments had significantly higher pH, K, Na and Mg content.

After the data were consolidated, pH and Na were significantly greater for D+B treatments one month after the applications of dung and beetles took place (Table 5). Likewise, pH was significantly higher six months after the applications took place where applied dung beetles were active. For D+B treatments, Mg content was greater for both sampling intervals but was only significantly greater compared to D, X and N treatments six months after the applications were placed. Cation exchange capacity was significantly greater six months after applications of dung and beetles for the X treatments compared to D+B, D and N treatments.

Table 4. Mean ± SE of soil parameters measured from four treatments; dung-and-dung beetles (D+B), dung only (D), control / no dung, no beetles (X) and naturally-occurring dung beetles (N). 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 application.

Soil properties	Post application 1							
	One month				Six months			
	D+B	D	X	N	D+B	D	X	N
pH	4 ± 0.30	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
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
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
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
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
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
S (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
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

	Post application 2			
	One month			
	D+B	D	X	N
pH	4.1 ± 0.1	4 ± 0.04	4.1 ± 0.04	4.1 ± 0.1
P (mg.kg^{-1})	41.2 ± 20.3	82.6 ± 17.7	63.4 ± 22.3	64 ± 19
K (mg.kg^{-1})	103.7 ± 23.7	76 ± 8	104.6 ± 13	93.8 ± 13.4
Ca (mg.kg^{-1})	331.7 ± 42.1	325.3 ± 49.3	290 ± 70.8	418.4 ± 133.6
Na (mg.kg^{-1})	10.7 ± 1.8	15.3 ± 3.6	9.4 ± 0.9	14.4 ± 1.7
Mg (mg.kg^{-1})	77.2 ± 18.3	65.7 ± 12.2	51.2 ± 11.5	74.1 ± 20
S (mg.kg^{-1})	36.7 ± 1.8	50.2 ± 8.6	46 ± 6.1	49.2 ± 7
CEC [cmol(+)/kg]	1.7 ± 0.1	1.8 ± 0.2	2.2 ± 0.8	2.4 ± 0.6

	Post application 3							
	One month				Six months			
	D+B	D	X	N	D+B	D	X	N
pH	**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})	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})	**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})	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})	**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})	**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
S (mg.kg^{-1})	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]	2.9 ± 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

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

Table 5. Consolidated mean ± SE of soil parameters measured from four treatments; dung-and-dung beetles (D+B), dung only (D), control / no dung, no beetles (X) and naturally-occurring dung beetles (N). Measurements were taken one- and six months after the applications.

Soil properties	Post applications							
	One month				Six months			
	D+B	D	X	N	D+B	D	X	N
pH	**4.70 ± 0.16*** **	3.99 ± 0.02	4.23 ± 0.04	4.2 ± 0.06	**4.53 ± 0.16** **	4.18 ± 0.07	4.14 ± 0.05	4.16 ± 0.07
P (mg.kg^{-1})	53.37 ± 9.91	82.62 ± 10.20	63.05 ± 9.18	61.46 ± 7.92	86.06 ± 14.44	92.32 ± 9.09	70.13 ± 7.31	53.19 ± 6.74
K (mg.kg^{-1})	175.66 ± 26.85	76.04 ± 4.62	129.81 ±12.37	114.36 ± 9.03	189.22 ± 30.95	123.75 ± 11.92	127.10 ± 14.20	112.20 ± 10.65
Ca (mg.kg^{-1})	402.65 ± 28.15	325.34 ± 28.44	381.27 ± 43.92	420.44 ± 49.06	387.88 ± 30.89	323.56 ± 24.55	392.50 ± 36.06	395.30 ± 33.64
Na (mg.kg^{-1})	**21.71 ±4.27***	15.30 ± 2.09	11.12 ± 1.22	15.02 ± 1.20	26.13 ± 6.12	14.36 ± 1.46	14.40 ± 2.23	14.10 ± 1.43
Mg (mg.kg^{-1})	140.25 ± 23.02	65.69 ± 7.06	65.13 ± 6.13	73.02 ± 8.33	**123.91 ± 26.26*** **	48.95 ± 2.14	56.02 ± 6.57	56.68 ± 4.59
S (mg.kg^{-1})	44.38 ± 4.48	50.23 ± 4.94	51.32 ± 7.93	50.91 ± 4.39	67.48 ± 9.14	77.73 ± 6.70	81.15 ± 8.42	73.21 ± 8.84
CEC [cmol(+)/kg]	2.32 ± 0.28	1.79 ± 0.10	2.95 ± 0.35	2.91 ± 0.32	5.39 ± 0.34	4.96 ± 0.33	**6.53 ± 0.38***	5.46 ± 0.27

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

3.6 Principal component 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 except for one D+B plot (Fig. 18a). Magnesium and K had the highest factor loading scores of -0.96 and -0.83, respectively (Appendix, Table K). 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 (Fig. 18b). 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 (Fig. 18c). 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. Similar patterns were observed one month, and six months post application 3 (Fig. 18d and 18e). 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).

After the data were consolidated, it was clear that D+B treatments were clearly separated from D, X and N treatments one- and six months after applications took place (Fig. 19a and 19b). One month after applications, crude protein content and Na levels for PC1 explained most of the data with factor loadings of 0.92 and 0.96, respectively. Protein, P and Na each had a factor loading score of 0.93 six months after the applications of dung and beetles took place for PC1.

Factor loadings for principal components 1 and 2 for the separate sampling intervals and the consolidated sampling intervals are indicated in the Appendix, Table K and Table L, respectively.

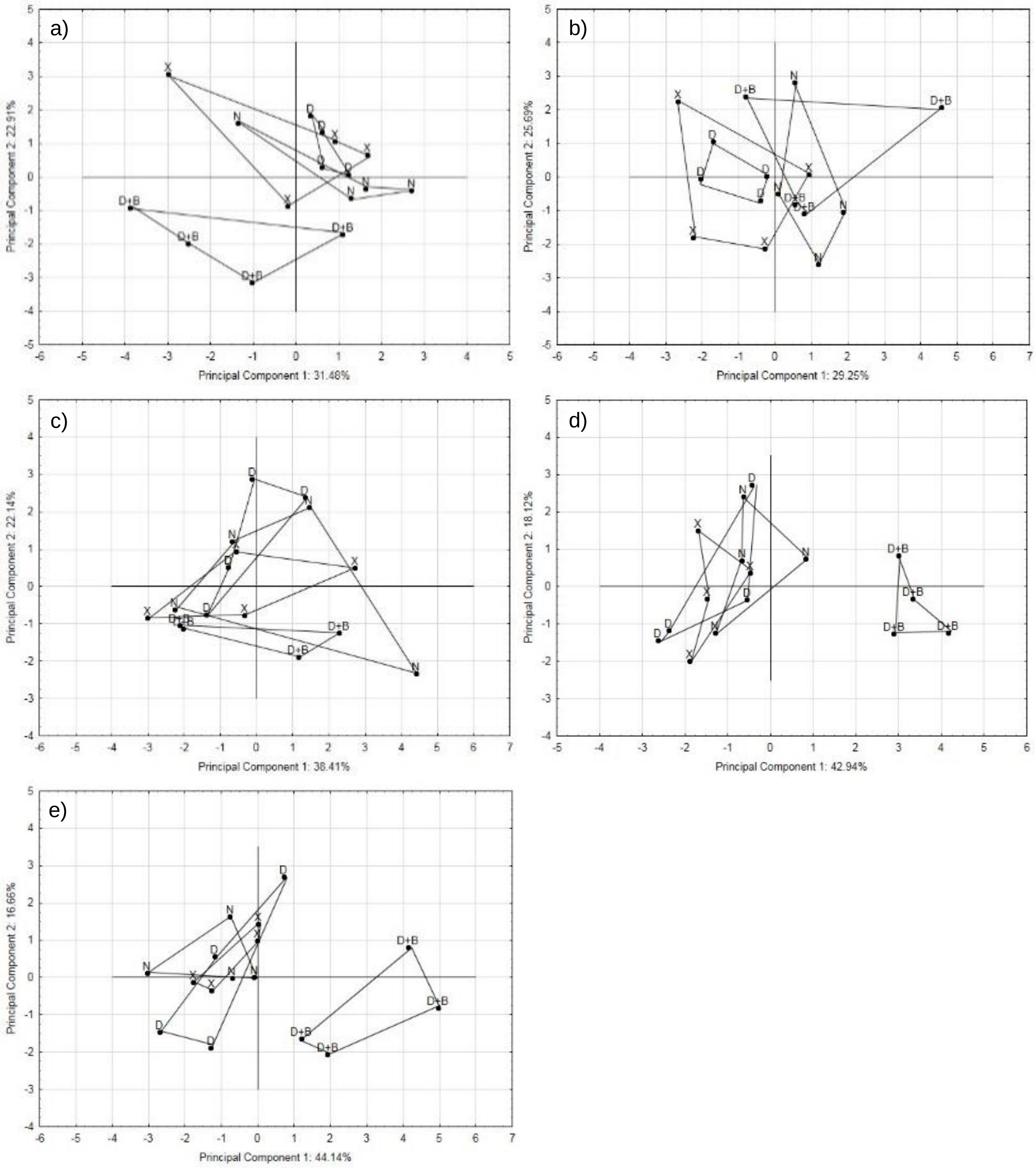

Figure 18. Principal component analyses (PCAs) of 11 variables for: dung-and-dung beetle (D+B); dung only (D); control / no dung, no beetles (X); and naturally-occurring beetles (N) treatments. Measurements were taken (a) one month and (b) six months post the first application of dung and beetles, (c) one month post the second application, and (d) one month and (e) six months post the third application.

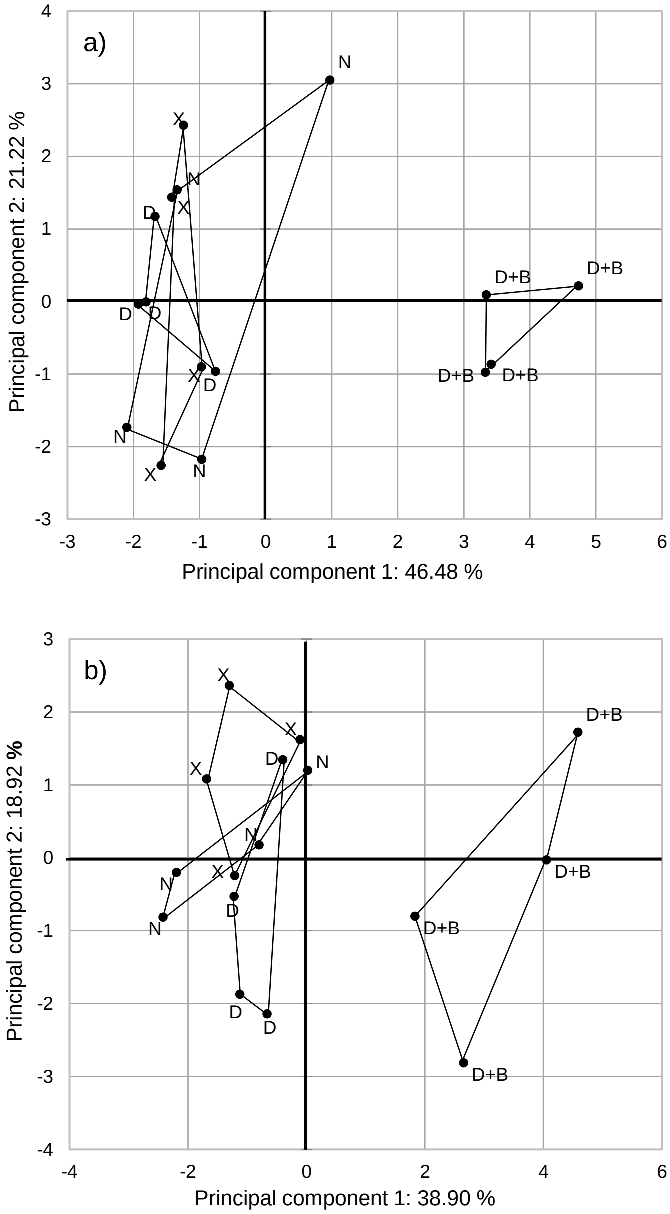

Figure 19. Consolidated principal component analyses (PCAs) of 11 variables for: dung-and-dung beetle (D+B); dung only (D); control / no dung, no beetles (X); and naturally-occurring beetles (N) treatments. Measurements were taken (a) one month and (b) six months post the applications of dung and beetles.

4. Discussion

This study provided evidence supporting many of the stated hypotheses, specifically 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, including treatments with naturally-occurring dung beetles. A month after each 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 be present in low numbers and may not be able to achieve a measurable improvement in soil and plant properties without augmenting their numbers with additional dung beetle applications. Furthermore, naturally occurring dung beetles seen were only of the small roller-type, and it is suspected that larger tunnelling dung beetles are more efficient at dung removal (Slade et al., 2007).

The most prominent result of this study was the increase in water infiltration rate where applied dung beetles were active. 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 are affected by soil compaction, therefore alleviating soil compaction is vital to improving soil hydraulic properties. After the data were consolidated, water infiltration rate differed significantly between and within treatments one month- and six months after applications took place, in other words, both the treatment and the time at which the sampling took place had a significant effect on water infiltration rate. It could be inferred that the climatic conditions for 2016 and 2017 impacted water infiltration rate. Extreme drought conditions were present in 2016 and most of 2017, which would have affected plant growth and in turn, water infiltration rate. Water infiltration rate increased for all treatments in 2017 and correlated with increased rainfall in the region.

Root growth is affected by soil compaction and low pH levels. Repeated measures ANOVA tests revealed that plant biomass yield was significantly greater one month after beetle and dung applications where applied dung beetles were active, and can be explained by dung beetle activity introducing plant roots to nutrients required for growing. Plant biomass yield was highly variable six months after beetle and dung applications, which could reflect the complex environmental conditions typical of reclaimed mined land. During the second 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 an increase 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 upper soil layer where most of plant rooting takes place. The results of this study suggested that dung beetles can alleviate soil compaction within the top 10 cm, but that the longevity of these effects may initially depend on the frequency of the applications, or until a self-sustaining population of dung beetles have established associated with cattle grazing on rehabilitated areas. Dung beetles were able to tunnel into soil compacted to a penetration resistance of more than 5 000 kPa. As dung beetle brood balls were observed at depths of 20 cm, it is likely that dung beetles can complete their lifecycles on post-mining soil. Brown et al. (2010) similarly showed that dung beetle activity decreases bulk density.

Soil pH was significantly higher for all plots with applied dung beetles and is biologically relevant for plant root growth. Decreased root size and root branching results in a decrease of nutrient uptake. Soil pH will decrease faster with time if the CEC of the soil is low (Brown and

Lemon, 2016), which was not reflected in the results; however, soil pH was much more variable for treatments with low CEC. Cation exchange capacity was significantly greater for the control / no dung, no beetle treatments six months after applications took place, which is contradictory to most literature regarding cation exchange capacity. Research has shown that high organic matter, soil pH and clay content increases soil CEC, yet the control had none of these characteristics. However, Ružicic et al. (2016) stated that low CEC values in the soil horizon is generally associated with maximum hydraulic conductivity, which could provide an explanation as to why D+B treatments had lower CEC as opposed to X treatments. The effect and trade-offs of CEC and hydraulic conductivity of soils should be investigated further, specifically on post-mining soils.

According to the PCAs, dung beetle activity also resulted in similar patterns in the data, particularly noticeable after the third application where D+B treatments were clustered together on the PCA plot. This suggests that dung beetle activity could improve soil parameters if dung and beetle 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 in treatment plots with applied dung beetles could be attributed to dung beetle activity. It is also clear that naturally-occurring dung beetles on the mine did not have a significant effect on any plant or soil parameters measured. The low natural abundance, small body size and dung-rolling behaviour of dung beetles on the reclaimed mine areas was probably why they were not able 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 increased by dung beetle activity six months after the third application. The second and third applications took place two months apart, which could suggest that more frequent applications of beetles and dung may be required to improve the condition of highly degraded soil in a variable environment.

CHAPTER 4: GENERAL DISCUSSION, CONCLUSIONS AND FURTHER RESEARCH RECOMMENDATIONS

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 that 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 sufficient dung and dung beetles are available. 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 a non-invasive, sustainable and complementary treatment approach to current mine reclamation practices. The results suggest that the introduction of dung beetles to rehabilitated areas should be considered as a key step in the reclamation process, especially for areas where the post-mining land is put to grazing use. 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 rate, soil penetration resistance and herbaceous plant biomass yield of 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 sampling interval. Acidic soil (with a pH 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 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. Establishing whether the dung beetles emerge as fully-functional adults was not part of the study by Venter et al. (unpublished).

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 soil layer to reshaped-mine spoils as dung beetles create tunnels that increase the water infiltration rate, potentially exposing underlying spoils to oxygen, creating an opportunity for acid mine drainage generation. In natural soils, *O. alexis* typically constructs tunnels to a maximum depth of 23 cm, and *O. alexis* brood balls were recorded at 20 cm in highly compacted soils exceeding a penetration resistance of 5 000 kPa (Edwards and Aschenborn, 1987; Venter et al., unpublished). A shallow soil layer (< 25 cm) could have 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 affected by soil bulk density. 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 water 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 scale. It was observed that the beneficial effects of 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 would affect the comparability of the two experiments but provides important insights into what could be expected with a lower 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, possible dust fallout from discard stockpiles and variation in soil depth. Therefore, results were less variable for simulated plots 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 tunnelling dung beetles be bred in a facility to attain the desired larger body size before being transferred to rehabilitated areas that have 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 the dung beetles present in an ecosystem are, the more ecological services they provide (Manning et al., 2016). Furthermore, it should be considered to include nutrient-dense organic supplements 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 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 plants need to regenerate their roots and shoots before the area can be grazed again (Dohi et al., 1991). High intensity grazing, if managed correctly, supports 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 in plant biomass yield was seen for D+B treatments compared with D treatments. The control (X treatments) out performed dung only treatments in terms of plant biomass yield, probably because the dung remained on the soil surface and prevented underlying grass from growing by restricting sunlight access.

Even though treatments with applied dung beetles only had 20.13% more biomass yield compared to the control (X treatments) and 19.66% more than N treatments, it may be a significant increase for a farmer. Miranda et al. (2001) 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 $kg.ha^{-1}$ N) which yielded 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^{-1})
D+B	793.83 ± 218.7
D	498.98 ± 134.21
X	634.06 ± 148.36
N	637.73 ± 158.78

2. Further research and recommendations

Due to the variability of climatic conditions experienced during this study, it is recommended that future research be undertaken over a longer period to obtain a comprehensive body of data. It is recommended that dung and dung beetle applications take place in spring, when seasonal conditions are favourable both for dung beetles and for microbe activities. 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 (rollers, tunnellers, and dwellers). 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 the unfavourable conditions of post-mining soils. The feasibility of combining dung beetle applications with high density grazing should be explored.

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. It would be important to investigate other interactions on post-mining soils, such as the influence of predation on dung beetle activity, because areas where dung beetle treatments could be applied to would likely not be covered in shade netting. Following the results in the current study, an additional project could examine if dung beetle activity can increase water infiltration rates on the slopes of rehabilitated discard dumps, and if erosion is affected by dung beetle activity on rehabilitated discard dumps.

Following research that shows that dung beetle activity can aid in post-mining rehabilitation of highly degraded land and that they are able to successfully reproduce, the costs of implementing dung beetle treatments should be calculated in detail. The costs would include the initial capture and breeding of the dung beetles, renting/building a facility for breeding, employing individuals to distribute dung beetles on the identified area, and monitoring the area to document any impacts.

REFERENCES

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. Appl. Soil Ecol., 29, 165–171.

Baudoin, M., Vogel, C., Nortje, K., Naik, M., 2017. Living with drought in South Africa: lessons learnt from the recent El Niño drought period. Int. J. Disaster Risk Reduct., 23, 128–138.

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, 1-12.

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.

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.

Bornemissza, G.F., 1976. The Australian dung beetle project 1965-1975. Aust. Meat Res. Committee Rev. 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, Ottawa.

Brown, J., Scholtz, C.H., Janeau, J-L., Grellier, S., Podwojewski, P., 2010. Dung beetles (Coleoptera: Scarabaeidae) can improve soil hydrological properties. Appl. Soil Ecol. 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., 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.

Carter, S., Gulati, M., 2014. Climate change, the Food Energy Water Nexus and food security in South Africa. Understanding the Food Energy Water Nexus. WWF-SA, South Africa.

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, 2007. Guidelines for the rehabilitation of mined land. Chamber of Mines of South Africa, Coaltech Research Association, 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.

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.

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.

Culliney, T.W., 2013. Role of Arthropods in Maintaining Soil Fertility. Agric. 3, 629 – 659.

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

Díaz-Raviña, M., Acea, J., Carballas, T.M., 1993. Seasonal fluctuations in microbial populations and available nutrients in forest soils. Biol. Fert. Soils. 16, 205-210. 10.1007/BF00361409.

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., 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 the introduction of additional dung-burying beetles (Coleoptera: Scarabaeidae) into New Zealand, New Zealand J. Agric. Res. 36, 163-171, DOI: 10.1080/00288233.1993.10427498

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

Edwards, P., Aschenborn, H., 1987. Patterns of Nesting and Dung Burial in *Onitis* Dung Beetles: Implications for Pasture Productivity and Fly Control. J. Appl. Ecol., 24, 837-851.

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.

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

Fincher, G.T., Monson, W.G., Burton, G.W., 1981. Effects of cattle faeces rapidly buried by dung beetles on yield and quality of coastal Bermuda grass. Agron. J. 71, 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 3, 1-18.

Fork, D.C., 1986. The control by state transitions of the distribution of excitation energy in photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 37, 335–361.

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.

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 Tillage Res. 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.

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.

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.

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

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.

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.

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

Ketterings, Q., Reid, S., Rao, R., 2007. Cation Exchange Capacity (CEC). Agronomy Fact Sheets Series, Fact Sheet 22. Cornell University Cooperative Extension.

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/

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.

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, Statistics South Africa 2012, Pretoria.

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

Limpitlaw, D., Briel, A., 2014. Post-mining land use opportunities in developing countries. J. South Afr. Inst. Min. Metall. 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.

Manning, P., Slade, E.M., Beynon, S.A., Lewis, O.T., 2016. Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. Agric. Ecosyst. Environ. 218, 87– 94.

Manning, P., Cutler, G.C., 2018. Ecosystem functioning is more strongly impaired by reducing dung beetle abundance than by reducing species richness. Agric. Ecosyst. Environ. 264, 9-14.

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.

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.

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. Agron. J. 98, 873 – 882.

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.

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. Int. J. Biodivers. Conserv. 7, 57-80.

Miranda, C.H., Santos, J.C., Bianchin, I., 2001. The role of *Digitonthophagus gazella* in pasture cleaning and production as a result of burial of cattle dung. Trop. Grassl. 22, 14-18.

Mittal, I.C., 1993. Natural manuring and soil conditioning by dung beetles. Trop. Ecol. 34, 150-159.

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 Tillage Res. 55, 87 – 97.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., Favila, M.E., 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biol. Conserv. 141, 1461-1474.

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.

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

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.

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.

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.

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.

Ružicic, S., Mileusnic, M., Posavec, K., Nakic, Z., Durn, G., Filipovic, V., 2016. Water flow and solute transport model of potentially toxic elements through unsaturated zone at regional wellfield Kosnica. Hydrol. Process. 30, 4113-4124.

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.

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, V., Sheoran, A.S., Poonia, P., 2010. Soil Reclamation of Abandoned Mine Land by Revegetation: A Review. Int. J. Soil, Sediment and Water 3, 1-20.

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.

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., Roslin, T., Santalahti, M., Bell, T., 2015. Disentangling the 'brown world' faecal-detritus interaction web: dung beetle effects on soil. Oikos. doi:10.1111/oik.02640

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.

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

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

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

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

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.

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.

Venter, G., Dabrowski, J., Scholtz, C.H., Truter, W.F., unpublished manuscript titled: Dung beetle assemblages on reclaimed coal mines in eMalahleni (South Africa), their environmental associations and tunnelling ability on compacted soils.

Viljoen & Associates, 2013. Arnot coal mine specialist soil assessment as part of the consolidated EMPR. Golder Associates, Johannesburg.

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.

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.

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

APPENDIX: SUPPLEMENTARY INFORMATION

Table A. ANOVA results for plant biomass yield, water infiltration rate and protein content measurements taken one month- and six months post the first application of beetles and dung, and one month and six months post the second application. Treatments were: dung-and-dung beetles (n= 4); dung only (n= 4); and control / no dung, no beetles (n= 4) [df=2].

	Post application 1								Post application 2							
	One month				Six months				One month				Six months			
	SS	MS	F	*p*	SS	MS	F	*p*	SS	MS	F	*p*	SS	MS	F	*p*
Plant biomass yield (g.m^{-2})	1910.8	955.39	1.90	0.21	0.91	0.46	33.65	0.000	16950	8475.2	8.78	0.008	5691	2845	0.92	0.43
Water infiltration rate (mm.h^{-1})	0.27	0.13	70.69	0.000	0.88	0.44	151.67	0.000	0.39	0.19	120.88	0.000	0.11	0.06	12.03	0.0003
Protein content (%)	0.12	0.01	2.45	0.14	0.22	0.11	0.09	0.91	0.15	0.08	0.04	0.96	0.02	0.01	2.45	0.14

Table B. Repeated measures ANOVA results for herbaceous plant biomass, water infiltration rate and protein content measurements taken one month- and six months post the applications of beetles and dung. Treatments were: dung-and-dung beetles (n= 4); dung only (n= 4); and control / no dung, no beetles (n= 4) [df=2].

	Post applications							
	One month				Six months			
	SS	MS	F	*p*	SS	MS	F	*p*
Herbaceous plant biomass (g.m^{-2})	15121.61	7560.81	5.98	0.02	43702.33	21851.17	5.41	0.03
Water infiltration rate (mm.h^{-1})	22874.90	11437.45	75.33	< 0.0001	27941.99	13970.99	118.72	< 0.0001
Protein content (%)	0.18	0.09	0.05	0.95	6.08	3.04	2.97	0.10

Table C. ANOVA F – and *p* values for soil penetration resistance measurements taken one month and six months post the first application of beetles and dung, and one month- and six months post the second application. Treatments were: dung-and-dung beetles (n= 4); dung only (n= 4); and control / no dung, no beetles (n= 4).

	Post application1				Post application 2			
Soil depth	One month		Six months		One month		Six months	
(cm)	F	*p*	F	*p*	F	*p*	F	*p*
1	1.49	0.28	5.21	0.03	1.77	0.23	1.21	0.34
2	2.16	0.17	6.29	0.02	8.48	0.009	11.78	0.003
3	1096	0.20	3.12	0.09	8.67	0.008	33.16	0.000
4	1.12	0.37	5.54	0.03	5.77	0.02	104.37	0.000
5	0.95	0.42	7.48	0.01	4.66	0.04	27.90	0.000
6	1.46	0.28	8.81	0.008	4.26	0.05	90.1	0.000
7	1.47	0.28	8.37	0.009	5.09	0.03	49.84	0.000
8	1.23	0.34	7.96	0.01	5.23	0.03	91.27	0.000
9	1.47	0.28	6.25	0.02	6.23	0.02	60.41	0.000
10	1.87	0.21	5.29	0.03	7.14	0.01	77.23	0.000
11	2.65	0.12	5.53	0.03	7.20	0.01	99.26	0.000
12	2.60	0.13	6.57	0.02	7.03	0.01	88.03	0.000
13	2.22	0.16	5.51	0.03	5.31	0.03	180.75	0.000
14	2.34	0.15	4.60	0.04	6.29	0.02	700.23	0.000
15	3.01	0.10	3.97	0.06	6.64	0.02	120.27	0.000
16	3.07	0.10	3.78	0.06	6.06	0.02	151.42	0.000
17	2.79	0.11	3.48	0.08	5.51	0.03	71.55	0.000
18	2.85	0.11	4.31	0.05	4.43	0.05	47.06	0.000
19	2.27	0.16	4.11	0.05	3.12	0.09	44.61	0.000
20	1.29	0.32	5.12	0.03	2.75	0.12	1.63	0.25

Table D. Repeated measures ANOVA F – and *p* values for soil penetration resistance measurements taken one month and six months post the application of beetles and dung. Treatments were: dung-and-dung beetles (n= 4); dung only (n= 4); and control / no dung, no beetles (n= 4).

	Post applications			
Soil depth (cm)	One month		Six months	
	F	*p*	F	*p*
1	1.31	0.32	5.49	0.03
2	1.45	0.29	29.05	0.000
3	1.18	0.35	11.40	0.003
4	2.67	0.12	9.46	0.006
5	3.98	0.06	9.09	0.007
6	5.32	0.03	8.34	0.009
7	5.18	0.03	11.11	0.004
8	4.70	0.04	10.77	0.004
9	5.02	0.03	11.84	0.003
10	6.48	0.02	10.71	0.004
11	9.28	0.01	10.96	0.004
12	9.96	0.005	10.74	0.004
13	9.59	0.006	8.41	0.009
14	10.74	0.004	9.90	0.005
15	15.04	0.001	9.94	0.005
16	17.07	0.001	8.70	0.008
17	15.14	0.001	9.53	0.006
18	13.18	0.002	9.63	0.006
19	8.28	0.01	7.63	0.012
20	5.64	0.03	7.15	0.01

Table E. Factor loadings of 11 variables for measurements in principal components 1 and 2 (PC 1 and PC 2) from the principal component analyses (Fig. 7).

	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 (mg.kg^{-1})	0.85	-0.26	-0.87	-0.10	0.36	-0.44	0.84	-0.01
K (mg.kg^{-1})	0.94	0.15	-0.93	-0.03	0.93	0.28	0.83	-0.40
Ca (mg.kg^{-1})	0.98	-0.09	-0.96	-0.03	0.84	-0.22	0.82	0.27
Na (mg.kg^{-1})	0.42	-0.33	-0.42	-0.75	0.82	-0.37	0.67	-0.11
Mg (mg.kg^{-1})	0.98	-0.04	-0.95	0.09	-0.90	0.15	0.93	0.18
S (mg.kg^{-1})	0.92	-0.08	-0.29	-0.89	0.81	0.10	-0.18	0.02
CEC [cmol(+)/kg]	0.90	-0.05	ND	ND	0.48	-0.03	0.83	-0.19

Table F. Factor loadings of 11 variables for measurements in principal components 1 and 2 (PC 1 and PC 2) from the consolidated principal component analyses (Fig. 8).

	Post applications			
	One month		Six months	
Variable	PC 1	PC 2	PC 1	PC 2
Water infiltration rate (mm.h^{-1})	0.67	0.17	0.86	-0.43
Herbaceous plant biomass (g.m^{-2})	0.58	-0.32	0.53	-0.35
Crude protein content (%)	0.60	0.19	0.63	-0.26
pH	0.32	-0.61	0.34	-0.67
P (mg.kg^{-1})	0.55	-0.41	0.88	0.25
K (mg.kg^{-1})	0.94	0.15	0.92	0.28
Ca (mg.kg^{-1})	0.79	-0.46	0.94	0.09
Na (mg.kg^{-1})	0.61	0.24	0.52	0.70
Mg (mg.kg^{-1})	0.79	-0.41	0.97	0.06
S (mg.kg^{-1})	0.56	0.70	-0.12	0.62
CEC [cmol(+)/kg]	0.74	0.51	0.82	0.05

Table G. ANOVA results for herbaceous plant biomass, water infiltration rate and protein content measurements taken one month and six months post the first application of beetles and dung, one month post the second application, and one month and six months post the third application. Treatments were: dung-and-dung beetles (n= 4); dung only (n= 4); no-dung-or-dung beetles (n= 4); and naturally-occurring dung beetles (n=4) [df=3].

	Post application 1								Post application 2				Post application 3							
	One month				Six months				One month				One month				Six months			
	SS	MS	F	*p*	SS	MS	F	*p*	SS	MS	F	*p*	SS	MS	F	*p*	SS	MS	F	*p*
Plant biomass yield (g.m^{-2})	112641	37547	4.75	0.02	2226.50	742.17	1.46	0.27	26798	8933	1.44	0.28	55189	18396	1.27	0.33	10853	3618	0.66	0.59
Water infiltration rate (mm.h^{-1})	0.08	0.03	3.54	0.05	53.84	17.95	1.92	0.18	320.82	106.94	5.43	0.01	0.17	0.57	5.02	0.02	828.7	276.2	5.76	0.01
Protein content (%)	1.65	0.55	1.05	0.41	1.65	0.55	1.05	0.41	0.02	0.007	2.3	0.13	8.1	2.7	3.19	0.06	2.6	0.88	0.68	0.58

Table H. Repeated measures ANOVA results for herbaceous plant biomass, water infiltration rate and protein content measurements taken one month- and six months post the applications of beetles and dung. Treatments were: dung-and-dung beetles (n= 4); dung only (n= 4); control / no dung, no beetles (n= 4); and naturally occurring dung beetles (n=4) [df=3].

	Post applications							
	One month				Six months			
	SS	MS	F	*p*	SS	MS	F	*p*
Herbaceous plant biomass (g.m^{-2})	162092.85	54030.95	5.003	0.02	4543.19	1514.40	0.51	0.68
Water infiltration rate (mm.h^{-1})	527.47	175.83	27.60	< 0.0001	387.64	129.21	3.80	0.04
Protein content (%)	5.89	1.96	4.14	0.03	8.94	2.98	3.50	0.05

Table I. ANOVA F – and *p* values for soil penetration resistance measurements taken one month and six months post the first application of beetles and dung, one month post the second application, and one month and six months post the third application. Treatments were: dung-and-dung beetles (n= 4); dung only (n= 4); no-dung-or-dung beetles (n= 4); and naturally-occurring dung beetles (n=4).

	Post application 1				Post application 2		Post application 3			
Soil depth (cm)	One month		Six months		One month		One month		Six months	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
1	2.73	0.09	3.17	0.06	6.86	0.006	9.28	0.002	1.44	0.28
2	2.33	0.13	7.68	0.004	13.71	0.000	6.02	0.01	1.80	0.20
3	11.07	0.000	4.81	0.02	18.67	0.000	12.19	0.001	0.82	0.51
4	17.25	0.000	2.69	0.09	20.63	0.000	4.77	0.02	1.01	0.42
5	33.28	0.000	2.14	0.15	35.76	0.000	11.28	0.000	6.11	0.01
6	63.58	0.000	2.04	0.16	29.49	0.000	18.42	0.000	2.35	0.12
7	71.21	0.000	2.88	0.08	21.62	0.000	17.99	0.000	2.09	0.16
8	47.75	0.000	1.89	0.19	18.41	0.000	12.36	0.000	2.25	0.14
9	35.18	0.000	2.48	0.11	11.77	0.000	6.53	0.007	2.96	0.07
10	40.09	0.000	1.28	0.33	8.06	0.003	6.70	0.007	2.16	0.15
11	37.10	0.000	0.15	0.93	5.59	0.01	2.42	0.12	2.49	0.11
12	22.65	0.000	0.73	0.55	2.95	0.08	1.23	0.34	2.44	0.11
13	13.45	0.000	0.70	0.57	3.06	0.07	2.36	0.12	2.34	0.13
14	8.12	0.003	0.32	0.81	1.44	0.28	2.38	0.12	1.66	0.23
15	7.27	0.005	0.17	0.92	0.92	0.46	0.78	0.53	1.90	0.18
16	6.98	0.006	0.37	0.77	1.05	0.41	0.89	0.47	1.03	0.42
17	5	0.02	0.51	0.68	0.92	0.46	1.73	0.21	1.20	0.35
18	4.93	0.02	1.08	0.39	0.50	0.69	1.28	0.33	0.45	0.72
19	3.90	0.04	1.50	0.26	0.72	0.56	1.26	0.33	0.21	0.89
20	5.25	0.02	3.04	0.07	0.80	0.52	0.88	0.48	0.17	0.91

Table J. Repeated measures ANOVA F – and *p* values for soil penetration resistance measurements taken one month and six months post the application of beetles and dung. Treatments were: dung-and-dung beetles (n= 4); dung only (n= 4); control / no dung, no beetles (n= 4); and naturally occurring dung beetles (n=4).

	Post applications			
Soil depth (cm)	One month		Six months	
	F	*p*	F	*p*
1	8.85	0.002	3.46	0.051
2	14.95	0.000	8.07	0.003
3	27.51	0.000	4.52	0.02
4	29.11	0.000	2.68	0.09
5	63.44	0.000	4.22	0.03
6	65.53	0.000	2.76	0.09
7	70.44	0.000	3.61	0.05
8	78.37	0.000	3.05	0.07
9	69.75	0.000	3.94	0.04
10	66.28	0.000	2.37	0.12
11	31.06	0.000	1.40	0.29
12	15.69	0.000	2.06	0.16
13	21.25	0.000	2.72	0.09
14	12.91	0.000	1.27	0.33
15	8.61	0.003	1.12	0.38
16	4.8	0.02	1.03	0.42
17	2.24	0.14	1.12	0.38
18	0.44	0.73	1.52	0.26
19	0.75	0.54	1.97	0.17
20	0.82	0.51	3.76	0.04

Table K. Factor loadings of 11 variables for measurements in principal components 1 and 2 (PC 1 and PC 2) from the principal component analyses (Fig. 18).

	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
S	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

Table L. Factor loadings of 11 variables for measurements in principal components 1 and 2 (PC 1 and PC 2) from the consolidated principal component analyses (Fig. 19).

	Post applications			
	One month		Six months	
Variable	PC 1	PC 2	PC 1	PC 2
Water infiltration rate (mm.h^{-1})	0,87	0,03	0,56	-0,48
Herbaceous plant biomass (g.m^{-2})	0,82	-0,10	0,09	0,29
Crude protein content (%)	0,92	0,03	0,93	-0,08
pH	-0,34	0,66	0,53	0,02
P (mg.kg^{-1})	0,80	0,13	0,93	-0,05
K (mg.kg^{-1})	0,71	0,08	0,80	0,30
Ca (mg.kg^{-1})	0,33	0,86	0,36	0,75
Na (mg.kg^{-1})	0,96	0,19	0,93	0,08
Mg (mg.kg^{-1})	-0,34	-0,35	-0,24	0,52
S (mg.kg^{-1})	-0,24	0,87	0,00	0,89
CEC [cmol(+)/kg]	0,61	-0,45	0,50	-0,21