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RESEARCH ARTICLE

Changes in soil microbial assemblages, soil chemistry, and vegetation composition associated with *Eucalyptus viminalis* dieback

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Abstract

Background and aims Tree dieback is increasing worldwide, threatening the biodiversity and functioning of many terrestrial ecosystems. Tree dieback is associated with multiple interconnected changes in community composition and ecosystem processes. These changes affect plant, fauna and soil microbial communities, and soil physical and chemical processes. However, these ecosystem changes associated with tree dieback have received little attention.

Methods We characterised ecosystem changes associated with dieback in the Australian tree, *Eucalyptus viminalis*. Dieback of *E. viminalis* is widespread and of unknown cause. Surveys were conducted in sites with a range of dieback severity to assess native and exotic plant communities. Soil was collected to assess soil chemistry and communities of arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (EMF), fungal plant pathogens and oomycetes.

Results Dieback in *E. viminalis* was associated with an increase in nitrate–N, soil pH, exotic plant cover and richness, AMF and plant pathogen richness (both fungi and oomycetes). Conversely, there was a negative relationship between dieback and ammonium-N, native plant richness and EMF richness. Dieback

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severity was also associated with reduced heterogeneity in community composition of both native and exotic plants as well as all microbial groups assessed. *Conclusions* Ecosystem changes associated with *E. viminalis* dieback are complex and likely interconnected via feedbacks between plants, soils, and microorganisms. If highly disturbed landscapes suffering tree dieback are depleted of healthy soil microbial communities and physicochemical properties, then natural or assisted revegetation programmes may have limited success unless microbial mutualists are reinstated through intervention.

Keywords Arbuscular mycorrhizal fungi · Canopy dieback · Ectomycorrhizal fungi · Oomycetes · Plant pathogens

Introduction

Forests and woodlands are some of the most prominent terrestrial ecosystems globally, covering over 30% of the land surface, providing habitat for more than half the world's terrestrial plant and animal species (Reid et al. 2005), storing approximately 45% of the carbon (Sabine et al. 2012) and providing a wide range of ecosystem services (Brockerhoff et al. 2017). In an era of climate change, conservation of forests and woodlands is increasingly urgent for maintaining human well-being, minimising biodiversity loss and reducing anthropogenic carbon emissions. Nevertheless, forest loss continues at ~ 5 M ha per year worldwide, albeit at reduced rates of decline than in previous decades (FAO 2020).

Tree dieback is one form of forest and woodland decline that is increasing worldwide, threatening the biodiversity and functioning of many terrestrial ecosystems (Allen et al. 2010; Hartmann et al. 2022). Tree dieback refers to the progressive loss of shoots, twigs, and branches, with many cases resulting in tree death (Houston 1992; Denman et al. 2022). This leads to gradual canopy loss of keystone tree species. Globally, tree dieback has been variously attributed to altered disturbance regimes (e.g., Jurskis 2005, 2016), drought (Steinkamp and Hickler 2015; Camarero 2021), nitrogen (N) deposition (Nakaji and Izuta 2017; Pardo et al. 2019), and pathogen and pest outbreaks (Allen et al. 2010, 2015; Hammond et al. 2022). Several cases of tree dieback have been caused by exotic pathogens such as *Phytophthora* species (Grünwald et al. 2008; Gómez-Aparicio et al. 2022), while others are strongly associated with insect outbreaks (Šturšová et al. 2014). The loss of mutualistic microorganisms such as mycorrhizal fungi, has also been linked to tree dieback, but causality remains unresolved (Egli 2011; Gehring et al. 2017). Mycorrhizal fungi can increase nutrient uptake, provide herbivore and pathogen defence, and confer drought resilience to plant hosts in exchange for carbon (Smith and Read 2008).

While some studies have been able to identify the probably primary triggers of tree dieback, the variety of factors implicated is indicative of the wide range of ecosystem processes associated with trees and the potential for complex feedback among them (Sapsford et al. 2017; Matusick and Fontaine 2020). For example, trees can directly influence soil chemistry via root activity and litter deposition, which in turn influence understorey plant communities (Ludwig et al. 2004; Abdallah and Chaieb 2012) and soil microorganisms (Prescott and Grayston 2013; Mueller et al. 2019; Beugnon et al. 2021). On the other hand, soil processes, microorganisms, and understorey plant communities can influence tree recruitment and vigour. For example, nutrient enrichment can promote vertebrate or invertebrate herbivory of canopy foliage (Bobbink and Hicks 2014), and soil microbes can facilitate acquisition of limiting resources and provide herbivore and pathogen defence (Smith and Read 2008).

Given these interactions, tree dieback is expected to be associated with multiple linked changes in community composition and ecosystem processes, including plant, fauna, and soil microbial communities, and soil physical and chemical processes (Anderegg et al. 2013; Karst et al. 2015; Steinrucken et al. 2016; Raghavendra et al. 2017; Betts et al. 2017; Fleming et al. 2021). Indeed, Horton et al. (2013) found a link between tree dieback, drought, increase in soil N

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availability, and a decrease in the abundance of ectomycorrhizal fungi (EMF) in Tasmanian temperate eucalypt forests. Nonetheless, few studies have undertaken an in-depth evaluation of the alterations to ecological communities undergoing tree dieback. Even though mycorrhizal fungi are crucial for tree and ecosystem health, their link to tree dieback has received little attention. Only a few studies could associate dieback with both declines in EMF and increases in arbuscular mycorrhizal fungi (AMF) (Egli 2011; Scott et al. 2013; Ishaq et al. 2013, 2018). Similarly, even though pathogen- and pest-driven tree dieback has been extensively studied, the consequences of tree dieback for native plant pathogen communities has seldom been addressed, even though dieback could be expected to promote native (Hopkins et al. 2018; Baldrian et al. 2023) or exotic (Flory and Clay 2013) plant pathogens. While it can be challenging to determine which environmental or ecological changes are the initial triggers of tree dieback, characterisation of the multiple ecosystem changes associated with tree dieback is an important step towards investigating causality and seeking restoration solutions.

To address this challenge, we aimed to characterise changes in native and exotic vegetation, soil chemistry, and soil microbial assemblages associated with dieback in the Australian tree, *Eucalyptus viminalis*. *Eucalyptus viminalis* woodlands are widespread in cooler regions of south-eastern Australia, and in parts of its distribution, trees are rapidly declining due to canopy dieback, with some cases leading to stand-scale mortality over broad landscapes (Ross and Brack 2015). Ross and Brack (2015) conducted an observational study of eight sites on an area subjected to severe dieback. While this study was unable to conclude the cause of this dieback, it has been linked to an endemic eucalyptus weevil (*Gonipterus* sp.) (Ross and Brack 2015). Jurskis (2016) alternatively proposed, albeit without empirical data, that *E. viminalis* dieback is caused by accumulation of soil available N in the absence of fires, which in turn changes soil physicochemical properties, vegetation structure, and native soil microbial communities (e.g., decline in EMF), leaving trees susceptible to drought, pests, and diseases. Both hypotheses, however, flag the potential role for ecosystem changes in *E. viminalis* dieback.

In the present study, we measured changes in plant and soil microbial community composition and soil chemistry along a gradient of *E. viminalis* dieback severity. We hypothesised that *E. viminalis* dieback would be associated with increased soil nutrient availability and exotic plant invasions (Horton et al. 2013; Jurskis 2016), a decline in EMF richness (Horton et al. 2013), and an increase in AMF and plant pathogen richness (Ishaq et al. 2018; Hopkins et al. 2018; Baldrian et al. 2023).

Materials and methods

Study area

The study was undertaken in the Monaro region of the Southern Tablelands of New South Wales, Australia, within an area encompassed by the townships of Cooma, Jindabyne, Bombala and Nimmitabel (~2,700 km^2; Table S1). The Monaro region is a plateau (700–1100 m) between the Snowy Mountains (west) and coastal escarpment (east), with a cool, temperate climate and an average annual rainfall of 500–700 mm (Dorrough et al. 2004). The region is dominated by temperate grasslands, predominantly on basalt soils and dominated by grass species within the *Themeda*, *Poa*, *Austrostipa* genera (Dorrough et al. 2004, 2020), interspersed with grassy woodlands on sedimentary and granitic soils that are dominated by *E. viminalis* along with *Eucalyptus rubida*, *Eucalyptus pauciflora* and *Eucalyptus stellulata* (Ross and Brack 2015). Since the mid-1800s, the region has been significantly modified through a combination of livestock grazing, fertiliser application, altered fire regimes and introduction of non-indigenous perennial pasture grasses (Dorrough et al. 2004, 2007, 2020). Native vegetation communities remain largely intact in remnants on private properties, Travelling Stock Reserves, Crown Reserves and National Parks.

Site selection and survey design

Altogether, we selected 46 woodland stands (hereafter termed sites) across the Monaro region that comprised an open, grassy woodland structure with an upper canopy dominated by *E. viminalis*, a sparse understorey of native shrubs, and a dense, species-rich ground layer of herbs and grasses. Sites were selected to represent a gradient of *E. viminalis* canopy dieback severity (Fig. S1). Sites with different levels of dieback severity were interspersed across

the region to limit confounding with vegetation and soil conditions which may have generated spatial autocorrelations.

Sites were also selected on the basis that they retained a standing canopy of mature *E. viminalis* trees. Sites with no canopy or evidence of previous canopy trees (i.e., dead trees) were excluded from sampling. We also excluded sites with significant signs of soil disturbance (e.g., feral animal diggings or erosion from livestock trampling, mechanical damage), fire damage, weed management (spraying, slashing, chipping), logging or other significant disturbances. We excluded areas containing farm infrastructure (e.g., roads, sheds) and heavily grazed areas such as stockyards or sheep camps. Although we could not control for grazing history at each site, we recorded the current grazing regime for each woodland (see site attribute Table S1).

The design of our surveys followed protocols used by the Australian Microbiome Initiative (Bissett et al. 2016) (https://research.csiro.au/ambsm/), including plot size, soil sampling, chemical analysis, and DNA extraction and sequencing of soil microbial taxa and downstream bioinformatics (details outlined below). Note, however, that the vegetation sampling methods described below were customised for this project and not aligned with Australian Microbiome Initiative workflows.

Vegetation survey

At each of the 46 sites, a single 25 m × 25 m quadrat (hereafter termed 'plot') was delineated in an area of woodland that was representative of the general characteristics and dieback condition of the surrounding vegetation. For each 25 m × 25 m plot, a full floristic survey was undertaken by recording the identity of all vascular plant species and assigning an estimate of percent foliage cover to each species using an 8-class Braun-Blanquet scale (modified from Tozer et al. 2010): 1 = rare, a single to a few individuals present, and cover < 5%; 2 = uncommon and cover < 5%; 3 = common and cover < 5%; 4 = very abundant and cover < 5%; 5 = 5% ≤ cover < 20%; 6 = 20% ≤ cover < 50%; 7 = 50% ≤ cover < 75%; 8 = 75% ≤ cover < 100%. The diameter at breast height (to nearest 10 cm using a hand-held tape measure), species identity and total number of individuals were also recorded for each tree standing > 2 m per plot. We also recorded the number, height (to nearest 10 cm using a hand-held tape measure) and identity of tree seedlings standing ≤ 2 m per plot. Surveys were undertaken during November and December 2021.

Ten smaller 1 m × 1 m quadrats (hereafter termed 'sub-plots') were then distributed across each 25 m × 25 m plot, haphazardly positioned within the canopy boundary of mature *E. viminalis* trees. Within each sub-plot, we visually estimated (to nearest 5%) the cover of litter (including leaves, strips of bark, fine twigs), coarse woody debris (larger twigs, branches, logs), exposed soil and the foliage cover of graminoids (combined for grasses, sedges, rushes, reeds, other grass-like monocots, e.g., *Lomandra*), herbs, climbers (including twiners, vines), shrubs (including eucalypts or other tree seedlings growing < 2 m tall) and trees (predominately eucalypts and *Acacia* species growing > 2 m tall).

Dieback severity assessment methods

Dieback severity of the resident *E. viminalis* woodland was assessed using a crown condition scoring method modified from Stone et al. (2008). First, each individual tree rooted within each plot and taller than 2 m was scored from "0" to "5" for each of five variables: (1) crown size and shape, (2) foliar density, (3) extent of dead branches, (4) epicormic growth, and (5) foliar damage (Table S2). Second, the five values were added together to derive a single crown condition score per tree that ranged from 0 (tree completely dead) to 25 (healthy tree with no signs of dieback). Third, a single crown condition score (also ranging from 0 to 25) was calculated per plot as the average of the individual tree scores. This average plot-scale crown condition score was then converted to a % measure of dieback severity, ranging from 0 (all trees completely healthy with no signs of dieback, equating to a crown condition score of 25) to 100 (all trees completely dead, equating to a crown dieback score of 0).

Soil sampling methods

Soil sampling occurred on the same day as the vegetation surveys, following the Australian Microbiome Initiative protocols. Within the centre of each of the ten sub-plots per plot, approximately 200 g

of soil was sampled at each of two depths: 0–10 cm (excluding leaf litter; hereafter 'upper soil depth') and 20–30 cm (hereafter 'lower soil depth'). A clean hand-trowel was then used to excavate a hole of 5 cm diameter to 10 cm depth, with soil placed in a clean stainless-steel bowl. The ten soil cores from each sub-plot were placed within the same bowl and thoroughly mixed to create a single 2 kg soil sample per plot. The process was repeated for the lower soil depth (20–30 cm depth) soil samples but using different equipment to avoid cross-contamination between soil depths. Equipment was cleaned with moistened paper towel, then sprayed liberally with commercial bleach (sodium hypochlorite 42 g/l), followed by 100% ethanol. After mixing the ten soil cores per sub-plot, a subsample destined for DNA analyses was placed in 50 ml tubes, while the remaining soil was placed within a clean plastic bag and sealed with a wire band. Both soil samples were placed in ice-filled containers during transport during the day, stored at −20 °C in the evenings during field work, and finally stored at −80 °C until chemical and DNA analyses.

We also recorded the broad geological context of each site as associated with either granite, basalt, metamorphic or sedimentary rock types (Table S1), according to current NSW 1500 K Simplified Surface Geology map layers (State Government of NSW and Department of Regional New South Wales 2024).

Soil chemical analysis

Soil samples were oven-dried at 40 °C for 24 h. Then, approximately 500 g dried soil was sent to the Soil and Plant Analysis Laboratory (CSBP; Bibra Lake, Western Australia) for chemical analysis. Plant-available phosphorus (P) and potassium (K) were measured by suspending soil in 0.5 M sodium bicarbonate solution at a 1:100 ratio adjusted to pH 8.5 for 16 h. Phosphorous concentration was then determined using a colorimetric method, while K concentration was determined using atomic absorption spectroscopy (Colwell 1963; Rayment and Higginson 1992). Organic carbon (OC) was determined by treating the soil with potassium dichromate solution and concentrated sulfuric acid. The number of chromic ions produced were then measured using a colorimetric method (Walkley and Black 1934). Ammonium-N and nitrate–N were measured by extracting them using 2 M potassium chloride solution and measured using a colorimetric method (Searle 1984). Soil pH was measured in calcium chloride at a 1:5 soil-to-solution ratio (Rayment and Lyons 2010).

DNA extraction and sequencing

DNA extractions, PCR amplification, and sequencing was performed by the Australian Genome Research Facility. DNA was extracted from 250 mg of soil material using the DNeasy PowerSoil Pro kit, respectively, (Qiagen, Carlsbad, USA). For each sample, 15 ng DNA were used to amplify the 18S rRNA, ITS1, and rps10 genomic regions for AMF, fungi, and oomycetes respectively (see Table S3 and Table S4 for primers used and PCR conditions). The first stage PCR was cleaned using magnetic beads, and samples were visualised on 2% Sybr E-Gel (Thermo-Fisher). A secondary PCR to index the amplicons was performed with the same conditions with Platinum SuperFi II mastermix (Life Technologies, Australia). The resulting amplicons were cleaned using magnetic beads, quantified by fluorometry (Promega Quantifluor) and normalised. The eqimolar pool was cleaned a final time using magnetic beads to concentrate the pool and then measured using a High-Sensitivity D1000 Tape on an Agilent 2200 TapeStation. The pool was diluted to 5 nM and molarity was confirmed using a Qubit High Sensitivity dsDNA assay (ThermoFisher). This was followed by sequencing on an Illumina MiSeq (San Diego, CA, USA) with a V3, 600 cycle kit (2 × 300 base pairs paired-end).

Bioinformatics

Following sequencing, adapters were trimmed using 'cutadapt' (Martin 2011), retaining only sequences that contained primers. For fungi, sequences were merged with flash2 (Magoč and Salzberg 2011). The ITS1 region was extracted from merged sequences with ITSx and sequences with ambiguous bases were removed (Bengtsson-Palme et al. 2013). For AMF and oomycetes, trimming, quality checks and sequence processing were done in VSEARCH v2.14.1 with default parameters unless otherwise stated (Rognes et al. 2016). Trimmed paired-end sequences were merged with a minimum of 10 bp overlap. Merged sequences were filtered using a maximum error rate of 0.1 and a minimum length of 200 and 300 bp for AMF and oomycetes, respectively.

For all microbial groups, sequences were dereplicated, denoised to zero-radius operational taxonomic units (zOTUs), and an abundance table was produced by mapping the filtered sequences to zOTUs using the UNOISE3 algorithm in USEARCH v11 (Edgar 2016). Then, sequences were clustered into operational taxonomic units (OTUs) at 97% identity for AMF and fungi, and at 100% identity for oomycetes (Foster et al. 2022) using the USEARCH 'cluster_fast' algorithm after sorting zOTUs by abundance. Then, consensus sequences were queried against their respective databases, depending on the target group: SILVA v138.1 for AMF (Quast et al. 2013); OomycetesDB for oomycetes (Foster et al. 2022); and UNITE v9.0 for fungi (Nilsson et al. 2019). This was done with the VSEARCH 'usearch_global' algorithm (Rognes et al. 2016). Taxonomy was assigned with a threshold of > 90% match and query cover of > 90% and selecting the best match.

The FUNGuild database was used to assign functional guild to each fungal OTU based on taxonomy (Nguyen et al. 2016). When an OTU had two possible guilds, it was classified as the most plausible guild based on the FUNGuild categorization. All OTUs that had three or more possible guilds were classified as undetermined, and they were dropped from guild-specific analyses.

Statistical analyses

All data were analysed, and figures were created, in R (R Core Team 2021). Prior to analysis, dieback severity was evaluated for potential spatial bias across the sampled region. A linear regression demonstrated that dieback severity declined significantly with increasing longitude (i.e., from west to east, $R^2 = 0.26$, $F = 15.1$, $P = 0.0003$, $n = 46$). Hence, our models were corrected for spatial correlation using the 'corSpatial' function from the *nlme* package (Pinheiro and Bates, 2024). Although most of our sites occurred on granite rock, some sites occurred on basalt, metamorphic, or sedimentary parental rock type. Hence, since we were not explicitly interested in testing hypotheses about microbial associations with geological context, we included parental rock type as a predictor covariate to account for this variation.

The initial denoised OTU abundance tables were rarefied to the smallest sequencing depth (17,451, 1,236, and 37,802 sequences for AMF, oomycetes, and fungi, respectively) to avoid sequencing depth bias (Dickie 2010). Then, all non-target sequences were removed from the dataset and not further analysed. For fungi, only OTUs that were assigned a guild of EMF or plant pathogens were further analysed, as they are the two guilds of interest.

Regression models

Regression analyses were undertaken to evaluate changes in plant species richness, microbial OTU richness, and plant cover with increasing dieback severity. Non-linear variation was examined by fitting linear, logarithmic, and quadratic functions to each relationship, bound by 95% confidence intervals. Best models were chosen based on Akaike information criterion (Zuur et al. 2009). Separate regression analyses were performed for native and exotic plant species and microbial guilds across all sites. For soil microorganisms, regressions between richness and dieback severity were performed separately for the two soil depths. For analyses of soil properties, each variable was square root-transformed to improve normal distributions of residuals and homogeneity of variance. Then, model selection was conducted as above but including soil depth as an interacting factor with dieback severity.

To visualise variation in plant and microbial communities across dieback severity, non-metric multidimensional scaling with Bray–Curtis dissimilarity of community matrix was used. To visualise species contributions to the ordination, two-way tables were constructed with the 'inkspot' function from the *rioja* package (Juggins 2015). Plots were ordered on the x-axis by increasing dieback severity, while individual taxonomic units were placed on the y-axis. Two-way tables summarise and present the raw community data as a powerful way of visualising the distribution of taxonomic units along environmental, spatial, or temporal gradients.

To test for changes in community composition of plants and soil microorganisms with increasing dieback severity, permutational multivariate analysis of variance were performed using 'adonis2' within the *vegan* package (Oksanen et al. 2024). To test homogenisation of plant and microbial communities with dieback severity, we created four categories of dieback severity: low (< 33%), moderate (33–66%), severe (67–99%), and eliminated (100%). Then

statistical differences among dieback categories were tested using 'betadisper' within the *vegan* package (Oksanen et al. 2024). To test which environmental variables correlated with plant and microbial communities across dieback severity, vectors of maximum correlation were calculated with the vector-fitting procedure using 'envfit' (9,999 permutations) within the *vegan* package (Oksanen et al. 2024). A biplot was drawn on the ordination to display the relationships between explanatory variables and ordination axes.

Results

Overview

Across the 46 plots, 218 unique vascular plant taxa were recorded, comprising 123 native and 95 exotic taxa. Average richness was 49 taxa per plot. Thirteen native and nine exotic plant taxa could not be routinely identified to species level due to lack of mature specimens, or degraded reproductive structures, and were thus grouped within genera for subsequent analysis (main examples: the graminoids *Poa, Austrostipa, Luzula, Hordeum, Avena, Lolium*; herbs *Geranium, Carduus, Oxalis, Medicago, Rumex, Wahlenbergia*). Hereafter, taxon richness is referred to as species richness.

Only four out of the 92 soil samples did not amplify any AMF, from which three were from the lower soil depth (i.e. 20–30 cm). There were 162 OTUs classified as AMF across all samples. These OTUs belonged to seven taxonomic families. There were 9,714 fungal OTUs belonging to 286 families (excluding AMF). From these OTUs, 383 and 234 OTUs were classified as EMF and plant pathogens, respectively. All 92 samples amplified EMF and plant pathogens. Oomycetes could not be amplified from 45 of 92 samples (~49%), from which 38 were from the lower soil depth. There were 284 OTUs belonging to oomycetes across all samples. These OTUs belonged to four and six taxonomic families and genera, respectively.

Patterns in soil chemistry

Regression models revealed that dieback severity was significantly associated with several soil properties, independent of soil depth (Fig. 1). With increasing dieback severity, nitrate–N and soil pH increased, while ammonium-N and litter declined. There was no relationship between dieback severity and P, K, or organic carbon at either soil depth. All soil properties presented higher values at the upper than the lower soil depth (Table S5).

Variation in plant richness across the dieback severity gradient

Dieback severity was associated with significant patterns in understorey vegetation characteristics (Fig. 2). Native plant richness was lower, and exotic plant richness was higher at higher dieback severity (Fig. 2a-b; Table S6). Native plant richness varied considerably where dieback was < 50% (with the highest richness levels detected at plots with 30–50% canopy dieback) then declined sharply as dieback exceeded 50%. In contrast, exotic plant richness increased significantly with dieback severity, reaching maximum richness at approximately 70% dieback.

There was no significant relationship between native plant cover and dieback severity (Fig. 2c, Table S6). In contrast, exotic plant cover showed a positive relationship with dieback severity (Fig. 2d, Table S6).

Variation in plant community composition across the dieback severity gradient

The composition of both native and exotic plant communities varied significantly with dieback severity (Fig. 3; Table S7). Native plant communities were highly variable amongst plots with low dieback severity (i.e. < 50%), while there was a marked reduction in variability in community composition with increasing dieback severity (Fig. 3a), which reflects a decrease in native plant richness at each plot (Fig. S2a). When using dieback severity as a categorical variable, native plants showed higher beta-diversity in sites with low dieback severity than in sites with severe dieback severity or where *E. viminalis* had been eliminated (Fig. S3a).

Almost 70% of native graminoid species were absent or rare from plots with > 50% dieback severity (Fig. S4). The notably exceptions were *Austrostipa* spp. and *Poa* spp., which were present throughout the entire dieback severity gradient. Roughly 50% of

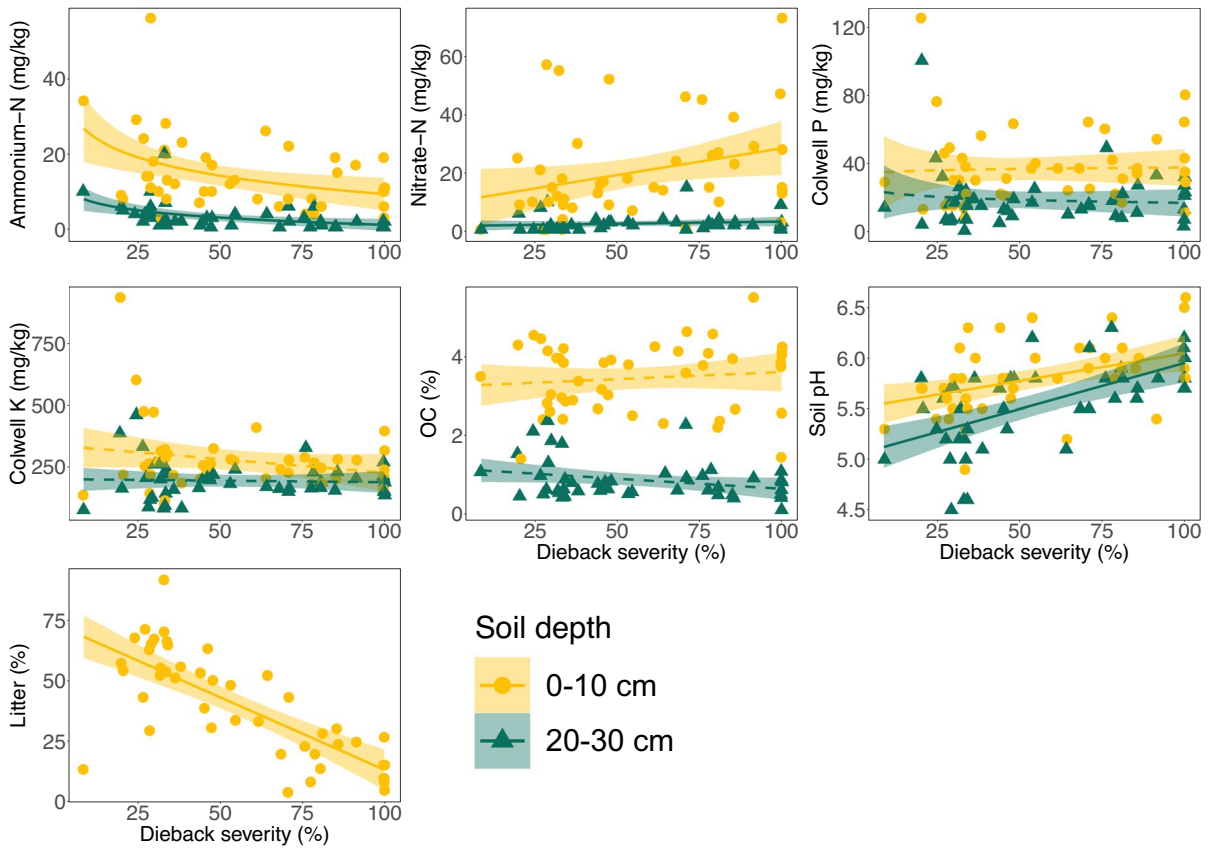


Fig. 1 Scatter plots depicting changes in each of six soil chemical variables across the *Eucalyptus viminalis* dieback severity gradient and between two soil sampling depths. The full lines indicate mean fitted values extracted from the best regression model. Dashed lines represent non-significant relationships. Ribbons represent 95% confidence intervals (depth: 0–10 cm = yellow; depth 20–30 cm = green). OC = organic carbon

native herbaceous species were absent or rare in plots with > 50% dieback severity, while the other 50% was present throughout the entire dieback severity gradient (Fig. S5). Almost 70% of native woody species were absent from plots with > 50% dieback severity (Fig. S6). Only one native woody species, *Melicytus dentatus*, was present in all plots with 100% dieback severity.

In contrast to native plants, changes in exotic plant communities (Fig. 3b) were reflected in the increase in richness and changes in cover across the dieback gradient (Fig. S2b; Fig. S7; Fig. S8). However, when using dieback severity as a categorical variable, exotic plants also showed higher beta-diversity in sites with low dieback severity than in sites with severe dieback severity or where *E. viminalis* had been eliminated (Fig. S3b).

Changes in community composition of both native and exotic plant species correlated with several environmental variables, most of which were shared between native and exotic plant communities (Fig. 3). The shared variables were dieback severity, tree seedling density, nitrate–N, litter, and the richness of EMF, plant pathogens, and oomycetes (Fig. 3, Table S8). Changes in native plant communities also correlated with changes in soil P, and tree density, while those of exotic plants also correlated with organic carbon (Fig. 3, Table S8).

Variation in microbial richness associated with dieback severity and soil depth

From the upper soil depth, there was a positive relationship between AMF richness and dieback severity,

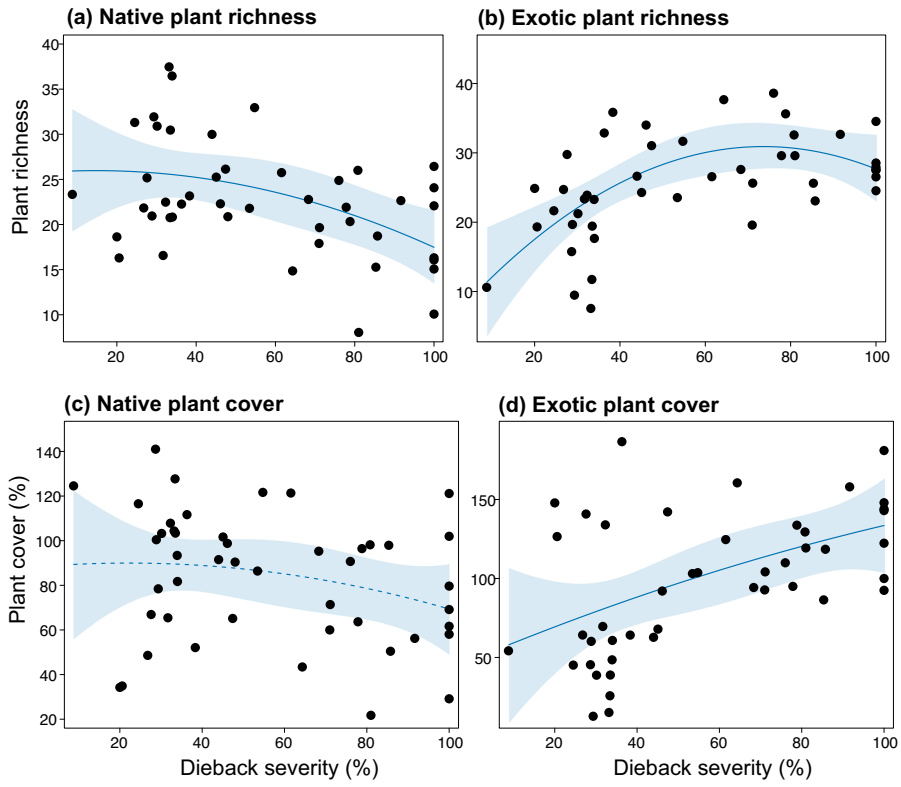


Fig. 2 Variation in (**a**, **c**) native and (**b**, **d**) exotic plant species richness (number of taxa per 25 × 25 m plot) and cover with increasing severity of *Eucalyptus viminalis* canopy dieback. The full lines indicate mean fitted values extracted from the best regression model. Dashed lines represent non-significant relationships. Blue ribbons represent 95% confidence intervals

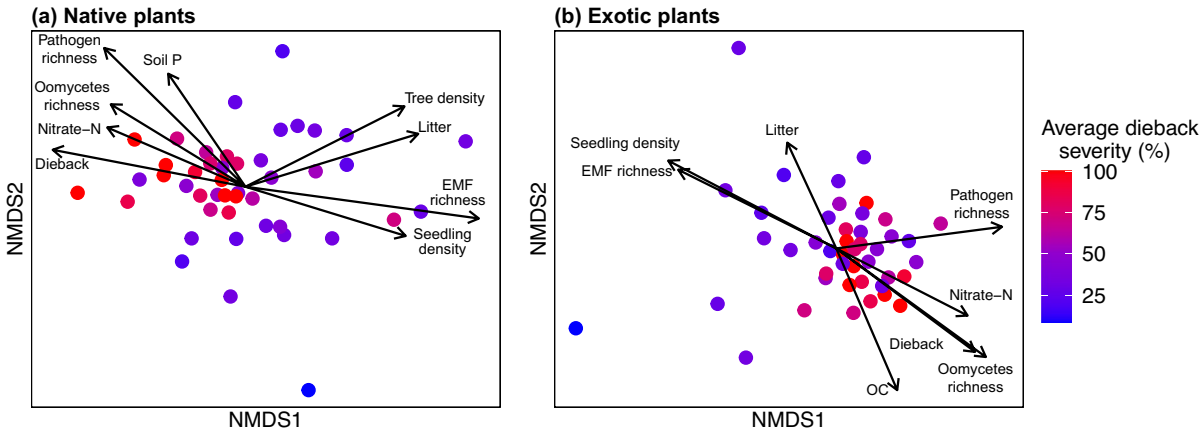


Fig. 3 Nonmetric multidimensional scaling ordination showing differences in community composition of (**a**) native (3D stress = 0.12), and (**b**) exotic plant species (3D stress = 0.11). Only the first two axes are shown. Colour of symbols represent the *Eucalyptus viminalis* dieback severity percentage. Vectors show variables selected from maximum correlation analyses (*Permutations* = 9,999) correlated with ordination. Vector length is proportional to their relative importance (Table S8)

while it was negative for EMF richness (Fig. 4a-b; Table S9). The relationship between the richness of both plant pathogens and oomycetes with dieback severity was quadratic (Fig. 4c-d; Table S9). From the lower soil depth, there was no relationship between AMF or plant pathogens with dieback severity (Fig. S9a,c; Table S9), but there was a negative and positive relationship between EMF and oomycetes with dieback severity, respectively (Fig. S9b,d; Table S9).

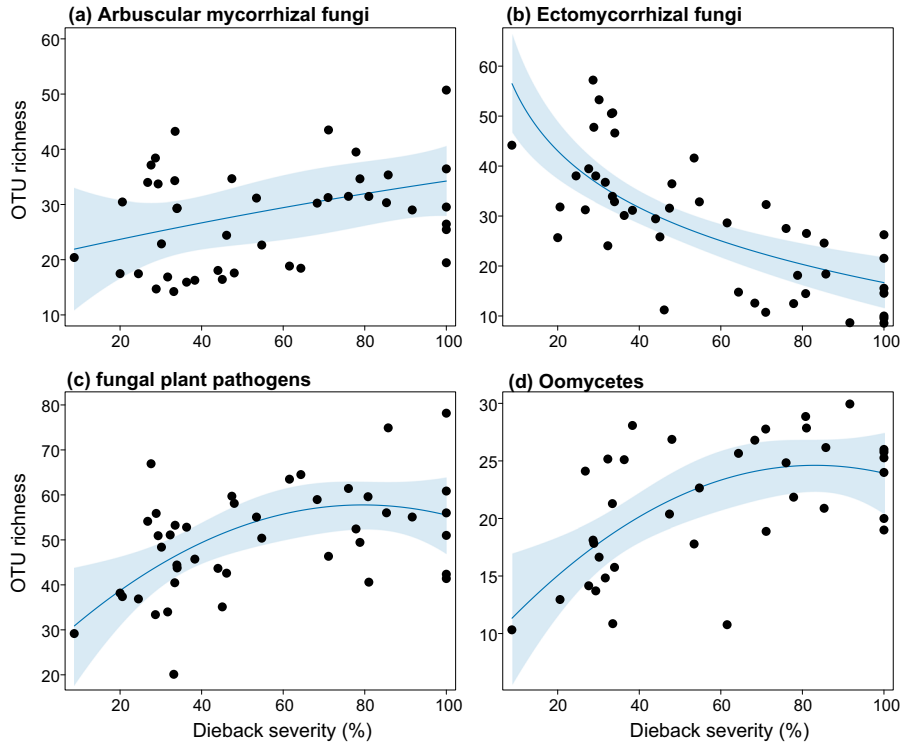


Fig. 4 Relationship between operational taxonomic unit (OTU) richness of (**a**) arbuscular mycorrhizal fungi, (**b**) ectomycorrhizal fungi, (**c**) fungal plant pathogens, and (**d**) oomycetes with increasing *Eucalyptus viminalis* dieback severity from the 0–10 cm soil sampling depth. Lines indicate mean fitted values extracted from the best regression model. Blue ribbons represent 95% confidence intervals

Richness of AMF was 1.3 times higher in the upper than the lower soil depth (Table S5). Richness of plant pathogens and oomycetes were two times higher in the upper than in the lower soil depth (Table S5). EMF richness did not differ between soil depths (Table S5).

Variation in microbial communities across the dieback severity gradient

From the upper soil depth, community composition of all four microbial groups (i.e., AMF, EMF, plant pathogens, oomycetes) changed with increasing dieback severity (Fig. 5; Table S10). Similar to plant communities, dieback severity also had a homogenising effect on communities of soil microorganisms (Fig. 5). Indeed, when using dieback severity as a categorical variable, all four microbial groups showed higher beta-diversity in sites with low dieback severity than in sites with severe dieback severity (67–99%), or where *E. viminalis* had been eliminated (100%, Fig. S3c-f).

These changes in composition were associated with several soil and vegetation variables, from which most were shared among all four microbial groups. These shared variables were dieback severity, litter, ground cover, bare soil, seedling density, number of trees, nitrate–N, soil pH, native plant richness, exotic plant richness, and exotic plant cover (Fig. 5; Table S11). The exceptions were ammonium-N that only correlated with changes in community composition of AMF and EMF, and tree density that correlated with changes in community composition of all microbial groups except oomycetes (Fig. 5; Table S11).

Changes in AMF community composition were further explored at the family level (Fig. S10). Acaulosporaceae and Gigasporaceae were rare or absent in sites with > 60% dieback severity. Claroideoglomeraceae and Diversisporaceae appeared to persist throughout the dieback severity gradient. For Glomeraceae and Endogonaceae, there was a turnover of OTUs across the dieback severity gradient. Lastly, unclassified AMF taxa were present across the entire dieback severity gradient.

Changes in EMF community composition were further explored at the order level (Fig. S11). All taxonomic orders decreased in richness and relative abundance with increasing dieback severity. Thelephorales, Cantharellales, and Russulales became

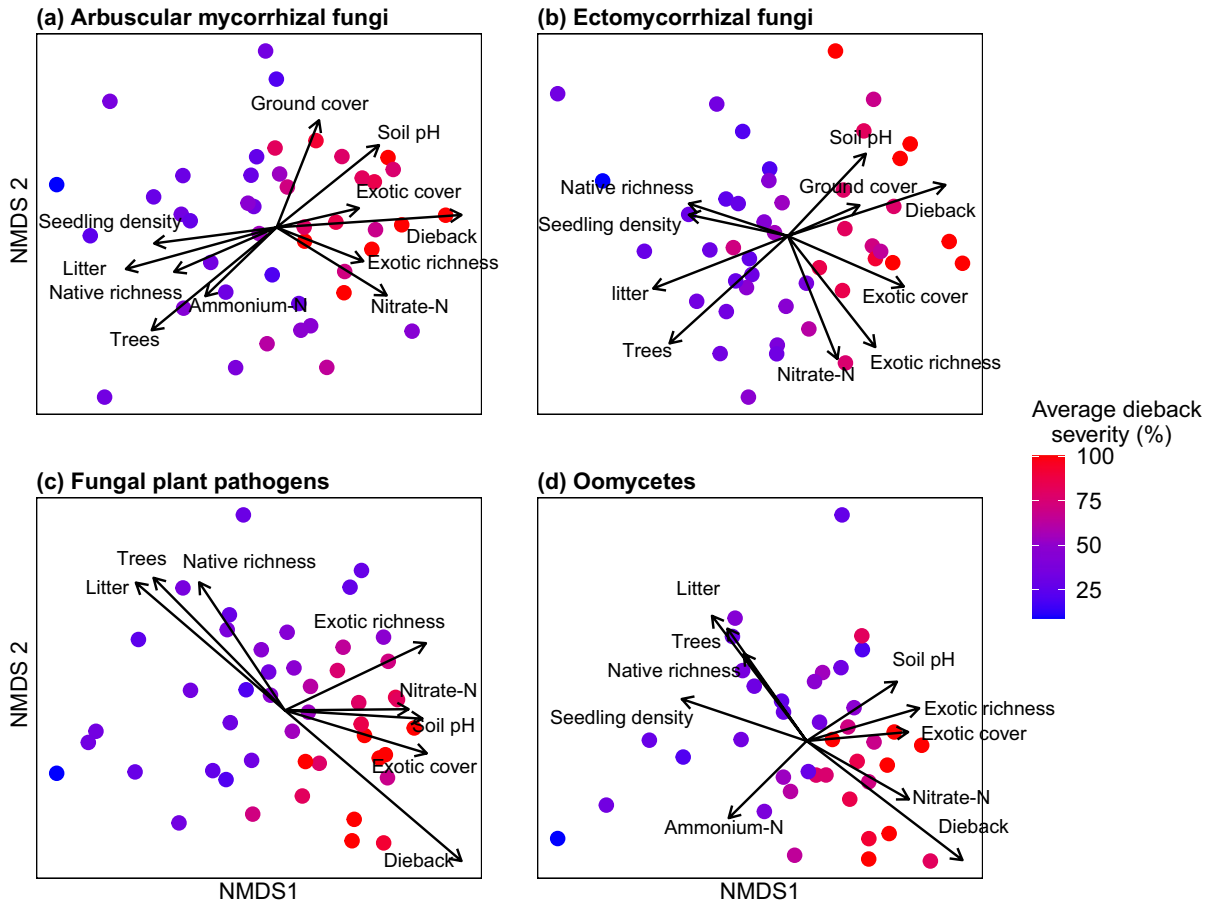


Fig. 5 Nonmetric multidimensional scaling ordination showing differences in community composition of (**a**) arbuscular mycorrhizal fungi (3D stress = 0.17), (**b**) ectomycorrhizal fungi (3D stress = 0.16), (**c**) fungal plant pathogens (3D stress = 0.14), and (**d**) oomycetes (3D stress = 0.18) from samples from the 0–10 cm soil sampling depth. Only first two axes are shown. Colour of symbols represent the dieback severity percentage. Vectors show variables selected from maximum correlation analyses (*Permutations* = 9,999) correlated with ordination. Vector length is proportional to their relative importance (Table S11)

rare or absent in > 60% dieback severity sites, while the other orders persisted but in reduced richness and relative abundance.

Changes in plant pathogen communities were further explored at the order level (Fig. S12). Glomerellales and Spizellomycetales were rare or absent in healthier sites and became more abundant and richer with increasing dieback severity. Pleosporales were present across the entire dieback severity but became more abundant with increasing dieback severity, while Capnodiales became less abundant.

Changes in oomycetes community composition were further explored at the genus level (Fig. S13). Both *Pythium* and *Phytophthora* showed a turnover of OTUs across the dieback severity gradient. *Phytopythium* and *Peronospora* were almost entirely absent in < 70% dieback severity sites, while *Aphanomyces* were only present in < 50% dieback severity sites.

From the lower soil depth, community composition of AMF, EMF, and plant pathogens changed with increasing dieback severity (Table S10; Fig. S14), while there were not enough samples with oomycetes present in the lower soil depth to conduct community composition analyses. These changes in composition were associated with several soil and vegetation variables, being dieback severity, number of trees, litter, soil pH, native plant richness, and exotic plant cover

common variables among the three microbial groups (Fig. S14, Table S11).

Discussion

In accordance with our hypotheses and with previous studies in other forest and woodland ecosystems, we found that *E. viminalis* dieback was indeed associated with an increase in nitrate–N, soil pH, exotic plant cover and richness, AMF and plant pathogen richness (both fungi and oomycetes), and with a decrease in ammonium-N, native plant richness, EMF richness, and litter cover. We also found that tree dieback was associated with changes in community composition of both native and exotic plants as well as all microbial groups assessed, which resulted in homogenised communities at higher levels of tree dieback severity.

Changes in soil chemistry

Our results are consistent with previous studies that have also found an increase in soil nitrate–N and pH linked to tree dieback (Anderson et al. 2010; Cobb et al. 2013; Klavina et al. 2016). However, we also found a decrease in ammonium-N with dieback severity, corroborating the results of a recent study which showed a decrease in ammonium-N concentration upon experimentally driven tree mortality in mesocosms (Jaeger et al. 2024). Although it is challenging to estimate soil available N, due to the volatility of both nitrate–N and ammonium-N, it is likely that altered soil properties, such as soil pH, might be regulating the decline in ammonium-N and increase in nitrate–N, since nitrification generally increases in less acidic soils (DeForest and Otuya 2020; Li et al. 2020; but see Li et al. 2018). For example, Orwig et al. (2008) found higher soil pH, nitrification, and N mineralisation rates in eastern hemlock forests infested with woolly adelgid than healthy forests.

Changes in plant communities

The homogenisation of native vegetation communities with tree dieback we observed has been found in other ecosystems (Rejžek et al. 2016; Rodríguez et al. 2017). For example, Nagel et al. (2019) found that there was a convergence of native plant species composition associated with *Abies alba* mortality; they attributed this to loss of rare species due to dieback promoting the recruitment of a subcanopy species, which in turn decreased light availability and increased soil pH. On the other hand, other studies have found that the response of native vegetation to tree dieback can depend on their life forms, with some species benefiting and others being hindered by tree dieback (e.g., Mitchell et al. 2016; Matuszkiewicz et al. 2024). In our study, however, the changes in native plant communities associated with dieback severity reflected a decrease in richness of all major life forms (i.e., graminoids, forbs, and woody native plant species). This decrease in native plant richness has also been previously found in another eucalypt woodland experiencing dieback, and authors attributed this to habitat degradation (i.e., canopy loss) (Shearer et al. 2009).

Reduced native plant richness and homogenisation could also result from suppression by exotic plant invasions (Dogra et al. 2010). This is consistent with our observations of increasing exotic plant cover with dieback severity, and two other studies linking understorey plant invasions to eucalypt decline (McDougall et al. 2002; Scarlett et al. 2013). Indeed, both studies found a positive relationship between dieback severity and exotic plant cover in eucalypt woodlands infested with the soil pathogen *P. cinnamomi*. Scarlett et al (2013) suggests this increase in exotic plant cover resulted from canopy clearing. Although many studies have evaluated the effects of ecosystem degradation on plant communities with a focus on plant provenance (e.g., Rojas et al. 2011), the associated changes in plant provenance (i.e., native versus exotic plants) with tree dieback specifically have been seldomly addressed.

Changes in soil microorganisms

Regarding mycorrhizal fungi, our results are consistent with three earlier studies that similarly suggest tree dieback tends to promote AMF richness and abundance but reduce that of EMF (Scott et al. 2013; Ishaq et al. 2018; Hopkins et al. 2018). For example, Ishaq et al. (2018) found that soil from *Eucalyptus gomphocephala* dieback sites harboured less EMF, but more AMF and pathogenic fungi than healthy sites. Authors interpreted these results as the decline in tree health is associated with loss of EMF, but causality was not possible to determine.

Previous studies have found that the response of EMF to N enrichment depends on their affinity to inorganic N, meaning nitrophobic EMF will decline while nitrophilic EMF will increase with N enrichment (Tahovská et al. 2020). In our study, however, all EMF taxa declined with dieback severity, including those described as nitrophilic. Indeed, we found no significant relationship between EMF richness and either ammonium-N or nitrate–N. This suggests that the decline in EMF we observed is likely driven by a decline in host availability, rather than increase in soil N. Exotic weeds invading eucalypt woodlands can maintain elevated available soil N and have been shown to be highly AMF-dependent (Albornoz et al. 2024). Thus, we propose that the plant species turnover from EMF-dominated (i.e., *E. viminalis*) to AMF-dominated (i.e., exotic weeds) is driving the increase and decrease in AMF and EMF richness, respectively. This is supported by the fact that 82% of the exotic plant species found in our study belong to taxonomic families that typically associate with AMF, while only 67% of native plant species do.

Several studies have evaluated changes in microbial groups associated with tree dieback, but our study is the first to our knowledge that incorporates AMF, EMF, along with plant pathogens consisting of both fungal and oomycetes assemblages. Indeed, only a handful of studies have focused on native oomycetes communities in forests and woodlands (Esmaeili Taheri et al. 2017; Fiore-Donno and Bonkowski 2021). Although these studies have typically not been in a context of dieback, three studies found higher fungal plant pathogen richness in relation to tree dieback (Steinrucken et al. 2016; Fukasawa et al. 2019; Wang et al. 2022), similar to our results for fungal and oomycetes pathogens. Our results are also in accordance with findings of higher oomycetes richness in disturbed than in undisturbed forests (Nardi et al. 2023). The authors attributed this result to canopy gaps increasing solar radiation and soil temperature, in turn increasing microbial activity.

Our findings do not provide evidence that any singular pathogen species directly caused the observed *E. viminalis* dieback patterns across the Monaro region of NSW. From all plant pathogens found in our study, only 19 OTUs were present in enough samples to be linked to *E. viminalis* dieback (i.e. > 70% of samples). From these, none matched a taxon known to cause adult tree dieback. However, we cannot rule out the possibility that certain oomycetes taxa may negatively impact recruitment and survival *of E. viminalis* seedlings. Furthermore, the decline in EMF we observed can potentially leave trees more vulnerable to an array of pathogens that normally would be deterred by EMF or non-declining trees (Branzanti et al. 1999; Desprez-Loustau et al. 2006; Corcobado et al. 2014). It is worth noting that no OTU found in our study matched *P. cinnamomi*. Meaning this pathogen is unlikely to be causing *E. viminalis* dieback, albeit being the cause of dieback in other eucalypt woodlands (e.g., Cahill et al. 2008; McDougall et al. 2024).

Ecosystem interactions and hypothesised pathways of dieback

The suite of ecosystem changes we observed in association with *E. viminalis* dieback likely involve cascading interactions, potentially including positive and negative feedbacks that can further promote dieback or ecosystem degradation. However, like most tree dieback studies, our data were insufficient to demonstrate causality. Nevertheless, by combining evidence of ecosystem change from our study with evidence from ecological theory and other studies discussed here, we hypothesise potential pathways linking the cascade of ecological change associated with *E. viminalis dieback* (Fig. 6).

While the changes in ecosystem processes and characteristics we observed in *E. viminalis* woodlands were significant, none stood out as being sufficiently substantial or independently driven to be an ultimate trigger of tree dieback. For example, changes in soil available N levels were relatively small, trees can persist with exotic understoreys, AMF are known to increase with exotic plants (Albornoz et al. 2023, 2024), and oomycete data pointed to no consistent pathogen that might trigger tree mortality. We thus propose that the changes we characterised could all potentially result from, rather than be the ultimate cause of, tree dieback. Instead, we propose the ultimate cause may reflect an external driver, such as increased moisture stress associated with climate change, leading to direct impacts on trees and indirect complex interactions between biotic and abiotic factors. We expand on this hypothesis in Fig. 6, with numbered steps in the pathway potentially explained as follows:

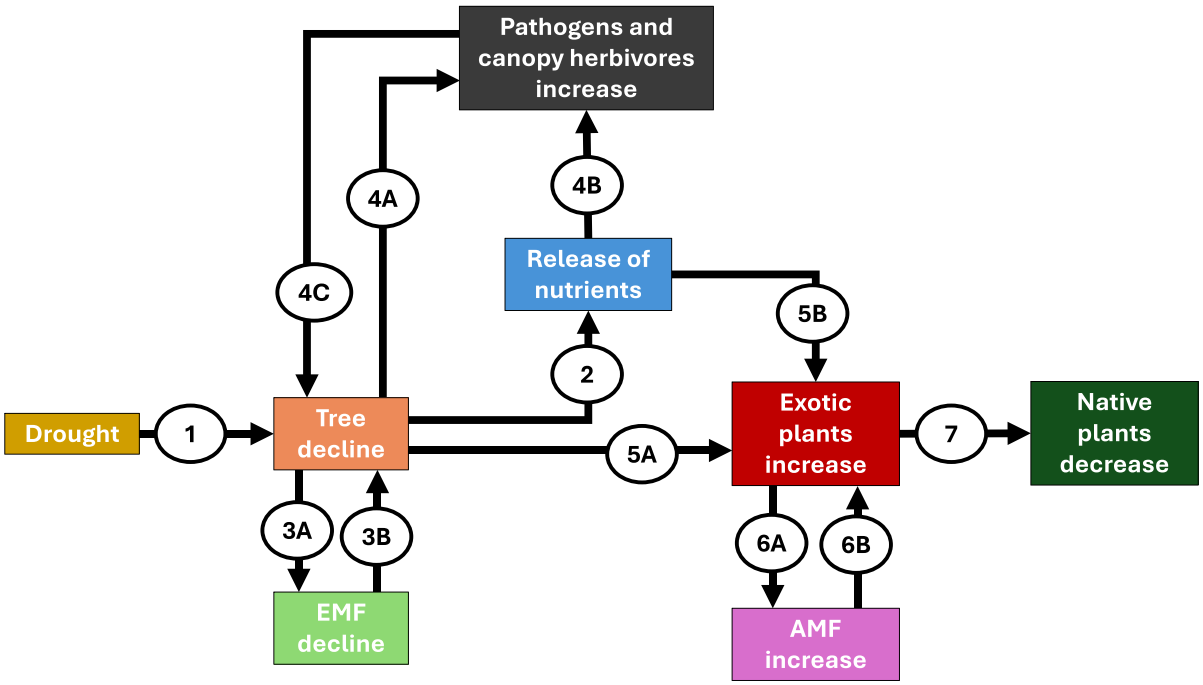


Fig. 6 Hypothesised pathway of cascading effects of *E. viminalis* dieback based on current evidence and ecological knowledge. Explanation of each pathway is described in the discussion section of the main text

1. *E. viminalis* populations across the Monaro region are at the edge of the species' lower rainfall range (Adams-Hosking et al. 2012). Hence, they are at risk due to increasing drought events and decreasing rainfall (Fig. 6.1), providing a potential ultimate trigger for *E. viminalis* decline. The climate associated stress may also trigger increased herbivory, with increasing winter minimum temperatures facilitating insect pest population growth (Pinkard et al. 2017).
2. Even though N deposition has been suggested as a potential cause of tree dieback (Jurskis 2005), increased soil N availability has also been found in forests experiencing dieback of known causes. For example, sudden oak death—a dieback disease caused by *Phytophthora ramorum*—increased soil nitrate–N (Cobb et al. 2013). Thus, the increased soil nitrate–N we observed may be a result of release of N from dying tree canopies and roots, and decreased nutrient uptake due to loss of fine roots (Fig. 6.2) (Scott et al. 2013; Deng et al. 2021).
3. Indeed, water-stress causes a decline in fine root density and longevity. In addition, defoliation decreases carbon allocated to microbial symbionts. Both the decline in fine roots and carbon allocation hinders EMF (Fig. 6.3A). However, EMF can also protect hosts against drought (Pickles and Simard 2017), pathogens (Branzanti et al. 1999; Corcobado et al. 2014), and herbivory (Gange et al. 2005). Thus, the decline of EMF may further promote *E. viminalis* decline via this indirect, positive feedback (Fig. 6.3B).
4. Declining *E. viminalis* may promote pathogens and herbivores that would not otherwise be able to infect healthy trees (Fig. 6.4A). Indeed, trees can produce allelochemical compounds to deter pathogens and herbivores, but excess N can decrease the production of these allelochemical compounds (Thomas et al. 2002), leaving the trees more susceptible to pathogens and herbivory (Fig. 6.4B). This increased enemy pressure can further promote *E. viminalis* decline (Fig. 6.4C).
5. Reduction in competition by *E. viminalis* due to canopy and root decline can promote exotic plant invasion (Fig. 6.5A). Increases in soil nitrate–N are also associated with exotic annuals in

other temperate eucalypt woodlands (Fig. 6.5B) (Prober et al. 2005; Prober and Wiehl 2011).

6. These plant invasions can, in turn, promote AMF richness in eucalypt woodlands (Albornoz et al. 2023, 2024), given these plants tend to be highly AMF-dependent (Fig. 6.6A) (Lekberg et al. 2013; Gomes et al. 2018). In turn, AMF can then further promote exotic plants via positive feedbacks (Fig. 6.6B) (Hilbig and Allen 2015).
7. Finally, plant invasions can ultimately supress native vegetation (Fig. 6.7) via increased competition and ecosystem modifications (Carboni et al. 2021; Matuszkiewicz et al. 2024).

Future studies could test these ecosystem interactions associated with tree dieback on adult trees. Manipulative rainfall exclusion (or enhancement) and nutrient enrichment experiments on adult trees, such as those of Van Diepen et al. (2007) and Nickel et al. (2018) are invaluable for elucidating the complex mechanisms behind tree dieback.

Conclusion

We have shown that the associated ecosystem changes with *E. viminalis* dieback are complex and likely interconnected via complex feedbacks. Regardless of the directionality between dieback severity and observed variables, if highly disturbed landscapes suffering severe tree dieback are depleted of healthy soil microbial communities and physicochemical properties, then natural vegetation regeneration from the seed bank or performance of establishing seedlings may be limited unless the microbial mutualists are reinstated as part of restoration practices (McDonald et al. 2016; Mueller et al. 2019; Merges et al. 2020). Mass planting of nursery-grown seedlings is a key method by which restoration practitioners aim to rehabilitate eucalypt populations lost to severe dieback in south-east Australia. However, high rates of seedling mortality (~ 50%, up to 95%, N. Taws Pers. Comms) can significantly hamper ecological restoration success. We suggest that an important factor in reduced establishment and growth of *E. viminalis* seedlings in severely dieback affected landscapes is a reduction of functional mycorrhizal communities, and this could be exacerbated by accumulation of pathogens and exotic plant invasions. Indeed, vegetation restoration may be limited without commensurate recovery of native mycorrhizal communities lost during degradation (Koziol et al. 2018). Finally, the management of undesirable microorganisms such as pathogens and 'weedy' AMF should be taken into consideration.

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Author contributions All authors contributed to the study conception and design. Field surveys and sample collections were conducted by Ben Gooden and Celeste Linde. Statistical analyses were conducted by Felipe E. Albornoz and Ben Gooden. The first draft of the manuscript was written by Felipe E. Albornoz and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All methodology is readily available in the manuscript. Should the manuscript be accepted, data supporting the results will be archived in Dryad and DNA sequences in NCBI SRA, and the data DOI will be included at the end of the article.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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