

Dung beetle activity improves herbaceous plant growth and soil properties on confinements simulating reclaimed mined land in South Africa

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Highlights

- Benefits of dung beetles are maintained on simulated reclaimed mine soils.
- Dung beetle activity improved water infiltration, plant biomass and soil nutrients.
- Dung beetles reduced soil compaction to an acceptable range for root development.
- Dung beetles offer a complementary biological approach to current reclamation efforts.

Abstract

Mining activities contribute greatly 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. 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 in dung to the plant root zone, which would otherwise remain on the soil surface and mostly volatilize in the absence of dung beetles. Furthermore, dung beetles create tunnels under dung pats, improving water infiltration rates, bulk density, soil aeration and pasture yields. The aim of the study was to determine whether these effects could be maintained on soil simulating reclaimed mined land, where very high rates of compaction may prevent tunnelling altogether. Three experimental treatments of dung + beetles (D+B), dung only (D) and control / no dung, no dung beetles (X) were applied twice over 2 years on 1 m³ experimental confinements. Various soil and herbaceous plant properties were assessed one and six months after each application of dung and beetles. Results obtained showed that water infiltration rate and plant biomass was significantly higher for all confinements containing dung beetles. Penetration resistance (soil strength) was significantly reduced for confinements with dung beetles. Magnesium and potassium levels in the soil were significantly higher for D+B treatments when compared to D and X treatments. In conclusion, results showed that dung beetles were able to maintain their activities in soils typical of reclaimed mine land, significantly improving soil properties and herbaceous plant growth. Incorporating the application of dung beetles to the conventional approach to rehabilitation has the potential to improve the efficacy of coal mine reclamation. This biological approach may also prove to be cost-effective over time as it provides a seasonal source of bioturbation, which does not disturb plant growth and reduces the requirements for soil rejuvenating tillage practices.

Keywords: dung beetle, mine reclamation, water infiltration, compaction, land degradation

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 (Tun et al., 2015). Poor soil conditions contribute to the decline in plant productivity (Passioura, 2002). Mining activities permanently alter the land's topography, drastically impairing land capability. In some countries, mine closure necessitates the return of land to viable land use capabilities such as agriculture (Limpitlaw and Briel, 2014). The effects of mining activities and wastes include the loss of grazing areas for animals and cultivated land, loss of agricultural production, water and air pollution, soil erosion, loss of biodiversity and geo-environmental disasters (Sheoran et al., 2010).

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

In developing countries, a common post-closure land use is low-intensity grazing (Limpitlaw and Briel, 2014). The nutritional value of plants is determined by its protein quantity, which is derived from the plants nitrogen content. Herbage feed-value becomes increasingly important when cattle are used to graze areas and turns into an expensive practice when fertilizers are needed to improve vegetation quality as crude protein content is otherwise too low. 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 (subfamily: Scarabaeinae) are classified by their predominant activity when processing dung. The three major functional guilds are telecoprids (dung beetles create a dung ball from a portion of a dung pat, roll the dung ball away and bury the dung ball at a different location from the dung source), endocoprids (dung beetles complete their entire lifecycle inside a dung pat) and paracoprids (dung beetles construct tunnels directly underneath dung pats, forming a continuous link to the dung source). An estimated 70% of southern Africa's approximately 780 species of dung beetles are tunnelers (Davis et al., 2008). The ecosystem services provided by dung beetles have been extensively reviewed by Nichols et al. (2008), stating that dung beetles play an important role in parasite suppression, secondary seed dispersal, nutrient cycling and plant growth enhancement.

Dung beetles enhance air permeability in soil, facilitate the transfer of nutrients in dung to soil, leading to an increase in herbage feed-value, biomass and nutritive value of the vegetation (Mittal, 1993; Bang et al., 2005). High-diversity assemblages of dung beetles are likely to improve functionality in a range of ecosystems, thereby contributing to ecosystem services (Manning et al., 2016). Tunnelling by dung beetles can improve various physical and hydrological aspects of soil by increasing water infiltration rates leading to higher soil moisture and reducing soil bulk density. Improved water infiltration rates result in reduced surface water runoff (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 soil-dung interface, subsequently increasing community- and function similarity. Dung beetles have been mass-reared and introduced into Australia, New Zealand, the United States of America and elsewhere for the purpose of dung burial, pest control and to facilitate pasture improvement in agro-ecosystems (Edwards, 2007; Bertone, 2005; Dymock, 1993; Bornemissza, 1976).

Most studies of the activities of dung beetles are undertaken to better understand their role and benefits in agro-ecosystems (Beynon et al., 2012, Farias and Hernández, 2017). No studies could be found that investigated their ability to maintain their activities and associated benefits in systems with extreme soil degradation, such as on reclaimed coal mines. In particular, soil bulk density rates on rehabilitated coal mines can be in excess of 1.8 g/cm^3 while those in agro-ecosystems are generally in the range of 1.1 to 1.5 g/cm^3 (Sheoran et al., 2010, Haigh and Sansom, 2007). Highly compacted soil may present a physical barrier to the tunnelling abilities of dung beetles. However, if they are able to maintain their activities under these conditions then dung beetles present a potentially valuable resource to be considered for improving reclaimed mined land conditions to further increase the range of viable land-use options. The objectives of this study were to determine whether dung beetles applied to reclaimed mine soils can maintain their beneficial activities by measuring (1) the properties of soil in terms of penetration resistance (kPa), nutrients (mg.kg^{-1}), pH, cation exchange capacity ($\text{cmol}(+).\text{kg}^{-1}$) 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 confinements. 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 yield and protein content, and that the effects will last at least six months after the applications of dung and beetles.

2. Materials and methods

2.1. The study site

The experiments were conducted at the University of Pretoria experimental farm, Gauteng, South Africa, at an altitude of 1308 m.a.s.l. (S25.752295, E28.252754). A total of 12 confinements were used, each measuring $1 \times 1 \times 1 \text{ m}$ built on the soil surface. The confinements were made of brick to prevent dung beetles from escaping as well as to limit

other invertebrates from entering the confinement. 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 in South Africa for rehabilitation purposes (Viljoen & Associates, 2013). The soil is classified as a sandy loam consisting of 77% sand, 6% silt and 17% clay. Preferential water flow along the edges of the confinements was reduced by constructing concrete ridges on the interior walls. A grass seed mixture, typically used in the mine rehabilitation process, was planted in the confinement and contained an equal mix of *Eragrostis tef* (Zuccagni) Trotter, *Chloris gayana* (Kunth) and *Digitaria eriantha* (Steud.). At the time of the study, the plant community was dominated by *D. eriantha* ($\pm 50\%$ ground cover) and *C. gayana* ($\pm 20\%$ ground cover). The mean annual temperature is 17.8 °C and precipitation is 697 mm for the area; however extreme drought conditions were present during the course of this study (Baudoin et al., 2017). All confinements were exposed to the same ambient environmental conditions.

2.2. Applications of dung and dung beetles

Dung and beetles were applied during autumn months in two separate applications within a period of 18 months. Three species of paracoprid (tunnelling) dung beetles were used in the treatments: *Euoniticellus intermedius* (Reiche, 1849), *Digitonthophagus gazella* (Fabricius, 1787) and *Onitis alexis* (Klug, 1835). These species have been successfully bred for introduction to other countries, and occur naturally in the Highveld of South Africa, where coal mining is concentrated. Beetles were collected from farms in the surrounding Highveld district. The variation in dung beetle body size was selected to ensure a range of tunnel widths and to reflect the body size range of dung beetles in the natural environment. All dung beetles were allowed to roam freely within the confinements where they were placed. Three treatments were applied to each of four replicated confinements: dung + beetles, dung only, and control / no dung, no beetles. All dung used in this study was collected from the same grass-fed,

drench-free cattle and was considered to be homogeneous across confinements as all dung was mixed before applications took place.

For the dung + beetles (D+B) treatment, each of the dung beetle confinements received a total application of five 1 kg fresh cattle dung pats placed on the soil surface and 100 dung beetles. The dung beetles were allocated as follows: 33 individuals of *E. intermedius*, 33 individuals of *D. gazella* and 34 individuals of *O. alexis*. Dung pats were applied in such a manner that all soil surface areas were covered in dung over time, with the first application placed alternatingly and the second application filling the areas that had not been exposed to dung. This was done due to the extreme drought conditions at the time of the study resulting in limited numbers of dung beetles available for the experiment.

The dung only (D) treatment consisted of five 1 kg fresh cattle dung pats placed on the soil surface in the manner described above with no dung beetles in order to study the effect of dung placement alone. The 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 repeated one month after each application of dung and beetles in May 2015 and May 2016 as well as six months after the applications (in September 2015 and September 2016) to determine the longevity of effects. The confinements were cleared of dung before the next application of dung and beetles took place.

2.3. Preparation of the experimental confinements

Each of the 12 confinements was covered with a 1 m³ 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 confinements. This was to prevent the movement of beetles in and out of the confinements.

2.4. Measurements

2.4.1. Herbaceous plant biomass and protein content

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

2.4.2. Soil properties and nutrient content

A 100 g sample from the top 20 cm of topsoil was collected randomly from each experimental plot and was analysed by Nvirotek Labs (NviroTek Laboratories (Pty) Ltd., South Africa) for the following: soil pH (1 to 2.5 ratio extraction with 1.0 M KCl ; determined with pH-meter [HI 223 pH-meter, Hanna Instruments (Pty) Ltd., South Africa]), and soil nutrient content including phosphorus (P; 1 to 7.5 ratio extraction with BRAY I extractant; determined colorimetrically), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) and sulphur (S; 1 to ten ratio extraction with 1.0 M ammonium acetate; determined by inductively coupled plasma analysis) as well as the cation exchange capacity (CEC; saturation by 1.0 M ammonium acetate, washed by ethanol and extracted with 1.0 M KCl; determined colorimetrically). All methods of the above-mentioned analyses were provided by Nvirotek Labs. 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.

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

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 $\text{mm}\cdot\text{h}^{-1}$ (Gregory et al., 2005). This method was repeated five times per plot.

2.5. Data analysis

Using XLSTAT version 2018.4 (Addinsoft, France), the data were analysed to ensure the assumptions for parametric tests were met. Data which were not normally distributed were log-transformed. Repeated measures analysis of variance (ANOVA) were used to compare group means for measurements taken one- and six months after the applications of dung and beetles. It was further used to determine whether herbaceous plant (biomass yield and crude protein content) and soil parameters (all soil properties and nutrient content data) differed significantly between and within treatments.

Results were reported as the mean \pm standard error (SE) one and six months after the applications of dung and beetles. Statistical significance was assumed at $p < 0.05$. A Principal Components Analysis (PCA) was used to detect groupings in the confinements using the measured soil (water infiltration rate, nutrient content, CEC, and pH) and herbaceous plant (biomass yield, and crude protein content) parameters to assess possible relationships among the variables. The factor loadings, following the PCA, were used as correlation coefficients between the variables and the principal components.

3. Results

3.1. Herbaceous plant biomass and protein content

Repeated measures ANOVA indicated that biomass yield was significantly greater (Table 1) for confinements with dung beetles (D+B) compared to confinements with only dung (D) and

reference confinements (X) for measurements taken one and six months after the applications of dung and beetles (Fig. 1A).

Biomass yield increased for all treatments six months post applications of dung and beetles. The results showed no significant difference between D treatments and X treatments for measurements taken one and six months post applications ($p > 0.05$). Herbaceous plant protein content was not significantly different among any of the treatments for measurements taken (Fig. 1B). Following the repeated measures ANOVA, no within treatment differences were observed.

Table 1. ANOVA results for herbaceous plant biomass, water infiltration rate and protein content measurements taken one and six months post the applications of dung and beetles. Treatments were: dung + dung beetles (n= 4); dung only (n= 4); and control / no dung, no dung beetles (n= 4).

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

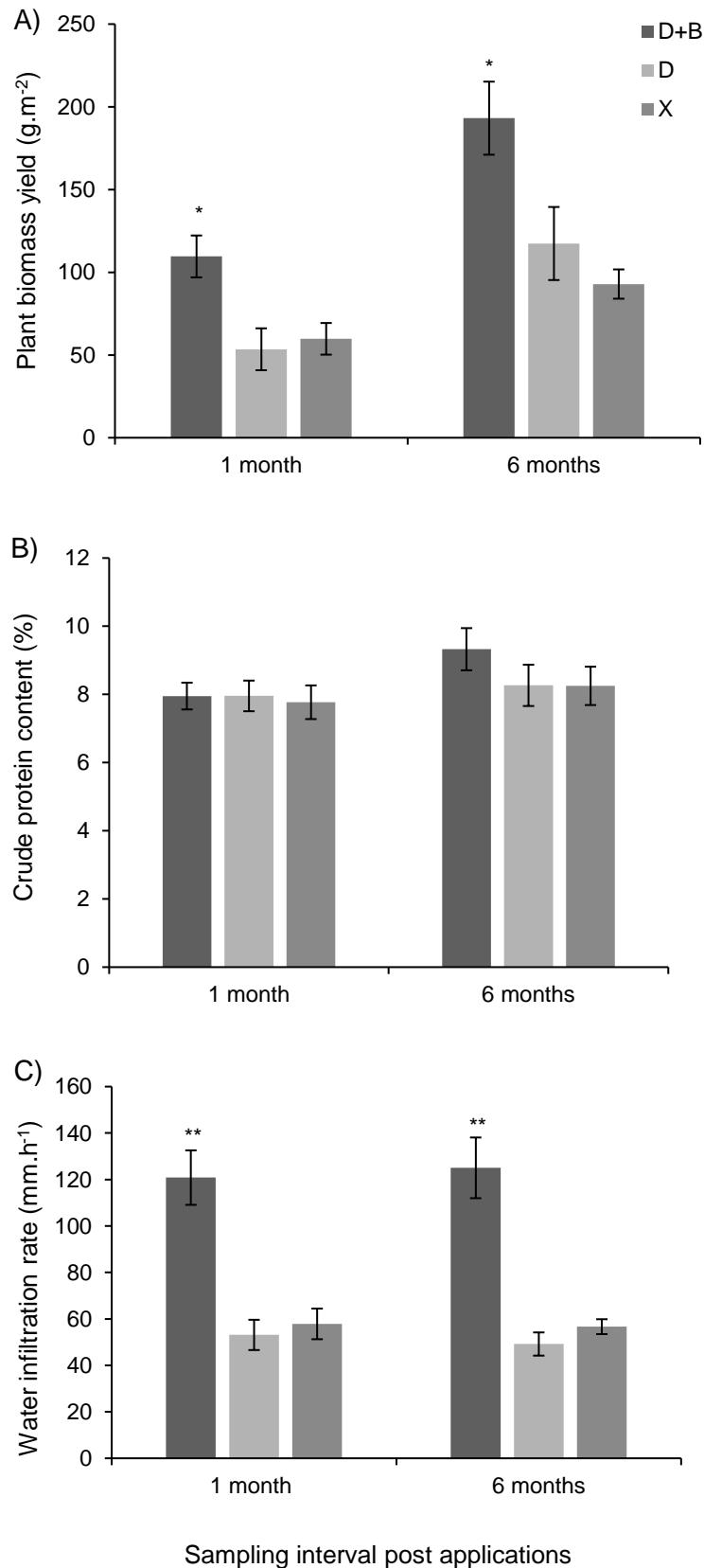


Figure 1. Mean \pm SE values for A – herbaceous plant biomass yield (g.m^{-2}), B – herbaceous plant crude protein content (%) and C – water infiltration rate (mm.h^{-1}) measurements taken one and six months post the applications of dung and beetles. Treatments were: dung + dung beetles (D+B; $n = 4$);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

3.2. Soil properties and nutrient content

Water infiltration rate was significantly higher for D+B treatments when measurements were taken one month after the applications of dung and beetles, as well as six months after applications ($p < 0.0001$). The repeated measures ANOVA indicated that there was no significant difference between D and X treatments for measurements taken one and six months after applications took place (Fig. 1C). The average water infiltration rate for all treatments remained similar six months after the applications were made.

Soil penetration resistance was significantly reduced for D+B treatments between 6 and 20 cm one month post applications (Fig. 2A; Table 2). D and X treatments had similar penetration resistance values and were more variable than D+B treatments.

Confinements with dung beetles had significantly lower penetration resistance for all depths measured six months after the applications of dung and beetles and no difference was observed between D and X treatments (Fig. 2B). At 20 cm, D and X treatments had similar values of 697.88 kPa and 692.33 kPa, respectively.

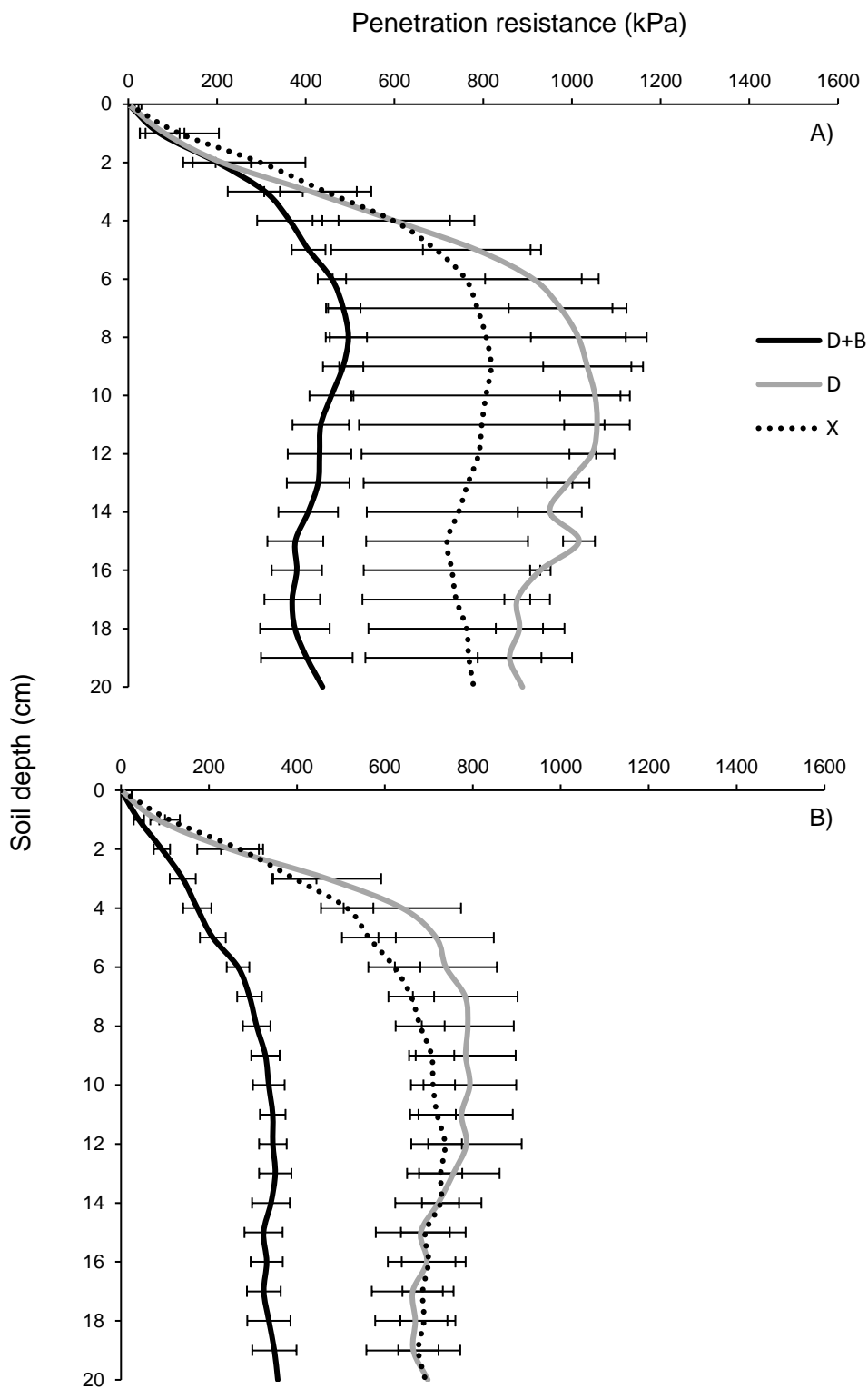


Figure 2. Mean \pm SE values for soil penetration resistance (kPa) measurements taken A – one month and B – six months post the applications of dung and beetles. Treatments were: dung + dung beetles (D+B; n= 4); dung only (D; n= 4); and control / no dung, no dung beetles (X; n= 4).

Table 2. ANOVA F – and p values for soil penetration resistance measurements taken one and six months post the applications of dung and beetles. Treatments were: dung + dung beetles (n= 4); dung-only (n= 4); and control / no dung, no dung beetles (n= 4).

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

According to repeated measures ANOVA results, no significant differences were observed one month after the applications (Table 3). Six months after the applications of dung and beetles, K, Mg and CEC were determined to be significantly greater for confinements where dung beetles were active ($p < 0.05$).

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

Table 3. Mean \pm SE of soil parameters measured from three treatments: dung + dung beetles (D+B; n= 4), dung only (D; n= 4), and control / no dung, no dung beetles (X; n= 4). Measurements took place one and six months post the applications of dung and beetles.

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

* - $p \leq 0.05$

No within treatment differences were observed according to the repeated measures ANOVA results.

3.3. Change of soil and plant variables in treatments

Principal Component 1 and 2 combined accounted for 62.04% of the total variation (Fig. 3A). The D+B treatments measured one month after the applications of dung and beetles took place indicate that the data were mainly distributed along Principal Component 1 (PC1). For PC1, measurements of K had a high correlation one and six months after applications with a correlation between 0.92 and 0.94, respectively. One month after the applications of dung and beetles, 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 70.76% of the total variation (Fig. 3B). 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 measurements taken one month post applications, water infiltration rate had a high correlation 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.

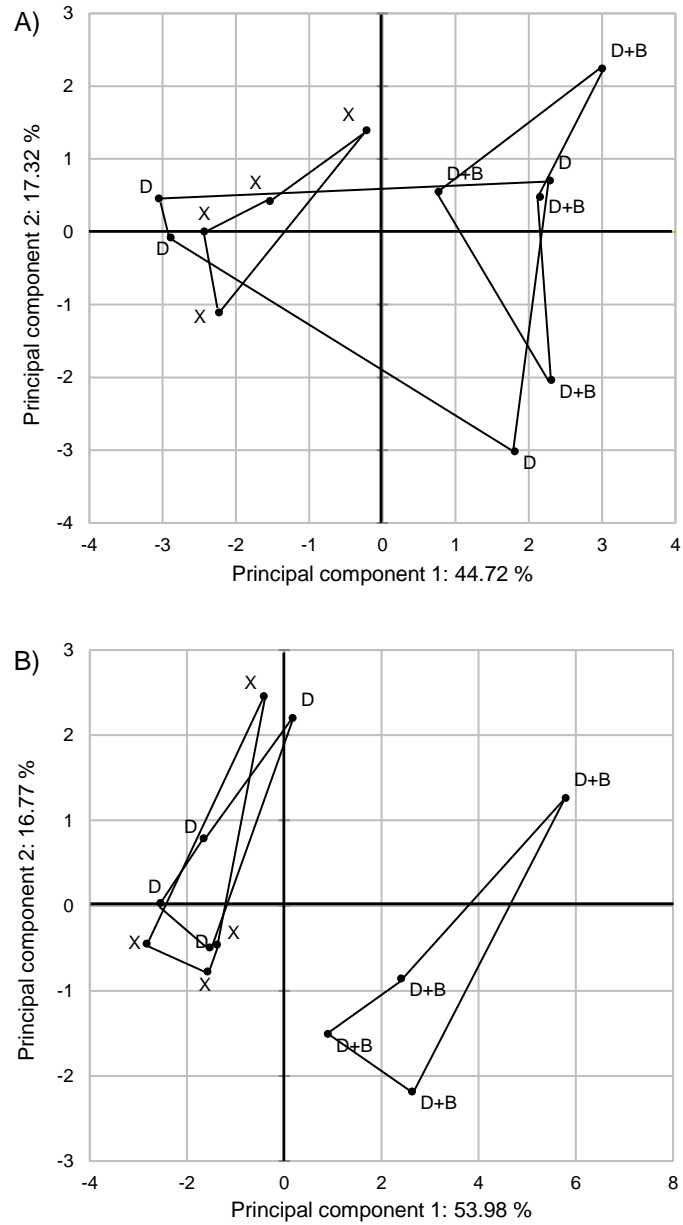


Figure 3. Principal component analyses (PCAs) for measurements taken A – one month and B – six months post the applications of dung and beetles. Treatments were: dung + dung beetles (D+B; n= 4); dung only (D; n= 4); and control / no dung, no dung beetles (X; n= 4). Variables were: herbaceous plant biomass ($\text{g}\cdot\text{m}^{-2}$); plant protein content (%); water infiltration rate ($\text{mm}\cdot\text{h}^{-1}$); soil nutrients; (P, K, Ca, Na, Mg and S); and soil properties (pH and CEC).

4. Discussion

The most noteworthy finding of this study was the higher rate of water infiltration seen for treatments containing dung beetles. High water infiltration rates may be problematic in post-mining soil as the water might seep through to the coal layer below the topsoil, increasing acid mine drainage seepage to groundwater. However, as the bottom of paracoprid tunnels are sealed off by brood balls, it is suspected that water infiltration rates are mainly limited to the plant root zone (Gaikwad and Bhawane, 2013). The Chamber of Mines of South Africa (2007) recommends 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. A study by Richardson and Richardson (2000) found that water infiltration and permeability was 129% deeper in presence of the activity of dung beetles, stressing the importance of applying a thicker topsoil layer to post-mining lands as dung beetles are naturally-occurring.

Similar to what other studies have found (Miranda et al., 2000; 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. Moreover, increased soil aeration associated with the dung beetle tunnels improves plant growth (Jones, 2005).

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

Percentage nitrogen content in vegetation has been found to increase significantly when dung beetles were active on a site (Bertone et al., 2006). In this experiment, the activity of

dung beetles did not have a significant effect on the crude protein content of the herbaceous plants for any of the treatments. An increasing trend (but not statistically significant) was seen six months post the applications of dung and beetles, with D+B treatments having greater crude protein content than D or X treatments. This could be explained by the relationship between N uptake and soil pH, whereby N needs to be mineralised to inorganic N for plant uptake and will not occur if the pH of the soil is low (<5.5; Mengel and Kirby, 2001).

Although the soil parameters of treatments with dung beetles had few significant differences when compared to D and X treatments, the results may be biologically meaningful. The pH in the soil on confinements with dung beetles increased after two applications of dung and beetles, possibly improving nutrient uptake (Jones, 2012). To obtain definitive results, measurements should be taken for a longer period and more samples should be taken per plot to improve the statistical power.

Magnesium ions, abundant in the soil containing dung beetles, play a vital role in photosynthetic organisms. Magnesium in dung is important for photosynthesis and movement of sugars within a plant (Silva and Uchida, 2000; Marschner, 1995). As Mg is one of the exchangeable cations mostly associated with CEC, confinements that had high Mg content were more likely to have higher CEC, as was observed six months after the applications of dung and beetles took place. The increase in CEC for treatments with dung beetles may be biologically meaningful, especially considering the large amounts of cations in the soil.

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

The practicality of using dung beetles to assist in alleviating poor soil conditions on reclaimed mined land is yet to be investigated. However, it is clear that on a small scale, dung

beetle activity has the potential to contribute towards reclaiming degraded land along with existing practices.

5. Conclusions and recommendations

No known studies have evaluated the effects of dung beetle activity on reclaimed mined land and their importance in aiding soil fertility of highly degraded environments. The activity of dung beetles greatly improves water infiltration rate, soil penetration resistance and herbaceous plant biomass yield of simulated reclaimed mined land. These parameters are highly impacted during the reclamation process, emphasising the benefits of incorporating dung beetles as a complementary strategy to improve rehabilitation efforts.

Future studies should utilise a similar experimental design under field conditions to determine whether the benefits of dung beetles can be maintained at larger spatial scales in order to improve post-mining land capabilities.

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