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Research Article

Disturbance of eucalypt forests alters the composition, function, and assembly of soil microbial communities

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Abstract

Forest disturbance has well-characterized effects on soil microbial communities in tropical and northern hemisphere ecosystems, but little is known regarding effects of disturbance in temperate forests of the southern hemisphere. To address this question, we collected soils from intact and degraded *Eucalyptus* forests along an east–west transect across Tasmania, Australia, and characterized prokaryotic and fungal communities using amplicon sequencing. Forest degradation altered soil microbial community composition and function, with consistent patterns across soil horizons and regions of Tasmania. Responses of prokaryotic communities included decreased relative abundance of Acidobacteriota, nitrifying archaea, and methane-oxidizing prokaryotes in the degraded forest sites, while fungal responses included decreased relative abundance of some saprotrophic taxa (e.g. litter saprotrophs). Forest degradation also reduced network connectivity in prokaryotic communities and increased the importance of dispersal limitation in assembling both prokaryotic and fungal communities, suggesting recolonization dynamics drive microbial composition following disturbance. Further, changes in microbial functional groups reflected changes in soil chemical properties—reductions in nitrifying microorganisms corresponded with reduced NO_3-N pools in the degraded soils. Overall, our results show that soil microbiota are highly responsive to forest degradation in eucalypt forests and demonstrate that microbial responses to degradation will drive changes in key forest ecosystem functions.

Keywords: 16S, bacteria, forest management, fungi, ITS, microbiome

Introduction

Anthropogenic forest degradation represents one of Earth's dominant land conversions and is altering forest ecosystem structure and function worldwide (Vitousek et al. 1997, Foley et al. 2005, Rudel et al. 2005). Indeed, over the past two centuries, Earth's land surface has lost approximately one-third of its forest cover, with much of the remaining forests subject to various forms of anthropogenic degradation (Ritchie and Roser 2021). Given the accelerating rate of forest degradation in the 21st Century (Drummond and Loveland 2010, Hansen et al. 2010), along with the critical role of forests in providing key ecosystem services that support human well-being (Millenium Ecosystem Assessment 2005), it is imperative to characterize the effects of forest degradation on terrestrial biodiversity and ecosystem functioning.

Forest ecosystem functions and services, e.g. carbon (C) storage and nutrient cycling, are primarily facilitated by microorganisms (e.g. prokaryotes and fungi) that inhabit soil (Fierer 2017). For example, prokaryotes perform several key biogeochemical functions such as nitrogen (N) fixation and nitrification while soil fungi are thought to be the predominant decomposers of recalcitrant compounds (e.g. lignin) (van der Heijden et al. 2008) and also influence plant productivity and nutrient cycling via mycorrhizal associations (e.g. Heděnec et al. 2023). Many prior studies have investigated effects of forest degradation on soil microbial communities, showing that past and present disturbances such as logging and conversion to agriculture can alter microbial community diversity, composition, and assembly (Jangid et al. 2011, Kohout et al. 2018, Mushinski et al. 2018, Osburn et al. 2019, 2021). These effects on microbial communities also have important implications for forest ecosystem functioning, e.g., forest disturbances have been linked to reduced microbial decomposition potential (Cardenas et al. 2015, Kohout et al. 2018) and increased abundance of taxa responsible for nitrogen (N) cycle processes such as nitrification and denitrification (Mushinski et al. 2018, Osburn et al. 2019). Functional changes in microbial communities following forest degradation have even been detected at a global scale, with a recent global meta-analysis showing increased abundance of rapidly growing copiotrophic (*r*-selected) bacterial taxa in degraded forest soils (Zhou et al. 2018). However, these studies are spatially biased in favour of tropical and northern hemisphere forests, while temperate forests of the southern hemisphere remain underrepresented. For example, the above-mentioned meta-analysis by Zhou et al. (2018) included data from 119 prior studies, but only three were from temperate southern hemisphere forests.

Temperate forests account for ~100 million ha of land in the southern hemisphere, ~57 million ha of which is found in southeastern Australia and Tasmania (Olson et al. 2001). These temperate Australian forests are dominated by *Eucalyptus* trees, which

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comprise 77% of the continent's total native forest area (Department of Agriculture, Fisheries, and Forestry 2018). While there is considerable prior work on soil microbial communities in eucalypt forests globally, much of it has focused on *Eucalyptus* plantations in tropical and subtropical areas of South America, Europe, and Asia, most of which is outside of the native range of these trees (Brockerhoff et al. 2013). These studies, predominantly conducted in south China, show that various *Eucalyptus* plantation characteristics and management strategies (e.g. stand age, understory removal, and nitrogen fertilization) can influence soil microbial communities (Cao et al. 2010, Wu et al. 2011, Zhao et al. 2013). Studies conducted in the native range of temperate *Eucalyptus* forests are less common, though one showed that *Eucalyptus* revegetation in Australia increased soil fungal:bacterial ratios (Carnovale et al. 2019), while others show that slow-growing oligotrophic (K-selected) bacteria are negatively impacted by postlogging fire in eucalypt forests of Tasmania, Australia (Ammitzboll et al. 2021, 2022). However, we know of only one prior study investigating effects of disturbance legacies (i.e. historical logging and wildfire) in Australian eucalypt forests, which focused on how disturbance affected microbial α-diversity and associations with environmental variables (e.g. soil physicochemical properties) (Bowd et al. 2022). As a result, key characteristics of soil microbial communities in currently forested, but anthropogenically degraded, eucalypt ecosystems of Australia remain largely uncharacterized.

While it is difficult to make specific predictions regarding soil microbial responses to forest degradation in temperate eucalypt forests, it should be noted that effects on microbial communities will be mediated by direct effects of forest degradation on soil physicochemical properties and vegetation characteristics (Fierer and Jackson 2006, Lauber et al. 2008, 2009, Goldmann et al. 2015). This is notable because soil and vegetation properties are quite distinctive in Australian *Eucalyptus* forests. For example, soils in Australia are among the oldest in the world, are often highly acidic, and are generally highly leached and very low in available nutrients, e.g. phosphorus (P) (Department of Agriculture, Fisheries, and Forestry 2021). In addition, *Eucalyptus* leaf litter contains high concentrations of phenolic acids and volatile organic compounds, which can impact soil microbial metabolism and community composition, e.g. by promoting the growth of methylotrophic taxa (Martins et al. 2013, McBride et al. 2020, 2022). Because of these unique soil and vegetation characteristics, Australian eucalypt forests likely host distinct soil microbial communities that may exhibit fundamentally different responses to forest degradation than those documented in other regions.

To investigate effects of forest degradation on soil microbial communities in these unique Australian *Eucalyptus* forest ecosystems, we collected soils from intact and anthropogenically degraded (historically burned and logged) eucalypt forests along an east–west transect across Tasmania, Australia. We then characterized prokaryotic and fungal communities using amplicon sequencing and compared microbial community composition, the relative abundance of key functional groups, and total microbial biomass in intact vs. degraded forests. We predicted that degraded forests would exhibit reduced total microbial biomass but would host increased relative abundance of microbial taxa that have been previously shown to benefit from soil disturbance, e.g. rapidly growing *r*-selected bacteria and nitrifying prokaryotes (Zhou et al. 2018, Osburn et al. 2019). Further, any changes in microbial community structure will also likely alter the frequency and types of potential ecological interactions among taxa, i.e. forest degradation may alter microbial co-occurrence network properties, as shown in prior work from other forested regions (Sun et al. 2017, Osburn et al. 2019). Therefore, we conducted network analysis of intact vs. degraded communities with the expectation that forest degradation would disrupt and reduce the complexity of microbial co-occurrence patterns. Finally, any observed shifts in microbial communities following forest degradation can likely be attributed to changes in the community assembly processes acting on those communities. For example, prior work from other regions has shown that land use change alters the relative importance of selection vs. stochastic processes (e.g. dispersal, drift) in assembling microbial communities by altering key soil properties (e.g. pH) and vegetation characteristics (Barnett et al. 2020, Osburn et al. 2021). Therefore, we used a null model approach to assess community assembly in intact vs. degraded soils with the expectation that *Eucalyptus* forest degradation would drive changes in the relative importance of selection, dispersal, and drift in assembling microbial communities.

Materials and methods

Study design and soil sampling

Soils were collected in February–April 2022 from intact and degraded wet eucalypt forests along an east–west transect across Tasmania, Australia (Fig. 1). Sites were split into four representative regions (Eastern, Central, Central-Western, and Western) that span wide gradients in climate and geology (Tyler 1992). For example, precipitation at our sites ranges from < 1000 to > 2000 mm annually (generally increasing from east to west; Table S1, Supporting Information), while bedrock geology transitions from primarily metamorphic quartz rocks in the west to primarily volcanic dolerite and basalt in the east (Tyler 1992, Rees and Cwynar 2010). Soil types also vary across our transect—soils were predominately Dermosols, though Organosols, Tenosols, Hydrosols, Rudosols, and Ferrosols were also present in some locations (Australian Soil Classification; Table S1, Supporting Information) (Williams 2012). Despite the significant environmental variation across our transect, we selected sites in all regions with similar dominant vegetation—all sites were temperate, wet eucalypt forests, and most had a *Eucalyptus obliqua* overstory, though *E. regnans* and *E. delegatensis* were present at some sites (Table S1, Supporting Information). Forest understories were primarily composed of *Pomaderris apetala*, with some sites also hosting *Acacia dealbata*, *Nothofagus cunninghamii*, or *Olearia argophylla* (Table S1, Supporting Information). Within each region, we identified four sites (16 sites total), two of which were categorized as 'intact' and two of which were categorized as 'degraded' (Fig. 1). Sites categorized as intact were relatively undisturbed with mature overstory *Eucalyptus* trees and well-developed understory vegetation (Fig. 1B). Degraded sites were highly fragmented forest remnants adjacent to timber plantations or agricultural land and all were burned ~15–50 years prior (Fig. 1C; Table S2, Supporting Information). Most of the degraded sites were also logged 20–80 years previously (Department of Natural Resources and Environment Tasmania), and therefore hosted younger trees with less developed understory vegetation. Complete details regarding site characteristics and land use histories are provided in Tables S1 and S2 (Supporting Information). Though our degraded sites are somewhat variable with regard to the timing and particular types of forest degradation represented, they all experienced significant physical disturbance as a result of anthropogenic activity in the recent past.

Within each of the 16 sites, we established three plots. Plots were generally located 10 m apart, though this distance was differ-

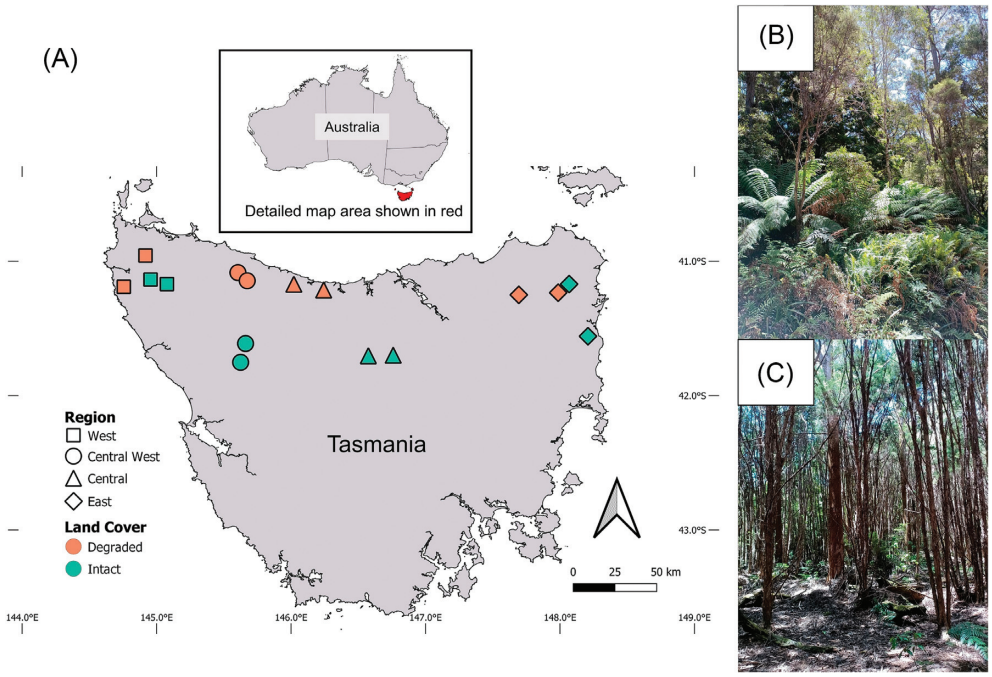

Figure 1. Map of Tasmania, Australia, showing locations of each of the 16 sites (A). Detailed site information is provided on Tables S1 and S2 (Supporting Information). Panels (C) and (D) show example photos of an intact forest site with developed understory (B) and a degraded forest site with young trees and regenerating understory (C).

ent in some sites (up to ~60 m apart) to avoid excessively boggy or rocky areas in those sites. Because of this variability in distances between plots, we explicitly included spatial distance into our statistical approach (see data analysis section below). At each of the three plots we removed the litter layer from a 50 cm^2 area of the forest floor and dug a small soil pit (to 5 cm mineral soil depth) with a sterilized trowel. We then separated the entire organic horizon and the top 5 cm of mineral soil into two separate plastic bags. This resulted in a grand total of 96 soil samples (4 regions × 4 sites per region × 3 sampling locations per site × 2 soil horizons per sampling location). Each of these individual 96 samples was subjected to the physicochemical and microbial analyses described in the sections below. Samples were transported from the field in a cooler with ice packs (transit time 3–6 h) and then stored at −20°C at the University of Tasmania prior to shipping. Samples were shipped frozen on dry ice to the University of Idaho where they were sieved (2 mm) and homogenized. Overall, our sampling scheme allowed for replicated soil characterization within the individual sites while also incorporating broad spatial representation of sites across the climatic and geologic gradients present in Tasmania. Our study thus represents an ideal opportunity to identify effects of eucalypt forest degradation on soil properties and microbial communities that are consistent across natural environmental variation.

Soil physicochemical analyses

We determined soil water content by mass loss after drying samples at 105°C for 24 h and measured pH in a 1:4 soil:DI H_2O slurry using a SevenCompact pH meter (Metter Toledo, Columbus OH, USA). To measure pools of available C, N, and P in soil, we performed an extraction with 0.05 M K_2SO_4 (1:5 soil:solution ratio). Slurries were shaken for 1 h and extracts were filtered with Whatman no. 1 filter paper. We analyzed extracts for extractable organic C (DOC) and total extractable N (TDN) on a Shimadzu TOC-L TNM-L analyzer (Shimadzu Instruments, Kyoto, Japan). We also analyzed extracts of for ammonium (NH_4-N) using the phenol–hypochlorite method (Weatherburn 1967), nitrate (NO_3-N) using the vanadium reduction method (Doane and Horwáth 2003), and orthophosphate (PO_4-P) using the AMP-malachite green method (Lajtha et al. 1999). As a metric of total microbial biomass, we extracted DNA from soils using a Qiagen PowerSoil Pro Kit (Qiagen, Valencia, CA, USA) and quantified DNA using the Quant-iT high-sensitivity fluorometric assay kit (Thermo Fisher Inc., Waltham, MA, USA), similar to previous studies (Fornasier et al. 2014).

Microbial community analyses

We characterized soil prokaryotic communities by amplicon sequencing of the V4 region of the 16S rRNA gene using the 515F/806R primer pair (Walters et al. 2016), and fungal communities by sequencing the ITS1 region using the ITS1f/2r primer pair (White et al. 1990), respectively. Amplicons were sequenced on an Illumina MiSeq with 250 bp paired-end reads. Raw reads were deposited in the NCBI archive under accession number PRJNA884366. We processed raw sequences using DADA2 (Callahan et al. 2016) and assigned taxonomy to unique sequences (i.e. amplicon sequence variants, ASVs) using the IDTAXA classifier (Murali et al. 2018) trained on the SILVA database for 16S (version 138.1) (Quast et al. 2013) and the UNITE database for ITS (version 8.3) (Abarenkov et al. 2010). Though the DADA2 ASV approach is not ideal for quantitative analysis of fungal α diversity, DADA2 performs well when assessing fungal community composition (Tedersoo et al. 2022), which was the primary goal of this study. After processing, we rarefied samples to 12,187 and 11,776 sequences per sample for 16S and ITS, respectively, to account for differences in sequence depth among samples. We then used picrust2 to generate putative functional profiles for 16S sequences (Douglas et al. 2020) and FungalTraits to assign functional guilds to fungal genera (Põlme et al. 2020). Complete details regarding PCR and am-

plicon sequencing protocols are provided in the Supplementary Information.

Data analyses

We performed all statistical analyses in R (R Core Development Team 2019). We conducted multivariate analysis of prokaryotic and fungal communities using the 'vegan' R package (Oksanen et al. 2019). We assessed effects of region, soil horizon, and land cover (i.e. intact vs. degraded forest), on soil communities using PERMANOVA with Bray–Curtis dissimilarities ('adonis2' function) and visualized community composition using principal coordinates analysis ('cmdscle' function, Bray–Curtis dissimilarity). Because we observed consistent effects of forest degradation on communities across regions and soil horizons (Fig. 2), for all additional analyses we aggregated samples across horizons and regions to focus on effects of forest degradation. To determine effects of degradation on individual variables (e.g. soil properties and relative abundances of taxa), we used linear mixed models with 'land cover' as a fixed effect and 'region' as a random effect. We ran mixed models using the 'lmer' function in the lme4 package (Bates et al. 2019). We assessed assumptions of normality of model residuals using Shapiro–Wilk tests and by visualizing quantile–quantile plots. When models did not meet assumptions, we used generalized linear models with gamma distribution and log-link function ('glmer' function, lme4 package) (Bolker et al. 2009).

To identify individual 16S and ITS ASVs that were responsive to forest degradation (i.e. differentially abundant ASVs), we used the 'exactTest' function in the edgeR package (Robinson et al. 2010). To quantify the relative importance of selection, dispersal, and drift processes on 16S and ITS community assembly in intact vs. degraded soil communities, we used a phylogenetic null model approach (Stegen et al. 2013). This approach is based on quantification of phylogenetic community turnover at short phylogenetic distances, i.e. the βNTI index, which, in turn, is based on alignment of the 16S and ITS sequences. While ITS alignments are unreliable for tree-wide analyses, they are accurate for analyses performed at short phylogenetic distances (Tedersoo et al. 2018a), such as the community assembly analyses presented here. We compared proportions of each assembly process between intact and degraded communities using Z-tests. Complete details regarding the community assembly models are provided in the Supplementary Information with method validation presented on Figures S1 and S2 (Supporting Information).

To compare prokaryotic and fungal ASV co-occurrence patterns between intact and degraded forest soils, we used network analysis. We constructed co-occurrence networks using SPIEC-EASI (Kurtz et al. 2015), a graphical Gaussian model approach that accounts for the compositional nature of amplicon sequence datasets when assessing statistical associations between ASVs. To improve the robustness of our networks, we only included 16S ASVs that occurred in a minimum of 20 samples and ITS ASVs that occurred in a minimum of 10 samples. We then used the igraph package (Csardi and Nepusz 2006) to visualize networks and to calculate two key network properties: 'degree' and 'betweenness', both of which were normalized for each respective network. Degree and betweenness are measures of the connectivity and centrality of a co-occurrence network, respectively, where degree is calculated as the number of edges per ASV and betweenness is calculated as the number of times an ASV is on the shortest path between two other ASVs. We compared distributions of normalized degree and betweenness values between intact and degraded networks using Kruskal–Wallis tests. We also compared the proportion of negative edges in networks between intact and degraded communities using Z-tests. Overall, these network metrics allowed us to identify differences in the frequency and types of potential ecological interactions in microbial communities between the intact and degraded forest soils.

To assess environmental drivers of microbial community composition, we used variation partitioning ('varpart' function, vegan package) (Peres-Neto et al. 2006). We considered soil properties, spatial factors, and vegetation characteristics as candidate drivers of microbial communities and tested the significance of individual partitions of variation using distance-based redundancy analysis ('dbrda' function, vegan package). Spatial factors were represented by principal coordinates of neighborhood matrix (PCNM) scores for each sample, which represent independent components of spatial variation among the plots and are based on a matrix of pairwise linear distances between the sites ('pcnm' function, vegan package) (Borcard and Legendre 2002). Vegetation characteristics were represented by a presence/absence matrix of the dominant over- and understory plant species at each sampling site. To quantify the importance of individual soil variables in accounting for variation in prokaryotic and fungal communities, we used hierarchical partitioning in the 'rdacca.hp' R package (Lai et al. 2022). For all analyses, data and R analysis scripts are available on figshare (Osburn 2023).

Results

Soil properties

As expected, soil physicochemical properties were distinct between organic horizon and mineral horizon soils; organic soils had higher microbial biomass, and C, N, and P pools, while mineral soils had higher C:N ratios (Table S3, Supporting Information). Soil pH was the only soil property that did not differ between soil horizons (Table S3, Supporting Information). Soil properties also exhibited regional variation, particularly in pH, extractable N, extractable organic C (DOC), and PO_4-P levels (Table S4, Supporting Information), likely reflecting variation in bedrock geology among sites. Averaged across regions and soil horizons, forest degradation also had clear effects on soil physicochemical properties—degraded forest soils had significantly higher pH and 32% higher microbial biomass than intact forest soils (Table 1). In contrast, intact forest soils had 38% higher DOC, ~4-fold higher NO_3-N, and ~11.5-fold higher PO_4-P than degraded forest soils (Table 1).

Microbial diversity and community composition

Forest degradation did not affect prokaryotic or fungal α diversity metrics, i.e. ASV richness and Shannon diversity (Table S5, Supporting Information). In contrast, forest degradation consistently influenced prokaryotic 16S and fungal ITS sequence (i.e. ASV) community composition (Fig. 2A and B), with directionally consistent effects on prokaryotic communities across all regions and soil horizons (Fig. 2A). The effects of forest degradation on 16S ASVs, however, were smaller in magnitude in forest soils from the eastern region, thus accounting for the significant land cover × region interaction (Fig. 2A). Fungal ITS ASV composition was less distinct between soil horizons but highly dissimilar among regions in the intact forest soils (Fig. 2B). However, forest degradation caused an apparent convergence in fungal ITS composition across regions, i.e. degraded soil fungal communi-

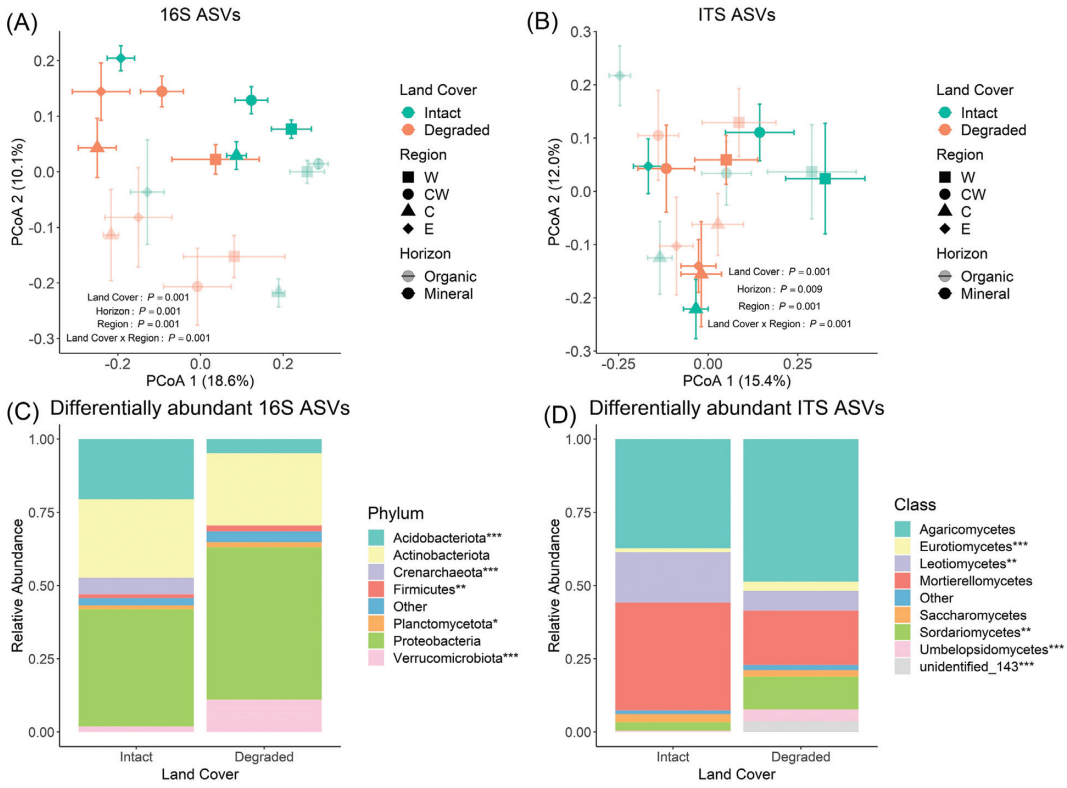

Figure 2. Effects of region, soil horizon, and forest degradation on 16S ASV community composition (A) and ITS ASV community composition (B). *P* values in (A) and (B) are from PERMANOVA with Bray–Curtis dissimilarities. Panels (C) and (D) show phylum level relative abundances of 471 differentially abundant 16S ASVs (C) and class level relative abundances of 323 differentially abundant ITS ASVs (D) identified by edgeR. In (C) and (D), statistical effects of forest degradation on the relative abundances of taxa were determined using mixed models with 'land cover' as a fixed effect and 'region' as a random effect. Asterisks next to taxa names indicate significant effects of land cover at the following levels: * $P < .05$, ** $P < .01$, and *** $P < .001$.

Table 1. Effects of forest degradation on soil physicochemical properties. Values are means followed by one standard error of the mean in parentheses. Statistical effects were determined using mixed models with 'region' as a random effect. Asterisks indicate significantly higher values at the following levels: * $P < .05$, ** $P < .01$, and *** $P < .001$.

Variable	Intact	Degraded
pH	4.23 (0.08)	4.89 (0.10)***
NO_3^- (μg N gdw^{-1})	15.2 (4.44)**	3.70 (0.79)
NH_4^+ (μg N gdw^{-1})	111 (13.8)	94.6 (14.8)
PO_4^- (μg P gdw^{-1})	22.0 (7.97)**	1.91 (0.61)
Total extractable N (μg N gdw^{-1})	159 (22.9)	112 (15.9)
Extractable DOC (μg C gdw^{-1})	416 (52.7)*	301 (40.4)
Extractable C:N	3.17 (0.20)	3.15 (0.25)
Microbial biomass (μg DNA gdw^{-1})	61.1 (5.43)	80.7 (7.52)*

ties appeared to form a distinct cluster (Fig. 2B), similar to 16S communities.

Aggregating 16S ASVs at the phylum level revealed effects of forest degradation on specific prokaryotic taxa. For example, degradation decreased the relative abundance of Acidobacteriota and increased the relative abundance of Verrucomicrobiota across all regions (Figure S3, Supporting Information). Taxonomic analysis of the 471 differentially abundant 16S ASVs identified by edgeR reinforced those patterns—differentially abundant Acidobacteriota were ~2.2-fold higher in the intact forest soils while Verrucomicrobiota were ~3.4-fold higher in the degraded forest soils. Differential abundance analysis also revealed negative responses of Crenarchaeota to forest degradation, which were 17-fold higher in the intact than degraded forest soils (Fig. 2C; Figure S4, Supporting Information).

Aggregating ITS sequences at the class level did not immediately reveal fungal taxonomic responses to forest degradation (Figure S5, Supporting Information). However, taxonomic analysis of 323 differentially abundant ITS ASVs did reveal patterns among responsive fungal taxa. In particular, differentially abundant Eurotiomycetes, Sordariomycetes, and Umbelopsidomycetes were 37%, 235%, and 463% higher in degraded forests, respectively (Fig. 2D), thus revealing positive responses of these taxa to forest degradation. Further, these positive responses were consistently observed across all regions of Tasmania (Figure S6, Supporting Information).

Microbial ecosystem functions

Taxonomic analysis of the 16S ASVs also showed effects of forest degradation on taxa that perform key soil functions. For example, the nitrifying class Nitrososphaeria within Crenarchaeota were 25-fold higher in intact forest soil communities (Figure S7, Supporting Information) and methane-oxidizing *Methylocella* were 6.8-fold higher in intact forest soil communities (Figure S8, Supporting Information). To gain additional support for these results, we predicted putative functional profiles for 16S sequences using picrust2 (Douglas et al. 2020) and summed the predicted gene abundances for the nitrification and methane oxidation pathways for each sample. The picrust2 results supported the taxonomic patterns—predicted nitrification genes had 55% higher relative abundance in the intact soils (Fig. 3A), which was also in line with the higher NO_3-N observed in those soils (Table 1). Similarly, predicted methane oxidation genes had 24% higher relative abundance in the intact soils (Fig. 3B).

To investigate fungal functions, we assigned fungal taxa to functional guilds using FungalTraits (Põlme et al. 2020), which assigned guilds to 77% of our ITS sequences (Figure S9, Supporting Information). The dominant guild was soil saprotrophs, which accounted for ∼60% of all ASVs (Figure S9, Supporting Information). Forest degradation did not affect the relative abundance of soil saprotrophs, but did alter the composition of soil saprotrophs, though the effects varied among different regions of Tasmania (land use × region interaction; Figure S10, Supporting Information) due to distinct responses of specific saprotrophic genera among the different regions (Figure S11, Supporting Information). Forest degradation did affect the relative abundances of two other groups of saprotrophs: litter saprotrophs, which were 2.6-fold higher in the intact sites (Fig. 3C), and unspecified saprotrophs, which were 2-fold higher in the intact sites (Fig. 3D). Degraded forest soils also had 3-fold greater relative abundance of animal parasites compared with the intact soils (Figure S9, Supporting Information).

Co-occurrence networks

Co-occurrence network analysis of 16S ASVs revealed distinct network topologies of prokaryotic communities between soils in intact and degraded forests (Fig. 4A and B, Table 2). The intact forest 16S network had more connections (i.e. edges) between taxa (494 vs. 270), and a higher proportion of negative edges (0.42 vs. 0.29), than the degraded forest 16S network (Fig. 4A and B, Table 2). The intact network also had more edges per ASV (i.e. 136% greater normalized degree; Table 2) and 125% greater normalized betweenness, indicating that taxa in the intact network were significantly more likely to be on the shortest path between two other taxa than taxa in the degraded network (Table 2). In general, these results indicate that the intact soil prokaryotic communities exhibited more connected, tightly clustered networks than the degraded soil communities. Network analysis of ITS sequences did not reveal effects of forest degradation on fungal co-occurrence patterns—intact and degraded ITS networks had similar topological properties with no significant differences in network statistics (Fig. 4C and D, Table 2).

Community assembly processes

Community assembly analysis indicated that 16S communities were shaped primarily by selection while ITS communities were shaped primarily by stochastic processes (i.e. dispersal and drift processes) (Fig. 5). Further, forest degradation altered the relative contributions of different assembly processes for both 16S and ITS communities. For example, degradation reduced the importance of homogeneous selection in assembling 16S communities and increased the contribution of stochastic processes, specifically drift and dispersal limitation (Fig. 5A). For ITS communities, forest degradation increased the contribution of dispersal limitation while decreasing the contribution of drift (Fig. 5B).

Environmental drivers of microbial responses

Variation partitioning analysis revealed that measured soil properties accounted for 29% of the variation in 16S communities, with 20% being independent of spatial variation and vegetation characteristics among sites (Fig. 6A). Spatial and vegetation factors independently accounted for 4% and 7% of the variation in 16S communities, respectively (Fig. 6A). For ITS, variation partitioning revealed that soil properties accounted for 16% of the variation in community composition, with 9% being independent of spatial variation and vegetation characteristics among sites (Fig. 6B). Spatial and vegetation factors both independently accounted for 6% of the variation in ITS communities (Fig. 6B). All partitions of variation were statistically significant for both 16S and ITS communities (distance-based redundancy analysis $P < .05$), though note that the significance of the interdependent partitions of variation cannot be tested. Considering contributions of individual soil variables, hierarchical partitioning revealed pH to be the most important soil property for both 16S and ITS communities, with extractable DOC and microbial biomass playing secondary and tertiary roles (Fig. 6C and D).

Discussion

Our study reveals influences of eucalypt forest degradation on soil microbial communities that were generally consistent across soil horizons and across regions of Tasmania, Australia. These patterns were particularly strong in prokaryotic communities—16S sequencing revealed clear differences in 16S ASV composition, taxonomic composition, and functional group abundances between intact and degraded forest sites. Importantly, these changes in 16S communities appear to be influencing ecosystem-scale forest processes—reductions in nitrifying archaea in degraded forest soils were accompanied by reduced NO_3-N pools, thus providing a mechanism for the reduced ecosystem-scale N mobility that has been observed in degraded eucalypt forests (Bowd et al. 2021). This altered ecosystem N cycling is relevant in the context of eucalypt forest management, as reduced N mobility may inhibit recovery of the degraded forests. We also observed reductions in methane-oxidizing bacteria in degraded forest soils, suggesting they function less effectively as methane sinks, a trend that has been observed in prior work (Cuer et al. 2018). Alternatively, this pattern may reflect increased availability of methane in the intact sites, though we did not observe differences in the relative abundance of methanogenic taxa. Regardless, our study reveals strong responses of prokaryotic communities to forest degradation in temperate eucalypt forests, with clear implications for the functions and services provided by these ecosystems.

We also observed dramatically reduced 16S network connectivity in degraded forest soil communities. While co-occurrence patterns cannot be directly interpreted as ecological relationships (Faust and Raes 2012), the substantially reduced frequency of associations between taxa we observed in the degraded 16S network does suggest that potential ecological interactions among taxa are disrupted by forest degradation in temperate eucalypt ecosystems. The reduced proportion of negative associations we

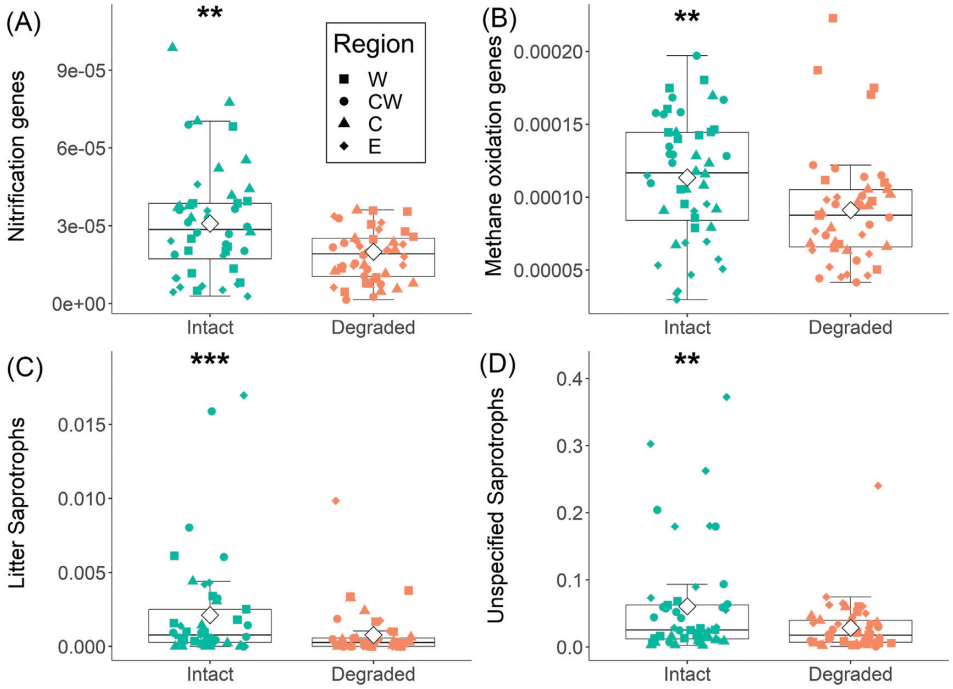

Figure 3. Relative abundances of genes involved in nitrification (A) and methane oxidation (B) and relative abundance of fungal taxa assigned to the litter saprotrophs guild (C) and the undefined saprotrophs guild (D). Putative functional gene abundances were predicted for 16S sequences using picrust2 (Douglas et al. 2020) while functional guilds were assigned to fungal genera using the FungalTraits database (Põlme et al. 2020). Relative abundances of taxonomic groups involved in nitrification and methane oxidation are shown on Figures S7 and S8 (Supporting Information) while relative abundances of all fungal guilds are shown on Figure S9 (Supporting Information). Asterisks indicate significantly higher relative abundance at the following levels: ** $P < .01$ and *** $P < .001$. *P*-values are from mixed models with 'land cover' as a fixed effect and 'region' as a random effect.

Table 2. Network metrics calculated for 16S and ITS co-occurrence networks constructed for intact and degraded forest soils. For normalized degree and normalized betweenness, values displayed are medians followed by interquartile ranges. Asterisks indicate significantly higher values at the following significance levels: *** $P < .001$. Proportion of negative edges was compared between intact and degraded soils using Z-tests, while distributions of normalized degree and betweenness values were compared between intact and degraded networks using Kruskal–Wallis tests.

	Network metric	Intact	Degraded
Prokaryotes	Nodes	195	188
	Edges	494	270
	Prop. negative edges	0.417***	0.285
	Degree	0.026 (0.021)***	0.011 (0.017)
	Betweenness	0.009 (0.015)***	0.004 (0.019)
Fungi	Nodes	98	103
	Edges	134	138
	Prop. negative edges	0.16	0.21
	Degree	0.021 (0.031)	0.020 (0.020)
	Betweenness	0.009 (0.052)	0.023 (0.045)

observed in the degraded soil 16S network indicates antagonistic interactions (e.g. competition) may be particularly impacted and that degraded soil communities may be less resilient to future environmental perturbation (Coyte et al. 2015). Our results also revealed differences in the relative importance of different community assembly processes in shaping 16S communities between intact and degraded forest soils. Specifically, degradation reduced the contribution of selection and increased the contribution of stochastic processes, primarily dispersal limitation, in assembling 16S communities. This identifies dispersal and recolonization dynamics as an important determinant of microbial community composition following forest disturbance in these ecosystems. Dispersal limitation may also be responsible for the differences in soil ecosystem functions we observed between intact and degraded forests—reductions in nitrifying and methane oxidizing taxa in the degraded soils may be due to incomplete recolonization of the degraded sites by these organisms.

The effects of forest degradation on prokaryotic communities can largely be attributed to differences in soil physicochemical properties between intact and degraded forest soils. For example, soil pH has long been known to be a dominant driver of soil bacterial community composition (Fierer and Jackson 2006, Lauber et al. 2009) and was the most important contributor to variation in 16S composition in our study. This identifies a key mechanism by which forest degradation altered prokaryotic communities—soils were substantially less acidic in degraded forests, thus account-

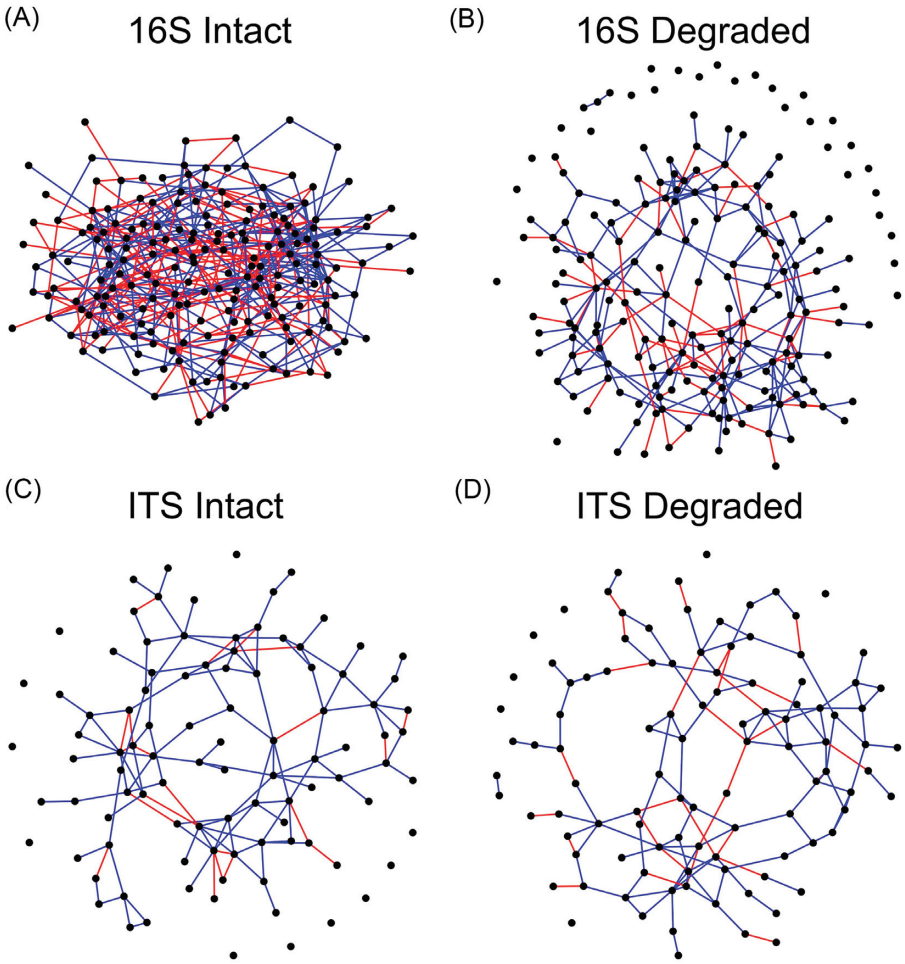

Figure 4. Co-occurrence networks constructed for 16S ASVs in intact (A) and degraded (B) forests and for ITS ASVs in intact (C) and degraded (D) forests using SPIEC-EASI (Kurtz et al. 2015). Nodes represent ASVs while edges (connections) represent significant associations between ASVs. Blue edges represent positive associations while red edges represent negative associations. Network statistics are presented on Table 2.

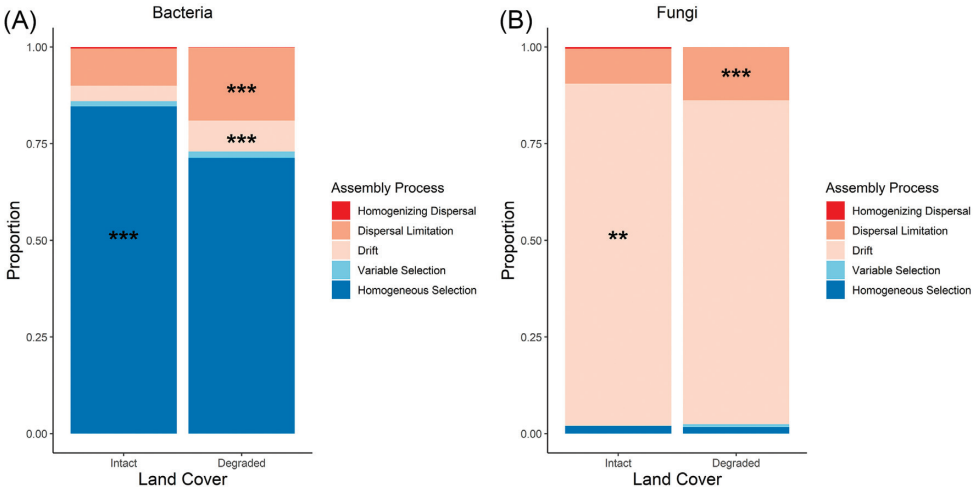

Figure 5. The relative importance of community assembly processes in 16S (A) and ITS (B) communities. Assembly processes were inferred using a phylogenetic null model approach (Stegen et al. 2013). Shades of blue indicate selection processes while shades of red indicate stochastic processes. Proportions of each process were compared between intact and degraded communities using Z-tests and asterisks indicate significantly higher proportions at the following levels: ** $P < .01$ and *** $P < .001$.

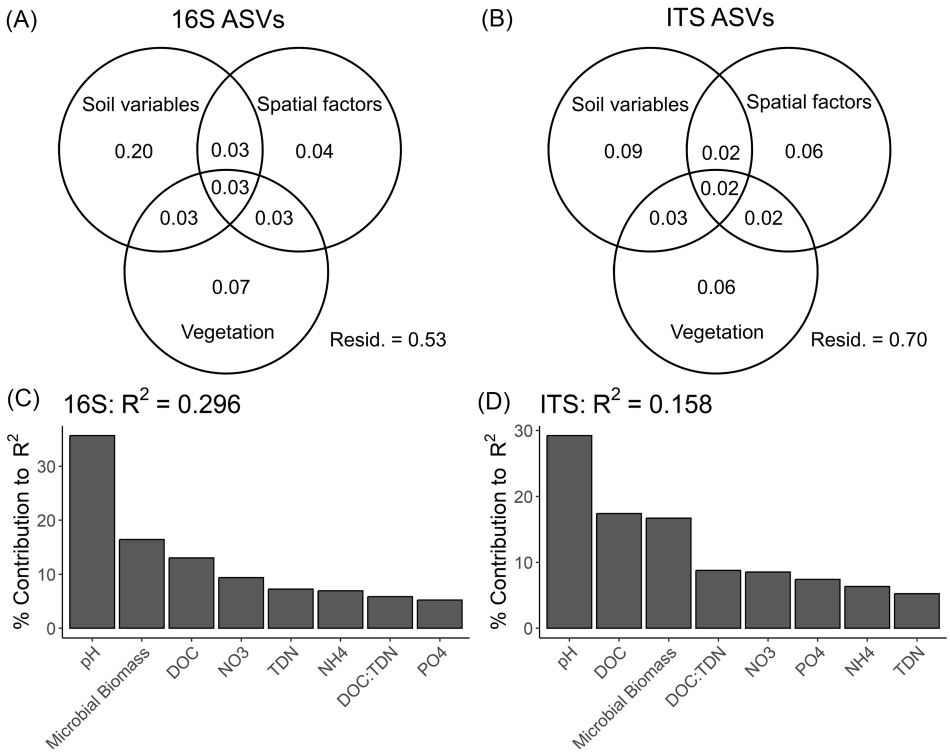

Figure 6. Variation partitioning results for 16S (A) and ITS (B) communities. Spatial factors are represented by PCNM scores for each sample. Significance of individual components was determined using distance-based redundancy analysis and all partitions were statistically significant (dbrda $P < .05$), though note that the significance of the interdependent partitions of variation cannot be tested. Panels (C) and (D) show the importance of individual soil variables (i.e. individual contributions to the total R^2 for soil variables) for 16S (C) and ITS (D) communities determined using hierarchical partitioning.

ing for the altered 16S communities we observed. Soil pH has also been shown to be an important driver of community assembly processes—acidic soils operate as a powerful environmental filter, increasing the importance of selection in assembling bacterial communities (Tripathi et al. 2018, Barnett et al. 2020), which is supported by the increased importance of homogeneous selection in the highly acidic soils of our intact sites. The greater pH observed in the degraded forest soils is very likely due to historical burning that occurred in those sites, which has also been observed in other eucalypt forests (Bowd et al. 2019, Ammitzboll et al. 2021). Extractable organic C (DOC) was also important in driving microbial community composition in our study and was significantly reduced in the degraded forest soils. This could be due to reduced organic matter inputs (e.g. leaf litter and root exudates) in the degraded forest soils or previous removal of organic matter from those sites. Additionally, soil physico-chemical properties not only structure microbial communities but may change in response to changes in microbial community composition. For example, the reductions in soil NO_3-N pools we observed likely reflect reduced abundance of nitrifying taxa in the degraded forest soils. Other soil chemical properties may also respond to shifts in microbial community composition—the differences in soil PO_4-P content we observed may reflect differences in microbial phosphatase enzyme production between intact and degraded communities. Many other feedbacks among anthropogenic degradation, microbial communities, and forest ecosystem functions likely exist, and identifying these relationships remains a priority for future research. Regardless, similar responses of soil chemical properties to historical disturbances have been observed in other Australian eucalypt forests, e.g. reduced organic C, NO_3-N, and PO_4-P (Bowd et al. 2019), suggesting that the microbial responses to forest degradation we report may be universally observed in these ecosystems.

Similar to prokaryotic communities, we observed changes in fungal ITS ASV composition, fungal taxonomic composition, and the relative abundances of some fungal functional guilds. The changes in fungal functional guilds potentially indicate reduced fungal saprotrophy in the degraded forest sites, where the relative abundances of litter and unspecified saprotrophs were significantly lower. These responses are likely due to reduced organic matter inputs or previous removal of organic matter (e.g. leaf litter) from the degraded sites. The negative responses of fungal saprotrophs and prokaryotic functional groups suggest that Eucalypt forest degradation is disrupting many aspects of microbial-mediated ecosystem function. Forest degradation also increased the importance of dispersal limitation in assembling fungal communities, reinforcing the importance of dispersal and recolonization processes in post-disturbance forest soil communities.

In contrast, some aspects of fungal communities did not respond to forest degradation. For example, fungal co-occurrence networks did not respond to forest degradation, nor did relative abundance of most fungal functional guilds, including the numerically dominant guild (soil saprotrophs) and ectomycorrhizal (ECM) fungi. The lack of observed mycorrhizal responses may be partly due to methodological limitations, as the ITS primer pair we used does not efficiently amplify arbuscular mycorrhizal (AM) fungi (Tedersoo et al. 2018b). *Eucalyptus* trees associate with both AM and ECM fungi and tend to transition from AM to ECM associations as the plants age (Chen et al. 2000). Therefore, there may be differences in the relative dominance of AM vs. ECM taxa in

forests of different ages with different timing/types of historical disturbance, though we are unable to assess this with our data. We also observed somewhat low relative abundance of ECM taxa in this study (~7% of sequences) compared with studies from other regions, e.g. northern hemisphere temperate forests, where ECM can comprise more than 30% of the soil fungal community (e.g. Veach et al. 2017, Osburn et al. 2019). This could be due to competition with AM fungi or poor representation of *Eucalyptus* ECM taxa in taxonomic and/or functional databases. Regardless, the lack of responses in some aspects of soil fungal communities may be due to higher variability and more unexplained variation in fungal than prokaryotic communities. Indeed, our variation partitioning analyses were able to explain less of the variation in fungal than prokaryotic communities (30% vs. 47%, respectively). Our inability to account for variation in fungal communities is in accordance with the predominance of stochastic processes in assembling fungal communities, which has been observed in multiple other studies (Powell et al. 2015, Wang et al. 2020, Osburn et al. 2021). These community assembly patterns corroborate our variation partitioning results—we observed greater relative contributions of spatial factors in accounting for variation in fungal communities, which can be interpreted as greater influences of biogeographic effects, e.g. dispersal and drift processes (Martiny et al. 2011). However, we did observe greater relative importance of vegetation characteristics in accounting for variation in fungal versus bacterial communities, which likely reflects the importance of plant–fungal interactions in driving fungal community composition (Peay et al. 2013).

We also note that locating appropriate sampling sites resulted in some intact and degraded forests being clustered spatially distant from one another, introducing the possibility that some of the observed effects of forest degradation were confounded with natural spatial variation. In addition, in some regions, the intact and degraded sites had slightly different vegetation characteristics (Table S1, Supporting Information), which may have introduced additional confounding influences. However, our results suggest that microbial responses were driven more strongly by anthropogenic forest degradation than by spatial and/or vegetation variation. For example, variation partitioning revealed that soil properties were more important than both vegetation and spatial factors for structuring both prokaryotic and fungal communities. In particular, communities were most strongly influenced by soil pH, which consistently responds to anthropogenic disturbance (i.e. fire) in eucalypt forests of Australia (Bowd et al. 2019, Ammitzboll et al. 2021). This, along with the generally consistent responses of microbial communities across all regions of Tasmania, supports our conclusion that anthropogenic forest degradation was the dominant driver of microbial communities in our study.

In general, as we predicted, microbial responses to forest degradation in temperate eucalypt forests appeared to be distinct from responses seen in other regions, e.g. northern hemisphere forests. For example, prior studies from other forested regions have shown increases in rapidly growing bacterial copiotrophs and decreases in slow growing oligotrophs following forest disturbance (Zhou et al. 2018, Osburn et al. 2019). In contrast, we observed no changes in the relative abundance of bacterial taxa commonly considered to be copiotrophic (e.g. Proteobacteria) and opposite responses of two bacterial groups that are commonly considered oligotrophic: Verrucomicrobiota, which responded positively, and Acidobacteriota, which responded negatively to forest degradation in our study. This suggests that anthropogenic degradation of eucalypt forests does not consistently select for particular microbial life history strategies and that some other characteristic of these taxa (beyond copiotroph vs. oligotrophy) is responsible for their different responses to forest degradation. For example, the lower abundance of Acidobacteriota in degraded soils likely reflects their general preference for more acidic environments. We also observed increased total microbial biomass in our degraded forest soils, which was contrary to our expectations and may be attributed to less acidic soil pH in the degraded soils, which likely increased overall microbial activity (Malik et al. 2018). Further, prior studies in northern hemisphere forests have observed increases in the abundance of nitrifying prokaryotes and increased NO_3-N pools following forest disturbance (Keiser et al. 2016, Lin et al. 2017), while we observed the opposite. One microbial response we observed, i.e. the reduced abundance of methane-oxidizing taxa in degraded soils, does appear to be consistent across southern and northern hemisphere forests (e.g. Sun and Badgley 2019), suggesting methane oxidizers may be universally sensitive to disturbance. Regardless, our results show that microbial communities in temperate eucalypt forests of the southern hemisphere will exhibit responses to disturbance that are largely distinct from responses observed in forests of other regions.

Overall, our study documents clear long-term effects of eucalypt forest degradation on soil microbial communities. These responses were generally consistent across the natural environmental variation present in Tasmania, Australia and across the range of disturbance types represented at our sites. Consistent effects of disturbance on prokaryotic communities were attributed to changes in key soil properties following degradation (e.g. pH), and appeared to drive altered forest ecosystem functions, e.g. reduced N mobility in degraded forest soils. We also observed effects of degradation on soil fungal communities, though these effects were less consistent, likely due to the high importance of stochastic processes in structuring fungal communities. Both prokaryotic and fungal communities, however, revealed the importance of dispersal processes (i.e. recolonization) in structuring soil microbial communities following degradation of these forests. Future studies should focus on identifying the characteristics and time scales of effects from specific disturbance types/management practices in temperate eucalypt forests (e.g. logging, fire, fragmentation, and agriculture conversion). Regardless, our results demonstrate clear and compelling responses of soil microbial communities to eucalypt forest degradation, with implications for the important functions and services provided by these ecosystems.

Authors' contributions

Ernest D. Osburn (Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing), Cooper Moon (Investigation, Writing – review & editing), Torrey Stephenson (Investigation, Writing – review & editing), Kawinwit Kittipalawattanapol (Investigation, Writing – review & editing), Menna Jones (Supervision, Writing – review & editing), Michael S. Strickland (Supervision, Writing – review & editing), and Laurel M. Lynch (Conceptualization, Funding acquisition, Supervision, Writing – review & editing).

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Supplementary data

Supplementary data are available at *FEMSEC* online.

Conflict of interest. None declared.

References

Abarenkov K, Nilsson RH, Larsson K-H *et al.* The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytol* 2010;**186**:281–5.

Ammitzboll H, Jordan GJ, Baker SC *et al.* Contrasting successional responses of soil bacteria and fungi to post-logging burn severity. *Forest Ecol Manag* 2022;**508**:120059.

Ammitzboll H, Jordan GJ, Baker SC *et al.* Diversity and abundance of soil microbial communities decline, and community compositions change with severity of post-logging fire. *Mol Ecol* 2021;**30**:2434–48.

Barnett SE, Youngblut ND, Buckley DH. Soil characteristics and land-use drive bacterial community assembly patterns. *FEMS Microbiol Ecol* 2020;**96**. https://dx.doi.org/10.1093/femsec/fiz194.

Bates D, Maechler M, Bolker B *et al.* lme4: linear mixed-effects models using "Eigen" and S4. CRAN, 2019.

Bolker BM, Brooks ME, Clark CJ *et al.* Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 2009;**24**:127–35.

Borcard D, Legendre P. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecol Modell* 2002;**153**:51–68.

Bowd EJ, Banks SC, Bissett A *et al.* Direct and indirect disturbance impacts in forests. *Ecol Lett* 2021;**24**:1225–36.

Bowd EJ, Banks SC, Bissett A *et al.* Disturbance alters the forest soil microbiome. *Mol Ecol* 2022;**31**:419–47.

Bowd EJ, Banks SC, Strong CL *et al.* Long-term impacts of wildfire and logging on forest soils. *Nat Geosci* 2019;**12**:113–8.

Brockerhoff EG, Jactel H, Parrotta JA *et al.* Role of eucalypt and other planted forests in biodiversity conservation and the provision of biodiversity-related ecosystem services. *Forest Ecol Manag* 2013;**301**:43–50.

Callahan BJ, McMurdie PJ, Rosen MJ *et al.* DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581–3.

Cao Y, Fu S, Zou X *et al.* Soil microbial community composition under eucalyptus plantations of different age in subtropical China. *Eur J Soil Biol* 2010;**46**:128–35.

Cardenas E, Kranabetter JM, Hope G *et al.* Forest harvesting reduces the soil metagenomic potential for biomass decomposition. *ISME J* 2015;**9**:2465–76.

Carnovale D, Bissett A, Thrall PH *et al.* Plant genus (*Acacia* and *Eucalyptus*) alters soil microbial community structure and relative abundance within revegetated shelterbelts. *Appl Soil Ecol* 2019;**133**:1–11.

Chen YL, Brundrett MC, Dell B. Effects of ectomycorrhizas and vesicular-arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. *New Phytol* 2000;**146**:545–56.

Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: networks, competition, and stability. *Science* 2015;**350**:663–6.

Csardi G, Nepusz T. The igraph software package for complex network research. *InterJournal* 2006;**1695**:1–9.

Cuer CA, Rodrigues R de AR, Balieiro FC *et al.* Short-term effect of *Eucalyptus* plantations on soil microbial communities and soil-atmosphere methane and nitrous oxide exchange. *Sci Rep* 2018;**8**:15133.

Department of Agriculture, Fisheries, and Forestry. Australia's State of the Forests Report. 2018.

Department of Agriculture, Fisheries, and Forestry. National Soil Strategy. 2021.

Doane TA, Horwáth WR. Spectrophotometric determination of nitrate with a single reagent. *Anal Lett* 2003;**36**:2713–22.

Douglas GM, Maffei VJ, Zaneveld JR *et al.* PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 2020;**38**:685–8.

Drummond MA, Loveland TR. Land-use pressure and a transition to forest-cover loss in the eastern United States. *Bioscience* 2010;**60**:286–98.

Faust K, Raes J. Microbial interactions: from networks to models. *Nat Rev Microbiol* 2012;**10**:538–50.

Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* 2006;**103**:626–31.

Fierer N. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat Rev Microbiol* 2017;**15**:579–90.

Foley JA, DeFries R, Asner GP *et al.* Global consequences of land use. *Science* 2005;**309**:570–4.

Fornasier F, Ascher J, Ceccherini MT *et al.* A simplified rapid, low-cost and versatile DNA-based assessment of soil microbial biomass. *Ecol Indic* 2014;**45**:75–82.

Goldmann K, Schöning I, Buscot F *et al.* Forest management type influences diversity and community composition of soil fungi across temperate Forest ecosystems. *Front Microbiol* 2015;**6**. https://dx.doi.org/10.3389/fmicb.2015.01300.

Hansen MC, Stehman SV, Potapov PV. Quantification of global gross forest cover loss. *Proc Natl Acad Sci USA* 2010;**107**:8650–5.

Heděnec P, Zheng H, Pessanha Siqueira D *et al.* Tree species traits and mycorrhizal association shape soil microbial communities via litter quality and species mediated soil properties. *Forest Ecol Manag* 2023;**527**:120608.

Jangid K, Williams MA, Franzluebbers AJ *et al.* Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. *Soil Biol Biochem* 2011;**43**:2184–93.

Keiser AD, Knoepp JD, Bradford MA. Disturbance decouples biogeochemical cycles across forests of the southeastern US. *Ecosystems* 2016;**19**:50–61.

Kohout P, Charvátová M, Štursová M *et al.* Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. *ISME J* 2018;**12**:692–703.

Kurtz ZD, Müller CL, Miraldi ER *et al.* Sparse and compositionally robust inference of microbial ecological networks. *PLoS Comput Biol* 2015;**11**:e1004226.

Lai J, Zou Y, Zhang J *et al.* Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.Hp R package. *Methods Ecol Evol* 2022;**13**:782–8.

Lajtha K, Driscoll CT, Jarrell WM *et al.* Soil phosphorus: characterization and total element analysis. In: *Standard Soil Methods for Long-Term Ecological Research*. New York: Oxford University Press, 1999, 115–42.

Lauber CL, Hamady M, Knight R *et al.* Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* 2009;**75**:5111–20.

Lauber CL, Strickland MS, Bradford MA *et al.* The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol Biochem* 2008;**40**:2407–15.

Lin L, Norman JS, Barrett JE. Ammonia-uptake kinetics and domain-level contributions of bacteria and archaea to nitrification in temperate forest soils. *Ecol Modell* 2017;**362**:111–9.

Malik AA, Puissant J, Buckeridge KM *et al.* Land use driven change in soil pH affects microbial carbon cycling processes. *Nat Commun* 2018;**9**:3591.

Martins C, Natal-da-Luz T, Sousa JP *et al.* Effects of essential oils from *Eucalyptus globulus* leaves on soil organisms involved in leaf degradation. *PLoS ONE* 2013;**8**:e61233.

Martiny JBH, Eisen JA, Penn K *et al.* Drivers of bacterial β-diversity depend on spatial scale. *Proc Natl Acad Sci* 2011;**108**:7850–4.

McBride SG, Choudoir M, Fierer N *et al.* Volatile organic compounds from leaf litter decomposition alter soil microbial communities and carbon dynamics. *Ecology* 2020;**101**:e03130.

McBride SG, Osburn ED, Lucas JM *et al.* Volatile and dissolved organic carbon sources have distinct effects on microbial activity, nitrogen content, and bacterial communities in soil. *Microb Ecol* 2022;**85**. https://dx.doi.org/10.1007/s00248-022-01967-0.

Millenium Ecosystem Assessment. *Ecosystems and Human Well Being: synthesis*. Washington: Island Press, 2005.

Murali A, Bhargava A, Wright ES. IDTAXA: a novel approach for accurate taxonomic classification of microbiome sequences. *Microbiome* 2018;**6**:140.

Mushinski RM, Zhou Y, Gentry TJ *et al.* Bacterial metataxonomic profile and putative functional behavior associated with C and N cycle processes remain altered for decades after forest harvest. *Soil Biol Biochem* 2018;**119**:184–93.

Oksanen J, Blanchet FG, Friendly M *et al.* vegan: community ecology package. CRAN, 2019.

Olson DM, Dinerstein E, Wikramanayake ED *et al.* Terrestrial ecoregions of the world: a new map of life on Earth: a new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *Bioscience* 2001;**51**:933–8.

Osburn ED, Aylward FO, Barrett JE. Historical land use has long-term effects on microbial community assembly processes in forest soils. *ISME Commun* 2021;**1**:1–4.

Osburn ED, McBride SG, Aylward FO *et al.* Soil bacterial and fungal communities exhibit distinct long-term responses to disturbance in temperate forests. *Front Microbiol* 2019;**10**. https://dx.doi.org/10.3389/fmicb.2019.02872.

Osburn ED. Data and R code from the Tassie forest degradation study. figshare, 2023. https://doi.org/10.6084/m9.figshare.21890712.v4.

Peay KG, Baraloto C, Fine PV. Strong coupling of plant and fungal community structure across western Amazonian rainforests. *ISME J* 2013;**7**:1852–61.

Peres-Neto PR, Legendre P, Dray S *et al.* Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology* 2006;**87**:2614–25.

Põlme S, Abarenkov K, Henrik Nilsson R *et al.* FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fung Diver* 2020;**105**:1–16.

Powell JR, Karunaratne S, Campbell CD *et al.* Deterministic processes vary during community assembly for ecologically dissimilar taxa. *Nat Commun* 2015;**6**:8444.

Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;**41**:D590–6.

R Core Development Team. R: a Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing, 2019.

Rees ABH, Cwynar LC. A test of Tyler's Line – response of chironomids to a pH gradient in Tasmania and their potential as a proxy to infer past changes in pH. *Freshwater Biol* 2010;**55**:2521–40.

Ritchie H, Roser M. *Forests and Deforestation*. Oxford: Our World in Data, 2021.

Robinson MD, McCarthy DJ, Smyth GK. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 2010;**26**:139–40.

Rudel TK, Coomes OT, Moran E *et al.* Forest transitions: towards a global understanding of land use change. *Glob Environ Change* 2005;**15**:23–31.

Stegen JC, Lin X, Fredrickson JK *et al.* Quantifying community assembly processes and identifying features that impose them. *ISME J* 2013;**7**:2069–79.

Sun S, Badgley BD. Changes in microbial functional genes within the soil metagenome during forest ecosystem restoration. *Soil Biol Biochem* 2019;**135**:163–72.

Sun S, Li S, Avera BN *et al.* Soil bacterial and fungal communities show distinct recovery patterns during forest ecosystem restoration. *Appl Environ Microbiol* 2017;**83**:e00966–17.

Tedersoo L, Bahram M, Zinger L *et al.* Best practices in metabarcoding of fungi: from experimental design to results. *Mol Ecol* 2022;**31**:2769–95.

Tedersoo L, Sánchez-Ramírez S, Kõljalg U *et al.* High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fung Diver* 2018a;**90**:135–59.

Tedersoo L, Tooming-Klunderud A, Anslan S. PacBio metabarcoding of Fungi and other eukaryotes: errors, biases and perspectives. *New Phytol* 2018b;**217**:1370–85.

Tripathi BM, Stegen JC, Kim M *et al.* Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. *ISME J* 2018;**12**:1072–83.

Tyler PA. A lakeland from the dreamtime the second founders' lecture. *Brit Phycol J* 1992;**27**:353–68.

van der Heijden MGA, Bardgett RD, Straalen NMV. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 2008;**11**:296–310.

Veach AM, Stokes CE, Knoepp J *et al.* Fungal communities and functional guilds shift along an elevational gradient in the Southern Appalachian Mountains. *Microb Ecol* 2017;**76**:156–68. https://dx.doi.org/10.1007/s00248-017-1116-6.

Vitousek PM, Mooney HA, Lubchenco J *et al.* Human domination of Earth's ecosystems. *Science* 1997;**277**:494–9.

Walters W, Hyde ER, Berg-Lyons D *et al.* Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *mSystems* 2016;**1**:e00009–15.

Wang P, Li S-P, Yang X *et al.* Mechanisms of soil bacterial and fungal community assembly differ among and within islands. *Environ Microbiol* 2020;**22**:1559–71.

Weatherburn M. Phenol-hypochlorite reaction for determination of ammonia. *Anal Chem* 1967;**39**:971–4.

White TJ, Bruns T, Lee S *et al.* Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols*. Amsterdam: Elsevier, 1990, 315–22.

Williams WD. *Biogeography and Ecology in Tasmania*. Berlin: Springer Science & Business Media, 2012.

Wu J, Liu Z, Wang X *et al.* Effects of understory removal and tree girdling on soil microbial community composition and litter decomposition in two *Eucalyptus* plantations in South China. *Funct Ecol* 2011;**25**:921–31.

Zhao J, Wan S, Fu S *et al.* Effects of understory removal and nitrogen fertilization on soil microbial communities in *Eucalyptus* plantations. *Forest Ecol Manag* 2013;**310**:80–6.

Zhou Z, Wang C, Luo Y. Effects of forest degradation on microbial communities and soil carbon cycling: a global meta-analysis. *Glob Ecol Biogeogr* 2018;**27**:110–24.

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