Methods

DNA samples were diluted to 10 ng/uL prior to PCR amplification. DNA samples were submitted to the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, NSW, Australia). Amplicons of the V4 region of the bacterial rRNA gene were generated using 515f (5’-GTGCCAGCMGCCGCGGTAA-3’; Caporaso et al., 2011) and 806r (5'-GGACTACHVGGGTWTCTAAT-3'; Caporaso et al., 2011). Amplicons to identify fungal taxa were generated using fITS7 (5’-GTGARTCATCGAATCTTTG-3’; Ihrmark et al. 2012) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White et al. 1990). All amplicons purified using the Agencourt AMpure XP system (Beckman Coulter, Lane Cove, NSW, Australia) and genomic libraries were prepared using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). Paired-end (2 x 251 bases) sequencing was performed on the Illumina MiSeq platform.

To process the DNA sequencing data, we used the approach described by Bissett et al. (2016) with a few modifications. Contigs were generated from paired-end reads using the 'fastq\_mergepairs' command in VSEARCH (version v2.3.4; Rognes et al., 2016) using a minimum overlap of 30 (for ITS sequencing) or 200 (for 16S sequencing) base pairs. Initial quality filtering removed DNA sequences containing ambiguous bases and/or homopolymers greater than eight bases in length. Sequences were kept for further analysis if they were within 200-470 (for ITS sequencing) or 251-255 (for 16S sequencing) base pairs in length and contained fewer than 0.5 expected errors. De novo operational taxonomic units (OTUs) at 97% sequence similarity were initially picked using numerically dominant sequences (observed at least two times) using the ‘-cluster\_smallmem’ command in VSEARCH. All quality-filtered sequences were mapped at 97% sequence similarity against representative sequences of these OTUs using the ‘-usearch\_global’ command in VSEARCH. Non-mapped sequences were subjected to a second round of de novo OTU picking, as above but only using sequences observed at least two times. All initially non-mapped sequences were then mapped against these newly picked OTUs, as above. Non-mapped sequences at this step represent singleton OTUs and were excluded from further analysis. Sequence read counts that were less than ten within individual samples were removed to reduce the likelihood of sequence reads being assigned to samples incorrectly.

Putative taxonomic identities for fungal and bacterial OTUs were generated using BLAST (v.2.6.0, Altschul et al. 1990) to compare representative sequences for each OTU to a reference database of gene sequences and taxonomic annotations (bacterial 16S rRNA: greengenes, DeSantis et al. (2006), version in output file; fungal ITS: UNITE Abarenkov et al. 2021, version in output file). Fungal ITS2 sequences were extracted using ITSx (Bengtsson-Palme et al. 2013, v1.1.3) for use during BLAST. Guilds of bacterial OTUs that were assigned to taxa were then inferred from Ho et al. (2017; copiotrophic - 'Actinobacteria', 'Bacteroidetes', 'Firmicutes', 'Betaproteobacteria', 'Gammaproteobacteria'; oligotrophic - 'Acidobacteria', 'Verrucomicrobia'). Trophic modes and guilds of fungal OTUs that were assigned to taxa were then inferred using FUNGuild (Nguyen et al. 2016; accession date in output file); only those assignments with ‘highly probable’ or ‘probable’ confidence were used in subsequent calculations.

Whole community and within guild diversity estimates were calculated as follows. Observed richness was based on OTU counts per sample remaining after random resampling of OTU tables at equal depth (samples with fewer than 5000 reads were excluded from calculations). Shannon diversity was calculated from resampled counts using the ‘diversity’ function from the ‘vegan’ package (Oksanan et al. 2020). Extrapolated (Chao1) richness was calculated using the ‘chao1’ function from the ‘fossil’ package (Vavrek 2011) and counts prior to resampling. Within guild relative abundance estimates represent the proportion of all reads in a sample that were assigned to that guild prior to resampling.

If included, predicted abundances associated with metabolic pathways were generated from representative 16S sequences for OTUs with the ‘picrust2\_pipeline.py’ script from PICRUSt2 v2.4.1 (Douglas et al. 2020). Before analyses, bacterial OTU tables were trimmed to include only those OTUs that made up at least 0.1% of reads in at least 5% of samples; the retained OTUs represented 54% of all reads in the resampled OTU tables. Phylogenetic placement of reads was performed using ‘SEPP’ (Mirarab et al. 2012), hidden state prediction using ‘castor’ (Louca and Doebeli 2018) and pathway inference using ‘MinPath’ (Ye and Doak 2009).

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