



# Fungal Communities Resist Recovery in Sand Mine Restoration

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The restoration of vegetation post-mining is particularly challenging in extreme conditions such as Mediterranean systems where soil moisture is limiting, soil temperature fluctuates dramatically, and soil carbon is very low. In such systems, soil microbial communities may play an important role in attenuating extreme conditions. Thus, vegetation establishment on such sites may be curtailed by depauperate soil communities. Soil fungal communities, in particular, are essential for nutrient turn over but we know very little about how these communities respond to mining and post-mining restoration. Fungi may be significantly affected by restoration practices. For example, the inclusion of deeper soil profiles (i.e., “overburden”) into restoration events is rare, but may expedite fungal community development. We studied a successional gradient of sand mine restoration in a former *Banksia* woodland in SW Australia to determine whether soil fungal communities recovered after 13 years. We also asked whether the inclusion of overburden into restoration sites improved soil fungal community development. Overall, fungal communities did not return to a pre-disturbance state by 13 years, nor did the inclusion of overburden affect their trajectory. Longer term studies are need to determine when, if ever, fungal communities are restored, and what effect this has nascent vegetation.

**Keywords:** fungi, sand mine, rehabilitation, mycorrhizas, plant pathogens, extreme conditions

## INTRODUCTION

Base raw material extraction, including sand and gravel, represents the largest and fastest growing share of the global mining industry (United Nations Environment Program, 2014). Sand extraction, in particular, represents a large ecological threat because sand resources are typically vegetated with unique, stress tolerant flora that have developed adaptations to local environments and may be difficult to restore (De Souza et al., 2013). Such soils are extremely low in organic material, highly leached, and can reach extremely high temperatures (Nussbaumer et al., 2016). Coadaptation between plants and soil microbes to cope with edaphic stresses is an essential part of the stability of all ecosystems, in particular these ecosystems which experience extreme environmental stress. Soil fungi, for example, have been shown to enhance plant drought tolerance (Gehring et al., 2017). Thus, rehabilitation of fungal rhizospheres may be an essential component of vegetation recovery, yet we know little about how to achieve it.

In general, mining of all types significantly reduces soil biota abundance and diversity (Ohsowski et al., 2012). Fungi are key determinants of soil physical, chemical and biological properties as they cycle nutrients, decompose organic matter, and form mycorrhizas with plants supporting their growth (De Boer et al., 2005; Gessner et al., 2010; Dickie et al., 2013). However, fungi are not considered or monitored in ecosystem restoration efforts as consistently as bacterial communities (Jones et al., 2018; Yan et al., 2018), despite the tight link between soil fungi and higher trophic levels (Stevens et al., 2018; Yan et al., 2018). Consideration of soil fungal communities is therefore an important aspect of successful sand mine restoration, but there are few guidelines for their successful restoration.

Soil fungal communities are sensitive to changes in abiotic conditions, therefore substrate conditions in a rehabilitation event may greatly affect the fungal establishment and persistence (Ohsowski et al., 2012). The most common and cost effective approach among mining operations is topsoil transfer from donor to recipient sites that have similar plant communities and ecosystem characteristics (Koch, 2007; Koch and Samsa, 2007). Direct transfer of topsoil (top 10 cm of soil profile) has been practiced for over 40 years, and while it is clearly important, it does not guarantee successful restoration (Koch, 2007). Biota are “inoculated” into the restored site via the topsoil—only if the important components of the soil food web are found within the top 10 cm of the soil profile. However, fungal taxa are well-known to be depth stratified (Rosling et al., 2003; Jumpponen and Jones, 2014), and may occur far below the top 10 cm of soil. This is particularly true in Mediterranean systems due to the great rooting depth of plants (Pickles and Pither, 2014). Unlike surface dwelling soil microbes, these fungal taxa would be less likely to disperse from neighboring, undisturbed sites, and their absence may hinder plant establishment. Including deeper layers of the soil profile in a restoration practice may improve fungal community re-establishment, and could improve restoration outcomes.

Overburden, in the context of sand mining, is the layer of substrate immediately below the top soil (10–100 cm). Overburden is typically not incorporated into restoration practice; however, it may contain a significant proportion of the rhizosphere which represents the most biologically active component in soil ecosystems (Rokich et al., 2001). For seasonally dry soils such as *Banksia* woodlands, where soil mining is commonly practiced, this layer can be considerably deeper than surface soils (Pickles and Pither, 2014). Failing to include this layer may delay or prevent the restoration of soil microbes, including fungi.

Presently, it is not known if incorporating rhizosphere soils into top soil improves restoration success. We evaluated fungal communities following restoration of a former sand mine location in a trial spanning 15 years, with and without the inclusion of overburden. We hypothesized that all soil fungal communities would become more similar to reference site over time, as fungal propagules from top soil transfer and dispersal from adjacent areas established over time (chronosequence study). Further, we hypothesized that the addition of overburden would hasten the recovery of soil fungal communities to

reference conditions compared to plots that did not receive overburden due to inclusion of taxa not present in surface layers (overburden study).

## METHODS

### Restoration Site

The *Banksia* woodlands are a floristic region in SW Australia with a Mediterranean climate and represents a global biodiversity hotspot of over 80% endemic species (Myers et al., 2000). Less than 35% of historical *Banksia* woodlands remain, due to large scale land clearing associated with rapid urbanization and to a lesser extent sand mining to support infrastructure development (Rokich, 2016). While much effort has been devoted to post-mine rehabilitation of this threatened ecosystem since the 1990s (Stevens et al., 2016), most restoration efforts achieve an unsatisfactory level of biodiversity return (Mueller et al., 2018).

In March 2016, soils were sampled from Hanson Construction Materials Gaskell Avenue Mine, Lexia, in southwestern Australia (32°14'35.4"S 115°46'38.5"E). The site is located in the *Banksia* woodlands ecosystem and has a history of 20+ years of rehabilitation. Reference soils samples were taken from sites adjacent to restored sites that were either restored using standard topsoil transfer protocols (Stevens et al., 2016), or amended with overburden. In total, we sampled 5 sites (2003, 2005, 2009, 2011, 2015), two of which had overburden amendments (2011, 2015) plus a reference site. For topsoil only (Stevens et al., 2016), fresh topsoil collected from the donor site was placed directly on top of the underlying substrate on the receptor site. For overburden plus topsoil profiles, soil 10–100 cm was freshly transferred from the intact donor site to the exposed underlying substrate of the mined receptor site, with fresh topsoil (the upper 0–10 cm) subsequently placed on top (Stevens et al., 2016). This profile mimics typical depth stratification of undisturbed reference soil profiles.

After topsoil addition, all restored sites were hand sown with a standard, locally sourced seed mix of species represented already in the seed bank (Mounsey, 2014). These included species routinely used in restoration and native to *Banksia* woodland such as *Allocasuarina fraseriana*, *Allocasuarina humilis*, *Banksia attenuata*, and *Banksia menziesii* and the under-story species *Acacia pulchella*, *Beaufortia elegans*, *Bossiaea eriocarpa*, *Gompholobium tomentosum*, *Hovea pun-gens*, *Hovea trisperma*, *Jackso-nia densiflora*, *Nemcia capitata*, and *Xanthorrhoea preissii* (Rokich et al., 2002).

### Reference Site Characteristics

The dominant canopy vegetation of *Banksia* woodlands is comprised of *B. attenuata* and *B. menziesii*, with *Eucalyptus todtiana* and *Nuytsia floribunda* occurring less frequently (Dodd et al., 1984). The understory is represented by woody species in Myrtaceae, Epacridaceae, Proteaceae, and Papilionaceae, and non-woody species in the Anthericaceae, Styliaceae, Haemodoraceae, Cyperaceae, and Dasyopogonaceae (Trudgen, 1997). The site is located within the Bassendean dunes, characterized by low-nutrient, leached acidic podzols with high acidity and low water holding capacities (McArthur and Bettenay, 1960; Dodd and Heddle, 1989). In general,

reconstructed soils in these sites have lower organic carbon content than native woodland. At our site, reference sites have been shown to have ~0.47% organic carbon (OC), and pH of 6.1, while restored sites have 0.37% OC and pH 7.4 (please refer to Benigno et al., 2013 for more details).

## Soil Sampling Chronosequence

For the chronosequence experiment, soils were sampled from standard topsoil transfer restoration events which occurred in 2003, 2005, 2009, 2011, 2015, as well as a reference site, in a space-for-time substitution design. In all rehabilitated sites, after topsoil addition, sites were hand sown with seeds from 12 common native species and planted with seedlings of three dominant plant species (*B. menziesii*, *B. attenuata* and *Eucalyptus totidiana*) (Dodd et al., 1984) as well as the seed mixture (above).

## Influence of Overburden Amendment

Soil samples were collected from two overburden amended sites (2011 and 2015) that also received standard topsoil addition. In addition, we sampled from the adjacent undisturbed site which included reference topsoil (top 10 cm) and reference overburden (10–100 cm). At each site, we randomly sampled five soil cores within a 10 m<sup>2</sup> plot. Each soil core was sampled adjacent to an *Acacia pulchella* shrub to standardize for differences in soil fungal composition. We chose to sample rhizosphere soil of *Acacia pulchella* because it is native to the area and occurred in all plots. Soils were then stored at 4°C and shipped to UBC Okanagan where DNA was extracted from soils using MoBio Powersoil (MAX, Qiagen Inc.).

## Fungal DNA Isolation and Identification Amplicon Preparation

Fungal ITS amplicons were generated using gITS86F (5' GTGARTCATCGARTCTTTGAA) and ITS4r (5' TCCTCCGCTTATTGATATGC) (Vancov and Keen, 2009), and sequencing adaptor barcoded gITS86F and ITS4r primers in the second round. All reactions contained 10 µl of 2X GoTaq Green Master Mix (Promega Corporation, Madison, WI), 2 µl forward primer (1 µM final concentration), 2 µl reverse primer (1 µM final concentration), 1 µl of template DNA, and water for a total volume of 20 µl. The thermocycler program for first round bacterial and fungal amplicon generation consisted of: 95°C for 4 min followed by 25 cycles of 95°C for 30 s, 53.4°C for 45 s, and 72°C for 2 min, with a final extension at 72°C for 5 min. For generating sequencing barcode adapted bacterial and fungal amplicons, the thermocycler program was the same except that 20 cycles were used with an annealing temperature of 65°C. After each round of PCR, amplicons were cleaned using an E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Norcross, GA), quantified using a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Mississauga, ON), and visualized on an agarose gel. Template-free and positive controls were included for all reactions.

## Sequencing

Barcoded amplicons were pooled in equimolar amounts and purified following agarose gel electrophoresis using an E.Z.N.A Gel Extraction Kit (Omega Bio-Tek) and, prior to sequencing on an Ion Torrent PGM (Life Technologies Inc., Carlsbad, CA), the library dilution factor was determined using an Ion Library Quantitation Kit. An Ion PGM Template Hi-Q OT2 400 kit on an Ion OneTouch 2 system was used to prepare DNA for sequencing, and enriched Ion Sphere Particles were quantified using an Ion Sphere Quality Control Kit. Sequencing was performed on a 316v2 chip with an Ion PGM Hi-Q View Sequencing Kit. Sequences were deposited in European Nucleotide Archive (accessions ERS3048307-ERS3048349). Demultiplexed FASTQ files were generated using Torrent Suite 5.0.5. *Split\_libraries\_fastq.py* in QIIME (v 1.9.1; Caporaso et al., 2010) was used to merge reads into a single FASTA file (phred quality score Q20, 3 low quality bases read truncation, zero Ns allowed, phred offset of 33). QIIME was used for open reference operational taxonomic unit (OTU) picking (*pick\_open\_reference\_otus.py*) and generating summary plots (*summarize\_taxa\_through\_plots.py*). Illegal characters were removed from the merged FASTA file before using the PIPITS pipeline (Gweon et al., 2015) for dereplication, removal of unique sequences, OTU picking, chimera removal, and taxonomy assignment with the *pipits\_funits* and *pipits\_process* commands.

## Data Analysis

Here we use the term phylotype to describe OTUs for the fungi identified using the above methodology. Differences in fungal phylotypes were assessed using parametric and multi-response permutation procedure (MRPP) using Bray-Curtis similarity matrices. To determine differences in phylotype richness, data were Box Cox transformed (MASS, Venables and Ripley, 2002) prior to ANOVA with site age as a fixed factor. MRPP is a permutation procedure, this process compares the occurrence of phylotypes in each soil group to multiple randomizations of the entire data set, determining whether the pattern observed is likely different to random. An A-value of than 0.3 is considered a significant effect size for ecological data (McCune and Grace, 2002). All values were FDR (false-discover rate) corrected to account for multiple comparisons. Communities were also compared using perMANOVA in Primer-E v6 (Anderson, 2001, Clarke and Gorley, 2006) to assess community level differences among years and overburden treatments, and to test for differences in dispersion. PERMADISP values are given for each comparison, indicating, when significant, that differences detected in the PERMANOVA are due to dispersion (heterogeneity) of the data rather than differences in fungal community structure. Indicator species analysis, a permutational procedure which determines which species occur in one soil type to the exclusion of all other treatments was performed in PC-ORD (McCune and Grace, 2002). All analyses were performed in R unless otherwise indicated (R 3.4.2, R Core Team, 2017).

## RESULTS

### Chronosequence Study

Did soil fungal communities become more similar to reference site over time?

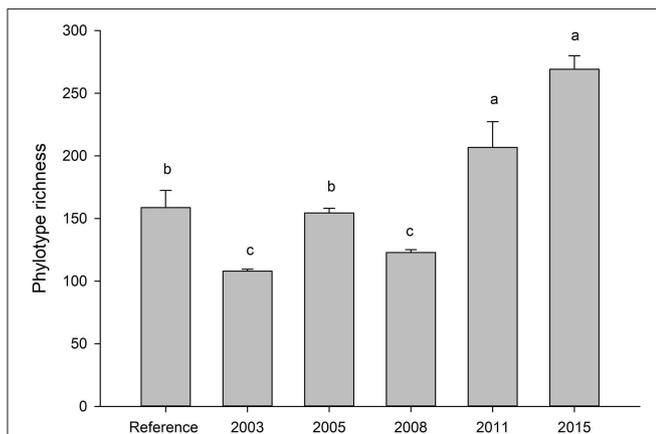
Fungal communities did not become more similar to reference sites over time, but reference soils were distinct from all other treatments (**Table S1**). Alpha diversity was highest in the most recently restored sites (2015) ( $p < 0.05$ ), and lowest in the oldest rehabilitation site (**Figure 1**). Concurrently, the proportion of phylotypes shared between with reference site also decreased over time (**Figure 2**). The most recently restored site shared the most (70%), while the oldest site (2003) shared the least (35%).

Across the chronosequence, fungal communities were significantly different ( $p = 0.001$ ) (**Figure 3**). The reference site was least similar to 2015 (Bray Curtis similarity 47.2) and most similar to 2003 (44.1), 2005 (44.2), and 2011 (44.3). Thus, there

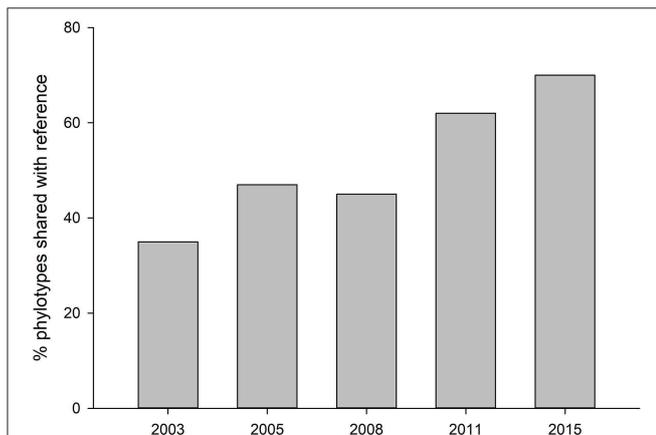
was no directional response for increasing similarity to reference site over time. In fact, variation within sites was often higher than between sites. The reference site had lowest within samples homogeneity (55.8) compared to all restored sites, meaning 2003 and 2008 were significantly less dispersed than the reference site ( $p = 0.02$  and  $0.08$ , respectively; **Table S1**).

In terms of composition, restored soils had decreased Eurotiomycetes (opportunistic plant pathogens) and increased Sordariomycetes (saprobes and plant pathogens) compared to reference soils (**Figure 4**, **Table S3**). Relative abundance of Agaricomycetes (saprobes and mycorrhizal fungi) varied between soils (**Figure S1**). The 2011 soils show a spike in a phylotype identified only as Agaricaceae (**Figure S1a**). This phylotype represents 8.3% of all sequences recovered for the 2011 soils. For the Dothidiomycetes (plant endophytes and pathogens; **Figure S1b**), younger restored soils show more sequences from this Phylum, particularly those identifying as *Capnodiales* (some plant pathogens), Tubeufiaceae (plant pathogens), and *Pleosporales* (saprobes/endophytes/parasites). Eurotiomycetes (**Figure S1c**) represent approximately 1/3 of all sequences recovered. These fungi were dominated largely by *Aspergillus* (saprobe), Trichocomaceae (saprobes found in extreme conditions), and *Chaetothyriales* (extremetolerant, saprobe/biotroph).

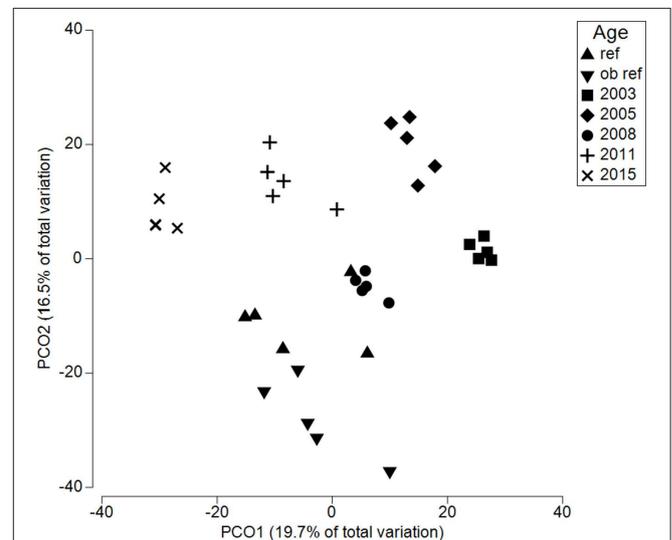
Many phylotypes detected in the reference soils were much less abundant in the restored soils, Leotiomycete (plant pathogens) phylotype relative abundance appeared to recover by 2008 (**Figure S1d**), with, and *Oidiodendron* (saprobe) sp. recovering but *Arachnopeziza* (saprobe) disappearing by 2015. In the Sordariomycetes (**Figure S1e**), a phylotype as *Sordariales* sp. was dominant in all soil, but less abundant in reference



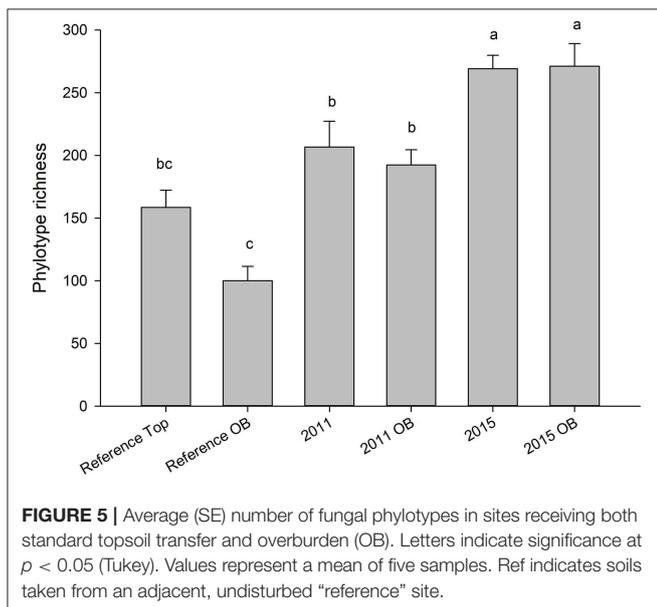
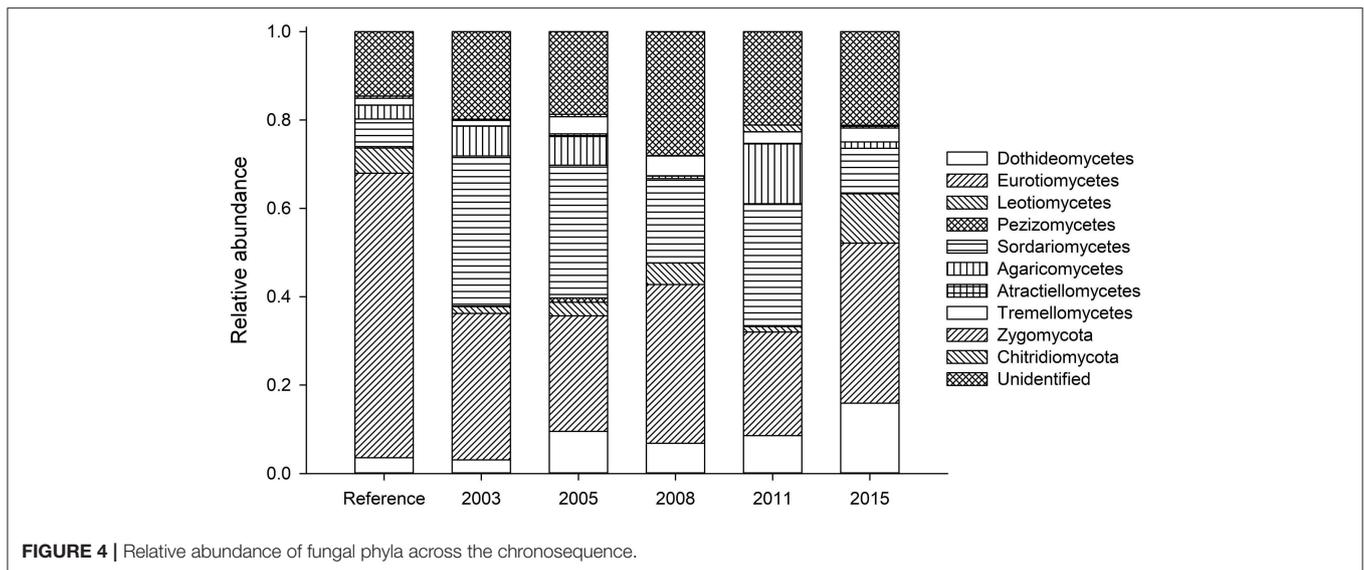
**FIGURE 1** | Average (SE) number of fungal phylotypes across the restoration chronosequence. Letters indicate significance at  $p < 0.05$  (Tukey). Values represent a mean of five samples. Ref indicates soils taken from an adjacent, undisturbed “reference” site.



**FIGURE 2** | Proportion of fungal phylotypes shared with reference site across the restoration chronosequence.



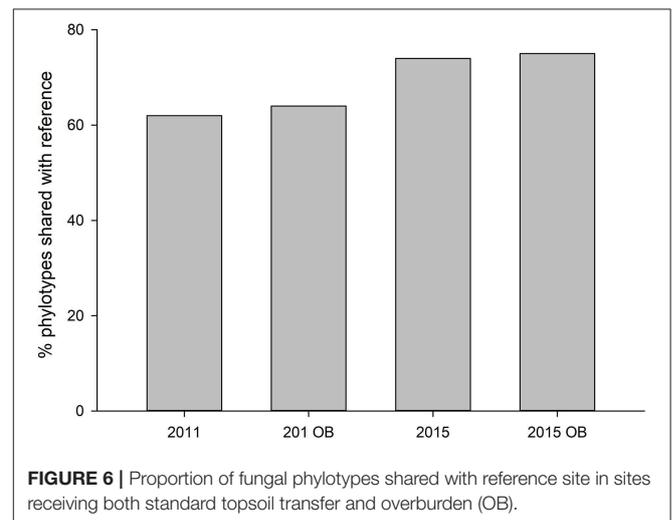
**FIGURE 3** | Principle coordinate analysis for soils along a restoration chronosequence. Each symbol represents the fungal community within a single soil core. Ref refers to an adjacent, undisturbed site. All sites are significantly different from each other ( $p < 0.0001$ ).



soils which was dominated by Coniochaeta (tree pathogens). Tremellomycetes (saprobe) was represented by only three phylotypes, but in different relative abundance to the reference site, by 2003 (Figure S1f).

### Overburden Study: Does the Inclusion of Overburden Increase the Similarity of Fungal Communities to Reference Site?

Both years, regardless of overburden amendment differed in terms of phylotype richness ( $p = 0.04$ ), and sites were significantly different from each other ( $p = 0.001$ ). The phylotype richness was highest in the most recently restored sites with overburden (271) but overburden amended sites did not differ



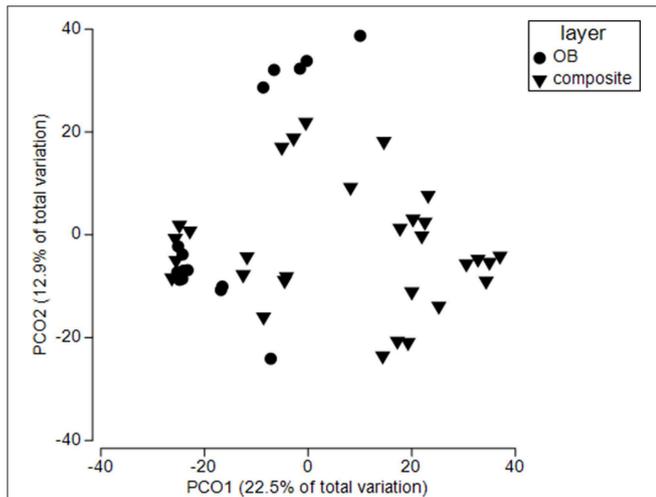
from topsoil only sites for 2011, 2015, or reference site ( $p > 0.05$ ) (Figure 5). The proportion of phylotypes shared with reference site was similar for overburden treatments in 2011 (62% overburden vs. 64%) and 2015 (75% overburden vs. 74%) (Figure 6).

Overburden inclusion significantly change fungal community composition ( $p = 0.001$ ) (Figure 7) but there was also an interaction between overburden and time of restoration ( $p = 0.01$ ). Overall, top soils were more similar to each other than overburden soil (Bray Curtis similarity 50.8 (top) vs. 46.9 (overburden)). This was most pronounced for reference sites (Bray Curtis similarity top 55.8 vs. 45.6 overburden) and less for 2015 sites [BC similarity 57.1 (top) vs. 54.2 (overburden)]. All sites showed significant pairwise differences among overburden and topsoil only amendments (see Table S2). Both reference and 2011 sites had similar levels of phylotype

turnover (dispersion) when comparing overburden to topsoil only sites ( $p = 0.82$  reference,  $p = 0.93$  2011), but 2015 overburden sites had more turnover than 2015 topsoil only sites ( $p = 0.07$ ; **Table S4**, **Figure 7**).

In terms of composition, there were few differences among overburden amended soils and those with topsoil only (**Figure 8**). The mycorrhizal fungus *Pisolithus* was more abundant in overburden reference soils. Also, *Gymnopilus allantopus*

(*saprobe*) was more abundant in overburden soils (2011) (**Figure S2a**). *Ceraporia*, a crust fungus, was extremely common in 2011 (topsoil only). There were fewer Dothidiomycetes in the reference overburden soil than the reference topsoil, and its abundance increased in restored soils (**Figure S2b**). Eurotiomycetes, on the other hand, were found more often in the reference soils relative to the restored soils (**Figure S2c**). Particularly Chaetothiales (non-lichen epiphytes), which comprised 30% of reference overburden samples. Leohumicola, a heat tolerant crust fungus, was an important component of the Leotiomycetes for reference overburden, but not top soil (**Figure S2d**). Reference overburden had among the lowest abundance of Sodiariomycetes (**Figure S2e**). Tremellomyces were almost completely absent from reference overburden, but common in other sites particularly 2015 (**Figure S2f**).



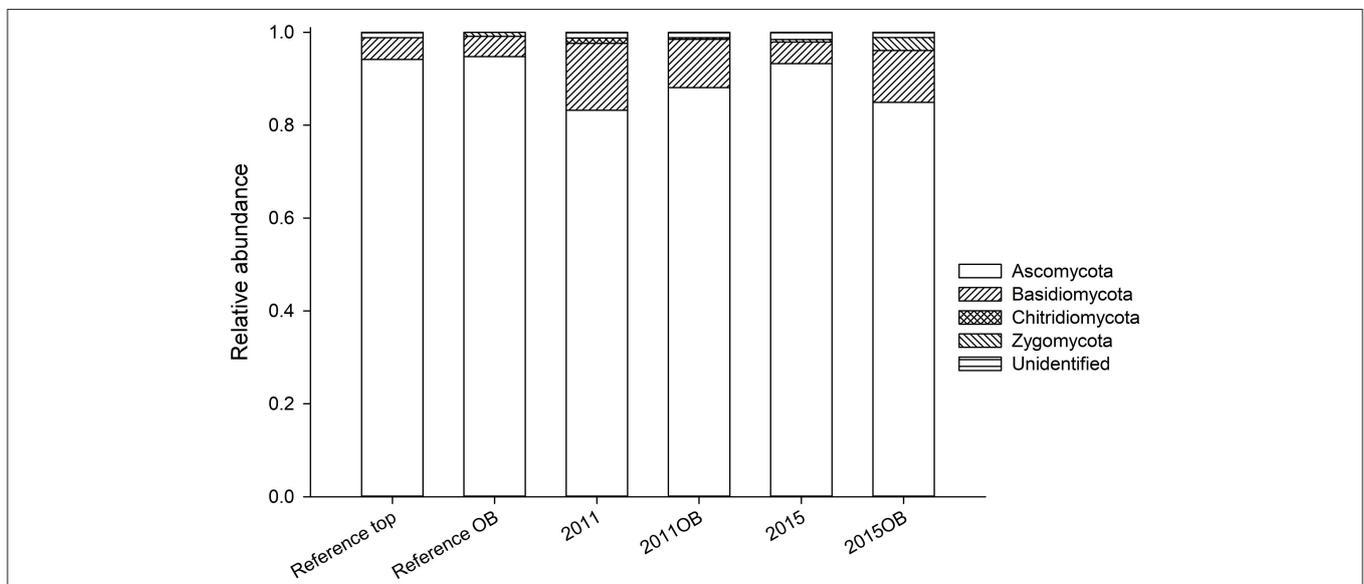
**FIGURE 7** | Principle coordinate analysis for soils in sites receiving both standard topsoil transfer and overburden (OB). Each symbol represents the fungal community within a single soil core. Ref refers to an adjacent, undisturbed site. All sites are significantly different from each other ( $p < 0.0001$ ), but sites responded differently to overburden treatment (site X overburden,  $p < 0.05$ ). See **Table S2** for more details.

## DISCUSSION

We investigated the recovery of soil fungal communities post-sand mining over time in former *Banksia* woodlands in SW Australia. After 13 years, restored soil fungal communities were significantly different from the reference site, indicating a lack of recovery. As we hypothesized, the addition of overburden significantly changed fungal communities, but, contrary to our hypothesis, did not result in a faster recovery back to the reference state.

### Were Older Sites Most Similar to Reference Sites?

We found little evidence that rehabilitated soils were approaching a reference state even 13 years post-rehabilitation. One study in a nearby system showed that the soil fungal community did not return to pre-bauxite mining conditions after 18 years



**FIGURE 8** | Relative abundance of fungal phyla in samples receiving topsoil transfer compared to those receiving overburden.

**TABLE 1** | Inferred ecological function based on indicator species identity (species found in these samples to the exclusion of others) found in undisturbed reference overburden and topsoil.

Number of sequences topsoil (10 cm)	Indicator species identification	Function/ecology/type/sequence source
1,347	Trichosporon	Oily yeast (can indicate stressful soil environment);denitrification
423	<i>Phaeococcomyces nigricans</i>	Tree/rock inhabiting black yeast
355	<i>Galerina</i>	Bryophyte associated fungi
117	Unknown	Unidentified endophyte of desert plant species
110	Herpotrichiellaceae	Sooty blotch or flyspeck apple disease, colonize waxy apple surfaces
102	<i>Pleosporaceae</i> sp.	<i>Verbascum lychnitis</i> endophyte (growing in calcareous industrial tailings)
58	Orbiliomycetes	Nematode trapping fungi that can be disease suppressive for <i>Trifolium repens</i> (White clover)
37	<i>Peniophorella</i>	Corticoid basidiomycete, wood inhabiting saprobe
27	<i>Acremonium</i>	Saprophyte, produces $\beta$ -lactam antibiotics, endophyte
7	Unknown	Melanized fungi from rock
<b>Overburden (10–100 cm)</b>		
21,508	Chaetothyriales	Sooty molds, epifoliar asymptomatic fungi that may infect sap sucking insects
17,201	Leotiomycetes	Numerous species, many cause plant disease such as powdery mildew
15,170	Chaetothyriales	Sooty molds, epifoliar asymptomatic fungi that may infect sap sucking insects
1,334	Leohumicola	Heat resistant hyphomyete
713	Cladophialophora	Black yeast-like fungus, associated with <i>Cenococcum sclerotia</i>
251	Russulaceae	Ectomycorrhizal family
179	<i>Amanita eucalypti</i>	Possibly ectomycorrhizal, thick-limbed death-cap, WesternAustralia
113	Sordariales	Perithecial ascomata-forming decay fungi
39	<i>Penicillium dravuni</i>	marine-derived algal species

(Banning et al., 2011), suggesting that our results are consistent for that area. In our study, recently transferred soils harbored significantly more phylotypes than reference soils, whereas the oldest sites had the fewest. The higher number of phylotypes early in succession could be due to greater fluctuations in the types of food available for the fungi (Frankland, 1998). Or it may reflect the overall species pool on which environmental filtering has yet to select suitable taxa. If species richness is due to a large species pool, our data support heavy environmental filtering which resulted in the lowest phylotype richness overall by 2003. Studies have shown decreased soil biodiversity and functioning post-mining (Gil-Sotres et al., 1992), while others show no change in richness (Yan et al., 2018). In this study it is not clear why numbers dropped below reference levels but indicates that either the community is still in flux or that reduced substrate complexity cannot support the same level of fungal diversity. Given that beta-diversity was also lowest in the oldest site (2013) and highest in the reference site, indicates a lack of complexity in restored sites may take a very long time to reach reference levels, if ever.

However, ecosystems rarely recover to reference conditions. In a meta-analysis of 400 studies, Jones et al. (2018) report a rate of recovery of 1.1–10% per year (with a median of 2.9%), with forest vegetation recovering most slowly than other ecosystems, and mining restoration recovery falling near the median of disturbance types considered. The relationship between soil community richness and ecosystem functions is well-demonstrated (i.e., van der Heijden et al., 2008), but it is

not yet clear if soil “recovery” can be evaluated through measures of soil microbial community metrics such as alpha-diversity or composition. Whether such soils achieve complete recovery may not be necessary for vegetation success, but this remains to be shown over long-term experiments which are lacking. A more appropriate recovery time-frame might be decades, rather than years.

## Did Overburden Change Recovery Trajectory?

We found no evidence for our hypothesis that overburden amendments would improve fungal community recovery. The reasons for this are not clear, and there has been no other investigations of the effect of overburden on soil fungal communities. In our study, soil collected from the “overburden” layer in reference sites differed from reference top soil, both in terms of phylotype richness and community composition. Contrary to our hypothesis, however, overburden reference soil was less diverse than reference top soil. This was surprising, given the lack of organic material and extreme temperatures of the top soil.

Whether our results reflect a general trend among dune soil systems is not clear. Little is known about fungal diversity in extreme soils (such as those found in *Banksia* woodlands), but fungal communities in other systems have been shown to be stratified based on soil depth (Jumpponen et al., 2010). Plants in arid ecosystems tend to root deeper, on average, than in temperate or tropical climates (Canadell et al., 1996; Pickles and

Pither, 2014), thus we would expect increased fungal activity in deeper, rhizosphere zones. We found some support for this with *Pisolithus*, a mycorrhizal fungus, found predominately in the reference overburden, and almost completely absent in the topsoil. Given that ectomycorrhizal plants show strong host preference (Dickie, 2007), lack of suitable host may prevent some plants from establishing. In our study, we identified indicator fungi unique to reference topsoil, including oily yeasts, non-lichenised rock inhabiting fungi, and fungi previously isolated or identified from challenging environments such as deserts or industrial tailings (Table 1). Indicator fungi unique to overburden soils were also mostly saprobes, yeast-like, and extremophilic, but contained two putative mycorrhizal fungal species. Restoration practices which increase the biodiversity of symbiotic fungi may hasten revegetation (Harris, 2009). To fully understand the importance of fungal taxa in restored soils, future studies must evaluate the relationship between fungal composition and plant establishment over long time scales.

## CONCLUSION

Restoration of soil fungal communities in a former sand mine did not recover to reference conditions over a 13-year time period. The inclusion of overburden in the restoration profile resulted in changes to the fungal community which may, over time, lead to different trajectories. However, given the paucity of data concerning the restoration of soil microbial communities in ecosystem restoration, these changes may not affect vegetation restoration. This study highlights the need for exemplar restoration projects that include appropriate balanced design and multiple suitable reference sites. Such sites will enable rigorous tests of key ecological management questions and are critical in ecological restoration research, where imperfect study systems are often the location of research simply because they are all that exist (Prober et al., 2019). Good design of monitoring programs will also be important for stakeholders (e.g., mining companies) that need to demonstrate restoration success to regulators.

Ultimately, evaluating fine-scale changes to soil microbial communities may not be the most effective predictor for restoration success. It may be that soil microbial communities

are functional even if they do not return to a “reference state.” Thus, studies should also consider functional responses, such as microbial biomass, substrate utilization and nutrient cycling. Longer experiments are needed, particularly in stressful conditions such as the Banksia woodland, where recovery may take longer than in mesic systems. Successful restoration efforts depend on additional research, particularly for understudied vegetation types and climates.

## DATA AVAILABILITY STATEMENT

The sequence data used to support the findings of this study have been deposited in the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>).

## AUTHOR CONTRIBUTIONS

MH, PN, and JS conceived of study. MH and PN collected field samples. BM and JV analyzed data. MG, LA, AW, MH, PN, JS, and JV wrote manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2019.00078/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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