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Biological conversion of low-grade coal discard to a humic substance-enriched soil-like material

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Abstract A mutualistic relationship between grasses, coal-degrading fungi, and arbuscular mycorrhizal fungi was proposed to account for the phyto-biodegradation of coal discard. In this study pot trial experiments were carried out to confirm transformation of the carbonaceous substrate, in the presence of a suite of coal degrading fungi and arbuscular mycorrhizal fungi, into a humic-enriched soil-like material in the *Cynodon dactylon*/coal rhizosphere. The results show that after 47 weeks of *C. dactylon* growth on coal discard the concentration of humics increased from (62.9 ± 1.5) to (112.1 ± 5.4) mg/kg. Substrate humic acid-like substance concentration positively correlated ($r^2 = 0.95$) with accumulation of above ground *C. dactylon* biomass. FTIR spectroscopy of the extracted humic-like substances confirmed both product identity and increased oxidation of the coal discard substrate. Substrate ash content and electrical conductivity declined coincident with an increase in humic acid-like substance concentration, which together reduced the intensity of acidity in the *C. dactylon*/coal discard rhizosphere. These observations support the proposal that biological oxidative degradation of coal discard leads to increased humic-like substance concentration and formation of a soil-like material. Results have profound implications for use of coal discard as an organic substrate to replace topsoil in phyto-bioremediation strategies for sustainable large-scale rehabilitation of coal discard dumps.

Keywords Biodegradation · *Cynodon dactylon* · Humic acid-like substances · *Neosartorya fischeri* · Coal discard

Abbreviations

ABTS	2.6-dimethoxyphenol, 2.2′[azino-*bis*-(3-ethylbonz-thiazoline-6-sulphonic acid) diammonium salt]
ECCN	EBRU Culture Collection Number
FTIR	Fourier transform infrared spectroscopy
HS	Humic acid-like substances
LAC	Laccase
LiP	Lignin peroxidase
MnP	Manganese peroxidase
RCBD	Randomized complete block design
TOC	Total organic carbon

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1 Introduction

Coal discard is low calorific residual material generated as a by-product of coal mining and processing and is not considered marketable by industry. The proliferation of unsightly and potentially hazardous coal discard dumps and tailings dams in particular, requires that the mining industry find new ways of treating waste. Indeed, several strategies do exist and include; (1) re-mining of dumped coal discard; (2) allowing coal discard dumps to be ameliorated naturally or by accelerating the process through re-

vegetation; and (3) disposing of coal discard in landfills (Nemerow and Agardy 1998). The disadvantage of such strategies is the capital investment and time required to achieve successful rehabilitation and an inability to guarantee elimination of this carbonaceous waste.

Phytoremediation is widely practiced by the industry and directed towards restoration of land that has been disturbed by coal mining or transformed into discard dumps (Leung et al. 2007; Maiti 2007; Juwarkar and Jambhulkar 2008). Establishment of vegetation cover has been an effective and socially acceptable implementation strategy for rehabilitation of discard dumps in South Africa (Cowan et al. 2016). However, current practice relies heavily on the import of topsoil to stabilize disturbed land, clad coal discard dumps, and support re-vegetation. Topsoil in South Africa is a scarce resource and is particularly limiting on land that has been mined. Consequently, topsoil must be sourced and transported to sites for rehabilitation. Furthermore, about 58% of South African soils contain less than 0.5% organic carbon and only 4% contain more than 2% organic carbon (Du Preez et al. 2011a, b). Thus, rapid mineralization of imported topsoil together with excessive cost of this practice has necessitated the search for an alternative rehabilitation technology.

The concept of fungal depolymerization and solubilization of low rank coal is not novel and has been investigated and reported (Cohen and Gabriele 1982; Ralph and Catcheside 1997; Hofrichter and Fritsche 1996, 1997; Hofrichter et al. 1999; Willmann and Fakoussa 1997a, b; Igbinigie et al. 2008; Sekhohola et al. 2013, 2014). However, most of the work on coal bio-solubilization is from laboratory based studies where processes are constrained and do not necessarily simulate what happens in the field. Consequently coal bio-solubilization has not been fully explored as a rehabilitation methodology for use at commercial scale. Even so, several studies clearly show that direct contact between metabolically active biocatalysts and substrate coal facilitates degradation of this recalcitrant material (Mukasa-Mugerwa et al. 2010; Tripathi et al. 2010; Klein et al. 2013; Oboirien et al. 2013; Sekhohola et al. 2014; Hazrin-Chong et al. 2014).

Recent studies in our laboratory on the biodegradation of coal discard within the rhizosphere of *C. dactylon* resulted in the development of a novel beneficiation strategy for rehabilitation of coal discard dumps (Cowan et al. 2016). *C. dactylon* was observed growing intermittently on dumps of bituminous hard coal discard that had not undergone any form of rehabilitation (Igbinigie et al. 2008, 2010). Further studies revealed the potential of a geologically weathered coal to sustain plant growth while investigation of the root zone indicated the presence of a diverse fungal flora. An extensive screening exercise followed and resulted in the isolation of among other fungi, the ascomycete *Neosartorya fischeri* (Igbinigie et al. 2008). This fungus was shown to actively degrade coal in a perfusion fixed-bed bioreactor, used to simulate the coal discard dump environment (Igbinigie et al. 2008), in the *C. dactylon*/coal rhizosphere (Igbinigie et al. 2010; Mukasa-Mugerwa et al. 2010), and in vitro (Sekhohola et al. 2014). Based on these findings, an integrated model was proposed to describe some of the interactions in the rhizosphere that transform coal discard to a humic acid-like substance (HS) enriched medium capable of supporting establishment of a healthy vegetation (Sekhohola et al. 2013). This model proposed, in no particular order, that; (1) grasses exude organic acids via the roots into the rhizosphere; (2) arbuscular mycorrhizas in association with roots utilize these organic acids to facilitate uptake of nutrients by the plant; (3) other microorganisms that may inhabit the coal environment also produce and/or utilize organic acids to degrade coal; and, (4) complex organics in the resulting humus material are further broken down by coal-degrading microorganisms to support plant growth. The present work further explores the mutualistic relationship between grasses, coal-degrading fungi, and arbuscular mycorrhizal fungi in the phyto-biodegradation of coal discard. A set of pot trial experiments was carried out to test the hypothesis that HS concentration in the *C. dactylon*/coal rhizosphere is indeed increased by the presence of laccase (LAC; EC 1.10.3.2)- and manganese peroxidase (MnP; EC 1.11.1.13)-positive coal degrading fungi. More specifically, these experiments were carried out to confirm transformation of the carbonaceous substrate into an HS-enriched soil-like material capable of supporting vegetation.

2 Materials and methods

2.1 Fungal culture maintenance and preparation of inoculum

A consortium of six coal degrading fungi from the EBRU culture collection was prepared using strains ECCN178, ECCN187, ECCN224, ECCN226, ECCN241 and ECCN243, which had been stored as mycelial plugs on 2.5% potato dextrose agar (PDA) in 50% glycerol (v/v) at −20 °C. *Neosartorya fischeri* strain ECCN84, isolated from different coal environments around South Africa (Igbinigie et al. 2008, 2010) and strains PPRI5328 (*Phanerocheate chrysosporium*) and PPRI4835 (*Coriolus versicolor*), obtained from the Agricultural Research Council (ARC, South Africa), were re-cultured on plates of 2.5% PDA at 26 °C. All fungi used as inoculum tested positive for production of the ligninolytic enzymes LAC and MnP on PDA with added 2.6-dimethoxy phenol, 2.2′[azino-*bis*-(3-ethylbonzthiazoline-6-sulphonic acid) diammonium salt] (ABTS) at 37 °C (Table S1) using the

protocols described by Pointing (1999). The fungal inoculum was prepared by washing and collecting spores from fungal mats (5 plates per strain) as described elsewhere (Mukasa-Mugerwa et al. 2010).

2.2 Substrate preparation

Coal discard and topsoil were sourced from mines in the Emalahleni coalfields, Mpumalanga Province, South Africa. The mine topsoil used in this study is classified as a technic (Witbank) form of South African soil (Fey 2010) belonging to the anthropic group that is equivalent to the WRB soil group comprised of anthrosols and technosols (IUSS Working Group 2006). Coal discard, typical of low-grade waste material from the Emalahleni mining region, comprised a mixture of roof coal and discard (calorific value of 8–10 MJ/kg) from the void after removal of high-grade coal. Mine topsoil and coal discard were milled and sieved to obtain a particle size of between 1 and 2 mm and where specified, combined 3:1 (v/v) to yield a homogeneous "mixed" substrate.

2.3 Characterization of mine topsoil, coal discard and coal discard/topsoil substrates

The basic physical and chemical characteristics of mine topsoil, the prepared coal discard/topsoil mix and coal discard were determined and are presented in Table 1. After oven drying (50 °C for 24 h), water holding capacity, electrical conductivity (EC), and pH were determined using deionised water in 1:1 and 1:5 substrate:solution ratios respectively according to the protocols described by Rayment and Higginson (1992). For each substrate, a saturated paste was prepared by adding de-ionized water to 100 g dry material and after 24 h the weight determined. Water holding capacity was calculated as percentage moisture content of the saturated paste where; % water holding capacity = (weight after water addition/weight of dry sample) × 100. For EC and pH, 100 mL de-ionized water was added to 20 g dry material which was mixed for 1 h and settled for 30 min. Conductivity and pH were measured at 25 °C respectively without disturbing the settled solids using an EC Testr 11 Dual range 68X 546 501 m (Eutech Instruments, Singapore) and a Hanna HI8 424 pH meter (Hanna Instruments, Woonsocket, RI).

Total organic carbon (TOC) was determined by direct combustion of 10 mg aliquots of material in a combustion furnace at 680 °C using an Apollo 9000 Total Organic Carbon Analyser fitted with a boat sampler (Model 183 Teledyne Tekmar, Mason, OH). For measurement of ash content, pre-weighed aliquots of dry substrate were placed in crucibles and heated at 900 °C for 5 h using a Carbolite CE muffle furnace (Carbolite, Hope Valley, UK), the mass of the residue determined, and ash content expressed as mass%. The HS concentration of each prepared substrate was determined as described below and the Fourier transform infrared (FTIR) spectra are presented in Fig. S1.

Table 1 Physicochemical properties of the prepared mine topsoil, coal discard/topsoil (3:1, v/v), and coal discard substrates

Parameter	Topsoil	Coal discard/topsoil (3:1, v/v)	Coal discard
Water holding capacity (%)	26.4 ± 0.2b	32.6 ± 0.2b	33.6 ± 0.2a
pH	6.4 ± 0.1a	4.3 ± 0.1b	4.2 ± 0.0c
Electrical conductivity (mS/m)	27.7 ± 0.6c	56.0 ± 2.0a	35.7 ± 2.5b
Ash (mass%)	93.7 ± 0.1a	71.2 ± 0.1b	55.5 ± 0.3c
Humic acid-like substances (mg/kg)	0.7 ± 0.2c	54.7 ± 2.8b	62.9 ± 1.5a
Total organic carbon (mg/kg)	1.4 ± 0.3c	7.2 ± 1.1b	10.3 ± 2.0a

Data are the mean ± SE of six determinations. The mass fraction of ash for each substrate was determined, multiplied by 100, and expressed as mass percentage. For each parameter, values followed by different letters are significantly different ($P \leq 0.001$)

2.4 Plant material, cultivation and sampling

To black plastic potting bags was added a layer of gravel and each bag then filled with 2 kg of substrate to which was applied 10 g arbuscular mycorrhizal fungi (Mycoroot Supreme, Mycoroot Pty Ltd., Grahamstown, South Africa) and 10 mL of coal-degrading fungal suspension (0.031 ± 0.002 g spore biomass mL^{-1}). The content of each potting bag was thoroughly mixed and irrigated. Control pots were filled with untreated coal discard. Subsamples of substrate from each pot were retained for characterization and for determination of initial HS levels. Seed of perennial grass *Cynodon dactylon* L. (0.06 g ≈ 200 seeds) was sown and lightly covered with moistened substrate (untreated coal discard control pots were not seeded), irrigated every 2 days for the first 8 weeks, and thereafter twice weekly using rainwater. Potting bags (6 per treatment) were arranged in a randomized complete block design (RCBD) in a polycarbonate-covered tunnel (Ulma Agricola, Spain) and growth allowed to proceed under ambient conditions. After 23 weeks (duplicate experiments initiated in February 2010), the above ground plant material was harvested from half of the potting bags and aliquots of the topsoil, coal discard/topsoil (3:1, v/v), and coal discard substrates sampled using a PVC auger (30 × 2 cm) inserted to a depth of 10 cm and the cored samples oven dried (50 °C × 24 h) prior to HS analysis. The residual topsoil, coal discard/topsoil, and coal discard substrate in each potting bag was thoroughly mixed and allowed to lie fallow for a further 24 weeks. Growth in the remaining potting bags was

allowed to proceed for a further 24 weeks under the conditions already described after which the substrate and plant material was harvested and analysed. Cumulative yield of *C. dactylon* was determined per treatment after freeze drying of the harvested above ground biomass.

2.5 Humic acid-like substance extraction and analysis

Humic acid-like substances (HS) were extracted and analysed using a method adapted from that described by Janoš (2003). To 2.5 g of oven dried material was added 0.1 M NaOH to a final volume of 100 mL and the mixture extracted on a rotary shaker for 24 h, centrifuged (Eppendorf bench top centrifuge 5810 R, Drücken, Germany) at 1252×*g* for 90 min at 10 °C, and the supernatant and pellet separated. The pH of the supernatant was adjusted to <1 using HCl and the HS precipitated by centrifugation and the pellet re-suspended in 0.1 M NaOH. HS in the re-suspended pellet were quantified by interpolation from standard curves for Leonardite-derived humic acids and peat-derived fulvic acids (purchased from the International Humic Substance Society, St. Paul, MN) after determining the absorbance using a Thermo Spectronic Aquamate UV–Vis scanning spectrophotometer (ThermoFisher Scientific, Waltham, MA) at 240 and 250 nm respectively.

Functional groups in HS extracts from substrate both before and after cultivation of *C. dactylon* were determined by FTIR spectroscopy using a PerkinElmer Spectrum 100 instrument (PerkinElmer, Waltham, MA) equipped with an attenuated total reflectance (ATR) accessory eliminating the need for mixing of samples with KBr. The ATR accessory, fitted with a diamond top-plate, has spectral range of 25,000–100 cm^{-1} and refractive index of 2.4 and 2.01 μ depth of penetration.

2.6 Statistical analyses

All data are representative of two independent experiments and are presented as the mean ± SE. Where specified data were analysed using either one-way analysis of variance (ANOVA) or linear regression (SigmaPlot Ver. 11; Systat Software, Inc., San Jose, CA) and significant differences between measurements for each treatment determined using the Holm–Sidak method ($P \leq 0.001$).

3 Results

3.1 Humic acid-like substance production in the rhizosphere: soilification

Following cultivation of *C. dactylon* on coal discard and coal discard/topsoil (3:1, v/v) in the presence of a suite of coal-degrading and arbuscular mycorrhizal fungi for 23 and 47 weeks respectively, a quantifiable and significant increase in concentration of extractable HS was observed (Fig. 1).

Substrate concentration of HS increased within the rhizosphere of *C. dactylon* cultivated on either coal discard or coal discard/topsoil (3:1, v/v) and this increase was quite dramatic after 47 weeks (Fig. 1). Although the concentration of HS (expressed as % change) had increased by 8.6% and 25.6% respectively in coal discard and coal discard/topsoil (3:1, v/v) after 23 weeks (Fig. 1a), one-way ANOVA revealed that % change values were not significant ($P = 0.077$). However, after 47 weeks of grass growth, % change in HS was significantly different ($P \leq 0.001$) for the coal discard and coal discard/topsoil (3:1, v/v) substrates at 77.8% and 59.5% respectively (Fig. 1b). The HS concentration of topsoil declined by approximately 40% at 23 weeks presumably due to rapid utilization and/or leaching and thereafter, remained unchanged (Fig. 1a, b). A very slight increase in HS content of the untreated coal discard control indicated that natural weathering of this substrate was not a major contributor to the soilification process during the course of this experiment.

Cumulative yield of *C. dactylon* from the inoculated topsoil, coal discard/topsoil (3:1, v/v) and coal discard substrates was determined after freeze drying of the harvested above ground biomass (Fig. 2). Biomass yield was greatest for the coal discard substrate, followed by the coal discard/topsoil (3:1, v/v) mix (Fig. 2a). Inoculated mine topsoil yielded the least above ground biomass.

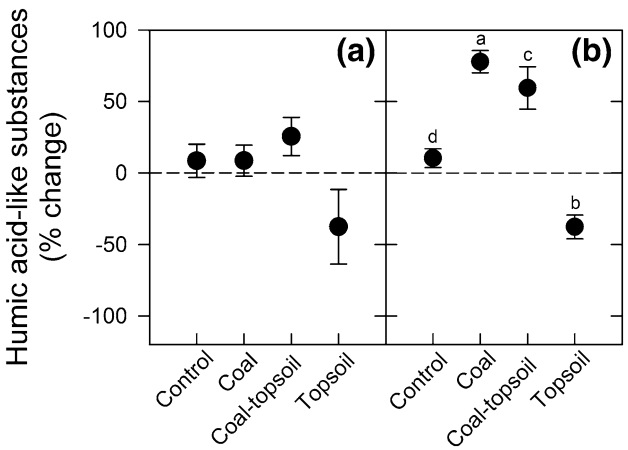

Fig. 1 Biodegradation induced changes in humic-like substance concentration of the coal discard, coal discard/topsoil (3:1, v/v) and topsoil substrates. Humic acid-like substances were extracted from untreated coal discard (control) and coal discard, coal discard/topsoil and topsoil 23 (**a**) and 47 (**b**) weeks after inoculation with mycorrhizal fungi, coal degrading fungi, and cultivation of *Cynodon dactylon*. Data are presented as mean ± SE ($n = 6$) and bars with different letters are significantly different ($P \leq 0.001$)

Interestingly, accumulation of above ground *C. dactylon* biomass correlated positively ($r^2 = 0.95$) with substrate HS concentration (Fig. 2b). Together, these results support the proposed mutualism between coal degrading fungi, arbuscular mycorrhizal fungi, and plants in the biotransformation of coal discard into a soil-like material. Indeed and as shown in Table 2, analysis of material from the *C. dactylon* rhizosphere after 47 weeks revealed that increased HS concentration coincided with reduced ash content, a decline in EC, and a rise in substrate pH (compare results in Tables 1, 2).

3.2 FTIR analysis of humic acid-like substances

A representative FTIR spectrum of the HS extracted from coal discard before inoculation with coal-degrading and mycorrhizal fungi and cultivation of *C. dactylon* compared to the spectrum of HS extracted after 47 weeks of treatment is shown in Fig. 3. Prior to fungal inoculation and cultivation of *C. dactylon*, the HS extracted from coal discard showed the presence of alkenes and methylene moieties (Fig. 3a). By comparison, the HS extracted 47 weeks after cultivation on the inoculated substrates were highly oxidized (Fig. 3b). The data revealed a broad band from 1800 to 3600 cm^{-1} indicative of the abundance of O–H and COOH groups coupled with peaks at 1702 and 1219 cm^{-1} confirming C=O stretching of various carbonyl groups including COOH and C–O stretching and O–H bending of COOH groups respectively. These findings confirm that the soil-like residue in the rhizosphere of *C. dactylon* cultivated on coal discard inoculated with coal degrading fungi and mycorrhizal fungi arises as a consequence of increased biological weathering.

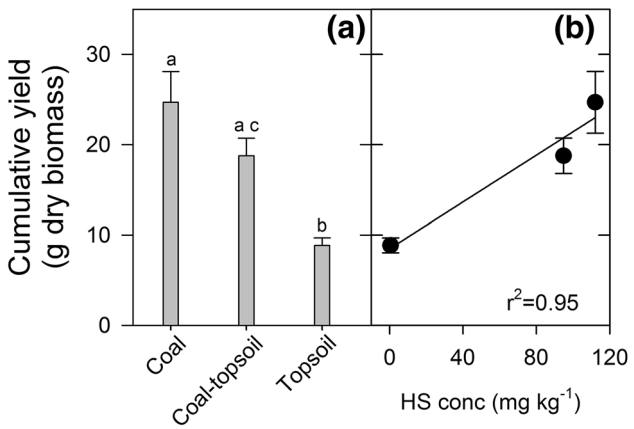

Fig. 2 Cumulative yield of above ground *Cynodon dactylon* biomass after cultivation on either coal discard, coal discard/topsoil (3:1, v/v) and mine topsoil inoculated with mycorrhizal and coal degrading fungi. The relationship between cumulative yield (**a**) and substrate HS concentration was determined by regression analysis (**b**). Data are presented as mean ± SE ($n = 6$) and *bars* with different letters are significantly different ($P \leq 0.001$)

Table 2 Physicochemical properties of the soil-like material from the rhizosphere of *Cynodon dactylon* cultivated on mine topsoil, coal discard/topsoil (3:1, v/v), and coal discard for 47 weeks in the presence of coal degrading fungi and mycorrhizal fungi

Parameter	Topsoil	Coal discard/topsoil (3:1, v/v)	Coal discard
Water holding capacity (%)	12.6 ± 2.3b	37.2 ± 2.7a	26.8 ± 0.7c
pH	6.2 ± 0.2a	5.2 ± 0.2bc	4.9 ± 0.2b
Electrical conductivity (mS/m)	12.7 ± 3.0	18.7 ± 3.3	20.5 ± 4.1
Ash (mass%)	95.6 ± 0.2a	57.8 ± 3.9c	43.2 ± 4.3b
Humic acid-like substances (mg/kg)	0.5 ± 0.1b	94.9 ± 8.4ac	112.1 ± 5.4a

Data are the mean ± SE of six determinations. The mass fraction of ash for each substrate was determined, multiplied by 100, and expressed as mass percentage. For each parameter, values followed by different letters are significantly different ($P \leq 0.001$)

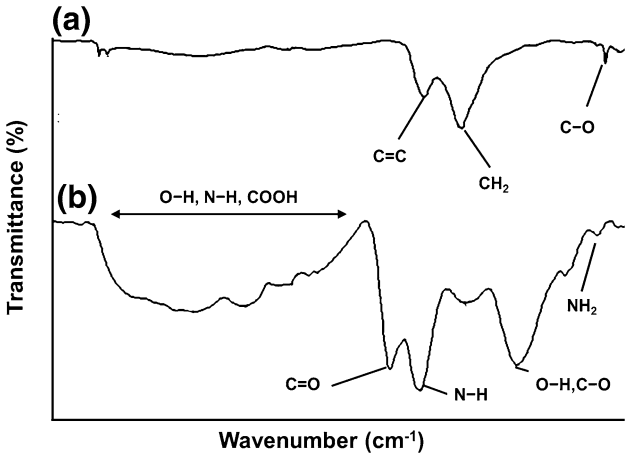

Fig. 3 Fourier transform infrared spectra of humic acid-like substances extracted from coal discard before (**a**) and 47 weeks after (**b**) inoculation with coal-degrading fungi, mycorrhizal fungi and cultivation *Cynodon dactylon*

4 Discussion

In the present study, the mutualistic relationship between grasses, coal degrading fungi, and arbuscular mycorrhizal fungi in the phyto-biodegradation of coal discard was studied. Results show that *C. dactylon* in the presence of coal degrading and arbuscular mycorrhizal fungi facilitated the biotransformation of coal discard into an HS-enriched

soil-like material. FTIR spectroscopy of the extracted HS confirmed both product identity and increased oxidation of the coal discard substrate. Substrate HS concentration correlated positively with yield of above ground biomass. In addition, physicochemical characterization confirmed that the material formed in the *C. dactylon* rhizosphere had reduced ash content, lower soluble salts concentration (i.e. EC), and reduced acidity. These results thus lend substantive support to the occurrence of a mutualistic oxidative degradation and/or biological weathering of coal discard by coal-degrading fungi in the *C. dactylon* rhizosphere.

It has been argued that the establishment of a healthy and productive vegetation cover can ameliorate most of the detrimental effects of stockpiled coal discard on the environment, including the human component. Cladding of discard dumps with perennial vegetation is assumed to bind and stabilize the substrate thereby reducing dust, reducing the ingress of oxygen and percolation of water to minimize erosion and, both acidic leachate formation and sediment loss (Truter et al. 2009). To this end, discard dumps and opencast spoil are conventionally treated with high levels of lime to negate any acid generating potential of the substrate, covered in a layer of topsoil (usually 50–100 cm), and re-vegetated using selected annual and perennial species. This approach brings with it specific problems that include acidification of the cover soil due to capillary rise of acid leachate formed gradually from the underlying discard. As a consequence, re-vegetation is often sporadic, substrate compaction ensues, and the cover vegetation eventually dies. This, coupled with the approach of sourcing and acquiring topsoil from remote locations, results in a rehabilitation strategy that is far from sustainable and outcomes that are less than desirable Cowan et al. (2016).

Early studies demonstrated the ability of ligninolytic fungi to degrade low rank coals in vitro through production of extracellular oxidative enzymes, predominantly lignin peroxidase (LiP), MnP and LAC (Hofrichter et al. 1999; Fakoussa and Hofrichter 1999; Zavarzina et al. 2004). The coal-degrading fungal strains used in the present study tested positive for the ligninolytic enzymes (i.e. LAC and MnP), which have been reported in several studies to be responsible for fungal breakdown of coal (Hofrichter et al. 1999; Zavarzina et al. 2004; Grinhut et al. 2007; Tao et al. 2010). Furthermore, studies in our laboratory on the fungal metabolism of coal discard confirmed that coal degradation by *Neosartorya fischeri* ECCN84, isolated from coal discard dumps, occurs coincident with elevated extracellular LAC (Sekhohola et al. 2014). It was previously postulated that fungal interaction with coal particles results in bioconversion of this substrate into a mixture of heterogeneous macromolecules that are mainly humic acids (Cohen and Gabriele 1982; Henning et al. 1997; Catcheside and Ralph 1999; Dong et al. 2006). Indeed, microorganisms isolated from coalmines have excellent potential for coal solubilization (Malik et al. 2017) and have been shown to catalyze release of complex organic moieties including polyaromatic hydrocarbons (Haider et al. 2015).

Soil humic substances support plant growth and appear to do so by stimulating development of soil microbial populations and enhancing uptake of essential nutrients and trace elements (Piccolo et al. 1993, 1997). This leads to enhanced plant growth, performance and ultimately increased quality and yield (van de Venter et al. 1991; Piccolo et al. 1993; Traversa et al. 2014). Indeed, FTIR analysis of the HS extracted from the residue after inoculation of coal discard and cultivation of *C. dactylon* revealed that these were similar to naturally occurring humic acids from compost (Traversa et al. 2014). Furthermore, the FTIR spectra of coal discard post biodegradation resembled that of the topsoil used in this study. The increase in oxygen-containing functional groups in the resultant soil-like material was taken to indicate onset of a fungal-plant catalysed soilification, which supported seed germination and plant growth (Fig. S2).

5 Conclusions

This study has confirmed that *C. dactylon* in the presence of coal degrading and arbuscular mycorrhizal fungi facilitates the biotransformation of coal discard into an HS-enriched soil-like material in the rhizosphere. Increased substrate HS concentration was linearly associated with increased recovery of above ground biomass, indicative of positive covariance between these two variables. The ability of the transformed substrate to support re-vegetation is in accordance with the proposal that in situ associations and mutualism, between plants and ligninolytic and mycorrhizal fungi, drive the phyto-biodegradation of coal discard. The potential to generate in situ a soil-like material from coal discard for use in rehabilitation of land disturbed by coal mining to reduce dependency on topsoil has profound implications. Future research will endeavour to optimise this process by evaluating associations between the coal degrading fungal consortium used in this study, arbuscular mycorrhizal fungi, and other plant species including mixed populations.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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