Habitat fragmentation in a Mediterranean-type forest alters resident and propagule mycorrhizal fungal communities

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Highlights

• Fragmentation alters propagule and resident mycorrhizal fungal community structure.
• Propagule communities are not resistant to habitat fragmentation.
• Changes in mycorrhizal fungal communities have effects on tree establishment health.
• Changes in mycorrhizal fungal communities may increase susceptibility to pathogens.

Abstract

Aims: Anthropogenic activities disturb forests and their associated mycorrhizal fungi. The combination of climate change and habitat fragmentation are linked to increased incidence of a canker disease in a Mediterranean-type forest tree in Western Australia. As changes in communities of mycorrhizal fungi could predispose these Mediterranean-type forest trees to decline, we investigated how two aspects of mycorrhizal fungal community structure, soil propagules and resident communities on mature trees, respond to habitat fragmentation.

Methods: Roots were collected from a forest tree (*Corymbia calophylla*) across a disturbance gradient. Soil collected from the same disturbance gradient was used in a glasshouse bioassay with *C. calophylla* as the bioassay host (i.e., soil propagule community). After four months, we harvested the seedlings and collected roots. DNA was extracted from the field roots (resident community) and glasshouse roots (propagule community), amplified with fungal specific primers, labelled with specific barcodes and subjected to 454 pyrosequencing.

Results: Mycorrhizal fungal community composition overlapped substantially between the soil propagule and resident communities. However, the resident community had greater mycorrhizal fungal richness than the soil propagule community. Habitat fragmentation had a

similar effect on each community structure: communities along highly fragmented areas had different community compositions than communities in a healthy forest.

Conclusion; With the increased mortality of *C. calophylla* forest trees in recent years along edge habitats, understanding the effects habitat fragmentation has on communities of mycorrhizal fungi will further elucidate host-mutualist interactions in these forest ecosystems. The changes in community composition of mycorrhizal fungal species in both propagule and resident pools can have cascading effects on future tree establishment and health by predisposing forest trees to other abiotic or biotic factors.

Keywords: DNA; Forest; Fungi; Plant-fungal interactions; Soil propagules

1. Introduction

Tree mortality is increasingly common in forest ecosystems and is often linked with anthropogenic disturbance (Seidl et al., 2017). Specifically, deforestation is occurring at an exceedingly alarming rate, resulting in highly fragmented forests interspersed among other land uses such as agriculture, mining and urban areas (Crockatt, 2012). Fragmentation can affect tree species diversity and overall forest structure as well as soil nutrient cycling through a reduction or change in aboveground vegetation and litter return (Xiao et al., 2017). Furthermore, edges along fragmented forests have different environmental conditions in comparison to their interior forest habitats (Forman and Moore, 1992). Changes in microclimate can increase tree mortality, damage and turnover (Laurance et al., 1998; Santo-Silva et al., 2016). Fragmented landscapes can also allow easier access for plant pathogens through animal and human movement (Barber et al., 2013; Tracey et al., 2014). Not only does fragmentation affect individual trees, but also the beneficial mycorrhizal fungi associated with tree roots; changes in the communities of mycorrhizal fungi can alter the interactions between mycorrhizal fungi and their tree hosts (host-mutualist interactions), thus affecting tree health (Cameron et al., 2013; Henry et al., 2016; Karpouzas et al., 2014; Pickles et al., 2012; Sapsford et al., 2017).

Mycorrhizal fungi are integral to tree health, and many trees would fail to establish without these fungal associations (Crocker et al., 2016; Das et al., 2016; Sapsford et al., 2017). Mycorrhizal fungi increase uptake of minerals and water through nutrient exchange, provide resistance to root pathogens and improve soil structure (Brundrett, 1991). Habitat fragmentation causes a decrease in the diversity and richness of both ectomycorrhizal and arbuscular mycorrhizal fungal communities (e.g., House and Bever, 2018; Hui et al., 2017; Schnoor et al., 2011). For example, ectomycorrhizal fungal communities associated with *Asteropeia mcphersonii*, endemic to Madagascar, differed between seedlings growing within a forest and those growing on the edge of the forest along mining roads (Henry et al., 2016). Changes in communities of mycorrhizal fungi could affect tree recruitment, especially for tree species dependent on mycorrhizal fungi (Tonn and Ibáñez, 2016).

Communities of mycorrhizal fungi exist as both propagules in the soil and those living on roots of resident trees within the forest (Miyamoto and Nara, 2016; Taylor and Bruns, 1999). Many studies focus on northern hemisphere forests and only on propagule or resident communities of mycorrhizal fungi. Knowledge of how both of these aspects of mycorrhizal fungal community structure respond to habitat fragmentation is lacking, especially in

southern hemisphere forest ecosystems. Propagules of mycorrhizal fungi can persist in the soil without roots and tolerate disturbances; this aspect of mycorrhizal fungal community structure is important as this community provides the inoculum potential of mycorrhizal fungal species after a disturbance (Brundrett and Abbott, 1995; Grogan et al., 2000). For example, 18 months post fire, the mycorrhizal fungal community colonizing *Pinus muricata* seedlings was dominated by species present as resistant inoculum in the soil prior to the fire (Baar et al., 1999). Propagule communities may be more sensitive to different types of disturbance and if so, establishment of tree species after disturbance could be limited (Izzo et al., 2006). How these two aspects of mycorrhizal fungal community structure respond to habitat fragmentation will determine the success of future establishment of tree species and their health, especially if they respond negatively to fragmentation. Changes in communities of mycorrhizal fungi and declines of certain mycorrhizal fungal species could predispose trees along fragmented edges to decline as a result of other abiotic or biotic factors (Sapsford et al., 2017).

Forests in southwest Western Australia (SWWA) provide an interesting case study area for determining the effects habitat fragmentation has on forest ecosystems. Forests in SWWA are highly fragmented due to activities such as agriculture, mining, forestry and urbanisation (Shepherd et al., 2002). Combined with introduced pathogens (Burgess et al., 2016), decreased annual rainfall and increased annual temperatures (Ruthrof et al., 2015), the region has experienced declines of many native tree species (e.g.,Matusick et al., 2013; Paap et al., 2017). Habitat fragmentation has negatively impacted *Corymbia calophylla* (family: Myrtaceae), a keystone tree species, in SWWA. *Corymbia calophylla* found along fragmented disturbed sites (i.e., along roadsides and in paddocks) have high incidence of a severe pathogenic fungus, *Quambalaria coyrecup*, which causes trunk and branch cankers in individuals (Paap et al., 2017, 2016). Other pathogens, such as *Phytophthora* spp., are also associated with highly cankered trees (Croeser et al., 2018; Paap et al., 2017). The combination of fragmentation and disease has caused the decline of *C. calophylla* in recent years (Paap et al., 2018).

Corymbia calophylla are mainly ectomycorrhizal fungal hosts but can have arbuscular mycorrhizal fungi associated with their roots (Brundrett and Abbott, 1995). We determined how both the soil propagule and resident root mycorrhizal fungal communities respond to fragmentation and how similar their responses were to fragmentation. As soil propagules may be more resistant to disturbance, we hypothesized communities of soil propagule mycorrhizal fungi would be different to communities of resident mycorrhizal fungi.

2. Materials and methods

2.1. Study site

Our study site was located in Pickering Brook, Western Australia (32° 01′ 27.2″S 116° 07′ 37.8″E; 264 m above sea level). The site consisted of a disturbance gradient from a highly disturbed area (road edge with anthropogenic disturbance) to healthy forest block (opposite side of road and distance from road) (Fig. 1). The gradient included five lateral transects (Table 1). The transect along the highly disturbed area (henceforth disturbed edge) consisted of a dominant stand of *C. calophylla*. The disturbed edge bordered a road and land designated into horticultural purposes. The other four transects were part of an intact forest: one along the road edge opposite the disturbed edge, one 50 m into the forest block and one 100 m and 200 m into the forest block, respectively. Each transect was 100 m in length and 3 m in width

and thus an area of 300 m^2. While floristically diverse, the dominant species in the forest site were *C. calophylla*, *Eucalyptus marginata* (family: Myrtaceae), and the midstory species *Xanthorrhoea preissii* (family: Asphodelaceae) and *Banksia grandis* (family: Proteaceae).

Fig. 1. A disturbance gradient in Pickering Brook, Western Australia where **(A)** the disturbed edge bordered land used for horticultural purposes and a paved road. **(B)** The forest edge was opposite the disturbed edge and bordered a paved road and forest. **(C)** Transects at 50 m, 100 m and 200 m within the forest were similar in forest structure and consisted of *Corymbia calophylla* (family: Myrtaceae), *Eucalyptus marginata* (family: Myrtaceae), and the midstory species *Xanthorrhoea preissii* (family: Asphodelaceae) and *Banksia grandis* (family: Proteaceae). An example of a cankered tree is indicated by the arrow.

Table 1. Stand information across five transects along a disturbance gradient: disturbed edge was a remnant stand of forest trees bordering horticultural land and a road, the forest edge was on the opposite side of the road and 50 m, 100 m and 200 m were distances into the forest block. Stand structure was similar at 50 m, 100 m and 200 m into the forest block and thus we have combined stand structure information for those three transects.

Transect	Total stem density (number stems per hectare)	Proportion of stems per hectare comprised of *Corymbia calophylla*	Total basal area (m²/ha)	Mean diameter at breast height (cm)	Cankers present (% of trees cankered)
Disturbed Edge	31	0.90	468.9	45.3	Yes (39.3)
Forest Edge	43	0.56	88.9	22.0	Yes (8.3)
50 m, 100 m and 200 m	35	0.23	72.9	24.9	No (0.0)

2.2. Resident myorrhizal fungal community

Ten *C. calophylla* trees from each transect were randomly selected (every 10 m the closest tree was sampled) and four soil samples (collected at cardinal points around the base of the tree) were taken from each tree in June 2014. The four soil samples from each tree were bulked (one sample from each tree for a total of ten bulked samples from each transect) and thoroughly mixed. *Corymbia calophylla* roots were picked out of each of the bulked soil samples (i.e., total 50 samples from all transects). We collected soil samples from the base of each tree which increased the probability of collecting *C. calophylla* roots; *C. calophylla* roots are identifiable by their distinct colour and form in comparison to other plant roots (S. J. Sapsford, personal observation). Once the roots were picked out, they were stripped of their fine roots: a lateral root is placed between the forefinger and thumb and stripped of the fine roots as the fingers are moved along the root. The stripped roots were placed into a Petri-dish for storage. New gloves and new Petri-dishes were used for each sample to prevent contamination between samples. The fine roots were ground using liquid nitrogen and 50 mg stored in 2 mL vials at -20 °C.

2.3. Glasshouse bioassay (measure of soil propagule community)

We placed the mixed bulked soil from each tree in every transect (i.e., 10 glasshouse replicates correspond to the soil collected from each tree) into 2.8 L free-draining polyurethane pots (P175STK, 2.8 L, Garden City Plastics, Canning Vale, Western Australia, 6155). An additional ten pots filled with pasteurised (steamed at 65 °C for three hours) river sand acted as controls for the glasshouse environment. The glasshouse controls received one-quarter of the recommended rate of Thrive (nitrogen : phosphorus = 5 : 1 ; all-purpose soluble fertilizer; Yates Australia, Padstow, New South Wales, 2211) twice during the four months of the experiment. Due to the lack of nutrients in pasteurised river sand, the addition of Thrive prevented death of the control plants. Three *C. calophylla* seeds (provenance Plantagenet collected from 34°39′11.8512″S 117°30′5.8356″E) were placed into each pot, and once germinated, thinned to one seedling per pot. Seedlings were chosen at random to be thinned. All pots were covered in high density polyethylene beads (Qenos Pty Ltd, Altona, Victoria, Australia) to reduce contamination by aerial spores. Pots were watered to container capacity every three days and harvested after four months. Fine roots were stripped from lateral roots and stored as described above. We used seedlings to bait the propagules (rather than direct sequencing of the soil) as we were interested in which species of mycorrhizal fungi were alive and able to colonise *Corymbia calophylla* tree roots.

2.4. DNA extraction and high-throughput sequencing

DNA was extracted from the ground forest and glasshouse roots using a PowerPlant Pro DNA isolation kit as per the manufacturer's protocol (now DNeasy PowerPlant Pro Kit from Qiagen, Victoria, 3148). Amplicon libraries were generated by amplifying DNA with the fungal specific primer fITS7 (Integrated DNA Technologies, Baulkham Hills, New South Wales, 2153) and the eukaryotic primer ITS4 as the reverse primer (Integrated DNA Technologies, Baulkham Hills, New South Wales, 2153) which amplified the ITS2 region (universal genetic barcode for fungi) (Ihrmark et al., 2012). Extraction controls (i.e., no root sample included in the extraction) were included and negative controls (i.e., water used in place of DNA) were run for each PCR sample. PCR amplification was conducted in an iCycler Thermal Cycler (Bio-Rad Laboratories Pty. Ltd., Gladesville, Victoria, New South Wales, 2115) in 25 µL reactions [2.5 µL of 25 mM MgCl$_2$ (Promega, Alexandria, New South Wales, 2015), 1.5 µL of 10 mM dNTPs (Promega, Alexandria, New South Wales, 2015), 1 µL of 10 mg mL^{-1} BSA (Fisher Biotec, Wembley, Western Australia, 6014), 0.5 µL of 10 µM pyro fITS7 (Integrated DNA Technologies, Baulkham Hills, New South Wales, 2153), and 0.125 µL of 5U µL^{-1} Go Taq Hot Start Polymerase (Promega, Alexandria, New South Wales, 2015) in buffer, 5 min at 94 °C, 30 cycles of (30 s at 94 °C; 30 s at 57 °C; 30 s at 72 °C); 7 min at 72 °C]. We double purified the amplicons using the Agencourt AMPure XP Bead PCR Purification protocol (Beckman Coulter Australia Pty Ltd, New South Wales, 2066), visualized the PCR products on 1 % agarose gels and pooled based on band intensity. Some glasshouse controls only produced faint bands, but this was equalised at the pooling stage. The emulsion PCR reactions were carried out according to the Roche GS Junior emPCR Amplication Method Manual Lib-L. The libraries were sequenced using Junior Genome Sequencer plates (454 Life Sciences/Roche Applied Biosystems, Nutley, NJ, USA). There was one library for forest roots and one for glasshouse roots (as sequencing was completed at different times). Individual barcodes were used to identify each sample (Shokralla et al., 2014).

2.5. Bioinformatic analysis

Sequence deconvolution such as quality control and clustering was carried out using the bioinformatics platform Sequence Clustering and Analysis of Tagged Amplicons (SCATA; scata.mykopat.slu.se) developed and maintained by the Swedish University of Agricultural Sciences in Uppsala (parameters were set as per Clemmensen et al., 2015). Specifically, reads with low mean quality (<20) and sequences with missing primers were removed. Primer sequences were removed and sequences passing quality control were then clustered into operational taxonomic units or OTUs. The threshold distance for clustering was set at 0.015 (corresponding approximately to species level for ITS; Nguyen et al., 2017), and minimum alignment for clusters (i.e., minimum length of pairwise alignment in the clustering process required to consider a sequence pair for clustering) was 0.85. Representative cluster sequences (OTUs) were identified by searching against the internally curated SCATA database (UNITE version 4; Clemmensen et al., 2015; Kõljalg et al., 2013) and through BLAST searches in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using Geneious (version R9 http://www.geneious.com/). All identifications provided by SCATA were rechecked on Genbank. Positive identifications were made at > 99 % and were otherwise conservative with assignment at genus (at least 95 %), family (at least 90 %), order (at least 80 %) or class level depending on % identical sites, query coverage, and where the reference came from (origin of specimen); the lower these rated the more conservative the assignment. Phylogenetic analysis was conducted on unknowns to provide correct classification. Positive

identifications were grouped either by order or family and then analysed using a nucleotide alignment consensus tree. By doing so, the unknowns could be assigned to a group based on where they were located on the consensus tree. This process in turn double checked that the assignments were correct. In each nucleotide alignment consensus tree, sequences (not from our dataset) were included that were 100 % identified for that group, i.e., curated by NCBI staff and/or augmented with data from the CBS (Centraalbureau voor Schimmelcultures, now Westerdijk Institute) isolate database. Final identities are considered phylotypes acknowledging their identity is based on sequence data rather than a living isolate. Any phylotypes found only in control samples were excluded from the analyses.

Ecological information of each phylotype was based primarily on FUNGuild (https://github.com/UMNFuN/FUNGuild) following the user's manual where assignments were made on ranks of 'probable' and 'highly probable' (Nguyen et al., 2016). Where there was no match or a phylotype was ranked as 'possible', literature searches were conducted and phylotypes assigned to a guild based on published literature. If more than one guild was applicable to a phylotype, then assignment was based on the most likely guild that would be found on tree roots. If no information was found than the phylotype was classified as an "unidentified fungus" guild.

2.6. Statistical analysis

All data were analysed in the program R version 3.4.0 (R Core Team, 2017), and all figures were developed using the package ggplot2 (Wickham, 2009). To determine mycorrhizal fungal richness (number of phylotypes) of each sample (i.e., soil samples collected from each tree in each transect (50 samples in total) in both in situ mature roots and glasshouse bioassay), operational taxonomic unit (OTU) of mycorrhizal fungal richness was rarefied based on the minimum number of sequences observed per sample using the rarefy function in the vegan package (Oksanen, 2015). Rarefied richness was modelled as a function of the covariates transect and community type (i.e., soil propagules (glasshouse bioassay) and resident community (roots of mature *C. calophylla* in forest)) using a linear mixed model with function lmer in the package lme4. We did not include an interaction term as we only had one disturbance gradient. Soil replicate (i.e., 10 soil samples within each transect) nested within transect was included as a random effect to incorporate possible dependency among replicates. Model assumptions were verified by visually inspecting residuals for assumptions of normality and homoscedasity (Zuur and Ieno, 2016). The best model was based on Akaike's Information Criterion (AIC) (Burnham and Anderson, 2004). Models with lower AIC values are considered more parsimonious explanations of the data than models with higher AIC values. We used the heat_tree function in the package Metacoder (Foster et al., 2017) to visualize the difference in taxonomic diversity between the resident and propagule mycorrhizal fungal communities.

To determine differences in mycorrhizal fungal community composition between soil propagules and resident mature forest communities, we used the Bray-Curtis dissimilarity index to calculate dissimilarly values from fungal OTU relative abundance data (Hellinger transformed). Bray-Curtis dissimilarity values were calculated with the vegdist function in package vegan (Oksanen, 2015). We preformed unconstrained ordination of plots using non-metric multidimensional scaling (NMDS) with function metaMDS in vegan to visualize compositional differences in mycorrhizal fungal composition between community type (i.e., soil propagules through bioassay and mature forest communities) and among transects. To support the NMDS analysis we used permutational multivariate analysis of variance

(PERMANOVA) on Bray-Curtis dissimilarity values testing differences in community composition among transects and between community type using the function adonis in vegan. We included a strata term of transect to constrain the permutations within each transect. We did not include an interaction term. The null hypothesis of a PERMANOVA is the centroids of the groups are equivalent for all groups (Anderson and Walsh, 2013). We repeated the above analysis on the incidence of each phylotype using the Jaccard dissimilarity index.

3. Results

3.1. OTU identification

The libraries from the forest roots and glasshouse roots were combined for deconvolution and 94.7 % of the sequences passed quality control. All PCR and extraction controls were sequenced and these were all negative (i.e., no DNA was sequenced and thus there was a low likelihood of cross contamination among samples). SCATA identified 1917 clusters and after removing doubletons and singletons, 1467 OTUs were identified. Of these, SCATA only positively identified 8.5 % (124); SCATA consists of internally curated databases and downloads from UNITE version 4 (https://unite.ut.ee/). Therefore, by using the BLAST function in Geneious version R9, all 1467 OTUs were identified to a taxonomic rank. Overall, 33.6 % OTUs were identified to genus, 58.5 % to family, 72.9 % to order, 88.6 % to class, 97.1 % to phylum, and 2.9 % to kingdom only. The 1467 OTUs were unique and corresponded to 1467 phylotypes (Supplementary Information Table S1).

3.2. Assignment of ecological niche

Phylotypes were grouped into six ecological guilds (arbuscular mycorrhizal fungi, ectomycorrhizal fungi, animal pathogens, plant pathogens, endophytes and saprotrophs) based on FUNGuild terminology (https://github.com/UMNFuN/FUNGuild) (Nguyen et al., 2016). An additional two guilds were added: unidentified fungus and non-fungal (Table S1). FUNGuild assigned 32.7 % of the OTUs (those with confidence ranking of 'highly probable' and 'probable') and the remaining 67.3 %, were assigned to one of the eight guilds mentioned above based on the current literature (includes those assigned as "unidentified fungus"). For this study, we focused only on phylotypes grouped in either ectomycorrhizal fungi or arbuscular mycorrhizal fungi guilds.

3.3. Mycorrhizal fungal communities

Out of the 1467 phylotypes, 261 were mycorrhizal fungi (all of which were included in all analyses). Of the 261 mycorrhizal fungal phylotypes, 87.4 % were ectomycorrhizal fungi and 12.6 % were arbuscular mycorrhizal fungi. Within the soil propagule community, we identified 164 of these phylotypes, whereas we identified 227 of the phylotypes within the resident community; 130 phylotypes were shared between the two different communities.

The most parsimonious model as indicated by AIC suggested that rarefied mycorrhizal fungal richness significantly (estimate of propagule= -1.222, SE = 0.394, t-value = -3.103, p = 0.002) differed between community type where richness was consistently higher within the resident community (estimated marginal mean ± SE; 4.97 ± 0.33) in comparison to the soil propagule community (3.74 ± 0.33) (Fig. 2). The additive effect of transect was dropped from the most parsimonious model but we included it in Fig. 2 to provide visual representation of the trend

across the disturbance gradient. Irrespective of transect, the abundance (proportion of reads) of each taxon was very similar across both community types with significant differences seen in only a few taxa: Peziales were more abundant in the propagule community and *Boletales* spp., *Sebacinaeae* spp. and *Cortinarius* spp. were more abundant in the resident community (Fig. 3, Table 2). Inclusive of transects, along the disturbed edge, there was an increase in the proportion of reads of Atheliaceae, Clavulinaceae, Sebacinaceae, Thelephoraceae and Tricholomataceae (Table 2). In contrast, the proportion of the arbuscular mycorrhizal fungal family Glomeraceae was higher within the forest than the disturbed edge (Table 2).
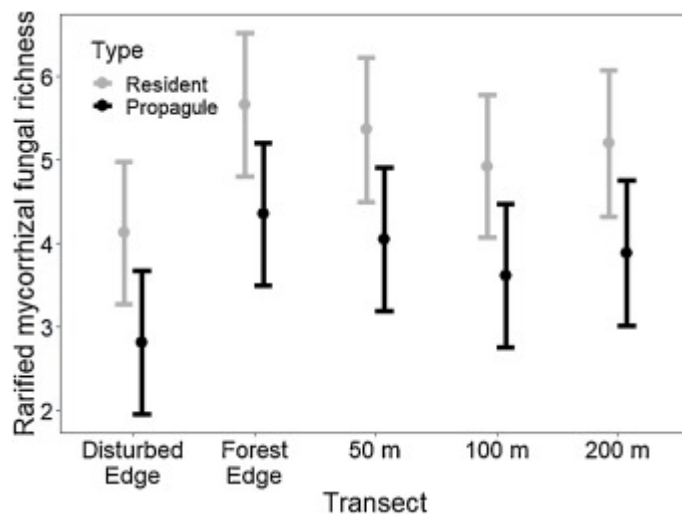

Fig. 2. Mean rarefied mycorrhizal fungal richness across a disturbance gradient: disturbed edge (i.e., remnant stand of *C. calophylla* bordering cleared land and a road), forest edge, and 50 m, 100 m and 200 m within a forest. Across the disturbance gradient two types of mycorrhizal fungal communities were measured: those present on resident roots of *Corymbia calophylla* and those present on seedlings in a glasshouse bioassay (i.e., soil propagules). Values are estimated marginal means ± standard error.
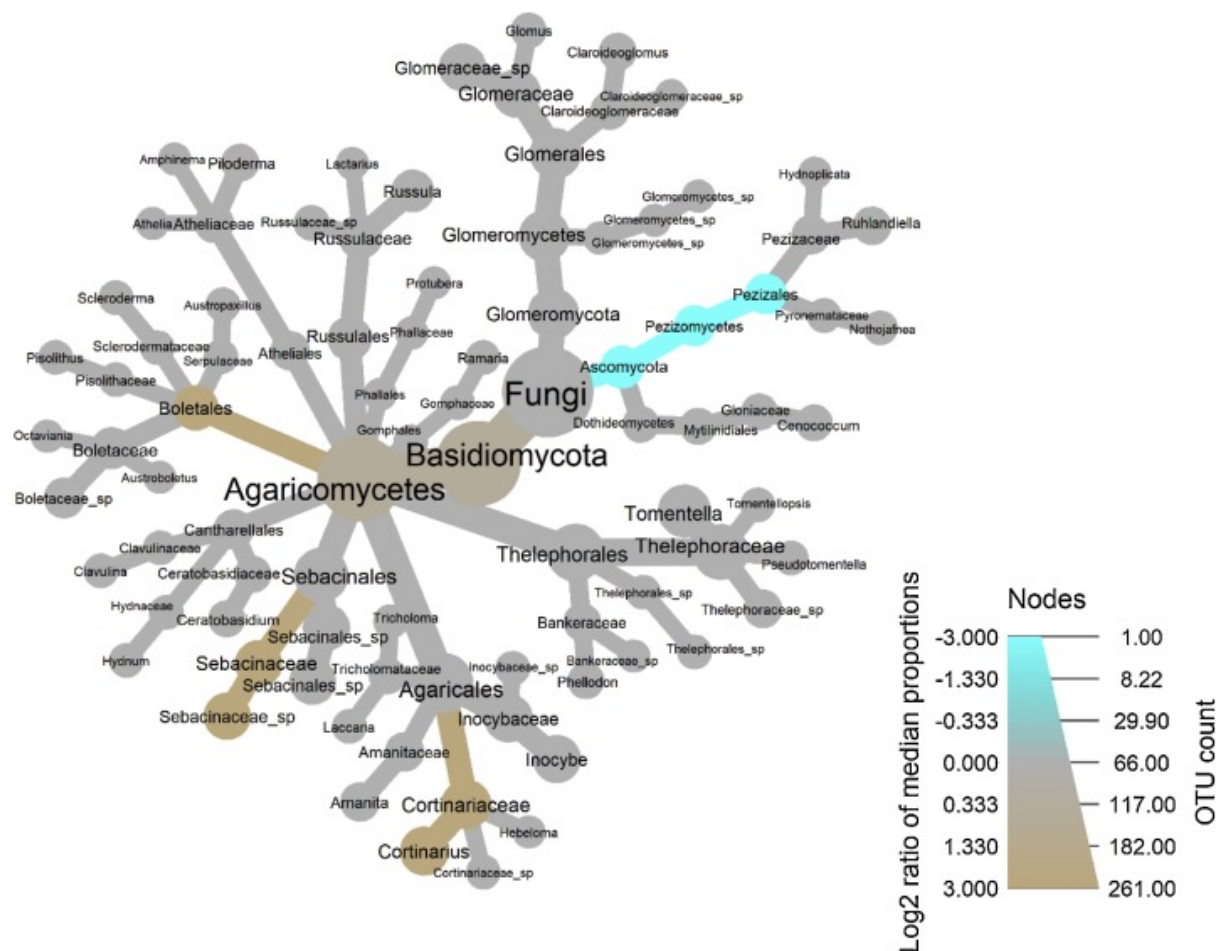
Fig. 3. Significant differences between proportion of reads observed in resident communities of mycorrhizal fungi found on *Corymbia calophylla* mature tree roots and those present on seedlings of *C. calophylla* in a glasshouse bioassay (i.e., soil propagules). The colour of each taxon represents the log-2 ratio of median proportion of reads observed in each community type. Significant differences were determined using a Wilcox rank-sum test followed by a Benjamini-Hochberg (FDR) correction for multiple comparisons. Taxa coloured in blue are more abundant in the propagule community whereas taxa coloured in brown are more abundant in the resident community. Taxa in grey showed no differences between resident and propagule communities

Table 2. Twenty-three families (plus one group not identified to family) of mycorrhizal fungal phylotypes (AMF = arbuscular mycorrhizal fungi, EMF = ectomycorrhizal fungi) found in both resident tree roots collected from a forest site in Western Australia and glasshouse roots baited from the same forest soils (i.e., soil propagules). N represents total number of phylotypes found in each family and the relative proportion (%) is the proportion of reads belonging to the family in question in each transect. The soil was collected along a disturbance gradient where the disturbed edge was a remnant stand of forest trees bordering horticultural land and a road, the forest edge was on the opposite side of the road and 50 m, 100 m and 200 m were distances into the forest block. Grey shaded rows indicate phylotypes not found as soil propagules in the glasshouse roots but found in resident tree roots.

Family	Total N	Resident Disturbed Edge	N	Forest Edge	N	50 m	N	100 m	N	200 m	N	Soil propagules Disturbed Edge	N	Forest Edge	N	50 m	N	100 m	N	200 m	N
Amanitaceae (EMF)	7	0	0	0	0	0	0	0.013	1	0.964	6	0	0	0	0	0	0	0	0	0	0
Atheliaceae (EMF)	7	12.424	4	0.161	2	0.407	3	0	0	0	0	0.222	2	0	0	0.006	2	0	0	0	0
Bankeraceae (EMF)	5	0	0	1.492	4	0.030	1	0.064	2	0.911	4	0	0	0.024	4	0.018	1	0.071	3	0.071	3
Boletaceae (EMF)	11	0.003	1	0.192	2	0.454	5	0.679	3	0.040	2	0	0	0	0	0	0	0	0	0	0
Ceratobasidiaceae (EMF)	4	0	0	0	0	0.128	2	0	0	0.010	1	0	0	0.014	1	0.038	2	0	0	0	0
Claroideoglomeraceae (AMF)	6	0.067	2	0	0	0	0	0.010	3	0.017	2	0	0	0	0	0.002	1	0.036	5	0	0
Clavulinaceae (EMF)	2	1.855	1	0.118	1	0.034	1	0	0	0.047	1	0.243	1	0.004	1	0.131	1	0	0	0.004	1
Cortinariaceae (EMF)	29	0.934	4	1.825	10	2.036	13	1.048	10	0.877	10	0.002	1	0.053	3	1.709	4	0.030	3	0.006	1
Glomeraceae (AMF)	26	0.155	3	0.279	10	0.030	4	0.077	5	0.622	11	0.034	4	0.200	8	0.671	18	0.293	7	0.152	12
Gloniaceae (EMF)	4	1.096	3	1.143	4	0.534	4	0.323	4	1.012	3	0.404	4	1.213	4	1.361	3	0.597	4	0.625	3
Gomphaceae (EMF)	2	0	0	0	0	0.094	1	4.651	1	0	0	0	0	0	0	0	0	0	0	0	0
Hydnaceae (EMF)	2	0.050	1	0.520	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inocybaceae (EMF)	26	0.356	3	3.898	14	0.057	5	0.037	5	0.030	3	0.653	6	2.898	8	1.685	2	0.320	7	0.038	3
Pezizaceae (EMF)	7	0	0	0.020	1	0	0	0.118	2	0.040	2	1.300	3	1.899	1	1.337	2	1.814	5	2.712	3
Phallaceae (EMF)	1	0	0	0	0	0	0	0	0	7.901	1	0	0	0	0	0	0	0	0	0	0
Pisolithaceae (EMF)	2	1.250	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyronemataceae (EMF)	2	0	0	0	0	0	0	0.212	1	0	0	0	0	6.577	2	1.493	1	7.526	2	8.260	2
Russulaceae (EMF)	16	0.309	2	1.529	12	0.450	7	0.286	6	0.074	5	0.053	2	0.269	9	1.387	7	0.051	4	0.107	6
Sclerodermataceae (EMF)	2	0.030	2	0.343	2	0.165	1	0.995	1	0.198	1	0	0	0	0	0	0	0	0	0	0
Sebacinaceae (EMF)	22	17.135	3	4.802	14	0.978	5	0.719	7	0.017	4	2.118	4	0.963	8	0.445	7	0.146	6	0.049	4
Other Sebacinales (EMF)	21	0.097	2	0.548	11	0.108	6	0.339	5	0.265	4	0.047	5	0.423	11	0.067	9	0.477	8	0.208	8
Serpulaceae (EMF)	2	0	0	0.020	1	0	0	0.104	1	0	0	0	0	0	0	0	0	0	0	0	0
Thelephoraceae (EMF)	48	5.908	8	2.702	25	4.359	17	4.722	21	1.242	17	18.767	8	3.727	11	13.158	13	2.443	14	1.774	8
Tricholomataceae (EMF)	4	0.047	1	0.010	1	0	0	0	0	0.151	1	6.072	2	0.144	1	0	0	0.269	1	0.089	2

There was substantial overlap between the composition of communities of mycorrhizal fungi, using relative abundance, between the soil propagules and resident roots (Fig. 4A), suggesting similar community composition between the two groups. However, there was a significant ($F_{1,91} = 4.735$, p = 0.0001) difference between the two structure types. There was also a significant ($F_{4,91} = 3.635$, p = 0.0001) effect of transect (Fig. 4A). Post hoc analyses showed significant (p adjusted = 0.01) differences among mycorrhizal fungal communities from the disturbed edge and all other transects, and no significant (p adjusted > 0.20) difference between pairs of transects within the forest (i.e., transect 2–5). A similar trend was seen when using incidence data (Fig. 4B): the composition of mycorrhizal fungal communities differed significantly ($F_{1,91} = 2.927$, p = 0.0001) between the two community structure types; however, there was extensive overlap between the two types of communities (Fig. 4B). There was a significant ($F_{4,91} = 3.299$, p = 0.0001) effect of transect (Fig. 4B). Post hoc analyses of the incidence data showed significant (p adjusted = 0.01) differences among all transect pairs except between 100 m and 200 m in the forest (p adjusted = 0.268).
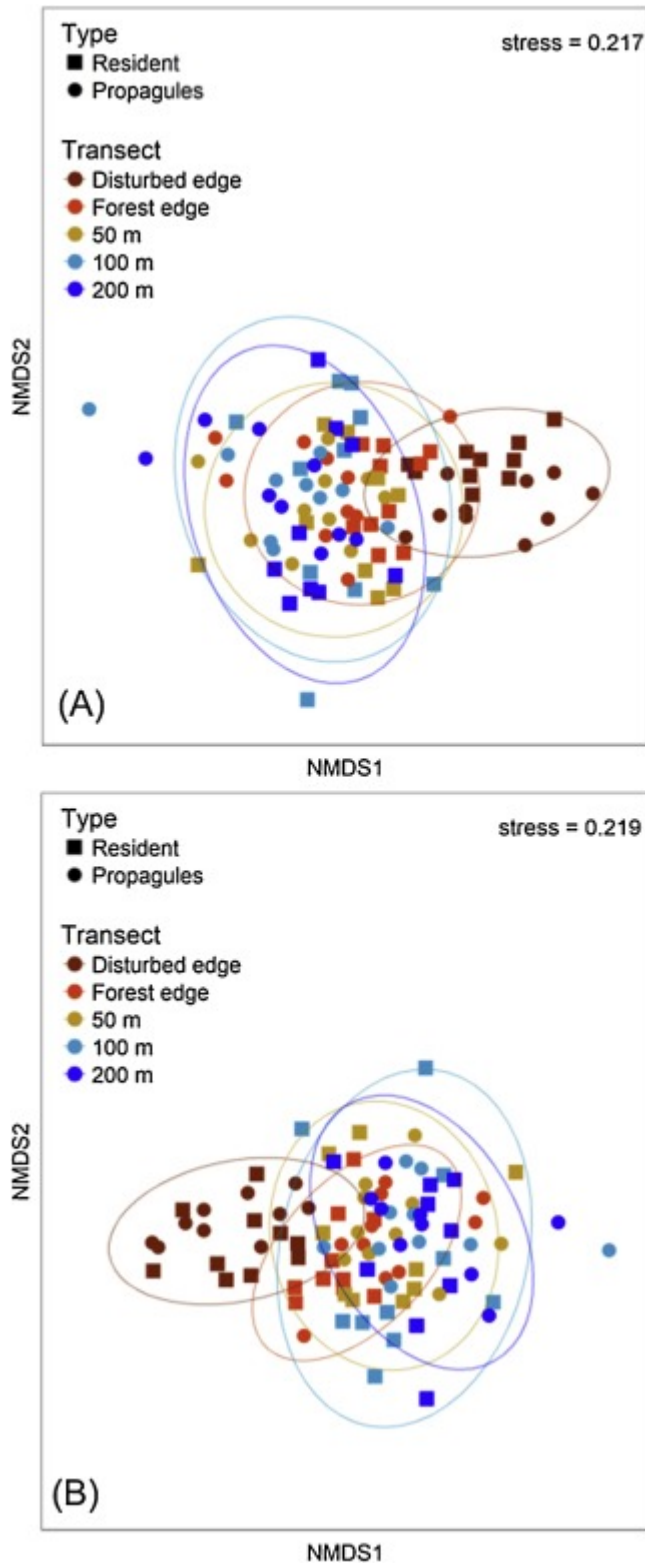
Fig. 4. Non-metric multidimensional scaling (NMDS) plot demonstrating the communities of mycorrhizal fungi associated with *Corymbia calophylla* across a disturbance gradient: disturbed edge (i.e., remnant stand of *C. calophylla* bordering cleared land and a road), forest edge, 50 m, 100 m and 200 m within a forest where **(A)** is the composition of mycorrhizal fungal communities represented by relative abundance (i.e., number of reads Hellingers transformed), and **(B)** is the composition of mycorrhizal fungal communities represented by incidence of phylotypes (i.e., presence-absence data). Across the disturbance gradient two types of communities were measured: those present on resident roots of *Corymbia calophylla* and those present on seedlings in a glasshouse bioassay (i.e., soil propagules).

4. Discussion

The community composition of the mycorrhizal fungi from our bioassay trial (i.e., propagule community) was similar to the community composition of the resident mycorrhizal fungi from the *C. calophylla* roots collected from our field site and both of these aspects of mycorrhizal fungal community structure responded in a comparable way to habitat fragmentation. The main difference between these two community structures was that more phylotypes were identified in the resident community and thus it had a slightly higher species richness. Differences in community composition between resident and soil propagule communities have been demonstrated in other forest ecosystems such as pine forests in the United States (Taylor and Bruns, 1999) and Japan (Murata et al., 2017). Interestingly, resident communities of mycorrhizal fungi on *Eucalyptus grandis* in Australia were predominantly ectomycorrhizal fungi whereas soil propagule communities colonizing *E. grandis* seedlings had extensive arbuscular mycorrhizal fungi (Adams et al., 2006). Due to the use of general fungal primers in our study, however, we were not able to confirm greater infection of arbuscular mycorrhizal fungi in seedlings in comparison to resident mature trees.

The phylotypes in greater abundance in the resident communities were in mycorrhizal fungal families often referred to as late succession fungi (e.g., Boletaceae and Cortinariaceae) which often fail to establish mycorrhiza on seedlings through spores or mycelial inoculum (Burgess et al., 1993; Jumpponen and Egerton-Warburton, 2005). Late-stage fungi colonize large, old trees and seedlings, but only when seedlings are in the presence of larger, older trees (Fleming, 1983). However, other factors such as physiological interactions between the fungi, the host plant, the environment, and the reproductive strategies of fungi affect colonization and establishment of fungi (Bergemann and Miller, 2002).

Habitat fragmentation altered both soil propagule and resident communities of mycorrhizal fungi. Species richness of each community was not significantly different across the disturbance gradient, but species composition changed across a disturbance gradient. In both resident and soil propagule communities, there was a greater proportion of ectomycorrhizal fungi belonging to the families Atheliaceae, Clavulinaceae, Inocybaceae, Sebacinaceae, Thelephoraceae and Tricholomaceae along the disturbed edges. In comparison, arbuscular mycorrhizal fungi were more abundant within the forest than along the disturbed edges. A similar finding was observed in *Eucalyptus gomphocephala* seedlings: a greater proportion of arbuscular mycorrhizal fungi were found in propagule communities in sites with declining canopies in comparison to sites with healthy canopies (Ishaq et al., 2018). In our study, the disturbed edge bordered horticultural land where nutrient fertilization would be common (Department of Primary Industries and Regional Development, 2016). Some species of mycorrhizal fungi respond strongly to disturbance and nutrient availability (Henry et al., 2016) and thus changes in soil nutrients can result in changes in community composition of these fungi (Ge et al., 2017; McBurney et al., 2017). However, we did not replicate our disturbance gradient in the current study and thus future work would need to explore this and possible mechanisms behind the effects of habitat fragmentation on communities of mycorrhizal fungi.

Communities of mycorrhizal fungi associated with *C. calophylla* in southwest Western Australia are relatively unknown, but in this study, we have also shown these communities are diverse with 23 families associated with this tree host. As we only looked at a single site, further replication would be needed to confirm this finding. In comparison, mixed pine and oak forests in England have up to sixteen families of mycorrhizal fungi associated with them;

however, the study on mixed pine and oak forests identified and sequenced individual mycorrhizal fungal morphotypes from tree roots from soil samples and thus used a different sampling method to our study (Suz et al., 2017).

In forest ecosystems threatened by both biotic and abiotic factors, changes in both resident and soil propagule communities could have long-term effects on the ability for re-establishment and restoration of these ecosystems. Both resident and soil propagule communities are necessary for mycorrhizal fungal colonization, whether through active mycelium or dormant structures. Previous work has demonstrated propagule communities are resistant to disturbance events such as fire, and post-fire plant communities are colonised via these resistant propagule communities. However, we have demonstrated propagule communities associated with *Corymbia calophylla* trees in a Mediterranean-type forest are sensitive to habitat fragmentation. The change in community composition of mycorrhizal fungi in both resistant and resident pools can have cascading effects on future tree establishment and health. *Corymbia calophylla* trees on fragmented edges have higher incidence of canker disease and a combination of fragmentation and changes in communities of mycorrhizal fungi could be decreasing the health of these trees, making them more susceptible to infection by *Quambalaria coyrecup*.

Declaration of Competing Interest

Acknowledgements

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