



Short communication

Effect of dung and dung beetle application on topsoil fungal assemblage of a post-coal mining reclamation land: Towards soil health improvement

Obinna T. Ezeokoli^{a,*}, Jessica Badenhorst^b, Adekunle Raimi^a, Jackie Dabrowski^{b,c}, Clarke H. Scholtz^b, Rasheed A. Adeleke^a^a Unit For Environmental Sciences and Management, North-West University, Potchefstroom, Private bag X6001, 2520, South Africa^b Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Pretoria 0002, South Africa^c Sustainability Research Unit, Nelson Mandela University, Private Bag x6531, George 6530, South Africa

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ABSTRACT

Soil management practices such as organic matter input can ameliorate soil health, including microbial communities that are crucial for ecological processes. In this study, the effect of successive application of cattle dung and/or dung beetle on the diversity and ecological guild of topsoil fungal assemblages on a post-coal mining reclaimed site was investigated. Three experimental treatments namely: dung plus dung beetles (dung + beetle), dung only (dung-only), and no dung, no dung beetle (control) was established on 4-m² subplots on a ≥16-year-old post-coal mining reclamation area. Following a 17-month experimental period, topsoil fungal assemblage was determined using next-generation sequencing of fungal ribosomal internally transcribed spacer 2 region. The highest operational taxonomic unit (OTU) richness and Shannon-Wiener diversity were obtained in the dung-only treatment while the lowest OTU richness and diversity was obtained in control treatment. Although not statistically significant, a trend towards lower OTU richness in the topsoil was observed with the addition of dung beetle compared to the only dung treatment. In multivariate space, fungal communities were differentiated between dung-only and control, suggesting the influence of dung addition. Overall, dung addition primarily improved topsoil fungal diversity and the abundance of beneficial plant-fungal symbionts important for nutrient mobilisation in the soil, with a trend towards lower diversity observed with dung beetle addition suggesting possible vertical displacement of organic matter and ecologically relevant fungal species to deeper soil depths.

1. Introduction

The restoration of ecosystem services to either pre-mining or specific land-use capabilities such as farming, grazing, wilderness, or wetlands is the goal of a land reclamation effort (Coaltech Research Association, 2018; Tanner and Möhr-Swart, 2007). A common reclamation approach in South Africa is the replacement of overburden material, topsoil and re-establishment of plant cover comprising a mixture of grass species (Coaltech Research Association, 2018; Tanner and Möhr-Swart, 2007). Soils in such reconstructed lands are anthropic and characterised by a mix of different soil horizons, shallow cover topsoil depth and high compaction that ultimately hampers land productivity (Mentis, 2020; Morgenthal, 2003; Schladweiler et al., 2005).

Several local recommendations have been proffered to minimise

such problems on reclamation lands. Such recommendations include, proper preservation of excavated topsoil, replacement of sufficient amount of topsoil to an adequate depth, minimising the use of heavy machinery to reduce compaction and the use of biofertilizers to augment loss of microbial diversity (Coaltech Research Association, 2018; Ezeokoli, 2020; Tanner and Möhr-Swart, 2007). The application of animal dung and dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) has also been suggested as a potential solution to alleviate some of these problems (Badenhorst et al., 2018; Dabrowski et al., 2019). For instance, Dabrowski et al. (2019) demonstrated that in highly compacted post-coal mining reclaimed lands, three species of dung beetle tunnelled into soils beyond a maximum penetration resistance of 5000 kPa. Similarly, Brown et al. (2010) reported that following a 48-hour of beetle activity, bulk density in the topsoil (0–10 cm) horizon of a heavily

Abbreviations: CCA, canonical correspondence analysis; ITS2, internally transcribed spacer 2; OTUs, operational taxonomic units; PCoA, principal coordinate analysis; PERMANOVA, permutational multivariate analysis of variance; PERMDISP, permutational multivariate analysis of homogenous dispersion.

* Corresponding author at: Unit For Environmental Sciences and Management, North-West University, Potchefstroom, Private bag X6001, 2520, South Africa.

E-mail address: ezeokoliobinna@gmail.com (O.T. Ezeokoli).

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grazed land was significantly reduced and such reduction was sustained for up to six months. Dung is widely used as a soil ameliorant in agroecosystems due to its nutrient content that supports the growth of diverse soil biota, including microorganisms and insects such as dung beetles (Frank et al., 2017). On the other hand, dung beetles, being coprophagous, increase dung aeration and pulverisation, facilitate the transport of dung-derived nitrogen into the soil, and influence uptake of dung-derived nitrogen by plants, microbial ammonification, nitrification, herbage growth, and pathogen suppression (Brown et al., 2010; Dabrowski et al., 2019; Hea et al., 2005; Nervo et al., 2017; Nichols et al., 2008).

The decomposition and mobilisation of plant nutrients by soil biota is a multispecies-driven process that involves a systematic interplay of roles between soil microfauna (e.g., microorganism) and macrofauna (e.g., dung beetles) (Ezeokoli, 2020; Ezeokoli et al., 2021; Gastauer et al., 2018; Slade et al., 2016). This interplay is embodied in the different trophic levels comprising the complex food webs, which dictate energy flow within the soil ecosystem (Taylor and Sinsabaugh, 2015). Knowledge of the impact of dung beetle and dung application on soil microbial communities and functions could advance the design of a post-mining soil reclamation protocol, which alleviates compaction problems and facilitates improved nutrient mobilisation and availability on reclaimed lands (Badenhorst et al., 2018). The objective of this study was to elucidate the influence of dung and dung beetle addition on the topsoil fungal diversity in a post-coal mining reclamation land. We hypothesised that dung beetle activity and dung addition positively affect the diversity, structure, and subsequently, the potential ecological roles of fungal communities in soils of a post-coal mining reclamation land.

2. Materials and methods

2.1. Study site

The study site was a reclaimed area of an opencast coal mine located in the coal-rich high veld of the Emalahleni, Mpumalanga Province, South Africa (24°0′–27°30′ S, 28°15′–32°5′ E). The study area is part of the grassland biome of South Africa and is characterised as a semi-arid climate, with annual precipitation of approximately 760 mm (Climate-Data.Org, n.d.). Reclamation on the study site was done at least 15 years prior, following the protocol briefly described in the introduction of this paper (also see Tanner and Möhr-Swart, 2007). Briefly, stored stockpiles of topsoil, subsoil and overburden material (carbonaceous materials and rocks) were replaced in reverse order of excavation. The site was then fertilized and seeded with a mixture of rehabilitation grass species, mostly *Eragrostis tef*, *Eragrostis curvula*, *Digitaria eriantha*, *Cynodon dactylon*, and *Chloris gayana* (Tanner and Möhr-Swart, 2007). At the onset of the study, *Eragrostis tef* was the dominant plant/species on the site and plant (aerial) cover was estimated at 60–80%.

2.2. Experimental plot design

Paracoprid (tunnelling) dung beetles used in this study were captured from farms located adjacent to the reclaimed mined lands using pit fall traps and maintained on cattle dung for two days. The dung beetles collected included *Euoniticellus intermedius* (Reiche, 1849), *Digitonthophagus gazella* (Fabricius, 1787), *Onitis alexis* (Klug, 1835), and *Copris mesacanthus* (Harold, 1878).

The experimental set-up was based on a modified version of the set-up used in Badenhorst et al. (2018). On the reclaimed site, 3 treatments were randomly set up on 4-m² sub-plots in quadruplicate replicates in March 2016. The treatments comprised: (1) cattle dung + dung beetles (hereafter referred to as “dung + beetle”), (2) cattle dung only (hereafter referred to as “dung-only”), and (3) no dung, no dung beetle control (hereafter referred to as “control”). Subplots with dung + beetle and dung-only treatments received 1 kg cattle dung pats per m². This dung application amount correlates with the natural dung deposition rate of

cattle based on Rosenberger et al. (1977), who found that a single wandering cattle in a natural setting will defecate approximately 2.5 kg of dung between 10 and 24 times a day. A total of 340 dung beetles were randomly applied in the dung + beetle treatment. The amount of dung beetle applied was based on observation from a previous study of the study area (Venter, 2018). Approximately the same amount of each species was applied per subplot (i.e., ~85 individuals of each species). The body sizes of the species ranged from 7 mm (*Euoniticellus intermedius*) to 20 mm (*Onitis alexis*). All sub-plots were covered with shade nets (mesh size 1.4 mm × 1.4 mm) to prevent the escape of dung beetles or colonisation by naturally occurring dung beetles. Following dung decomposition, dung beetles and/or dung were applied to the respective subplots on two subsequent occasions in the summer of 2017 (February and April).

2.3. Soil sampling

Soil samples were aseptically collected from each 4-m² experimental plot at the end of September 2017. At this time, cattle dung applied to treatment plots dung-only and dung + beetle had decomposed. Because the goal was to ascertain the impact of dung and dung beetle addition on a broader scale, sampling was largely influenced by the collection of a representative sample rather than directly at the positions where dung pats were placed. Representative soil samples were collected by pooling equal amounts of “soil cores” collected at the four corners and centre of the 4-m² plots. For fungal community analyses, soil samples were aseptically collected from a 0–10 cm depth, immediately placed on ice and stored at –70 °C in the laboratory until processing. Soil samples for analyses of soil physicochemical parameters were collected up to a depth of 15 cm as part of a parallel yet unpublished study and determined using standard methods as previously described (Badenhorst et al., 2018).

2.4. Fungal community and guild analyses

Total soil DNA was extracted using the ZR Soil Microbe DNA kit (Zymo Research, CA, USA) according to the manufacturer's instruction. The fungal internally transcribed spacer 2 (ITS2) region was amplified, sequenced, and analysed in silico as described in Supplementary Text S1. Briefly, fungal ITS2 was sequenced on the Illumina MiSeq (Illumina Inc., San Diego, CA, USA), bioinformatics analysis to determine operational taxonomic units (OTUs) was performed using the Quantitative Insight into Microbial Ecology version 2 (QIIME2; <https://qiime2.org/>) against the UNITE ITS 97% reference (<https://www.arb-silva.de/>). Alpha diversity indices, including OTU richness, Shannon-Wiener diversity index, and dominance (defined as the ratio of individuals belonging to the most abundant species relative to the overall number of members in the community) were determined using either QIIME2 or R software v.4.03 (<https://www.R-project.org>). A Venn diagram to visualise OTU distribution between treatments was constructed using DeepVenn (<http://www.deepvenn.com/>) and functional guild was analysed using FUNGuild (<http://www.stbates.org/guilds/app.php>).

2.5. Statistical analysis

All statistical analyses were performed using R software. The Kruskal-Wallis H test was used to test the difference in alpha diversity between treatments, with pairwise posthoc comparisons performed based on Bonferroni-adjusted *p* values. Relationship between alpha diversity and soil properties was determined using the nonparametric Spearman's rank correlation.

For beta diversity analyses of fungal communities, relative OTU counts were first log₁₀-transformed [$\log_{10}(x) + 1$, where $x > 0$] and differentiation among treatments in multivariate space determined based on Bray-Curtis dissimilarities. Significant differences in multivariate space was determined using the permutational multivariate

Table 1
Selected soil physicochemical properties of the different treatment subplots.

	Treatments		
	Dung-only	Dung + beetle	Control
pH (KCl)	4.29 ± 0.21 ^{ab}	4.86 ± 0.36 ^a	4.24 ± 0.13 ^b
P (Bray 1) (mg kg ⁻¹)	85.31 ± 14.25	104.16 ± 38.84	67.05 ± 20.22
K (mg kg ⁻¹)	95.26 ± 16.14 ^b	266.17 ± 41.33 ^a	111.39 ± 25.66 ^b
Na (mg kg ⁻¹)	11.61 ± 3.20 ^{ab}	36.76 ± 19.59 ^a	10.12 ± 2.01 ^b
Ca (mg kg ⁻¹)	298.51 ± 92.62	392.62 ± 85.4	355.82 ± 85.97
Mg (mg kg ⁻¹)	49.04 ± 8.47 ^b	180.58 ± 61.23 ^a	59.35 ± 19.11 ^b
Exchangeable acidity (%)	16.80 ± 11.47 ^a	1.27 ± 2.54 ^b	17.1 ± 3.50 ^{ab}
S-value (cmol(+) kg ⁻¹)	2.19 ± 0.57 ^b	4.28 ± 0.81 ^a	2.59 ± 0.66 ^{ab}
Bulk density (g cm ⁻³)	0.98 ± 0.10	0.96 ± 0.06	0.94 ± 0.06
CEC (cmol(+) kg ⁻¹)	5.49 ± 1.03	5.60 ± 0.94	5.76 ± 0.74
Clay (%)	19.00 ± 3.46	17.00 ± 2.58	25.5 ± 12.37
Silt (%)	17.04 ± 1.95	17.42 ± 0.64	26.57 ± 21.67
Sand (%)	63.97 ± 2.70 ^a	65.58 ± 2.17 ^a	47.93 ± 10.71 ^b
Texture	Sandy loam	Sandy loam	Sandy clay loam

Values are mean ± standard deviation of quadruplicate ($N = 4$) samples. Values with different superscript letters across rows are significantly different (Kruskal-Wallis Bonferroni-adjusted $p < 0.05$). CEC, cation exchange capacity; S-value, the sum of the exchangeable cations (Ca, Mg, Na and K); dung + beetle, dung + dung beetle; control, no dung, no beetle.

analysis of variance (PERMANOVA). Furthermore, the influence of soil physicochemical properties on fungal communities were determined by a canonical correspondence analysis (CCA) using a stepwise model. Constraining variables with multicollinearity (variance inflation factor > 10) were excluded from the final CCA model.

To determine fungal phylotypes (genus level) which were

significantly different between treatment plots, differential abundance testing was performed using DESeq (Anders and Huber, 2010) implemented in the web-based MicrobiomeAnalyst (Dhariwal et al., 2017); low abundance phylotypes with fewer than 10% abundance and 10% variation in the interquartile range were eliminated from the dataset.

3. Results

3.1. Physicochemical properties

Across all treatments, the soils were acidic (pH, 4.22–4.86) and had a bulk density ranging from 0.94 to 0.98 g·cm⁻³ (Table 1). The textural class was sandy loamy in the dung-only and dung + beetle treatment while the control treatment was sandy clay loam. Significant ($p < 0.05$) differences in physicochemical properties were only observed for pH, K, Na, Mg, exchangeable acidity, S-value (sum of the exchangeable cations Ca, Mg, Na and K) and proportion of sand particles (Table 1); pH, K, Na, Mg, and proportion of sand particles were lower in the control treatment compared with the dung-only and/or dung + beetle treatments.

3.2. Diversity and compositional differentiation of fungal OTUs

Following ITS2 extraction, quality filtering, merging, chimera and singleton removal, 411,099 high-quality sequences were obtained across all datasets ($N = 12$; 3 treatments each in quadruplicate replicates). Normalization of sequence reads to 15,600 sequences per sample was sufficient to capture the diversity in all samples (Supplementary Fig. S1). A total of 1004 OTUs were obtained across all samples, with the highest mean OTU richness and Shannon-Wiener index of diversity (H') obtained in the dung-only treatment (observed OTUs = 312; $H' = 6.6$), and the lowest mean OTU richness and H' obtained in the control treatment (observed OTUs = 190; $H' = 5.8$; Fig. 1a and b). OTU richness

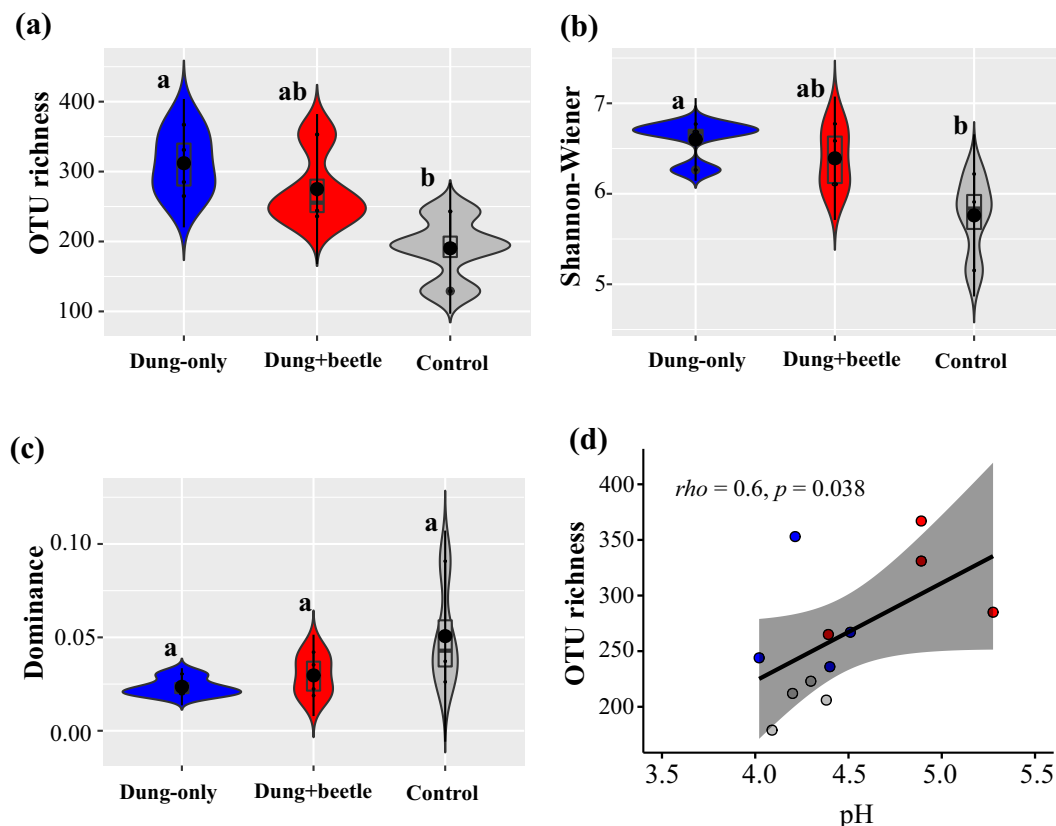


Fig. 1. Alpha diversity metrics of fungal assemblage. (a) Observed OTUs. (b) Shannon-Wiener index (c) Dominance. (d) Relationship between observed OTUs and pH. Shaded circles in the violin plots (a–c) are mean values. Control, no dung, no dung beetle; Dung + beetle, dung and dung beetle.

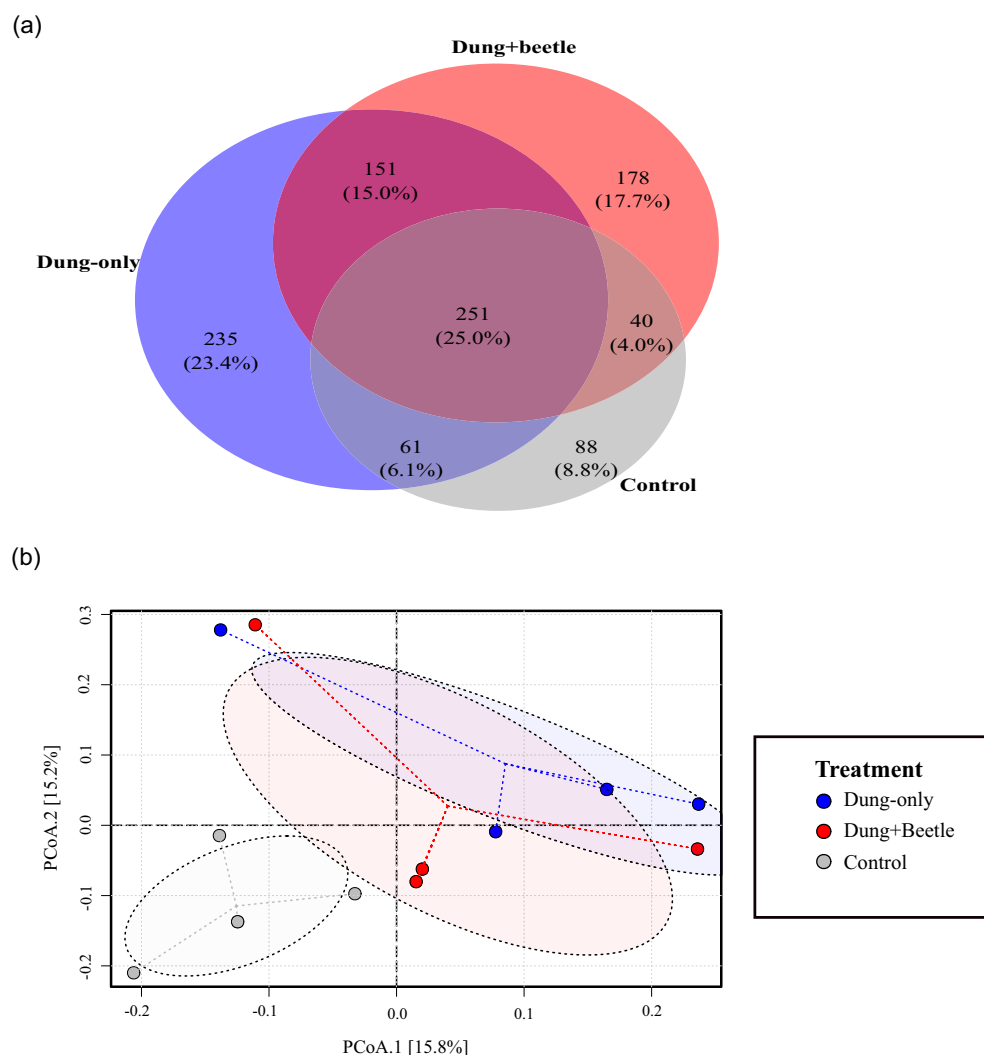


Fig. 2. OTU distribution (A) and PCoA plot of the Bray-Curtis dissimilarities between treatments (B). Venn diagram was plotted using the sum of OTUs present in at least one of the four replicates of each treatment. PCoA plots are based on the presence or absence of fungal OTUs. Dotted lines in the PCoA plot show the distance of every sample to its group centroids in multivariate space, while ellipses show 95% confidence intervals (standard error) in multivariate space around group centroids. Differences in multivariate space between treatments are not significant (PERMANOVA, $R^2 = 20.8\%$, $p = 0.0954$; PERMDISP, $p = 0.871$). Control, no dung, no dung beetle; Dung + beetle, dung and dung beetle; PCoA, principal coordinate analysis.

and H' was significantly different ($p < 0.05$) only between dung-only and control treatments (Fig. 1a and b); no differences were observed between the dung-only and dung + beetle treatments. No differences in the dominance index were observed among all treatments (Fig. 1c). OTU richness across treatments was positively correlated with soil pH (Spearman's $\rho = 0.62$; Fig. 1d).

Evaluation of OTU distribution showed that 251 of the 1004 OTUs (25.0%) were common among all three treatments; 235 OTUs (23.4%) were unique to dung-only treatment, 178 OTUs (17.7%) were unique to dung + beetle treatment, and 88 OTUs (8.8%) were unique to the control treatment (Fig. 2a). Principal coordinate analysis showed that the fungal community of dung-only treatment was differentiated (at 95% confidence interval) from the control treatment; no clear differentiation between the dung-only and dung + beetle treatments was observed (Fig. 2b). However, PERMANOVA analysis showed no significant differences between treatments ($R^2 = 20.88\%$, $p = 0.095$).

3.3. Relatively dominant and differential abundant phylotypes

Taxonomically, OTUs were classifiable to 7 phyla; Ascomycota was the most relatively abundant for each treatment (dung-only, 77%; dung + beetle, 80%; control, 74%), followed by Basidiomycota (dung-only, 11%; dung + beetle, 10%; control, 6%; Fig. 3a). Mortierellomycota was highest in the control treatment (5%) compared to dung-only (3%) and dung + beetle (2%) treatments. Glomeromycota relative abundance was

higher in the dung-only treatment (2%) compared to other treatments (dung + beetle, 1%; control, <1%; Fig. 3a).

A total of 287 OTUs were obtained at the genus taxonomic level from all samples. Of these, 31 genus-level phylotypes had at least 1% mean relative abundance in at least one treatment (Supplementary Table S1). Across all treatment, *Didymella* was the most abundant phylotype (dung-only, 8.71%; dung + beetle, 7.34%; control, 11.95%). *Westerdykella* was relatively least abundant in the control treatment (0.01%) compared to the dung-only (1.12%) and dung + beetle (0.99%) treatment. *Umbelopsis* was relatively more abundant in the control treatment (1.19%) compared to other treatments (dung-only, 0.52%; dung + beetle, 0.48%; Supplementary Table S1).

Based on differential abundance testing, 11 genus-level phylotypes had relative abundances that differed significantly across the treatments (false discovery rate-adjusted $p < 0.05$) among treatments with only seven of these classifiable at the genus taxonomic rank (Fig. 3b). Based on the CCA model, none of the measured soil physicochemical properties significantly influenced the fungal OTU-level communities across all treatments ($p > 0.05$; Supplementary Fig. S2).

3.4. Ecological guild of fungal communities

A total of 525 OTUs (52.3%) were matched to guilds. The trophic mode of fungal communities was mostly saprophytic with the highest relative abundance of saprophytes observed in dung + beetle (40.3%)

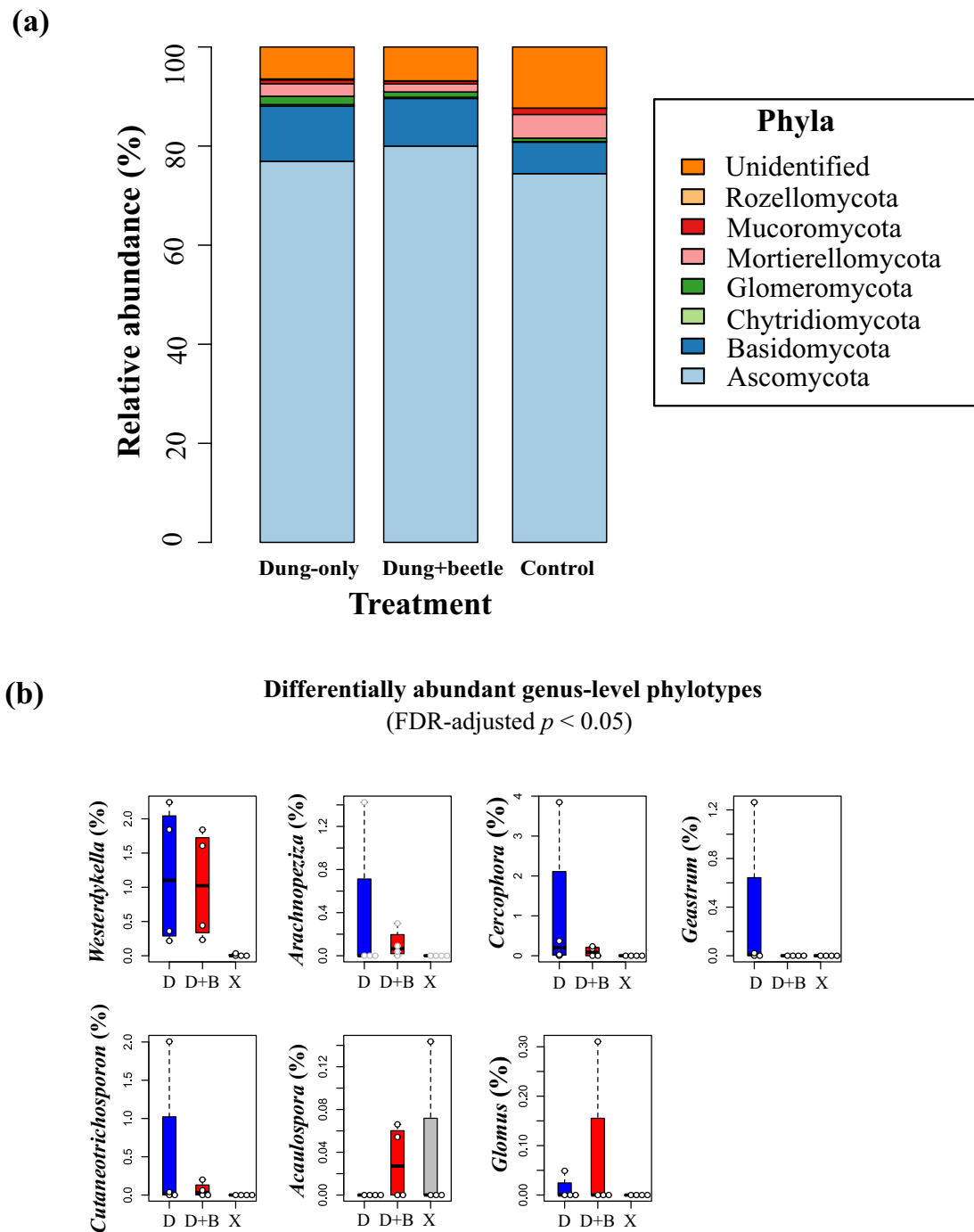


Fig. 3. Relative abundance of fungal assemblage phylotypes. (a) Phylum. (b) Differentially abundant genera (FDR-adjusted $p < 0.05$) computed using DESeq. Control (or “X”), no dung, no dung beetle; D, dung-only; Dung + beetle (or “D+B”), dung and dung beetle; FDR, false discovery rate.

compared with other treatments (Fig. 4a). The highest relative abundances of pathotrophs and symbiotrophs were observed in the dung-only treatment (31.8% and 3.9%, respectively). Of the symbiotrophs, arbuscular mycorrhizal fungi were the most abundant across all three treatments; 84.7% in dung-only treatment, 79.3% in the dung + beetle treatment, and 53.4% in the control treatment (Fig. 4b). The proportion of dung saprophytes were highest in the dung-only treatment (6.2%) followed by the dung + beetle treatment (1.0%) and control treatment (0%; Fig. 4c). The pathotrophs in all treatments were mostly plant pathogens; 95.5% in dung-only treatment, 99.2% in the dung + beetle treatment, and 99.7% in the control treatment. Animal pathogens were highest in the dung-only treatment (3.9%) and least in the control

treatment (0%; Fig. 4d).

4. Discussion

Novel investigations into soil management practices that are capable of improving soil quality and health could advance the design of sustainable post-mining soil reclamation and management protocols. In this study, the highest fungal richness and diversity was observed in the dung-only treatment, a clear differentiation (at 95% confidence interval) was obtained between dung-only and control treatments, and the trophic mode of fungal communities was mostly saprophytic.

The highest fungal richness and diversity observed in the dung-only

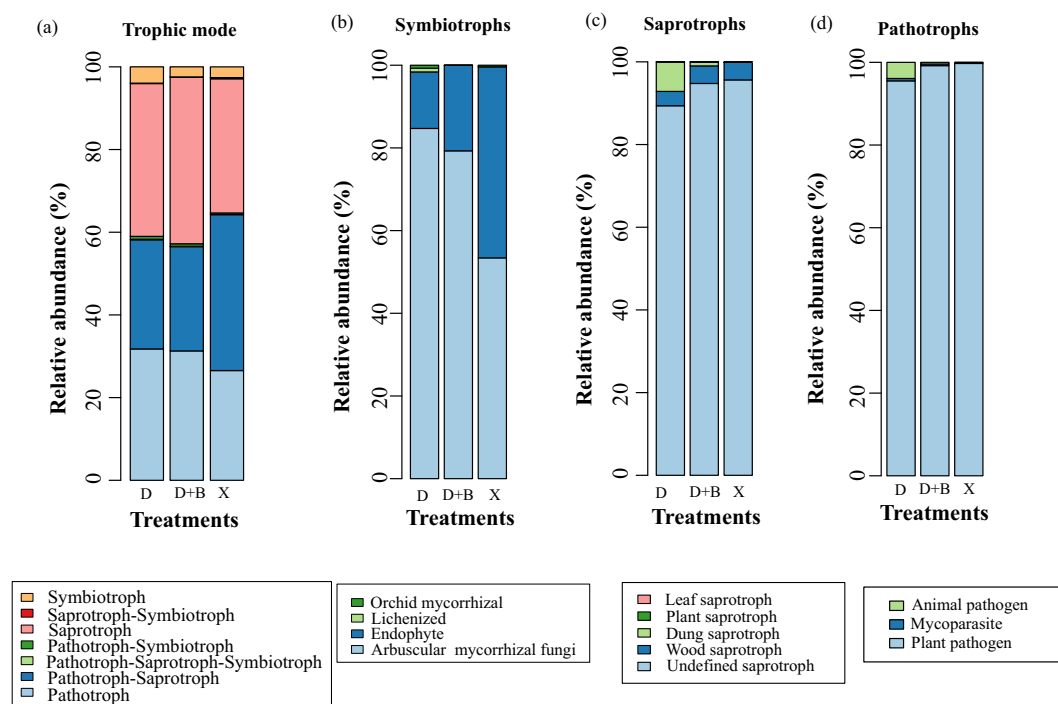


Fig. 4. Trophic mode and the ecological guild of fungal communities. (a) Trophic mode. (b) Symbiotrophs. (c) Saprotrophs. (d) Pathotrophs. Ecological guilds are based on confidence rankings of “highly probable” and “probable”. Control (or “X”), no dung, no dung beetle; D, dung only; Dung + beetle (or “D+B”), dung and dung beetle.

treatment suggests that dung addition improved the topsoil (10 cm) fungal richness and diversity. However, a trend for lower fungal diversity in the 0–10 cm depth was observed with the addition of dung beetles (i.e., dung + beetle treatment), although not significant. This observation may indicate vertical displacement of dung and dung-associated fungi into lower depths with dung beetle activity, but further studies are needed to evaluate this. Dabrowski et al. (2019) showed that *Digitonthophagus gazella* dung beetle tunnelled up to a depth between 18.67 and 20.3 cm in highly compacted soils on adjacent reclaimed land. In addition, the reduced fungal diversity may be linked to dung beetles feeding on the fungal biomass as a source of nitrogen (Holter and Scholtz, 2007; Frank et al., 2017); however, dung beetle activity has been reported to accelerate fungal growth in dung and dung balls (Yokoyama et al., 1991). The lower fungal richness and diversity in the control treatment is a function of the lack of dung inputs; reclamation lands typically have low organic matter contents to support soil biota and thus require ameliorants to improve soil health and quality for crop cultivation (Badenhorst et al., 2018; Ezeokoli et al., 2020).

The observed differentiation in multivariate space between dung-only and control treatments as well as between dung + beetle and control treatments, suggest that the soil fungal assemblage is influenced primarily by dung addition, followed by dung beetle activity. Different studies have shown that dung influence soil ecosystem processes and metabolic activities of macrofauna and microflora assemblages (Frank et al., 2017; Shang et al., 2020). Such influence is in part due to the strong link between microbial assemblages and soil chemical changes (Rousk et al., 2010), as evident with our observation of a significant ($p < 0.05$) positive correlation between pH and OTU richness.

Shifts observed in the fungal composition between treatments (Supplementary Table S1) may have ramifications for ecological restoration since microorganism play vital ecological roles. For example, the genera *Coniochaeta* and *Chaetomium*, which were highest in the dung-only treatment, are important drivers in carbon and nitrogen cycling (Challacombe et al., 2019). Similarly, the Glomeromycota phylum, which was most abundant in the dung-only treatment compared to other

treatments, comprise beneficial plant root symbionts, arbuscular mycorrhizal fungi, which are cosmopolitan in soil ecosystems and colonise 80% of terrestrial plants (Tedersoo et al., 2014); they form a specialised structure called arbuscules that are effective in nutrient exchange and offer various benefits to the host plants, including nutrient and water uptake, thereby contributing as major drivers of the terrestrial ecosystem's primary productivity (Ezeokoli et al., 2020; Taylor et al., 2015). Notably, species of the genus *Didymella*, which are known to be pathogenic (Aveskamp et al., 2008), had the lowest relative abundance in the dung + beetle treatment, suggesting that dung beetle activity contributed to their reduction; dung beetle activity has been shown to reduce pathogen incidence (Jones et al., 2015; Nichols et al., 2008).

The restriction of the experiment to a single site, a lack of a blocking variable to the experimental design, a lack of assessment of the fungi within the dung applied to the soils, and non-evaluation of the impact of the repetitive application of dung pats to the study outcomes all constitute limitations of this study. It is important to note that the results may have been influenced by plot selection bias and the well-known heterogeneity of the soil environment; the impact of these variables needs to be further investigated before strong recommendations can be made in this regard. In addition, further studies investigating the fungal assemblage at deeper depths (i.e., beyond 10 cm) as well as studies which will relate dung broad ball depths information with the soil fungal profile at the maximum dung beetle burrowing depth may help provide deeper insights.

In conclusion, this study showed that the application of dung as a land management practice significantly improved the diversity of topsoil fungal species on a post-mining reclamation land. Although the addition and activity of dung beetle may improve soil hydrological properties as shown in previous studies, the trend towards lower fungal diversity on addition of dung beetles compared with the dung-only treatment may suggest that extensive activity of dung beetles could lead to vertical displacement of dung organic matter and dung-associated beneficial fungal species to deeper depths. As such, important trade-offs between improved soil hydrological properties and

nutrient availability in the topsoil could be established by increasing the rate of dung addition to compensate for those removed by dung beetles. Overall, dung and dung beetle-combined application, if well managed, may be a viable and sustainable way of improving soil quality and fungal biodiversity on post-mining reclamation sites.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2023.104804>.

CRediT authorship contribution statement

RAA and JD conceptualised this study. JB and JD setup and maintained the experimental site. JB and OTE generated physicochemical and high-throughput sequencing data, respectively. OTE performed the bioinformatics analysis and wrote the first draft of the manuscript together with RAA, AR and JB. RAA, JD and CHS supervised the study and reviewed the manuscript drafts. All authors read and approved the final draft of the manuscript.

Declaration of competing interest

The authors have no conflict of interests to declare.

Data availability

Paired-end sequence reads from this study are available in the Sequence Read Archives of the National Centre for Biotechnological Information under the accession number PRJNA684427 (<http://www.ncbi.nlm.nih.gov/bioproject/684427>).

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