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# Arbuscular mycorrhiza status of gold and uranium tailings and surrounding soils of South Africa's deep level gold mines. II. Infectivity

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**Abstract**

An AMF infectivity study and spore viability assessment was performed on substrata obtained from gold and uranium mine tailings dumps ('slimes dams') in the North West and Free State provinces of South Africa. Three slimes dams in each region were categorized as recently vegetated (RV), old vegetated (OV) and never vegetated (NV), and dams then divided into five zones based on elevation above ground level, steepness and broad chemical differences. Rhizosphere samples were collected from two of three plant species common to all sites; *Eragrostis curvula*, *Atriplex semibaccata* and *Cynodon dactylon,* as well as from bare areas, in order to allow comparisons across all site categories because of the rarity of the grasses on the lower slope of NV dams. Infectivity was determined by the mean infection percentage method from a bioassay of the substrata using *Eragrostis curvula* cv Ermelo as a host plant. There was no difference in total infectivity between North West and Free State substrata, but within regions, there were differences in infectivity between rehabilitation ages, between zones, and between rhizosphere and bare areas. Toepaddock substrata and veld soil produced the highest total infection levels overall. On both dams and veld, total arbuscular levels differed between rhizosphere and bare substrata, and the percentage of arbuscules (max. 15.4%) and vesicles (max. 22.0%) as a proportion of total infection structures was low. A low correlation between infectivity and total spore numbers was also found. Spore numbers and the numbers of viable spores increased with zone down the slimes dams to the veld, and also differed within zones between rhizosphere and bare substrata with marked interactive effects. Substratum organic matter (SOM) levels differed between regions, and between zones within the North West region increasing with distance down the slopes to the veld, and were strongly correlated with total spore numbers. Substratum pH values and most AMF parameters were positively correlated in the order of RV > OV > NV dams, indicating that natural colonization of acidic NV sites by AMF is at very low rates, and that AMF colonizing RV slopes are not surviving the transition from RV to OV, with the associated increase in acidity, conductivity and decline in plant cover. Substratum conductivity differed between zones in both regions, with minor interaction between region and zone, and was negatively correlated with pH, AMF infectivity and total spore numbers. Our findings demonstrate that the ameliorant effects of liming and irrigation on substratum pH and conductivity are short-lived, but despite the physico-chemical constraints, a significant measurable AMF inoculum potential does exist on all substrata. Amelioration of tailings with organic matter and use of acid and salt-tolerant AMF would be expected to contribute to more persistent AMF communities and vegetation on gold and uranium slimes dams.


*Keywords:* Gold and uranium mine rehabilitation; Inoculum potential; Plant growth; Soil microflora

## 1. Introduction

An aim of many rehabilitation programmes is to accelerate the dominance of plant populations by perennial, mycotrophic species in order to create more stable plant communities. Increased mycorrhizal activity is a feature of succession (Allen, 1991; Smith et al., 1998) and this relationship can be used as a management tool for reclamation of disturbed and polluted sites. For example, on land polluted by lead ore mining in Slovenia, Regvar et al. (2006) identified two grasses, *Calamgrostis varia* and *Sesleria caerulea*, as the most suitable

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candidates to drive secondary succession based on their mycorrhizal status and ability to tolerate polluted sites. Disturbed sites and fertilized sites in general tend to attract ruderal non-mycotrophic or facultatively mycotrophic plants, which preclude the introduction of mycorrhizal propagules (Reeves et al., 1979). In contrast, on toxic (polluted) soils and gold slimes dams the more common naturally-colonizing plants are not ruderals, but comprise slower-growing perennial species (Weiersbye et al., 2006). However, the high-input grassing regimes used on gold and uranium slimes dams in South Africa actively introduce ruderal species and exotic pasture grass species. The low mycotrophy of these plant forms would be further exacerbated by the high fertilization regimes, inhibiting the development of suitable mycorrhizal symbioses (i.e. those that are tolerant to worsening substrate conditions in the long-term) (Eason et al., 1999). A survey of a chronosequence of slimes dams found that less than 5 percent of ruderal species introduced during grassing persisted once fertilization and irrigation ceased, although there was a high diversity of naturally-colonizing perennial plant species (Weiersbye et al., 2006).

A parallel survey of the arbuscular mycorrhiza (AM) status of plants on gold and uranium slimes dams in the North West province was undertaken by Straker et al. (2007). Five indicator host plant species were sampled from recently vegetated (RV), old vegetated (OV) and never vegetated (NV) slimes dams, and from different zones (the top, lower slopes, retaining wall and toepaddocks) as well as the surrounding natural soils ('veld'). Root AM-colonization parameters (total, hyphal, vesicular, arbuscular) and AM spore status were assessed. All plants sampled were mycorrhizal, and there was an impoverished AM status on slimes dam slopes compared with slimes dam flat areas and natural soils, together with a trend in AM status of RV > OV > NV. The study concluded that the flat, polluted soils around slimes dams and the flatter areas of OV and NV slimes dams were sources of more acid-tolerant AM fungal (AMF) inoculum.

The Straker et al. (2007) survey represented single observations of plant and substratum AM status in late summer and did not provide a reliable indication of the inoculum potential (i.e. infectivity) of the substrata. Moreover, inferring AM status from spore counts is notoriously problematic due to the clumped, uneven distribution of spores which necessitates very high replication for the distribution to approach normality (Walker et al., 1982). For these reasons, it was essential to corroborate the observations of Straker et al. (2007) by performing infectivity assays on substrata from the same sites and along the same gradients, and in addition, to replicate the study in two different mining regions. Infectivity assays are a more accurate measure of the AMF inoculum potential of a substrate since they incorporate the infective ability of all propagules: spores, soil mycelium, root fragments, auxiliary cells and sporocarps (Read et al., 1976). The aim of this study was therefore to undertake infectivity assays and relate these to physico-chemical characteristics of the substrata, so that informed decisions can be made on augmenting AMF to aid slimes dam rehabilitation, either by introducing AMF inoculum or inoculated secondary succession plants.

## 2. Materials and methods

### 2.1. Slimes dam sites

The gold and uranium slimes dams sampled were situated in the North West and Free State provinces. Slimes dams in the two provinces were differentiated in terms of climate, soils and vegetation biome and, to a lesser extent, substrate chemistry as previously described (Witkowski and Weiersbye, 1998; Weiersbye et al., 2006; Straker et al., 2007). Three dams in each region were categorized into recently vegetated (RV—fertilization and irrigation had ceased >1.5<3 years previously), old vegetated (OV—fertilization and irrigation had ceased >4<8 years previously) and never vegetated (NV—no record of dams ever having been ameliorated). Each slimes dam was further sub-divided into zones for sampling: the lower berm, lower slope, retaining wall, toepaddocks and surrounding veld (Straker et al., 2007). As far as was possible sampling was conducted on similar aspects.

### 2.2. Sampling of plant rhizosphere and bare areas

Sampling with a soil auger was conducted in late summer 2001, at the same time of year as the surveys of Straker et al. (2007). Samples were collected from the rhizosphere of two host plants common to all dams, *Eragrostis curvula* (Schrad.) Nees and *Atriplex semibaccata* R. Br., but if either of these species was absent within a particular site it was replaced with a rhizosphere sample from *Cynodon dactylon* (L.) Pers., another ubiquitous species. All three species had been observed to be mycotrophic to some extent by Straker et al. (2007). Since vegetation was negligible on the lower slopes of NV dams, bare patches were sampled from all sites to allow for valid comparisons across all categories of dams. In each zone, on each dam, three samples (±1 kg, depth of 0–20 cm) were collected at distances of 100 m along the slope contour for each host and bare patch. Three replicate rhizosphere samples were created by bulking sets of three rhizosphere samples from a sampling point, irrespective of host plant species.

### 2.3. Substratum chemistry

Conductivity, salinity and pH were assessed on fresh substratum samples, and organic matter on air-dried (40 °C) samples, using standard methods (Anderson and Ingram, 1993).

### 2.4. Spore counts and viability determination

Spore density in susbtrata was determined as described by Straker et al. (2007) after spore extraction from 50 g subsamples. Spore viability was assessed using the 2,3,5-triphenyl tetrazolium chloride (INT) method of Walley and Germida (1995) for AMF spores, with the following modifications: freshly harvested spores were incubated at 24 °C for 72 h with continuous agitation, after spore walls had been rendered more permeable through 10 s ultrasound. The deposition of red

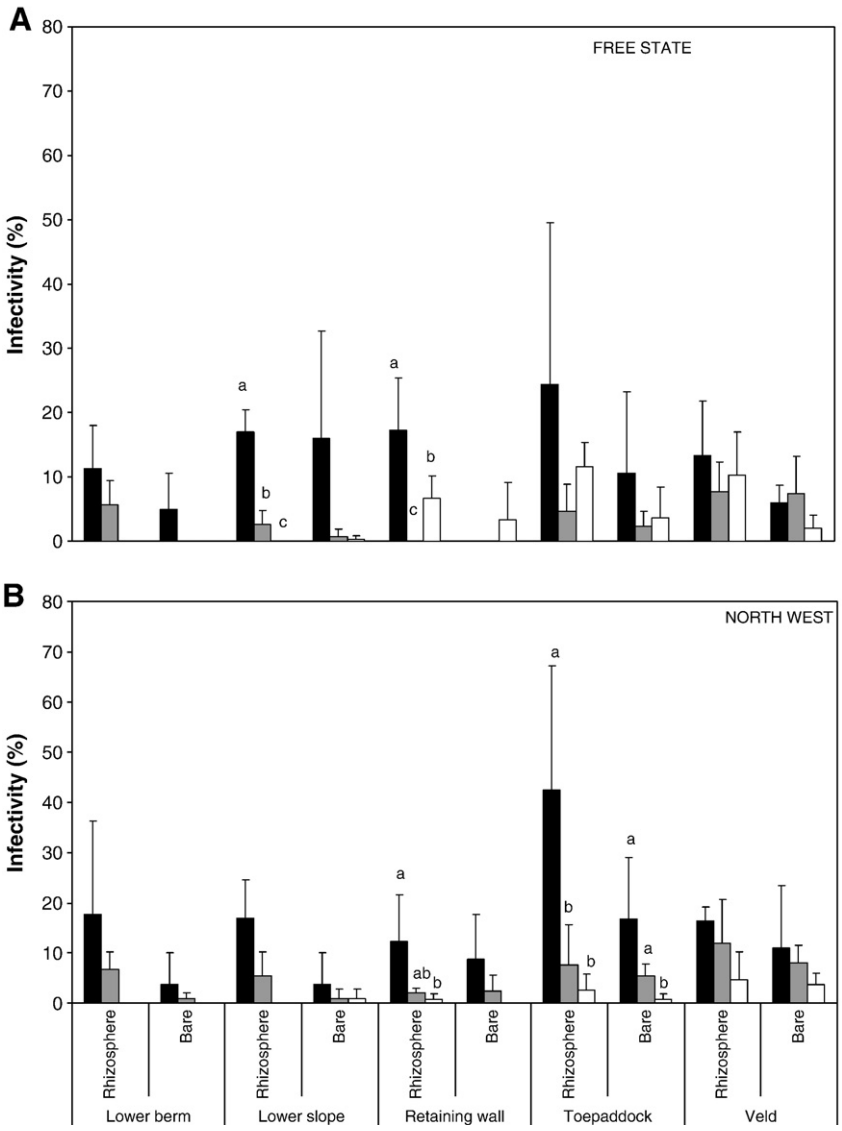


Fig. 1. Total infectivity (sum of vesicles, arbuscules and hyphae) from bioassay of rhizosphere and bare soil derived from five zones in dams of three different rehabilitation ages (RV ■; OV ▩; NV □) in two provinces: (A) Free State, (B) North West. Values are the means of three replicates (±SD) and different letters represent significant differences ( $p<0.050$, L.S.D.) between dams. (RV=recently vegetated; OV=old vegetated; NV=never vegetated).

formazan within spores (indicating respiration) was assessed using transmitted light microscopy (for less-pigmented spores), and fluorescence microscopy (λ excitation=488 nm, λ emission=600–650 nm) to detect formazan fluorescence inside pigmented spores (Weiersbye and Straker, 1997).

### 2.5. Substratum infectivity assay

The infectivity assay used was a modification of the mean infection percentage (MIP) method (Moorman and Reeves, 1979). A dilution series was not created for the substratum samples since infection levels were expected to be low (Straker et al., 2007). However, to improve on the accuracy of the MIP method of just scoring the percentage of root segments showing any sign of infection, or the constraints associated with Most Probable Number (MPN) assays (Adelman and Morton, 1986), the gridline intersect method of assessing infection levels was used. The Moorman and Reeves (1979) assay measures the primary ingress from propagules, as well as secondary spread from established infection units, unlike the infection unit method (Franson and Bethlenfalvay, 1989) which measures primary infection units formed from single entry points prior to commencement of secondary spread. Although the latter method provides a 1:1 correspondence between number of propagules and number of infection points, it is laborious, and was not considered suitable for substrata likely to have a low inoculum potential.

*E. curvula* cv. Ermelo, an indigenous C4 grass cultivar with a fibrous root system, was grown from seed under greenhouse conditions in 9 cm diameter plastic pots containing substratum and autoclaved (121 °C), acid-washed river sand in a ratio of 3:1 ($n=5$ per sample). Pots were randomized and watered daily to

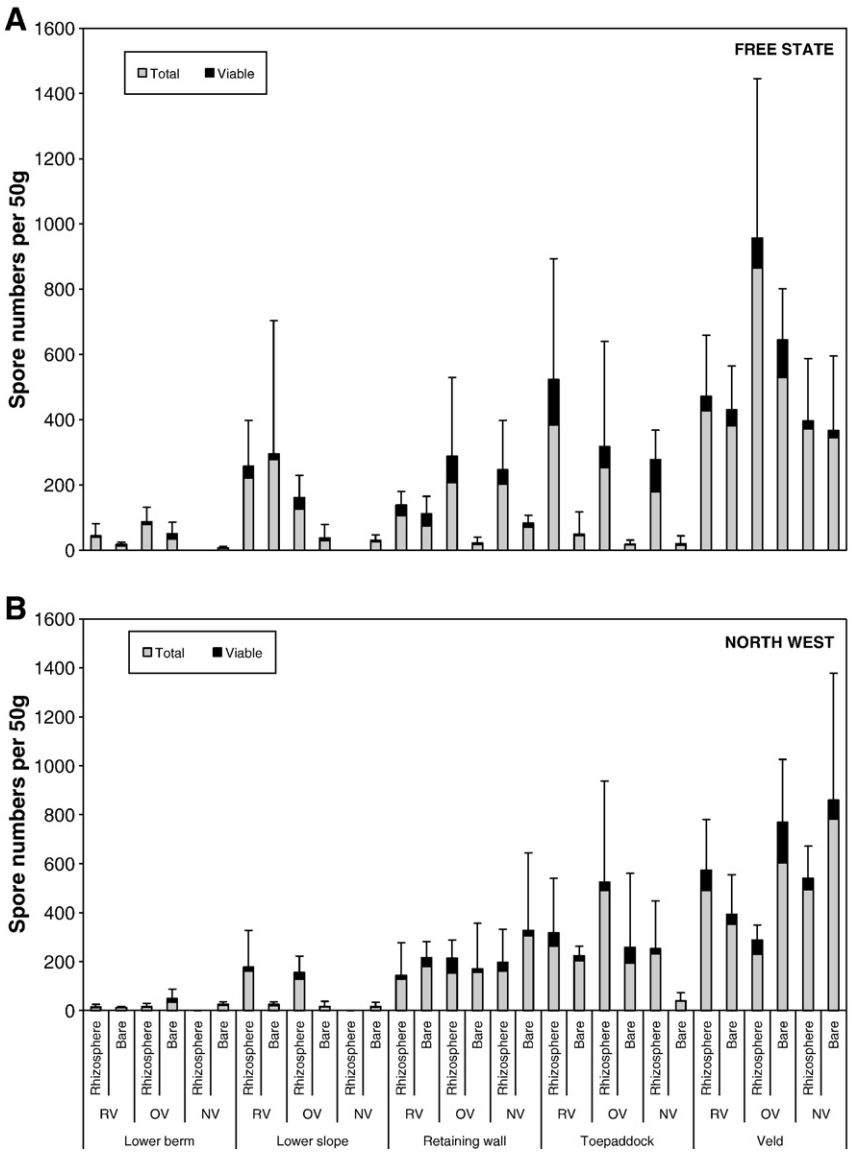


Fig. 2. Total and viable AMF spore numbers in rhizosphere and bare soil derived from five zones in dams of three different rehabilitation ages in two provinces: (A) Free State, (B) North West. Values are the means of three replicates (±SD of total spore numbers). No significant differences were found between dams. (RV = recently vegetated; OV = old vegetated; NV = never vegetated).

80% field capacity. After germination, seedlings were thinned to 10 per pot. Straker et al. (2007) observed that slimes-polluted soils (toepaddock) had a higher AM status than the tailings dams themselves, although not as high as unpolluted soil (veld). Therefore an extra set of pots of *E. curvula* grown in toepaddock substratum ($n = 5$) was destructively harvested every 15 days in order to monitor infection levels. When levels in these plants reached 40–50% (after 50 days), the entire experiment was terminated and all plants harvested.

### 2.6. Assessment of AMF colonization levels

Roots were stored in 45% (v/v) ethanol and cleared and stained using the method of Koske and Gemma (1989), omitting the bleaching step, as the roots were not pigmented. Intraradical status of arbuscules, vesicles, hyphae and total colonization was determined by the modified intersect method of McGonigle et al. (1990) with a count of at least 100 intersections per sample.

### 2.7. Statistical analyses

Data were analyzed using SAS (SAS Institute, 1985) by four-way ANOVA (rhizosphere/bare patches; zone; rehabilitation age; region), three-way ANOVA (rhizosphere/bare patches; zone; rehabilitation age) and one-way ANOVA followed by Fisher's L.S.D test (differences between rehabilitation age of dams). All percentage data were arcsin transformed prior to statistical analysis, and data are presented as untransformed means and standard deviations.

## 3. Results

### 3.1. Total infection levels

There were no differences in total infection values of slimes dams between North West and Free State regions, but there were differences between rehabilitation ages ($p<0.001$), between rhizosphere and bare areas ($p<0.001$), and between zones ($p=0.003$) in both regions, with interactions between region and rehabilitation age ($p=0.010$). In the Free State there was also an interaction between rehabilitation age and zone ($p=0.049$). RV dams exhibited the highest infection levels overall (Fig. 1), with OV dams generally producing higher levels than NV dams, although there were some exceptions for the Free State region. The toepaddocks and veld soil generally produced the highest total infection levels for both regions, although levels were similarly high for the lower slope and the retaining wall of the RV dams of the Free State (Fig. 1).

### 3.2. Individual fungal structures

Hyphal infection levels had similar patterns as for total infection (data not shown), but with no interaction between rehabilitation age and zone in the Free State. Arbuscular levels differed between rhizosphere and bare substrata in both Free State ($p=0.042$) and North West regions ($p=0.005$), with an interaction between rhizosphere/bare and zone for the North West region ($p=0.044$). In the North West region, levels of vesicles differed between rehabilitation ages ($p=0.042$), and for the Free State region there was a three-way interaction between rehabilitation age, rhizosphere/bare and zone ($p=0.031$). The percentage of arbuscules and vesicles as a proportion of the

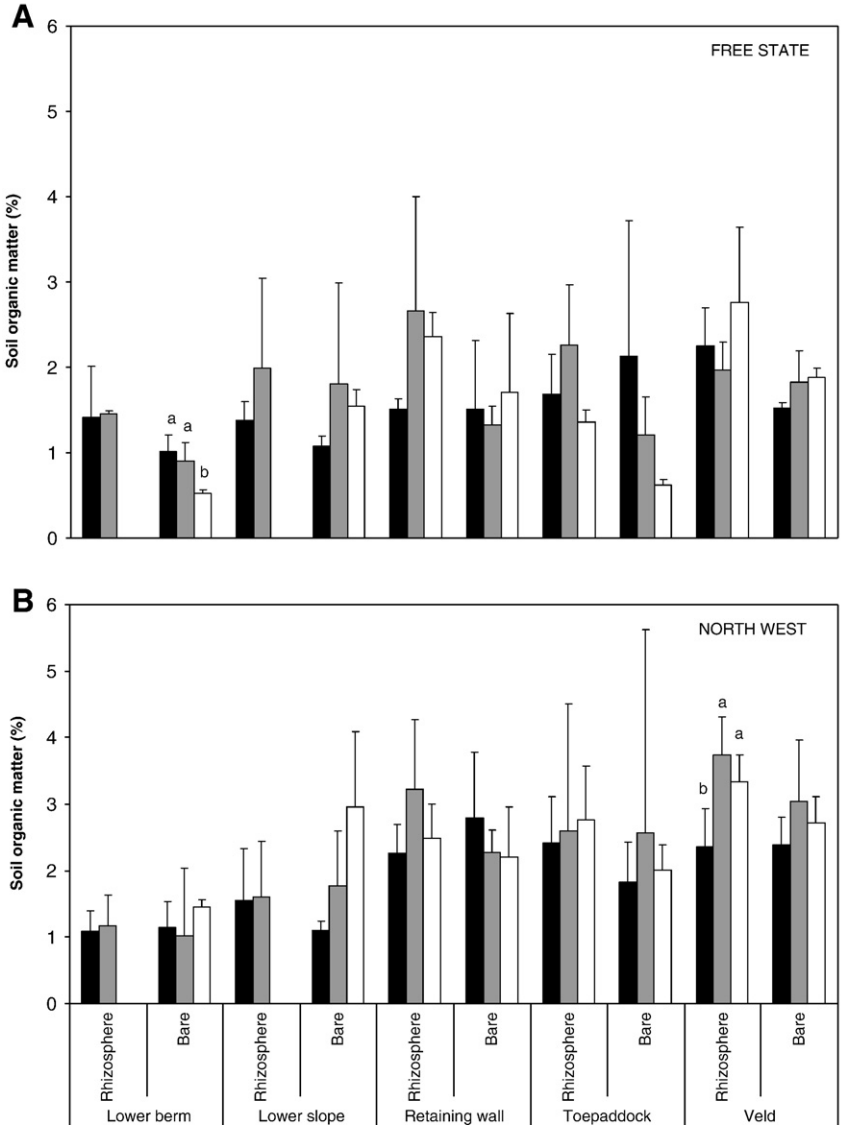


Fig. 3. Soil organic matter (SOM) in samples of rhizosphere and bare soil from five zones in dams of three different rehabilitation ages (RV ■; OV ▩; NV □) in two regions. Values are the means of three replicates (±SD) and different letters represent significant differences ($p<0.05$, L.S.D.) between the dams. (RV = recently vegetated; OV = old vegetated; NV = never vegetated).

total infection structures was low; arbuscules never exceeded 15.4% of the total and vesicles never exceeded 22% of the total. There was a strong relationship between percentage infectivity and percentage hyphae ($r^2=0.984$) as hyphae were the dominant infection structures observed.

### 3.3. Spore numbers

There were differences in spore numbers between rhizosphere and bare substrata ($p<0.001$), and between dam zones ($p<0.001$) within regions, with interactions between rhizosphere/bare and zones for both regions ($p=0.031$) and an interaction between region and zone ($p<0.001$). Spore numbers increased downslope in the order of berm<slope<retaining wall<toepaddocks<veld, being very marked for the North West region (Fig. 2). The differences between rhizosphere and bare areas are marked in the lower slope of both regions, and between in the retaining wall and toepaddocks of the Free State region as well (Fig. 2).

### 3.4. Spore viability

Spore viability differed between slimes dam zones in both regions ($p<0.001$), and between rhizosphere and bare substrata only in the Free State ($p<0.001$) (Fig. 2). There were also interactions between rhizosphere/bare and slimes dam zones for both regions (Free State: $p=0.002$ and North West: $p=0.031$). The distribution pattern for viable spores between zones, and between rhizosphere and bare areas (Fig. 2), was correlated with that of total spore numbers ($r=0.623$; $p<0.001$. $n=168$). Generally, highest viable spore levels were found in the veld and toepaddock zones around OV dams but the rhizosphere of

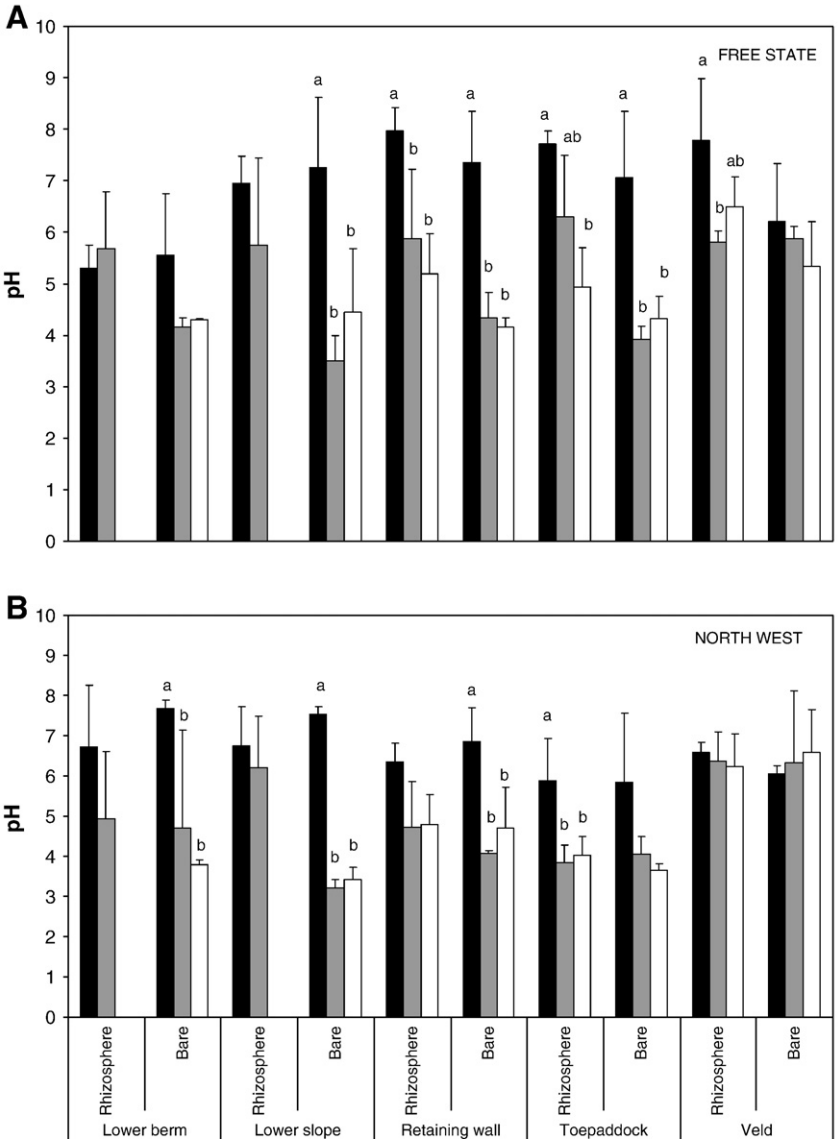


Fig. 4. The pH (aq) of rhizosphere and bare soil derived from five zones in dams of three different rehabilitation ages (RV ■; OV ▩; NV □) in two provinces: (A) Free State, (B) North West. Values are the means of three replicates (±SD) and different letters represent significant differences ($p<0.050$, L.S.D.) between dams. (RV=recently vegetated; OV=old vegetated; NV=never vegetated).

the RV Free State dams also produced high levels. The viable spores numbers as a proportion of total spores in the Free State ranged from 6% to a maximum of 56% (in the rhizosphere of the toepaddock of NV dams), whereas the range in the North West was from 4% to a maximum of 40% (in the bare areas of the lower berm of OV dams) (Fig. 2). No particular zonation of viable spore numbers as a proportion of total spores emerged.

### *3.5. Levels of substratum organic matter (SOM), pH and conductivity*

The concentration of SOM in slimes dams differed between regions ($p<0.001$), and between zones for both regions ($p<0.001$). There were only differences between rhizosphere and bare substrata ($p<0.001$) in the Free State, with interactions between rehabilitation age and zone ($p=0.037$). There was a general increase in SOM from lower berms down slopes to the veld soils, but this trend was more apparent in the North West (Fig. 3). Although SOM levels correlated with total spore numbers ($r=0.418$; $p<0.001$; $n=168$), they were not correlated with total infectivity levels.

The pH of the sampled slimes dams differed between regions ($p=0.012$); between rehabilitation ages ($p<0.001$); between zones ($p<0.001$), and, for the Free State only, between rhizosphere and bare substrata ($p<0.001$) (Fig. 4). There were interactions between region and rehabilitation age ($p<0.001$); between region and zone ($p=0.036$); between region and rhizosphere/bare substrata ($p<0.001$); between rehabilitation age and zone ($p<0.001$), and in the Free State only, between rhizosphere/bare substrata and zone ($p=0.003$). In the Free

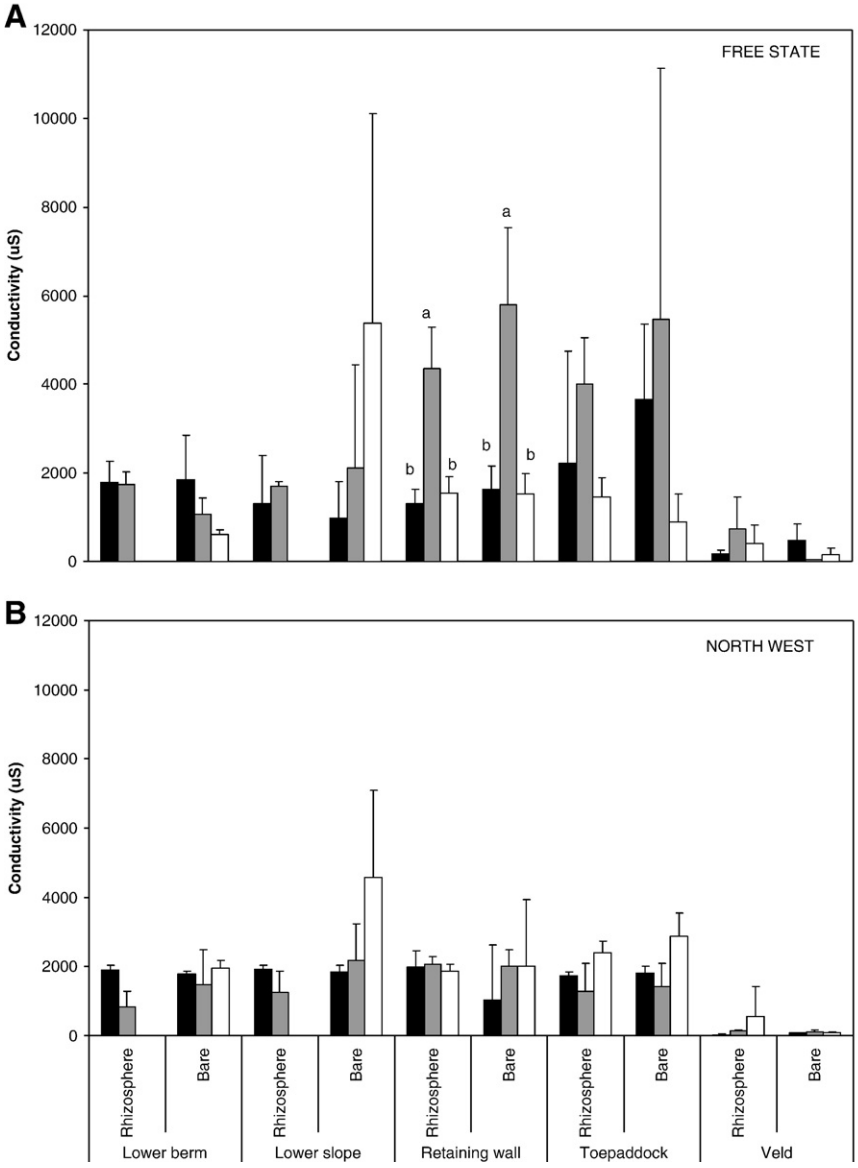


Fig. 5. Conductivity of samples of rhizosphere and bare soil from five zones in dams of three different rehabilitation ages (RV ■; OV ▩; NV □) in two regions. Values are the means of three replicates (±SD) and different letters represent significant differences ($p<0.05$, L.S.D.) between the dams. (RV = recently vegetated; OV = old vegetated; NV = never vegetated).

State, RV dams had pH values of 7–8 in most areas (i.e. the bare areas of the lower slope, in the retaining wall, the toepaddocks and the rhizosphere excluding the lower berm and bare of the veld). These pH values were much higher than those of OV and NV dams, which ranged from 3.5–4.5 (in the bare areas of the lower berm, lower slope, retaining wall and toepaddock) to 5.0–6.0 (in the rhizosphere of the lower berm and slope, retaining wall and toepaddock, and in the veld). Apart from the more alkaline pH of the rhizosphere in the veld surrounding the RV dam, all veld soil pH values in the Free State were between 5.5 and 6.5 (Fig. 4).

The pattern of slimes dam pH in the North West region was similar to that of the Free State, although slimes dam pH values were generally lower, and veld soil pH higher, than in the Free State. High pH levels of 7.0–8.0 were again found on RV dams in the bare areas of the lower berm and slope and retaining wall, with a pH of 6.0 in the toepaddocks of RV dams (Fig. 4). In general, the pH of NV and OV dams in the North West was lower than on RV dams, ranging from 3.5–4.5 on the lower berm and slope to 4.0–5.0 on the retaining wall and toepaddock. The pH of the veld soils around all three dams in the North West was between 6.0 and 7.0 (Fig. 4). For both regions combined ($n=168$), substratum pH was weakly correlated to percent infectivity ($r=0.240$; $p=0.002$), percent hyphae ($r=0.310$; $p<0.001$), total spore numbers ($r=0.192$; $p=0.013$) and number of viable spores ($r=0.230$; $p=0.003$), and inversely, to conductivity ($r=-0.332$; $p<0.001$).

The conductivity of gold slimes dams and polluted soils is due largely to sulphate concentration, and to a lesser extent, to chlorides, and may be a deterministic factor influencing AMF infectivity. Substratum conductivity differed between zones in both regions ($p<0.001$), with interactions between region and zone ($p=0.043$), and between region and rehabilitation age ($p=0.049$). Conductivity was lowest in the veld soils, and highest in the retaining wall and toepaddocks (presumably due to the vertical leaching of salts), with the retaining walls of OV dams of the Free State having higher conductivity values than all other dams and zones (Fig. 5). These differences patterns translated into weak, negative correlations between conductivity levels and infectivity ($r=-0.158$, $p=0.040$) and conductivity levels and total spore numbers ($r=-0.349$, $p<0.001$).

## 4. Discussion

Straker et al. (2007) found that AM parameters were influenced by host species on gold slimes dams, and that there were interactions between host species and broad substratum type. Interactions between host species and rehabilitation age, and zones, also influenced root colonization, with the forbs *Asparagus laricinus* and *Asclepias fruticosa* being more mycotrophic on gold slimes dams, and the grass *C. dactylon* and forb *A. semibaccata* being more mycotrophic in veld soils. However, the latter two species were more common on all sites, and therefore we used their rhizosphere samples for infectivity bioassays, together with that of a ubiquitous grass, *E. curvula*. The infectivity levels, as measured by root colonization, differed between rehabilitation ages, between zones and between rhizosphere and bare areas in both the North West and Free State provinces. In general, RV dams had the highest infectivity levels followed by OV and then NV dams. Thus, the infectivity assay confirms the root colonization patterns observed by Straker et al. (2007). These authors also observed the contribution of arbuscules and vesicles to total root colonization to reach maxima of 3% and 48% respectively, whereas in this study the infectivity assays produced maxima of 15.4% and 21.8% respectively. These two sets of values are not directly comparable as they arise out of different growth situations, but the consistently higher proportion of hyphae and lower proportion of arbuscules may indicate that the AMF populations of gold slimes dams are dominated by species producing Paris type infections (Smith and Read, 1997). Both studies concurred in finding spore densities to be in the order of berm < slope < retaining wall < toepaddocks < veld. Straker et al. (2007) also found strong influences of rehabilitation age on spore density—which may have been a function of their more highly-replicated study within one region. However, temporal differences in reproductive phases can also be affected by local environmental perturbations. For example, exposure to excessively high temperatures can negatively affect sporulation, viability and rates of spore germination (Nadarajah and Nawawi, 1987), while anthropogenic soil acidification is known to greatly change the species composition of AMF in pasture ecosystems (Wang et al., 1993). We found that spore viability as a proportion of total spore numbers was consistently low with little difference between zones or regions, suggesting that constraints to spore viability are common to all sites: these constraints would also include potentially toxic levels of a number of metals, metalloids or radionuclides (Weiersbye et al., 1999).

The weak positive correlations between pH and some AMF parameters (percentage infectivity, percentage hyphae, spore and viable spore numbers) in this study suggest a positive response of AMF to increasing pH which on RV and OV dams would have been created by the addition of agricultural limes and compost during slimes dam amelioration (Witkowski and Weiersbye, 1998). In a container-trial, Siqueira et al. (1984), similarly found that the addition of dolomitic limestone increased root colonization, inoculum potential, spore germination and germ tube growth of two introduced species of AMF, but also that there were significant differences in response between the two species. It appears that the response of AMF to soil pH is highly variable and may be indicative of different ecotypes. For example, some AMF perform poorly in acid soils, whereas others perform poorly after liming, some improve plant growth in limed soils, and some are effective without liming (Entry et al., 2002). In addition, anthropogenic soil acidification can cause declines in inoculum potential (Clapperton and Parkinson, 1990), reduction in root colonization (Hutchinson et al., 1999) and sometimes complete elimination of AMF (Danielson and Visser, 1989). More field experiments are required to understand the growth requirements of acid-tolerant AMF in gold slimes dams.

The trend of increasing SOM with distance down slimes dam slopes towards the veld was also observed by Witkowski and

Weiersbye (1998) and is a result of erosive processes that may also transport AMF propagules downslope. We found there to be a correlation between total spore numbers and SOM. SOM levels are likely to affect AMF through influences on nutrition as well as ameliorating substrate pH and conductivity. M. Forbes and C.J. Straker (unpublished data) demonstrated a strong positive relationship between both total and available phosphorus and SOM in slimes amended with different concentrations of compost. Johnson et al. (1992) have shown that in some environments, soil organic content is equally as important as plant host species in regulating species composition of AMF communities. In addition, Smith et al. (2000) reported that P uptake by external hyphae can vary greatly between fungi and that the efficiency of transport across the symbiotic interface may be a rate-limiting step. Thus, the correlation between spore numbers and SOM observed in this study may reflect shifts in individual AMF species density as a result of these species—different responses to *P*-availability and *P*-availability would be expected to influence subsequent sporulation patterns.

We found there were significant differences in spore numbers between rhizosphere and bare soil samples, especially in the Free State. The rhizosphere refers to the region of soil subject to the influence of plant roots and is characterized by intense microbial activity. The external mycelium constitutes up to 90% of total mycelium in established mycorrhizas, and AMF runner hyphae grow distantly from points of colonization extending up to 8–20 km/l soil (Marschner, 1995). Thus, it is quite probable that runner hyphae extended beyond the 'rhizosphere' areas sampled in this study into the 'bare zone'. However, sporulation during early colonization appears to be related to colonized root length (Olsson et al., 1997, 1999) and since host roots stimulate germination of spores and subsequent colonization, there would be a greater likelihood of finding higher concentrations of spores in the rhizosphere than in bare areas.

Little is known of the effects of sulphate salinity on AM biology. Brown and Bledsoe (1996) found suppression of AMF colonization in *Jaumea carnosa* in an upland site compared with a marine site, probably due to osmotic stress associated with seasonal chloride salinity extremes. Generally, there appears to be a negative relationship between AMF colonization and soil chloride salinity. Excessive concentrations of sodium and chloride can inhibit germination of glomalean spores and hyphal elongation (Estaun, 1989; McMillen et al., 1998). In seeds derived from trees growing on acid mine drainage, Weiersbye and Witkowski (2007) found that a combination of high chlorides and sulphates exerted a stronger negative influence on seed mass, viability and germination percentages than high sulphates alone. In this study, weak, but significant negative correlations were found between conductivity and percentage infectivity and spore levels, suggesting a detrimental effect of conductivity on AMF inoculum potential.

This study showed that despite the constraints on development of functional AM communities imposed by the physico-chemical characteristics of slimes, and by vegetation programmes reliant on non-AM species, the AMF inoculum potential is still measurable by means of an infectivity assay. The infection levels recorded varied between 0% and 50% after 50 days. In the Moorman and Reeves (1979) study, infectivity in a highly disturbed soil was 10% at a similar harvest time, but 80% in an adjacent undisturbed soil. However, the strength of this inoculum potential in slimes dams is strongly correlated with dam zonation (slopes are subject to erosion rates that limit the long-term persistence of potential host plants, as well as AMF colonization), region (characteristics related to climate, biome, underlying soils, landscape setting and available vegetation propagules), proximity to the rhizosphere, and in particular the age of the dams, with older and non-vegetated dams having lower infection potentials. This last pattern indicates that although AMF do get introduced during present grassing programmes, they diminish with time as the slimes revert to acidic conditions. In the case of NV dams the immigration of AMF onto the dams is minimal, although such AMF as are present must be highly tolerant of adverse conditions. AMF levels were also found to be significant on the flat areas of OV dams where SOM is retained (Straker et al., 2007), and a more perennial vegetation develops over time (Weiersbye et al., 2006). This understanding needs to be exploited when designing holistic rehabilitation programmes that consider the essential contribution of soil microflora to creating and maintaining sustainable systems. Requena et al. (2001) demonstrated, in a desertified Mediterranean ecosystem, that inoculation with native AMF and rhizobial nitrogen-fixing bacteria can enhance the establishment of key plant species, and also increase soil fertility and quality (soil nitrogen, SOM, formation of stable soil aggregates, and nitrogen transfer from N-fixing to non-fixing plants). Vivas et al. (2003) co-inoculated red clover with a strain of *Brevibacillus,* a *Plant Growth Promoting Rhizobacterium* (PGPR), and an AMF mixture isolated from lead-polluted soil in Hungary and obtained enhanced plant growth, nitrogen and phosphorus accumulation, nodule formation, mycorrhizal infection and lead accumulation in plants grown on the polluted soils.. Addition of organic materials to soils has been a common rehabilitation practice to improve the physical properties of degraded soils, and AMF applied with compost has been shown more effective in improving soil physical properties of a semi-arid Mediterranean soil than AMF plus inorganic or other organic treatments (Celik et al., 2004). The presence of AMF and organic matter contributes to enhancing plant survival in gold and uranium tailings. Over a three-year period, AMF- and rhizobia-inoculated seedlings of five legume tree and forb species had higher establishment rates in gold tailings than un-inoculated plants at compost additions of between 0.5% and 2% dry mass (equivalent to 1.3% organic matter). At compost additions exceeding 5%, AM and rhizobia inoculation had no influence on plant survival (I.M. Weiersbye, C.J. Straker and E.T.F. Witkowski, manuscript). Taken together, these studies point to the essential components of successful rehabilitation of gold slimes dams: reduced slope angle to stem the loss of organic resources; application of organic composts rather than inorganic chemical amendments; communities of acid-tolerant and mycotrophic perennial plants, including legumes; and inoculation of sites with acid-tolerant AMF, N-fixing bacteria and PGPR so that the community can function after the effects of lime and fertilizers have declined. These harsh substrata have clearly imposed selection pressures which have selected for strains of tolerant

AMF that can be isolated and used to inoculate appropriate plant species on newly vegetated dam sites. Since AMF show considerable functional diversity (Johnson et al., 2003; Munkvold et al., 2004) inoculation with mixed strains of AMF would be more beneficial than single strains. Many of these components have already been introduced into gold mine rehabilitation practices in South Africa and elsewhere.

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