

# Do dieback-affected *Eucalyptus pauciflora* subsp. *niphophila* have fewer roots colonised by ectomycorrhizae than healthy trees?

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## Abstract

*Eucalyptus* dieback is a severe phenomenon impacting the health of *Eucalyptus pauciflora* subsp. *niphophila* (snow gum) in Kosciuszko National Park. Ectomycorrhizal (ECM) colonies within the feeder roots of species such as *E. pauciflora* subsp. *niphophila* can greatly improve tree health in light of dieback stress. We investigated if there was greater ECM presence in healthy *E. pauciflora* subsp. *niphophila* than in those suffering from severe dieback. Feeder root samples were compared between healthy *E. pauciflora* subsp. *niphophila* stands in Perisher Valley and dieback-affected *E. pauciflora* subsp. *niphophila* stands 500 m from Perisher Valley. Across the sites, 42.92% of healthy feeder roots from 13 trees were colonised by ECM, compared to 32.77% of dieback-affected feeder roots in 13 trees. However, this difference was not statistically significant. Interestingly, a post hoc observation noted dieback trees had fewer available feeder roots. Although this study was unable to demonstrate if fewer available feeder roots were a cause or effect of dieback, it was unsurprising that fewer roots sustain trees in poorer health condition, such as those affected by dieback. These novel findings stress the need for further research into the causes of widespread dieback in *E. pauciflora* subsp. *niphophila*, including a microbiome study to classify ECM samples as a function of fungal diversity and a more robust analysis of root counts to better understand the dieback phenomena.

**Running Title:** Ectomycorrhizae in dieback-affected *Eucalyptus pauciflora* subsp. *niphophila*

**Key Words:** mycorrhizae, feeder roots, snow gum, Kosciuszko National Park

## Introduction

Tree dieback, the chronic deterioration and premature death of tree stands (Jurksis & Turner 2002; Ross & Brack 2015), threatens global tree health, while its causes and possible mitigation methods remain 'enigmatic' (Ishaq *et al.* 2016). Each tree experiences a range of dieback symptoms at different times (Ross & Brack 2017). Dieback has been observed in many species, including *Eucalyptus* species through parts of Eastern Australia (Jurksis & Turner 2002). Dieback in *Eucalyptus* has three stages of classification marked by symptom severity: Stage I, mild; Stage II, severe; and Stage IV, dead (Ward-Jones 2020). The phenomenon of dieback has recently extended through vulnerable Australian alpine areas,

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including Kosciuszko National Park (KNP), NSW, here it may prove irreversible, given that higher altitudes to which species can migrate are limited (Sritharan *et al.* 2021).

Previous literature hypothesised that dieback in *Eucalyptus* species may be attributed to abiotic factors, biotic factors or both, with multiple stressors accelerating the process (Ross & Brack 2015). These include wood-boring *Phoracantha longicorn* (longicorn), infrequent burning, imbalances in soil nitrogen and essential nutrients, drought stress, and root fungal pathogens (Jurskis 2016; Ross & Brack 2015, 2017). According to a theory proposed by Ross and Brack (2015) for dieback in *Eucalyptus viminalis* (manna gum), faced with severe dieback symptoms, trees re-direct nutrients towards the growth of epicormic shoots to conserve resources; however, this incidentally increases epicormic shoot palatability, leaving Eucalypts highly vulnerable to secondary defoliator attacks (Ross & Brack 2015, 2017). Thus, a positive feedback loop develops, gradually leading to irreversible tree health decline across a dieback-affected landscape.

One unexplored dieback-related factor is how changes to soil microbiology and root functioning can either impair or improve responses in tree health to dieback. Mycorrhizal fungi form an important nutritional symbiosis with host plants at their roots (Kariman *et al.* 2014). Mycorrhizae are vital for increasing root surface area for efficient nutrient exchange, particularly in recovery from stress (Launonen *et al.* 1999), improving plant nutrient and water uptake in exchange for carbon as part of the symbiotic process (Tedersoo & Bahram 2019; Kariman *et al.* 2014; Scott *et al.* 2013). Ectomycorrhizae (ECM) is a key type of mycorrhizae for tree-stress protection, with 6,000 identified species across 30 plant lineages (Tedersoo & Bram 2019; Lilleskov *et al.* 2011). ECM forms a sheath-like mantle on the outside of feeder roots, providing them with outer protection; mycorrhizae improves water uptake, fights drought stress, increases root biomass and defends host plants against soil pathogens (Tedersoo & Bahram 2019; Kariman *et al.* 2014; Adams *et al.* 2006; de Witte *et al.* 2017; Weidlich *et al.* 2020). ECM almost entirely dominates feeder roots in mature Eucalypts (Teste *et al.* 2020).

In *Eucalyptus* species, both improved and declining tree health may be associated with ECM presence. Ishaq *et al.* (2013) demonstrated the importance of mycorrhizae presence in mitigating stress faced by *Eucalyptus gomphocephala* (tuart) seedlings. Examining the proportion of ECM and arbuscular (AM) fungi colonisation, the authors found a 'probable link' between increased ECM presence and healthier tree canopies (Ishaq *et al.* 2013). This work is supported by Scott *et al.* (2013), who found that declining *E. gomphocephala* seedlings had significantly lower fine root counts and ECM presence. Horton *et al.* (2013) also concluded that ECM species richness was lowest where *Eucalyptus delegatensis* (alpine ash) was severely declining. Thus, improved tree health can predict increased ECM composition within host roots. However, it is unknown if greater ECM presence is linked to better tree health – or, conversely, if lesser ECM facilitates dieback, or even if dieback is linked to a decline in ECM composition (Sapsford *et al.* 2017). For instance, inoculation of ECM colonies in *Castanea* (chestnut) improved plant growth and recovery from water stress (Aryal *et al.* 2021). Addressing this causality dilemma will aid in understanding how ECM presence may mitigate dieback.

This study focuses on the health of *Eucalyptus pauciflora* subsp. *niphophila* (snow gum), an endemic Eucalypt found in high and dry altitudes in the Kosciuszko National Park (Halter *et al.* 1997; Worboys & Good 2011). Dieback in *E. pauciflora* subsp. *niphophila* is currently vast, visually prevalent and escalating in severity throughout KNP. However, dieback in the species is critically understudied, and knowledge of how severe dieback symptoms impact its health and functioning is limited (Ross & Brack 2017). We aimed to determine if there was greater ECM presence in healthy *E. pauciflora* subsp. *niphophila* than in those suffering from severe Stage II dieback. We hypothesised: (1) healthy *E. pauciflora* subsp. *niphophila* trees would have a greater feeder root ECM presence than dieback-affected (Stage II) *E. pauciflora* subsp. *niphophila* trees, due to the many benefits of ECM in hosts. We predicted:

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(2) this would present as a strong statistical difference in ECM presence between healthy and dieback-affected trees; thus, (3) if healthy trees were associated with greater ECM presence, a lack of ECM colonies could be subsequently associated with dieback-affected trees.

## Methods

Data from feeder root samples were analysed to observe ECM presence and post hoc root count between healthy and dieback-affected *E. pauciflora* feeder roots.

### *Site description*

Root samples were collected from 30 November to 2 December 2021 under dry conditions. Samples were collected from two sites in Perisher Valley, NSW (36.24'35"S, 148.24'43"E, 25° slope, elevation: 1,760m ASL) and another two sites 500 m from Perisher Valley. All sites had similar surrounding understory cover with grasses and small woody shrubs (such as *Hovea montana*), elevation (~1,800m), terrain and slope (25°–28°). Each site had some degree of disturbance and dieback presence, identified by consistency in epicormic branches.

### *Root sample collection*

Root samples were collected from two mature populations of *E. pauciflora* subsp. *niphophila*. Trees were selected on the basis of dieback scores, with their diameter at breast height (DBH) varying from approximately 10 cm to 50 cm. The first population included 13 living *E. pauciflora* subsp. *niphophila*, scored as suffering from Stage II dieback at the Perisher Valley sites (n = 13). We chose to collect feeder roots from trees suffering from Stage II dieback, as this was the most visually abundant and easiest to identify (whereas Stage I is mild, with minor bark cracking and red weeping trunk holes; (Ward-Jones 2020). We identified Stage II dieback from the formation of progressive horizontal galleries, defoliated sparse canopy cover, dull leaves, presence of epicormic shoots and severe bark peeling (Ward-Jones 2020). The second population of *E. pauciflora* subsp. *niphophila* included 13 living trees at sites 500 m from Perisher Valley. These trees were scored as healthy, with no or minor dieback symptoms (n = 13). At each tree, after scrub was cleared, approximately 50 feeder roots were collected per sample by shovelling two to three holes around the base of the trunk, 10 to 30 cm deep by 30 cm wide, and 1 m from the base of the trunk. Feeder roots were defined as roots  $\leq 2$  mm diameter (Scott *et al.* 2013), with *E. pauciflora* subsp. *niphophila* roots identified as a dark brown, smooth and thin. Prior to counting, samples were labelled, bagged and stored at 10°C.

### *Ectomycorrhizal identification*

To assess for ectomycorrhizal presence, feeder roots from each tree sample were examined under a stereo microscope using a root assay, following a method of identification similar to that employed by Ishaq *et al.* (2013) and Agerer (2001). Observer blinding was performed using a letter code for each tree. Three root branches were randomly selected from each sample, severed and rinsed, cut into three sections each, and placed onto a petri dish. Ten random sections of the root on each dish were assessed in the same field of view (FOV) to determine presence of ECM on feeder roots. ECM presence was defined by a cream-coloured, velvet-like sheath appearance on short, fat feeder roots (Tendersoo & Bahram 2019). Suberised roots (roots absent of ECM) were defined as clear, long and thin. Per dish, the number of ECM colonised feeder roots and un-colonised suberised roots were counted with notes made for unusual appearances.

### *Data analysis*

Data were recorded in Microsoft Excel. A sum of the number of colonised roots per tree was calculated. A post hoc total root count per tree was found by adding up colonised and uncolonised ECM presence counts for individual trees. A percentage of colonised or uncolonised roots relative to the total root count per tree was calculated (TCS). Samples were then unblinded. Across all trees, the percentage of colonised roots for healthy affected trees was calculated (HCS), and repeated for dieback-affected tree samples (DCS):

$$HCS \text{ or } DCS (\%) = \frac{TCS \text{ per healthy (or dieback affected) tree}}{Total \ TCS \text{ across all samples}} \times 100$$

For both HCS and DCS, mean, minimum, maximum, standard deviation (SD) and standard error (SE) were calculated. In R, boxplots for ECM presence and root counts for healthy and dieback-affected *E. pauciflora* subsp. *niphophila* were created. A Shapiro-Wilk normality test assessed normal distributions of ECM presence and root counts. A Mann Whitney Wilcoxon U-Test examined differences in ECM presence and root counts between healthy and dieback-affected *E. pauciflora* subsp. *niphophila* populations.

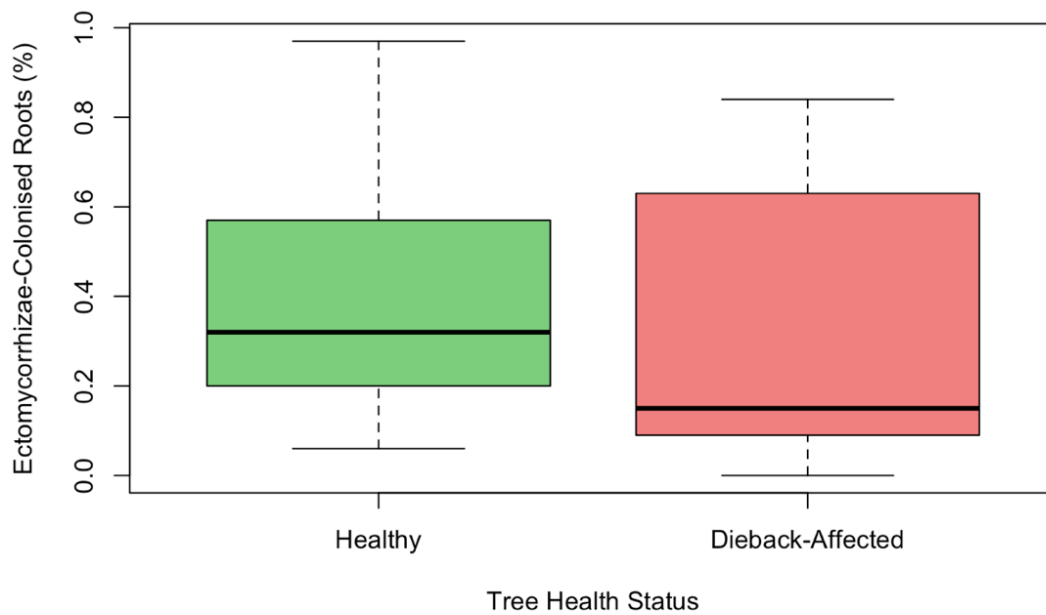
## Results

### *General observations*

We repeatedly noted that dieback-affected tree root samples had very few feeder roots available to examine microscopically. Feeder roots in the dieback-affected trees were observationally sparse and spread out across the main root, compared to dense feeder root presence in healthy feeder root samples. It took much longer to search for and collect dieback-affected feeder roots, as these trees had notably very few roots available, with some trees possessing no roots at all.

### *Ectomycorrhizal presence*

Of 13 healthy *E. pauciflora* subsp. *niphophila* individuals examined, healthy feeder roots contained greater numbers of ECM. On average, 42.92% of healthy feeder roots examined were colonised by ECM, with a minimum of 6% and a maximum of 97% (SD  $\pm$  0.29, SE  $\pm$  0.08; Figure 1). Of 13 dieback-affected *E. pauciflora* subsp. *niphophila* individuals examined, dieback-affected feeder roots contained fewer numbers of ECM. On average, 32.77% of dieback-affected feeder roots were colonised by ECM, with a minimum of 0% and a maximum of 84% (SD  $\pm$  0.32, SE  $\pm$  0.09; Figure 1).

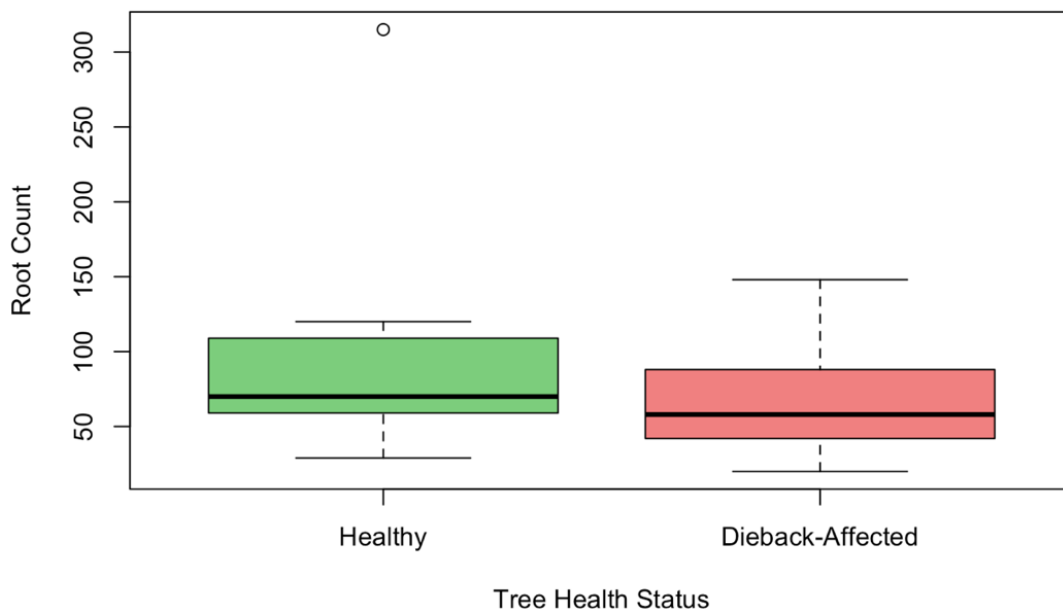


**Figure 1.** Comparison of the percentage of ectomycorrhizal colonised feeder roots between healthy ( $n = 13$ ) and dieback-affected ( $n = 13$ ) root samples across four populations of *E. pauciflora* subsp. *niphophila* in Perisher Valley, NSW.

The percentage of ECM colonisation in feeder root samples was compared to the health status of their respective *E. pauciflora* subsp. *niphophila* tree (healthy or dieback-affected). Assessing for normality of the ECM colonisation data, a Shapiro-Wilk Normality test recorded a significant p-value ( $W = 0.886$ ,  $p = 0.008$ ). This indicated that ECM colonisation data were not normally distributed between healthy and dieback-affected feeder root samples. A non-parametric Mann Whitney Wilcoxon U-Test, comparing unpaired samples within a non-normal distribution (van Emden 2008) found a non-significant p-value ( $W = 112$ ,  $p = 0.166$ ), indicating a non-significant difference in ECM colonisation between healthy and dieback-affected *E. pauciflora* subsp. *niphophila* feeder roots.

#### Root counts

A post hoc trend was observed in feeder root counts. Dieback-affected *E. pauciflora* subsp. *niphophila* root samples contained notably fewer feeder roots than healthy *E. pauciflora* subsp. *niphophila* (Figure 2). Noting that an outlier of 315 roots was included from a healthy tree; healthy samples had a mean count of 95 feeder roots ( $n = 13$ ,  $SD \pm 72.01$ ,  $SE \pm 19.97$ ; Figure 2) compared to a mean count of 68.46 feeder roots in dieback-affected samples ( $n = 13$ ,  $SD \pm 39.01$ ,  $SE \pm 10.82$ ; Figure 2). Assessing for normality of the root counts, a Shapiro-Wilk Normality test recorded a significant p-value ( $W = 0.748$ ,  $p = 2.611e-05$ ). A non-parametric Mann Whitney Wilcoxon U-Test found a non-significant p-value ( $W = 111$ ,  $p = 0.1823$ ), indicating a non-significant difference in root counts between healthy and dieback-affected *E. pauciflora* subsp. *niphophila* feeder roots.



**Figure 2:** Comparison of root counts between healthy ( $n = 13$ ) and dieback-affected ( $n = 13$ ) root samples across four populations of *E. pauciflora* subsp. *niphophila* in Perisher Valley, NSW. An outlier in healthy tree samples is included.

## Discussion

This study aimed to identify and compare ECM presence between healthy *E. pauciflora* subsp. *niphophila* and dieback-affected (Stage II) *E. pauciflora* subsp. *niphophila*. As ECM presence was, on average, common to both healthy and dieback-affected *E. pauciflora* subsp. *niphophila* feeder roots (Figure 1), we successfully met this aim. We also aimed to determine if there was greater ECM presence in healthy *E. pauciflora* subsp. *niphophila* than in those suffering from severe dieback, hypothesising that healthy *E. pauciflora* subsp. *niphophila* trees would have greater ECM presence than dieback-affected trees (1). However, despite healthy *E. pauciflora* subsp. *niphophila* feeder roots containing a greater percentage of ECM colonies than dieback-affected feeder roots, this difference was not statistically significant, rejecting our initial hypothesis (1) and prediction (2) (Figure 1). This suggests that an abundance of ECM colonies is not directly associated with healthier *E. pauciflora* subsp. *niphophila*. It may also indicate that a lack of ECM colonies may not be directly associated with dieback-associated decline in *E. pauciflora* subsp. *niphophila*, contrary to our prediction (3).

These results contrast with observations of previous studies where a higher proportion of ECM colonies was associated with healthier trees in *E. gomphocephala* (Ishaq *et al.* 2018; Ishaq *et al.* 2013), as well as with healthier canopies in a comparison of healthy and declining *E. gomphocephala* (Scott *et al.* 2013). In the present study, it was generally observed that trees severely affected by dieback possessed far fewer roots that were harder to find than healthy trees. This was evidenced in the minimum ECM presence count for dieback feeder roots, as well as the gap in mean root counts between healthy and dieback-affected samples (Figures 1 & 2). It is important to note that this difference was not statistically significant. However, there was an abundance of healthy feeder root samples to analyse, as demonstrated by the inclusion of a root count outlier within healthy samples (Figure 2). As root area correlates with tree canopy size, *Eucalyptus* species suffering from more severe dieback symptoms have reduced capacity to allocate resources to roots (Scott *et al.* 2013), a possible contributor to reduced root biomass, just as we observed.

Sapsford *et al.* (2017) found that both abiotic and biotic stressors can lead to a loss of ECM diversity and functioning. This loss, according to Ishaq *et al.* (2018), negatively impacts the health of other *Eucalyptus* species such as *E. gomphocephala*. Thus, mean discrepancies in available feeder roots between our dieback-affected and healthy samples may explain our null result. Our results suggest that dieback-affected trees may possess fewer roots; however, further testing is needed. We predict that a lack of roots may negatively impact trees' nutrient and water uptake. This result is particularly alarming, as reduced root biomass can contribute to rapid tree decline by impairing root functioning (Jurskis & Turner 2002). Therefore, we can surmise that dieback-affected trees may reduce their root abundance in response to dieback-related stressors. However, to confirm this interpretation, additional formal and statistical tests are necessary, particularly as our root count results were not significantly different. These tests must include a wider range of samples, as in Ishaq *et al.* (2018), as well as a multi-factor ANOVA, as in Adams *et al.* (2006). We could expect this testing approach to produce more robust and potentially statistically significant results. These tests should also identify if (and how) reduced root abundance impacts root and tissue functioning of the entire *E. pauciflora* subsp. *niphophila* tree.

Our post hoc, visually observed lack of root abundance in dieback-affected trees is significant in relation to ECM prevalence. Many lineages of host plants have evolved to select more efficient root fungi and thus extract greater benefits from their symbiosis (Kiers *et al.* 2011). Host plants rewarded ECM colonies on particular roots with greater nutrients in exchange for increased carbohydrate uptake by those colonies; the two partners reward each other (Kiers *et al.* 2011). However, hosts cannot discriminate between fungal partners once colonisation is finalised (Kiers *et al.* 2011). Where multiple trees are faced with dieback-related stressors (for instance, carbohydrate depletion and *P. longicornis* wood boring), trees are unable to selectively preference efficient ECM colonies, and instead direct their nutrients towards growing epicormic shoots (Ross & Brack 2015). As a trade-off response, ECM presence subsequently reduces and is replaced by less-efficient arbuscular mycorrhizal (AM) fungi (Ishaq *et al.* 2018, Ishaq *et al.* 2013; Tedersoo & Bahram 2019). Therefore, in dieback-affected trees, the reallocation of essential resources to epicormic shoots may lead to reduced ECM presence as a trade-off response to dieback symptoms. Thus, it is unsurprising that fewer roots are required to sustain dieback-affected trees. This conflicts with our prediction that if healthy trees have greater ECM presence, a lack of ECM colonies could thereby contribute to dieback symptoms (3). Findings from Simard *et al.* (2012) support this conclusion, as feedback loops via networks of mycorrhizae and their hosts heavily influenced forest structure and function in response to stressors. Further, altered nutrient compositions across NSW dieback sites gradually eliminated root mycorrhizae, impairing root functioning and accelerating dieback (Jurskis & Turner 2002).

The ECM variation in *E. pauciflora* subsp. *niphophila* observed in this study may be a response to dieback-related reductions in root abundance, rather than a cause of dieback itself. This is likely to have been confirmed if we had examined more feeder roots in equal numbers across healthy and dieback-affected trees. However, we cannot demonstrate that dieback-related decline in *E. pauciflora* subsp. *niphophila* samples was the sole contributor to reduced ECM presence. While disturbance can impact ECM presence (Iordache *et al.* 2009), we observed dieback symptoms across trees in all sites, with all sites located within proximity to ski resorts. The difficulty in attributing a cause or contributor to ECM reduction in *Eucalyptus* species similar to *E. pauciflora* subsp. *niphophila* is supported by many well-evidenced examples (Ishaq *et al.* 2018; Scott *et al.* 2013; Sapsford *et al.* 2017). In addition, ECM presence is not only influenced by host health and may also be influenced by microbiome competition (Adams *et al.* 2006). Further, declines in ECM presence were attributed to non-dieback-related stressors such as temperature, respiration acclimation, changes to soil enzymes, drought stressors and climate change (Malcolm *et al.* 2009; Nickel *et al.* 2018). Stress induced by one change, such as differences in ECM presence, is not the sole cause of dieback (Ross & Brack 2017). Therefore, we can suggest that dieback does not

present equally across a site, as shown by variance in ECM presence between individuals (Figure 1).

Our study could have used more robust methods across a greater breadth of sampling sites. For instance, Ishaq *et al.* (2018) studied four trees in each of 12 sites, collecting two soil cores 5 m from each tree. By comparison, we only examined 13 trees in two locations, shovelling shallow pits of soil at the base of each tree. Further, we did not explicitly account for age differences between trees, which also may impact ECM presence (Ishaq *et al.* 2018). In the future, to include root count as a co-variate, we would need to extract intact soil cores from tree samples of a standardised age, thus efficiently quantifying root counts per tree (Sapsford *et al.* 2017). Furthermore, a future study could classify ECM samples as a function of fungal diversity, rather than examine the presence or absence of ECM alone. Powell and Rillig (2018) note that richness is the most efficient measure of fungi functional diversity, while classifying ECM into distinct categories is important when analysing function (Agerer 2001). Using a microbiome study to examine ECM fungal diversity may also be useful, as successfully demonstrated by Ishaq *et al.* (2018). To establish a more robust method, a future microbial study could involve a visual examination of root feeders after root staining, as well as calculation of the percentage of ECM colonisation using a gridline intersect method, as in Vierheilig and Piché (1998). This study could also use metabarcoding to describe specific variations in ECM species among healthy and dieback-affected trees (Sapsford *et al.* 2017). Future research could additionally involve inoculation of *E. pauciflora* subsp. *niphophila* seedlings with ECM communities and monitoring of any changes to comparative health factors (such as seedling survival, growth rate, and biomass) over three months, as in Valdés *et al.* (2019).

Knowledge of how ECM interacts with a dieback-affected *E. pauciflora* subsp. *niphophila* host is useful, particularly as damage in one part of the tree quickly moves around the rest of it (Scott *et al.* 2013). Changes to ECM communities significantly impact entire ecosystem functioning and tree survival through the vital influence of mycorrhizal networks on host structure and functioning (Horton *et al.* 2013; Simard *et al.* 2012). Therefore, our unexpected finding of no significant difference between dieback-affected and healthy *E. pauciflora* subsp. *niphophila* is alarming given the apparent accelerating rate of dieback throughout the KNP. Despite inconclusive results with our predictions, our null finding also indicates a lack of understanding of dieback in *E. pauciflora* subsp. *niphophila*, highlighting the need for further timely and thorough exploration of this subject.

## Conclusions

There was no significant difference in feeder root ECM prevalence between dieback-affected and healthy *E. pauciflora* subsp. *niphophila* trees. However, as root count was reduced in dieback-affected tree samples, this potentially indicated a reallocation of resources to other parts of the tree. Thus, we highlighted the novel finding that dieback-affected trees may reduce their root abundance as a response to dieback-related stressors, potentially linked to reduced ECM presence. Given the rapid spread of dieback in the KNP and a conspicuous lack of knowledge on the subject, further research on dieback and ECM fungal diversity in *E. pauciflora* subsp. *niphophila* is urgently needed.

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## References

- Adams F, Reddell P, Webb MJ, Shipton WA (2006) Arbuscular mycorrhizas and ectomycorrhizas on *Eucalyptus grandis* (Myrtaceae) trees and seedlings in native forests of tropical north-eastern Australia. *Australian Journal of Botany* **54**, 271–281. doi:10.1071/BT05028
- Agerer R (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* **11**, 107–114. doi:10.1007/s005720100108
- Aryal P, Meiners SJ, Carlswald BS (2021) Ectomycorrhizae determine chestnut seedling growth and drought response. *Agroforestry Systems* **95**, 1251–1260. doi:10.1007/s10457-020-00488-4
- de Witte LC, Rosenstock NP, van der Linde S, Braun S (2017) Nitrogen deposition changes ectomycorrhizal communities in swiss beech forests. *The Science of the Total Environment* **605–606**, 1083–1096. doi:10.1016/j.scitotenv.2017.06.142
- Halter R, Sands R, Ashton DH, Nambiar EKS (1997) Root growth of subalpine and montane eucalyptus seedlings at low soil temperatures. *Trees* **12**, 35–41. doi:10.1007/PL00009694
- Horton BM, Glen M, Davidson NJ, Ratkowsky D, Close DC, Wardlaw TJ, Mohammed C (2013) Temperate eucalypt forest decline is linked to altered ectomycorrhizal communities mediated by soil chemistry. *Forest Ecology and Management* **302**, 329–337. doi:10.1016/j.foreco.2013.04.006
- Iordache V, Gherghel F, Kothe E (2009) Assessing the effect of disturbances on ectomycorrhiza diversity. *International Journal of Environmental Research and Public Health* **6**, 414–432. doi:10.3390/ijerph6020414
- Ishaq L, Barber PA, Hardy GESJ, Calver M, Dell B (2013) Seedling mycorrhizal type and soil chemistry are related to canopy condition of *Eucalyptus gomphocephala*. *Mycorrhiza* **23**, 359–371. doi:10.1007/s00572-012-0476-5
- Ishaq L, Barber PA, Hardy GESJ, Dell B (2018) Diversity of fungi associated with roots of *Eucalyptus gomphocephala* seedlings grown in soil from healthy and declining sites. *Australasian Plant Pathology* **47**, 155–162. doi:10.1007/s13313-018-0548-x
- Jurskis V, Turner J (2002) Eucalypt dieback in eastern Australia: a simple model. *Australian Forestry* **65**, 87–98. doi:10.1080/00049158.2002.10674859
- Jurskis, V (2016) ‘Dieback’ (chronic decline) of *Eucalyptus viminalis* on the Monaro is not new, unique or difficult to explain. *Australian Forestry* **79**, 261–264. doi:10.1080/00049158.2016.1236427
- Kariman K, Barker SJ, Finnegan PM, Tibbett M (2014) Ecto- and arbuscular mycorrhizal symbiosis can induce tolerance to toxic pulses of phosphorus in Jarrah (*Eucalyptus marginata*) seedlings. *Mycorrhiza* **24**, 501–509. doi:10.1007/s00572-014-0567-6
- Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuysse P, Jansa J, Bücking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**, 880–882. doi:10.1126/science.1208473
- Launonen TM, Ashton DH, Keane PJ (1999) The effect of regeneration burns on the growth, nutrient acquisition and mycorrhizae of *Eucalyptus regnans* F. muell. (Mountain Ash) seedlings. *Plant and Soil* **210**, 273–283. doi:10.1023/A:1004609912315
- Findlay *et al.*: Ectomycorrhizae root colonisation in dieback-affected snow gums

- Lilleskov EA, Hobbie EA, Horton TR (2011) Conservation of ectomycorrhizal fungi: Exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology* **4**, 174–183. doi:10.1016/j.funeco.2010.09.008
- Malcolm GM, López-Gutiérrez JC, Koide RT, Eissenstat DM (2009) Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi. *Global Change Biology* **15**, 2333–2333. doi:10.1111/j.1365-2486.2009.02036.x
- Nickel UT, Weikl F, Kerner R, Schäfer C, Kallenbach C, Munch JC, Pritsch K (2018) Quantitative losses vs. qualitative stability of ectomycorrhizal community responses to 3 years of experimental summer drought in a beech–spruce forest. *Global Change Biology* **24**, e560–e576. doi:10.1111/gcb.13957
- Powell JR, Rillig, MC (2018) Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. *The New Phytologist* **220**, 1059–1075. doi:10.1111/nph.15119
- Ross C, Brack C (2015) *Eucalyptus viminalis* dieback in the Monaro region, NSW. *Australian Forestry* **78**, 243–253. doi:10.1080/00049158.2015.1076754
- Ross C, Brack C (2017) Monaro dieback: simple answers are too simple. *Australian Forestry* **80**, 113–114. doi: 10.1080/00049158.2017.1311762
- Sapsford SJ, Paap T, Hardy GESJ, Burgess TI (2017) The ‘chicken or the egg’: which comes first, forest tree decline or loss of mycorrhizae? *Plant Ecology* **218**, 1093–1106. doi:10.1007/s11258-017-0754-6
- Scott PM, Shearer BL, Barber PA, Hardy GESJ (2013) Relationships between the crown health, fine root and ectomycorrhizae density of declining *Eucalyptus gomphocephala*. *Australasian Plant Pathology* **42**, 121–131. doi:10.1007/s13313-012-0152-4
- Simard SW, Beiler KJ, Bingham MA, Deslippe JR, Philip LJ, Teste FP (2012) Mycorrhizal networks: mechanisms, ecology and modelling. *Fungal Biology Reviews* **26**, 39–60. doi:10.1016/j.fbr.2012.01.001
- Sritharan MS, Hemmings FA, Moles AT (2021) Few changes in native Australian alpine plant morphology, despite substantial local climate change. *Ecology and Evolution* **11**, 4854–4865. doi:10.1002/ece3.7392
- Tedersoo L, Bahram M (2019) Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes. *Biological Reviews of the Cambridge Philosophical Society* **94**, 1857–1880. doi:10.1111/brv.12538
- Teste FP, Jones MD, Dickie IA (2020) Dual-mycorrhizal plants: their ecology and relevance. *The New Phytologist* **225**, 1835–1851. doi:10.1111/nph.16190
- Valdés RC, Villarreal RM, García FG, Morales SG, Peña SS (2019) Improved parameters of *Pinus greggii* seedling growth and health after inoculation with ectomycorrhizal fungi. *Southern Forests* **81**, 23–30. doi:10.2989/20702620.2018.1474415
- van Emden HF (2008) ‘Statistics for terrified biologists’. (Blackwell Publishing: Malden, USA)
- Vierheilig H, Piché Y (1998) A modified procedure for staining arbuscular mycorrhizal fungi in roots. *Journal of Plant Nutrition and Soil Science* **161**, 601–602. doi:10.1002/jpln.1998.3581610515
- Ward-Jones J (2021) ‘Spotting snow-gum dieback; Guide for recording incidental observations of snow-gums exhibiting dieback symptoms using the ArcGIS Survey123
- Findlay *et al.*: Ectomycorrhizae root colonisation in dieback-affected snow gums

application'. (Fenner School of Environment and Society, The Australian National University: Canberra, Australia)

Ward-Jones J (2020) 'Dieback of subalpine snow gums, *Eucalyptus pauciflora* subsp. *niphophila* in Perisher Valley, Kosciuszko National Park: a description of symptoms and landscape drivers'. (Master's thesis, The Australian National University: Canberra, Australia).

Weidlich EWA, Mito PT, Furtado ANM, Ferst LM, Ernzen JP, Neves MA (2020) Using ectomycorrhizae to improve the restoration of neotropical coastal zones. *Restoration Ecology* **28**, 1324–1326. doi:10.1111/rec.13284

Worboys GL, Good RB (2011) 'Caring for our Australian Alps catchments: summary report for policy makers'. (Department of Climate Change and Energy Efficiency: Canberra, Australia)