

2023

An investigation into how tree characteristics and species composition associated with veteran and ancient trees influences invertebrate species

Clapp, B.

Clapp, B. (2023) 'An investigation into how tree characteristics and species composition associated with veteran and ancient trees influences invertebrate species', *The Plymouth Student Scientist*, 16(2), pp. 224-252.

<https://pearl.plymouth.ac.uk/handle/10026.1/21838>

The Plymouth Student Scientist
University of Plymouth

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

An investigation into how tree characteristics and species composition associated with veteran and ancient trees influences invertebrate species

Brandon Clapp

Project Advisor: [Dr Sophie Fauset](#), School of Geography, Earth and Environmental Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA

Abstract

Veteran and ancient trees have distinctive characteristics that act as microhabitats: such as tree cavities and deadwood. These trees and their microhabitats are keystone features for specialised communities of invertebrates, fungi, and epiphytes. However, due to limited species data, there are still uncertainties around the relative importance of veteran and ancient trees and their associated microhabitats on these invertebrate species. This investigation aims to assess how the tree characteristics of veteran and ancient trees affect the species richness and composition of the three taxa (i) invertebrates, (ii) fungi, and (iii) epiphytes. Epiphytes, fungi, and invertebrates on the tree trunk were recorded and identified; pitfall traps were also installed and collected after 48 hours for identification in the lab. The most significant tree host characteristics that impacted species richness and community structure were the tree size, measured as the diameter at breast height (m) and the number and quality of tree cavities found only on veteran and ancient trees. Due to the low sample size, the ancient tree was combined with veteran trees for data analysis. Tree size significantly affected the variation in the number of microhabitats between tree species, accounting for 68% of the variation, suggesting that larger trees that are generally older will provide more microhabitats. Although the ancient and veteran trees combined had a higher diversity of invertebrates with a mean of three compared to mature trees with two, it was not statistically significant. Furthermore, tree size and the number of microhabitats did not significantly impact the variation associated with invertebrate species richness. Tree cavities benefit invertebrate species, with the wood mould providing stable levels of moisture, pH, and temperature. However, this was not observable due to the invertebrates being in diapause during the sampling period.

Key words: Microhabitats; characteristics; species richness; composition, trees

Introduction

Ancient and veteran trees are both known for their rich and specialized biodiversity, showing distinctive characteristics like tree cavities, deadwood, epiphytic plants, and sap runs, which create essential microhabitats utilized by a wide range of invertebrate species (Thygeson *et al.*, 2017). Although all ancient trees are veteran, not all veteran trees are ancient; however, with these trees sharing the same characteristics, researchers and conservationists often use these terms interchangeably (Woodland Trust, 2008).

Tree hollowing leads to the formation of cavities in ancient trees due to age, passing beyond maturity compared to trees of the same species. In contrast, veteran trees can develop similar characteristics at a mature stage due to adverse growing conditions like drought (Woodland Trust, 2008; Kirby and Watkins, 2015). Unfortunately, scientists still have uncertainties about the natural processes that form tree cavities due to them being difficult to analyse in the field (Remm and Löhmus, 2011). However, according to Wetherbee *et al.* 2022 and Cuff *et al.* 2020, these hollows are likely formed by the digestion of lignin and cellulose of the heartwood by specialised heart-rot fungi, which is exposed either due to age or the physical environment. Once the heartwood weakens, cavities develop through the action of heart-rot fungi, invertebrates, and the physical breakdown of the decaying wood (Kirby and Watkins, 2015). This process can continue for centuries, so tree cavities in ancient trees tend to be larger, more structurally complex, and more valuable than those found on veteran trees (Wetherbee *et al.*, 2022).

These hollows are often referred to as keystone vegetation features (Remm and Löhmus, 2011), providing specialised communities of invertebrates with stable temperatures and levels of moisture and pH, creating a very valuable microhabitat (Müller *et al.*, 2014; Sebek *et al.*, 2013). During the development of tree cavities, wood mould consisting of the decomposed wood, insect frass, leaf litter, and excrement starts to form in the bottom of the tree cavity producing a nitrogen-rich environment and contributing to the stable microclimate (Schauer *et al.*, 2017; Kirby and Watkins, 2015). Taxonomically, cavity mould is very diverse, supporting obligative groups including beetles (Coleoptera) and flies (Diptera) as well as facultative species such as spiders (Araneae), springtails (Collembola), mites (Acari) and millipedes (Diplopoda) (Kirby and Watkins, 2015). The term obligative refers to species like the violet click beetle (*Limonicus violaceus*), which is dependent on these cavities and wood mould during their lifecycle like for breeding, whereas facultative species do not rely on these features but still benefit (Ranius *et al.*, 2009; Cuff *et al.*, 2020).

The term saproxylic refers to species dependent on dead woody material at some stage during their life cycle; similarly, epixylic species like mosses and lichen prefer to colonize deadwood substrates (Stokland *et al.*, 2012). Saproxylic species play a critical ecological role within terrestrial ecosystems, with fungi and invertebrate species acting as the primary agents for wood decay, which is essential for nutrient recycling and ecosystem functioning (Stokland *et al.*, 2012; Nieto and Alexander, 2010). This investigation looks at both fungal groups, including Basidiomycota, the most crucial group of saproxylic fungi and Ascomycota, which also contains many wood-inhabiting fungi (Stokland *et al.*, 2012). Basidiomycetes produce their spores

externally, while ascomycetes produce them internally within a sac (An Australian Government Initiative, 2012).

Despite their value, veteran and ancient trees have experienced a rapid decline in their population worldwide due to several factors, including urbanization, intensification of agriculture, lack of appropriate tree planting, edge effects and habitat fragmentation, as well as traditional silviculture (Müller *et al.*, 2014; Nolan *et al.*, 2020). Although this impacts all forest species, the reliance on deadwood habitats by saproxylic species makes them highly susceptible to anthropogenic land-use change caused by the following two drivers (Belcik *et al.*, 2018). Intensified rates of deforestation in temperate regions to make space for agricultural pastures have significantly decreased populations of saproxylic species by reducing the abundance of veteran and ancient trees in addition to the volume and availability of deadwood resources (Belcik *et al.*, 2018; Webster and Jenkins, 2005; Stokland *et al.*, 2012). Forest management in Europe has also been proven to reduce the quality and availability of cavities and deadwood habitats, reducing the overall heterogeneity through the planting of monocultures and application of other traditional silviculture practices like short harvesting periods (Lassauce *et al.*, 2011; Stokland *et al.*, 2012). The reductions in variations between sites through simplifying tree composition, age and stand structure have negatively impacted the quality of deadwood habitats (Belcik *et al.*, 2018). Due to these drivers, mortality rates in veteran oak trees have reached 1% per annum (Kirby and Watkins, 2015), exceeding the limit of 0.5% recommended by (Gibbons *et al.*, 2008). Enhanced mortality and silviculture in Europe have caused a reduction in veteran tree distribution and habitat availability for many obligative endangered species like the violet click beetle. As a result, 14% of saproxylic beetle species are experiencing a declining population, and 11% of them are already classified as threatened on the IUCN Red List (Nieto and Alexander, 2010). Many of these species are endemic and rely on specific conditions provided by the varying tree cavity and deadwood habitats. Therefore, further declines in habitat availability and veteran and ancient tree populations could potentially lead to the extirpation or extinction of these populations (Ranius *et al.*, 2009).

Although the trend of veteran trees is expected to be declining throughout Europe, with studies in Finland showing that veteran broadleaf trees over >150years old have declined since the 1970s, it is difficult to assess these trends with studies conducted within several countries over a small spatial scale (Jacobsen *et al.*, 2023; Henttonen *et al.*, 2019). As shown in (Figure 1) with Europe having the lowest forest cover (millions of ha), there have been significant efforts by the European Union to reforest these areas by extending the forest cover within Europe by 17.5 million ha between 1990-2015. Despite this being beneficial, secondary forests cannot simply replace the previous habitats, with studies by Henttonen *et al.* 2019 and Belcik *et al.* 2018, suggesting this has not had a significant impact on increasing the number of large trees or the availability of tree cavities and deadwood habitats needed to support saproxylic organisms.

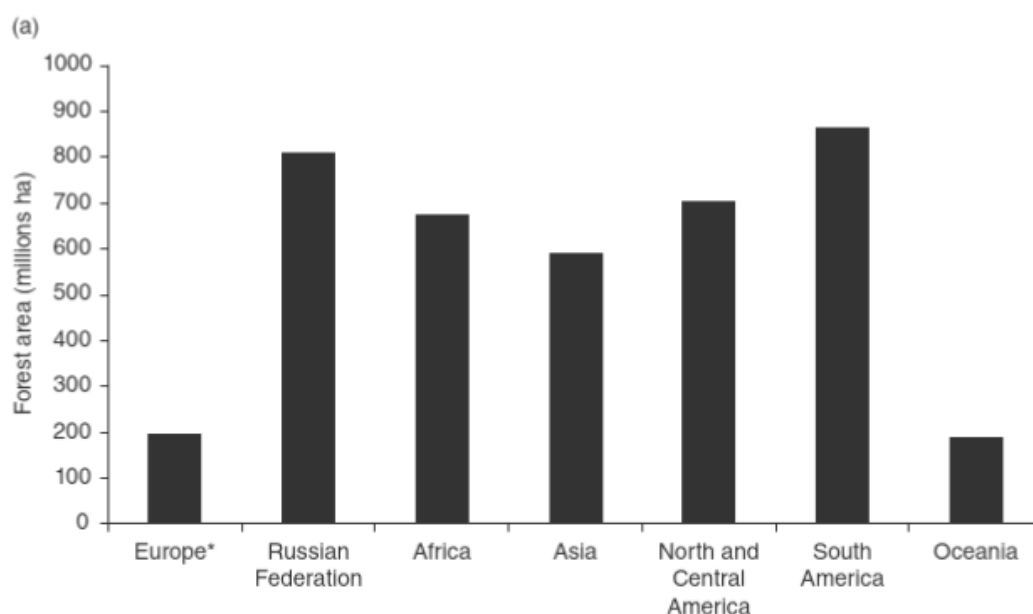


Figure 1: European forest cover (millions of hectares) compared to the other continents and Russia (Kirby and Watkins, 2015)

In partnership with the Woodland Trust and the Ancient Tree Hunt, the Ancient Tree Inventory is a citizen science recording project where people can record and map the location, species, type, and circumference of a notable, veteran, or ancient tree. Containing over 170,000 tree records, this extensive database is recognised by Natural England and the Forestry Commission as a source of information when looking at the impact of development on these trees (Ancient Tree Forum, 2017; Nolan *et al.*, 2020).

It is commonly accepted between studies that deadwood habitats and tree cavities are beneficial and relied upon by many obligative fungal and invertebrate species such as saproxylic beetles. However, with these studies neglecting facultative species and other taxa: including springtails and epiphytic bryophytes, the importance of the habitats associated with veteran trees for facultative species still needs to be determined due to the limited dataset. (Corral *et al.*, 2021). In addition, even though the trends are commonly accepted, further assessment is required to analyse the importance of deadwood habitats using species data (Müller *et al.*, 2014). Therefore, this investigation aimed to record the presence of facultative species by quantifying species richness using an adaptation of the veteran tree's initiative specialist survey method. This initiative is the standard operating procedure for surveying ancient and veteran trees and their characteristics within the UK; however, this method groups invertebrates to gather a ranking known as a summary of the associate score (Magrath, 2018). With this method of grouping invertebrates, it would not be able to assess the trends of veteran trees on the facultative species. Therefore, this investigation decided to divide the invertebrates into individual taxa at an order level like Coleoptera and Collembola, with the presence of invertebrates given a value of (0) not present or (1) present.

This would allow the investigation to assess the importance of deadwood habitats and tree characteristics using data on species richness and composition but also enhance the otherwise lacking dataset for the facultative species.

This investigation aimed to assess and quantify how invertebrate species richness and community structure consisting of obligative and facultative species is influenced by the species composition and characteristics associated with veteran and ancient trees compared to mature trees. Following an adaptation of the veteran tree's initiative specialist survey method (Ancient Tree Forum 2020), the objectives were to: (1) Assess how species richness and community composition of invertebrates, epiphytes and fungi varies between veteran and mature trees due to the presence of microhabitats; (2) Quantify the influence of tree size on the number of microhabitats and assess the impact on species richness and community composition; and (3) Assess how invertebrate species richness and composition associated with the tree is influenced by the presence of epiphyte and fungal species.

Hypotheses

- (1) Veteran trees will have a higher species richness than mature trees.
- (2) Microhabitats will have a positive relationship on the three classification groups: (A) Invertebrates; (B) Fungi; and (C) Epiphytes.
- (3) Diameter at breast height will have a positive influence on the three classification groups: (A) Invertebrates; (B) Fungi; and (C) Epiphytes.
- (4) Invertebrate species richness will be positively influenced by the presence of fungi and epiphytes.

Methodology

Sampling sites

The investigation was conducted within three selected areas within Plymouth, Devon, UK. Analysing the Ancient Tree Inventory (ATI) database published by the Woodland Trust, the following locations consisting of Beaumont Park, Ham Woods and Manadon Woods was selected (Figure 3, Table 1). These locations were selected based on their presence of comparable tree species and types (Woodland Trust, 2023).

Beaumont Park

Often known as squirrel park, Beaumont Park is situated within central Plymouth and is used by families and dogwalkers as an area of recreation. This area contains many mature and veteran turkey oaks planted by Plymouth City Council (Plymouth City Council, n.d^a).

Ham Woods

Situated in a steep valley near the Burrington Industrial Estate, this location consists of several habitats, including woodlands, rough grasslands, marshes, and hedgerows, expanding over 35.9 hectares. Ham Woods is also a designated Local Nature Reserve and is managed by Plymouth City Council (Plymouth City Council, n.d^b).

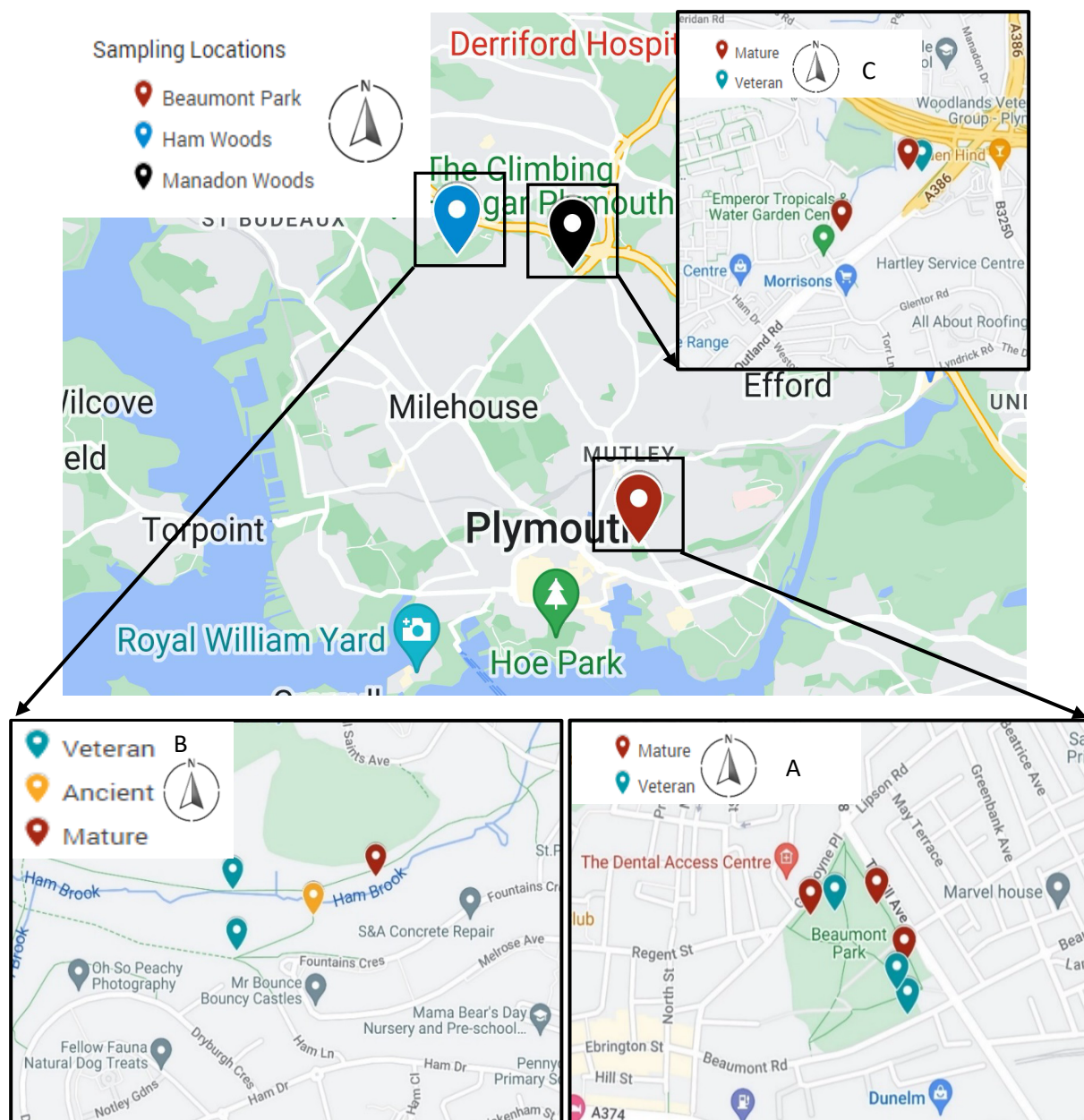


Figure 2: The three sampling locations consisting of Beaumont Park (A), Ham Woods (B) and Manadon Woods (C) located throughout Plymouth, Devon, UK. Map data: © 2023 Google (Google Maps, 2023).

Table 1: Easting and Northing co-ordinates, soil characteristics, total number of trees surveyed, and the total species richness of fungi, epiphytes and invertebrates associated with the trees recorded at the three sampling sites (*British Geological Survey, 2023*)

| | Beaumont Park | Ham Woods | Manadon Woods |
|--|--|--|--|
| Easting | 248611 | 246744 | 248098 |
| Northing | 54833 | 57799 | 57558 |
| Soil | Free draining, slightly acidic loamy soils (Soilscape 6) | Free draining, slightly acidic loamy soils (Soilscape 6) | Free draining, slightly acidic loamy soils (Soilscape 6) |
| Total trees recorded | 6 | 4 | 3 |
| Total veteran & ancient trees | 3 | 2 | 2 |
| Total invertebrate species richness | 12 | 12 | 7 |
| Total fungal species richness | 2 | 4 | 5 |
| Total epiphyte species richness | 18 | 18 | 8 |

Manadon Woods

Located next to the A386, Manadon Woods is a small woodland dominated by pedunculate oak and common beech trees. According to the Ancient Tree Inventory, this location is one of the few areas in Plymouth with a known sighting of an ancient tree, however for this investigation, it was considered too small to be an ancient tree, so it was classified as a veteran.

Data inputting, co-ordinates, and tree distance

Using GPS Pathfinder Office, a data dictionary was created with the aid of Richard Hartley (Appendix 1). The dictionary was then exported onto a Trimble® Geo 7X Global Navigation Satellite System, which stored the collected data in the field. However, the primary purpose of this device was to record the coordinates measured as the longitude and latitude of the recorded trees with a small marginal error of a few centimetres to show the distribution of recorded trees. After all the trees had been recorded, the coordinates were converted from longitude and latitude to easting and northing and plotted onto the map (Figure 1). In addition, this device was also used to create waypoints to try and locate the selected recorded trees on the Ancient Tree Inventory database and find the locations of the planted pitfall traps. The Trimble® Geo 7X also comes with an integrated laser rangefinder, which was used to record the distance of the nearest tree of the same tree type regardless of its species. This was done by pointing straight ahead towards the trunk of the selected target whilst standing upright next to the tree trunk of the recorded tree.

Tree Species, type, and microhabitats

Using the Ancient Tree Inventory (ATI), the following three species were selected: (i) pedunculate oak; (ii) turkey oak; and (iii) common beech. These species were

chosen due to their variations in characteristics, with the oak species likely to be older and have a larger diameter than the common beech trees. This investigation was a ground-based survey; therefore, the only microhabitat feature sampled was the individual tree cavity counted on the tree trunk. These cavities were also used to classify the trees, with veteran and ancient trees showing symptoms of cavities, unlike the mature trees. In addition, an ancient tree was defined as a significantly large veteran tree. A similar number of veteran and mature trees were sampled, collecting data from six trees each; however, with the low sample size for the ancient tree, it was combined with the veteran tree for data analysis. In total, thirteen trees were recorded.

Diameter at Breast Height (DBH)

To assess the impact of tree size on invertebrate species richness, the diameter at breast height (DBH) was taken using a 5m girthing tape. The circumference was recorded at a selected level of 1.5m from the ground level unless any distinctive features were present such as a tree burl which would skew the data. In this scenario, the circumference was recorded slightly below 1.5m to get an accurate representation. The values were then divided by pi (π) to convert the circumference values into the diameter.

Fungal species richness

To assess the importance of deadwood habitats for wood-inhabiting basidiomycetes and ascomycetes fungi, visible fruiting bodies on the tree trunk or underneath the canopy were identified down to a species level using a Collins fungal identification guide (Buczacki, Shields and Ovenden, 2012). However, some species were also identified using the online app PictureThis, which uses Artificial intelligence to identify species down to a family level (PictureThis, n.d.).

Epiphyte species richness

Epiphytes consisting of ferns and mosses found colonizing the tree trunk were identified down to a species level using identification keys; unfortunately, the exact source used cannot be recalled. This investigation also assessed the lichen species on the tree trunk, which was identified down to a family level using PictureThis (*PictureThis, n.d.*). Although this method is limited, it could still differentiate between lichen species allowing this investigation to assess species richness and community structure.

Invertebrate species richness

Veteran and ancient trees are sensitive habitats; therefore, this investigation used non-destructive sampling methods to survey invertebrate species richness and community composition. The first method was to conduct a five-minute hand search of the recorded tree, using a 10-20x magnifying glass to locate invertebrates on the tree trunk. Invertebrates were then collected using a pooter and identified using an AIDGAP identification guide. Species were then identified to their highest level possible with these resources, which in this case was an order level, except for red spider mites (*Tetranychus urticae*). The species richness score of each species was given a score of (0) not present or (1) present; the species were then all added for a total species richness score for that tree. If applicable, this search focused on any tree cavities present to further assess these microhabitats' importance and differentiate between mature and veteran trees.

Pitfall traps were installed underneath the tree canopy to quantitatively sample epigeal invertebrate species present near the base of the tree (Leather, 2005). Pitfall traps were red disposable cups with a dimension of 8x8x10cm, planted inside a small hole created using a bulb corer. After levelling the soil around the disposable cup, 33% was filled with a concentrated saline solution, with approximately 35.7g of salt used for each trap. This preservative was used instead of alternatives like ethylene glycol due to the short sampling period and lower toxicity. A few drops of washing-up liquid were added to the solution to break the surface tension, allowing invertebrates to sink to the bottom. Mammal corridors were installed into the pitfall traps by placing small sticks or stones at the bottom of the trap to reduce the chance of bycatch of small mammals. Rainfall covers consisting of small plant potholders were installed 3-4cm from the soil surface to allow free access to the traps while preventing sample dilution or loss.



Figure 3: Equipment consisting of concentrated saline solution, a rainfall cover, washing up liquid and a bulb corer used to install a pitfall trap in Beaumont Park.

Due to time restraints, specimens caught with the pitfall traps were collected after 48 hours and transported back to the lab using leak-buster sampling pots. After completing a laboratory risk assessment and being trained by Jane Akerman, specimens were transferred from the saline solution into a Petri dish filled with industrial denatured alcohol (IDA 95) solution to remove the salt crystals formed on the invertebrates. The Petri dishes were then placed underneath a Leica S9 i microscope with a magnification of 55x to identify the invertebrate specimens to the highest taxonomic level possible using AIDGAP identification keys (Figure 4).

Species were identified to a variety of taxonomic levels, including: (Order) Collembola, Coleoptera; (Suborder) Adephaga (Phylum) Nematoda; (Class) Pauropoda; and (Species) *Arcitalitrus dorrieni*, *Chordeuma proximum*. Species richness was quantified by combining the data collected from hand-searching and pitfall traps.



Figure 4: Beetle larvae (Coleoptera) identified underneath a Leica S9i microscope.

Normality testing

An Anderson-Darling normality test was conducted to assess the distribution of each variable. Invertebrate species richness was normally distributed; therefore, the differences in means between the two tree types were tested using a two-sample t-test. However, Epiphyte and fungal species richness were deemed not normally distributed, so a Mann-Whitney U test was conducted instead to test the differences between the medians between veteran and mature trees.

Ordination (Nonmetric multidimensional scaling)

Nonmetric multidimensional scaling (NMDS), a form of ordination, was used to condense and visualize the diversity and data between the species communities consisting of fungi, epiphytes and invertebrates recorded on the veteran and mature trees within a low dimensional space (Coding Club, n.d.). This ordination was conducted due to null values within the species communities and the flexibility to choose any similarity distance; in this case, Bray-Curtis dissimilarity was used because the null values do not impact it. Ordination can also plot environmental variables like tree distance to assess which variable drives the observed differences in species composition.

Generalized linear mixed modelling

To quantify the influence of predictor variables, including microhabitats and diameter at breast height on species richness, a linear mixed model was conducted in R studio due to the non-independence and multiple grouping factors within the dataset, which makes it difficult to disentangle the variables (Hajduk, 2022). Unlike simple regression models, mixed models can also incorporate random categorical grouping factors to get an improved estimate of the variation caused by the predictor variable. The three random variables are location, tree species and tree type. Their total variation (%) was calculated by adding the variation value for the residual and random variable, then dividing the random variable by the total variation and multiplying by 100.

Variables excluded from data analysis

This methodology was also designed to record many other variables in the field that could impact species richness and community structure. However, these variables either did not directly correlate to the central aims of this investigation or insufficient data were collected to conduct an appropriate analysis.

1. Weather conditions
2. Temperature (°C) (Kestrel 3000)
3. Tree form such as coppiced, pollard, maiden
4. Light intensity (Lux) (Lux meter)
5. Epiphyte percentage cover (10x10cm quadrat)
6. Tree distance (Integrated laser rangefinder on the Trimble Geo-7x)

Results

Species richness and tree type

Figure 5 shows a higher range of invertebrates associated with veteran trees (mean = 2.6 ± 1.5 SD) than mature trees (mean = 2.2 ± 2.1 SD). The difference is not statistically significant between the two tree types (two-sample t-test, $df = 8$, $t = -0.39$, $p = 0.708$); therefore, we accept the null hypothesis stating that species richness will not be higher on veteran trees compared to mature and reject hypothesis 1. Figure 5 also indicates that veteran trees have a higher epiphyte species richness (mean = 3.7 ± 1.7 SD) than mature trees (mean = 3.0 ± 0.6 SD). Like the invertebrates, there is also no significant statistical difference for epiphyte species richness (Mann-Whitney U, $W = 45.50$, $p = 0.357$) and fungal species richness (Mann-Whitney U, $W = 41.00$, $p = 0.132$) between the two tree types

Community structure of veteran and mature trees

Species abundance and presence vary between veteran and mature trees, with veteran trees being plotted closer together, meaning the overall species community is more similar compared to mature trees, which are more clustered and far apart (Figure 6). Environmental gradients that could influence species composition were plotted against the ordination, however they were deemed to be insignificant (Table 2).

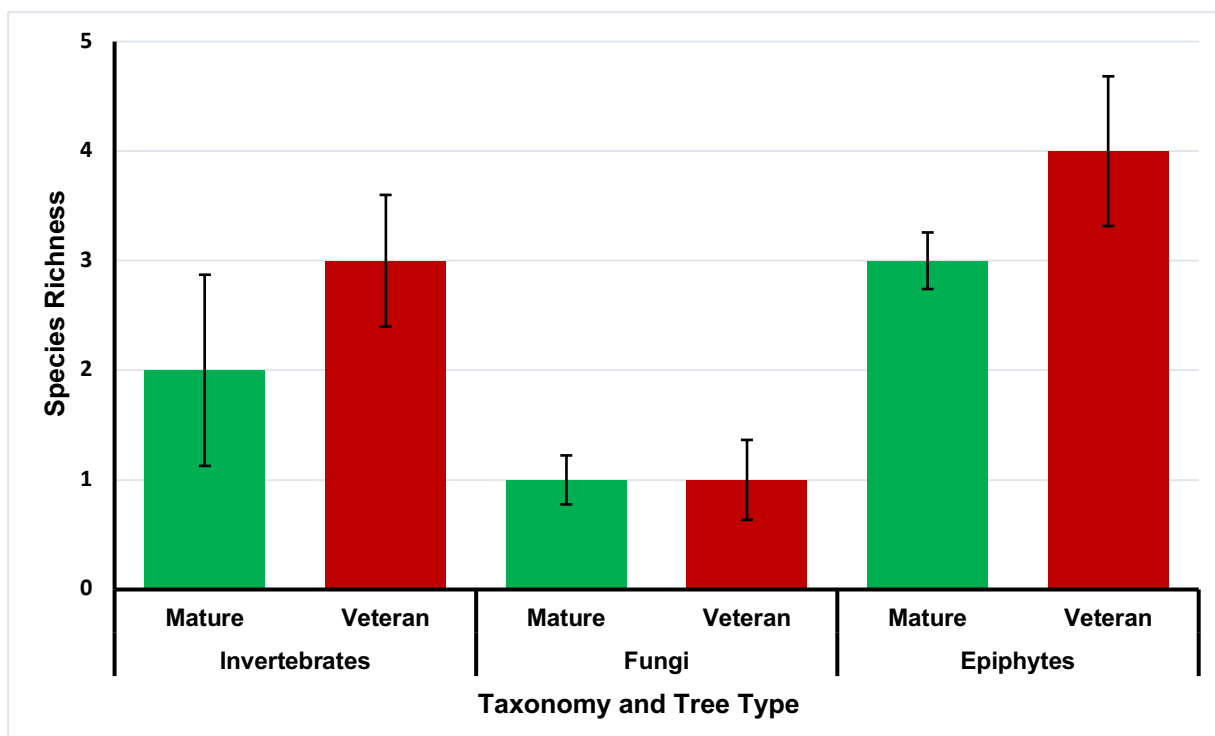


Figure 5: Mean species richness for the three classification groups with standard error bars between veteran and mature trees.

Table 1: The effect and significance of environmental variables on species communities associated with the thirteen veteran and mature trees.

| Environmental gradients | P- value | R- squared |
|---------------------------|----------|------------|
| Tree species | 0.340 | 0.228 |
| Tree distance | 0.558 | 0.109 |
| Temperature | 0.340 | 0.340 |
| Light intensity (lux) | 0.428 | 0.157 |
| Diameter at breast height | 0.526 | 0.112 |
| Microhabitats | 0.301 | 0.211 |

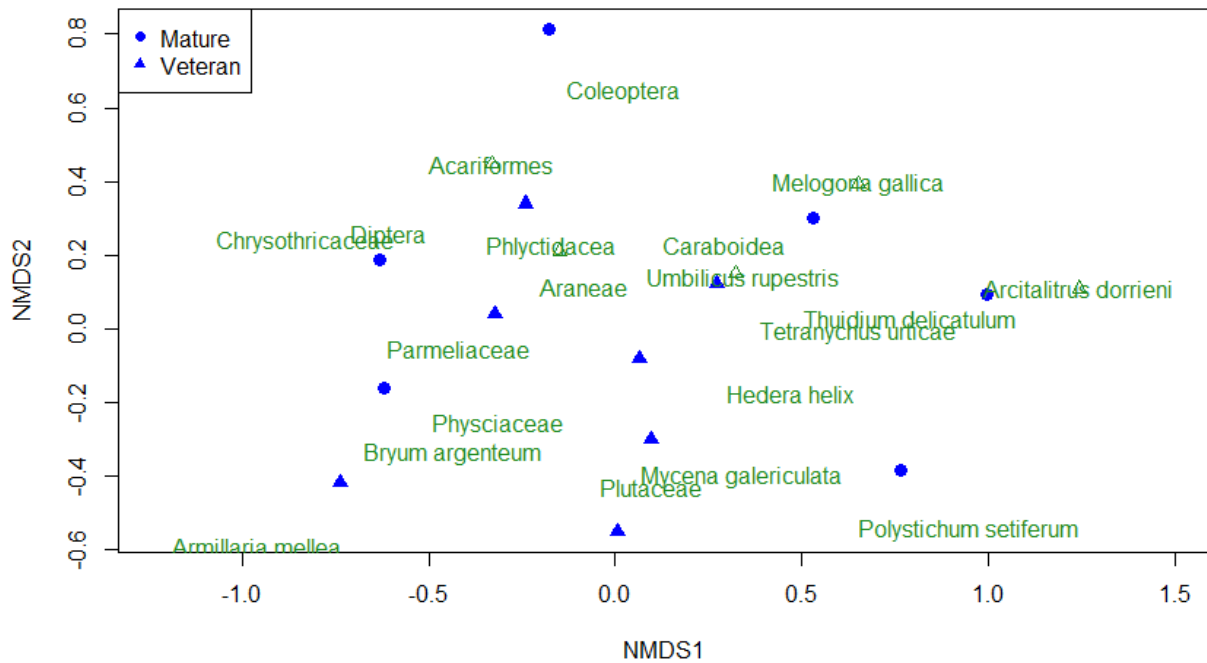


Figure 6: Nonmetric multidimensional scaling (NMDS) plot of species communities supported by the thirteen recorded trees consisting of mature, veteran and ancient trees (ancient tree combined with veteran). Due to formatting issues, green triangles were used to illustrate species that shared the same composition as another species, which caused R studio to overlap the text.

Invertebrate species richness, microhabitats, and diameter at breast height

After conducting a linear mixed model and realizing the random variables have no impact on the influence on invertebrate species richness, a simple linear regression analysis was performed to quantify the variation caused by the predictor variables microhabitats and diameter at breast height. The regression model (Figure 7A) indicates a weak positive relationship between microhabitats and invertebrate species richness, with microhabitats accounting for 15% of the variation. However, this test proved not to be statistically significant; therefore, the null hypothesis stating that microhabitats will not positively influence invertebrate species richness was accepted, and hypothesis 2A was rejected ($R^2 = 0.149$, $df = 11$, $p = 0.193$).

There is also a weak relationship between diameter at breast height and invertebrate species richness, with the diameter accounting for 27% of the variation (Figure 7B). However, this test was also proved not to be statistically significant, so the null hypothesis stating that there will be no positive relationship between invertebrate species richness and diameter at breast height was accepted, and hypothesis 3A was rejected ($R^2 = 0.270$, $df = 3$, $p = 0.128$).

Fungal species richness, microhabitats, and diameter at breast height

After performing another linear mixed model, it was discovered that random variables do not affect the variation between the number of microhabitats and fungal species richness. A simple linear regression (Figure 8A) indicates a weak relationship between the two variables, with microhabitats accounting for 36% of the variation. This test also proved statistically significant, so the null hypothesis was

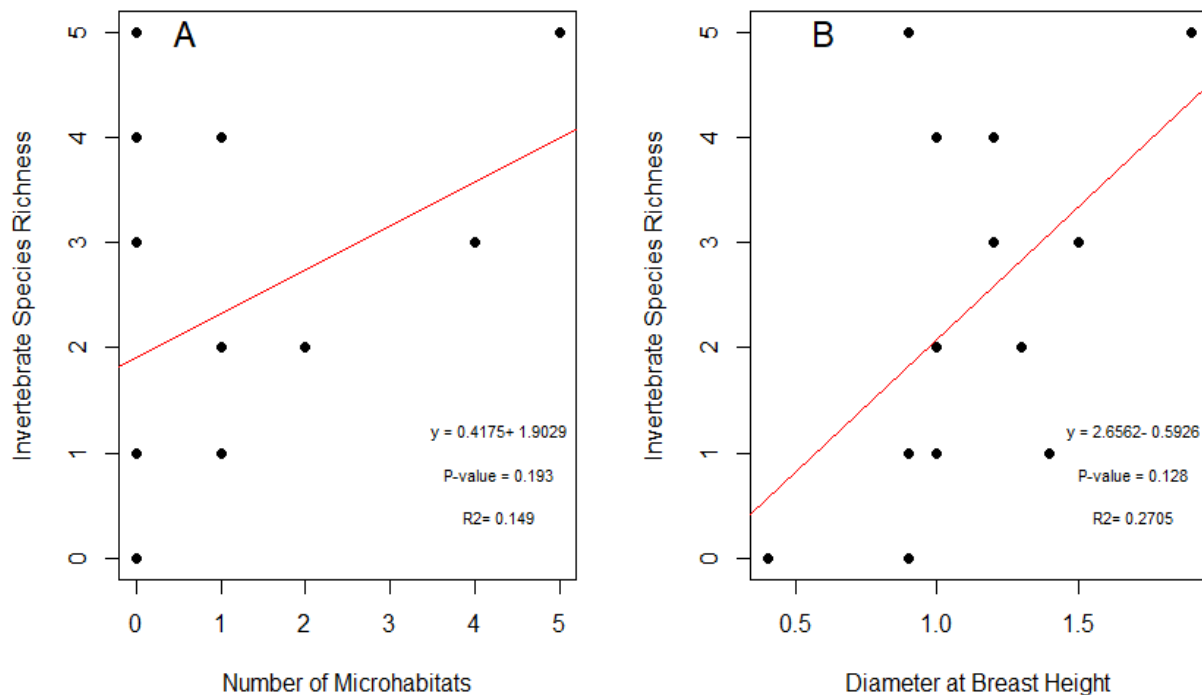


Figure 7. Linear regression models showing the effect of tree characteristics on invertebrate species richness. A: Weak positive relationship between the number of microhabitats and invertebrate species richness. B: Weak positive relationship between diameter at breast height and invertebrate species richness.

rejected, which states that there will not be a positive relationship between fungal species richness and microhabitats. Instead, hypothesis 2B was accepted ($R^2=0.355$, $df = 11$, $p = 0.031$).

A weak relationship was also found between breast height and fungal species richness, with the diameter at breast height accounting for approximately 31% of the variation (Figure 8B).

This test was proved to be statistically significant, so the null hypothesis stating that fungal species richness and diameter at breast height will not have a positive relationship was rejected, and hypothesis 3B was accepted ($R^2=0.311$, $df = 11$, $p = 0.047$). However, the random variable tree species was found to have a significant impact on the test, accounting for 69% of the variation.

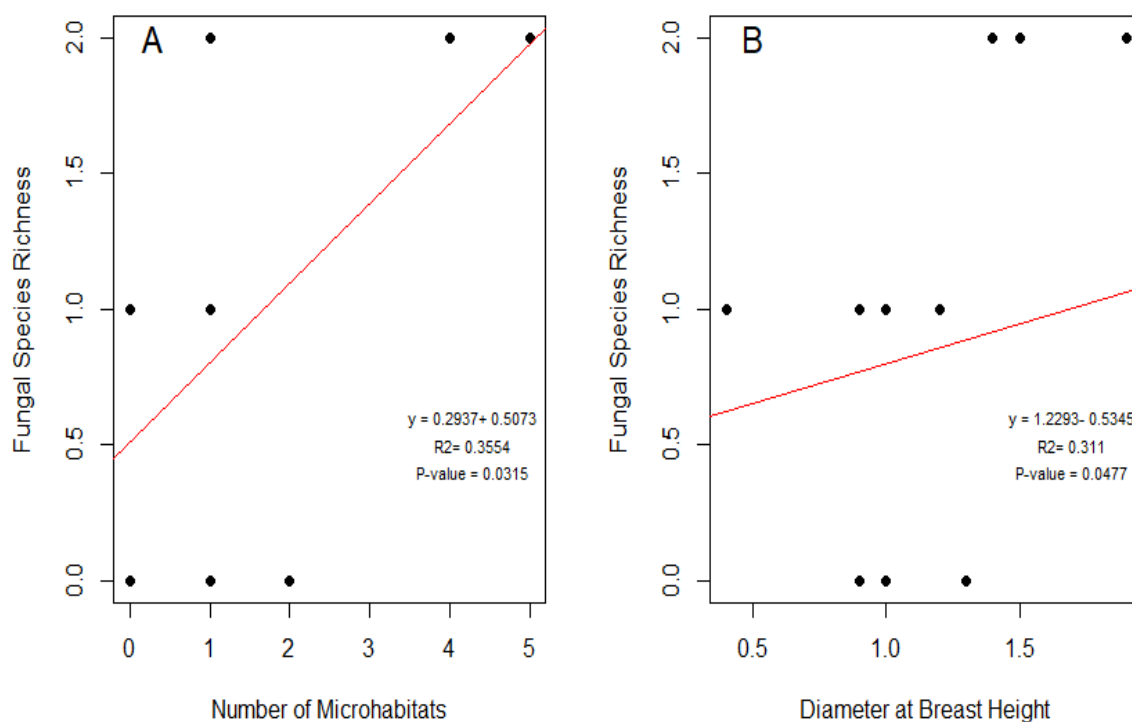


Figure 8. The relationship between fungal species richness and tree characteristics. A: Weak positive relationship between fungal species richness and the number of microhabitats. B: Weak positive relationship between fungal species richness and diameter at breast height.

Epiphyte species richness, microhabitats, and diameter at breast height

A linear mixed model indicates that tree type significantly impacts epiphyte species richness and microhabitats with a variation of 92%; however, this is likely due to the nature of the study since tree type was classified based on the presence of microhabitats. Figure 9A indicates a weak relationship between the two variables, with microhabitats accounting for 44% of the variation. This test proved to be statistically significant; therefore, the null hypothesis stating that the presence of microhabitats does not positively influence epiphyte species richness was rejected, and hypothesis 2C was accepted ($R^2 = 0.444$, $df = 11$, $p = 0.047$).

Figure 9B shows a weak relationship between diameter at breast height and epiphyte species richness, with the diameter accounting for 32% of the variation. This test was also found to be significant, so the null hypothesis stating that diameter at breast height does not positively influence epiphyte species richness was rejected and instead hypothesis 3C was accepted ($R^2 = 0.323$, $df = 11$, $p = 0.042$). Results from the linear mixed model indicate that all three random effects impact the variation with tree species accounting for 33%, location for 22% and tree type for 34%.

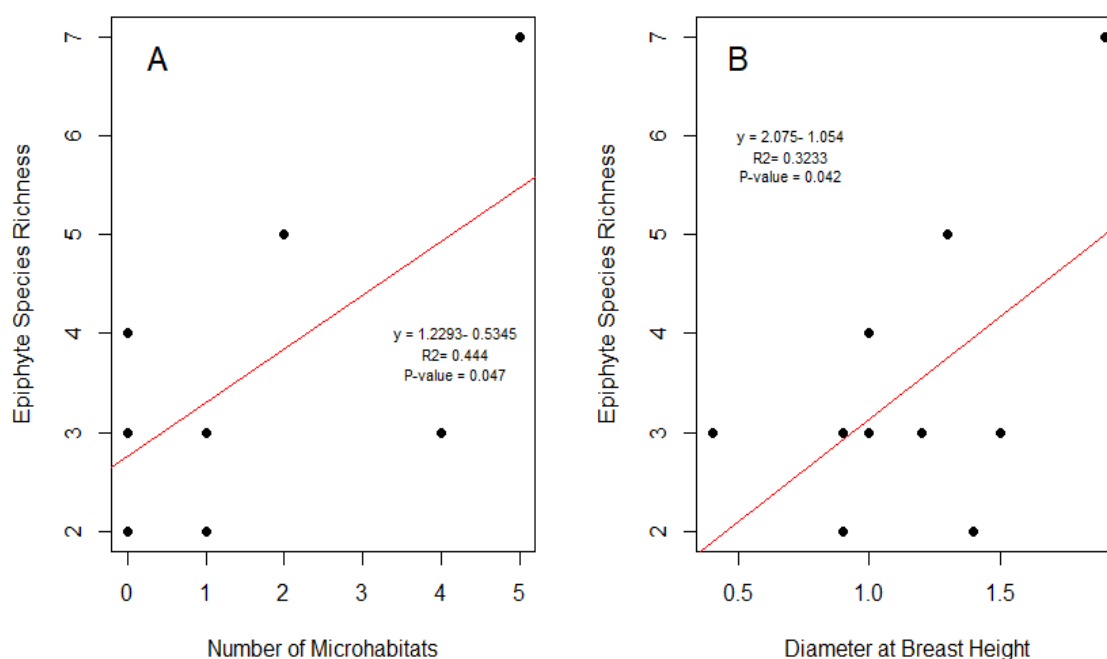


Figure 9: The effect of tree characteristics on epiphyte species richness. A: Weak positive relationship between the number of microhabitats and epiphyte species richness. B: Weak positive relationship between the diameter at breast height and epiphyte species richness.

Influence of fungi and epiphytes on invertebrate species richness

After conducting another linear mixed model, it was concluded that the presence of fungi only has a minor influence on invertebrate species richness with a variation of 8% (Figure 10A). However, this test proved not statistically significant; therefore, the null hypothesis stating that invertebrate species richness will not be positively influenced by the presence of fungi on the tree or underneath the canopy was accepted. Therefore, hypothesis 3A was rejected ($R^2 = 0.0798$, $df = 11$, $p = 0.349$). Random variables were also found not to have any influence on the variation.

The same process was performed between invertebrate species richness and epiphytes, and it was discovered that there is a weak relationship between the two variables, with epiphytes accounting for 25% of the variation (Figure 10B). Like the last test, this proved not to be statistically significant; therefore, the null hypothesis stating that the presence of epiphytes will not increase invertebrate species richness was accepted, and hypothesis 3B was rejected ($R^2 = 0.2538$, $df = 11$, $p = 0.253$). In addition, this test was also not influenced by the three random variables.

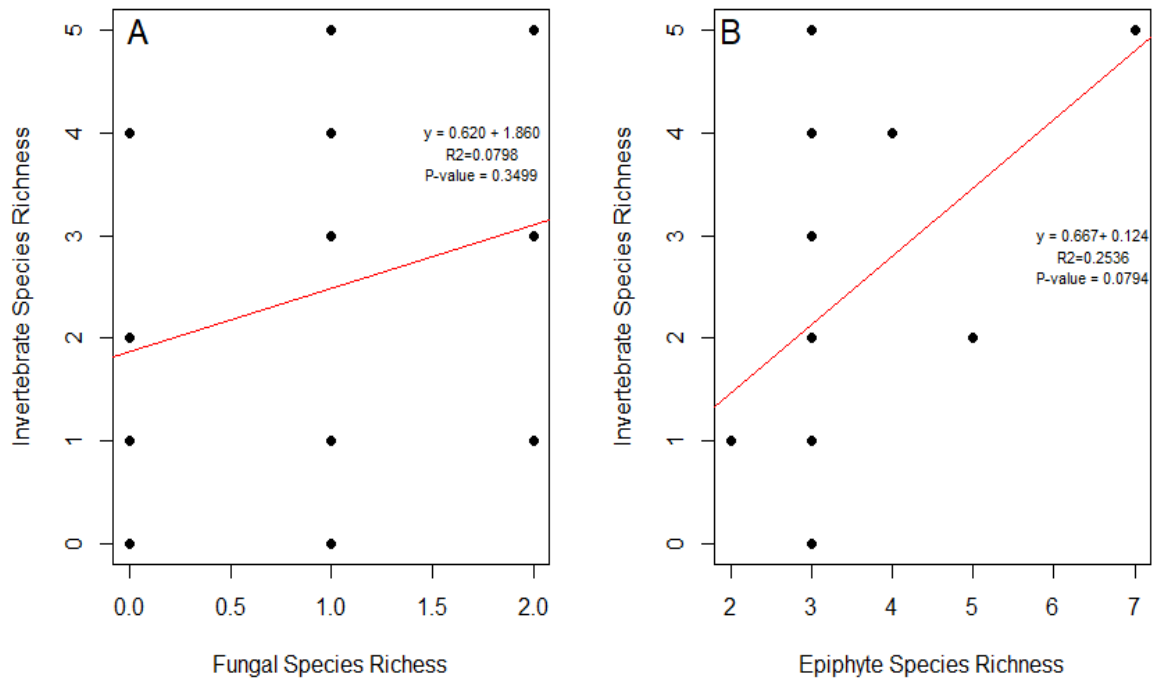


Figure 10: The effect of fungal and epiphyte diversity on the invertebrate species richness across the thirteen recorded trees. A: Weak positive relationship between fungal species richness and invertebrate species richness. B: Weak positive relationship between epiphyte species richness and invertebrate species richness.

Influence of tree size (diameter at breast height) on the number of microhabitats

A linear mixed model was also conducted to assess the relationship between the diameter at breast height on the number of microhabitats. By analysing Figure 11, there is a strong relationship between the two variables accounting for 68% of the variation. This test also proved to be statistically significant; therefore, the null hypothesis stating that the diameter of breast height does not positively influence the number of microhabitats was rejected, and instead, hypothesis 4 was accepted ($R^2 = 0.683$, $df = 8$, $p = 0.0009$). Random variables were also found to impact the results, with tree species accounting for 27% of the leftover variation and location accounting for 13%.

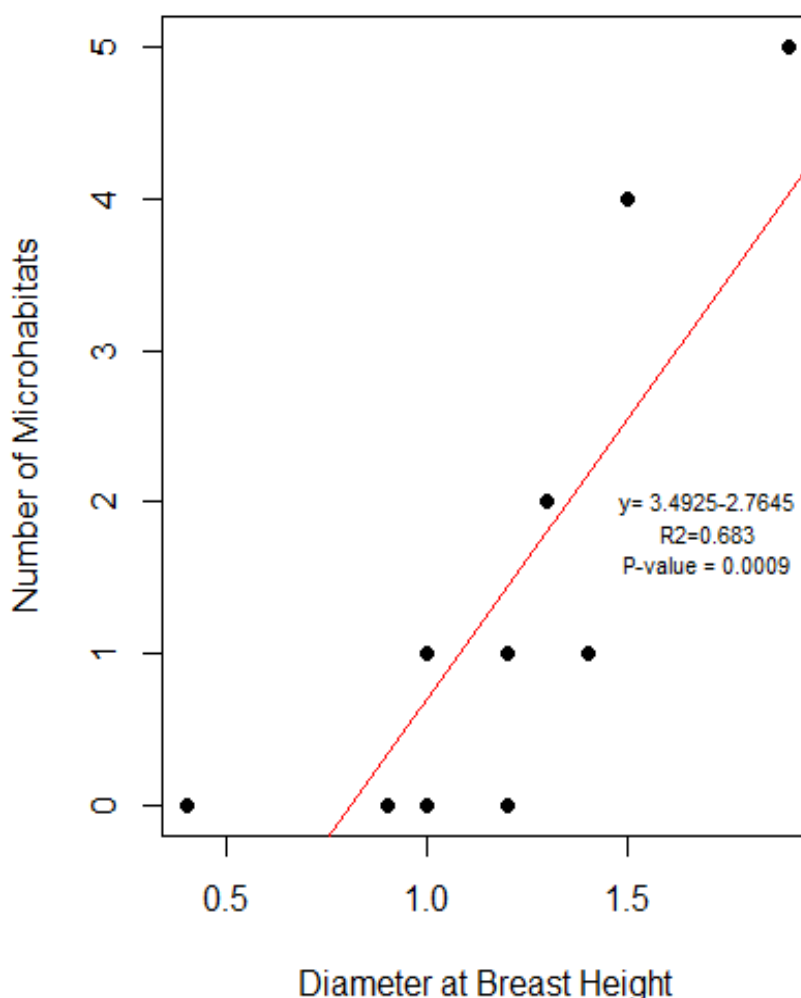


Figure 11: A linear regression model showing that the diameter at breast height has a significant effect on the number of microhabitats.

Discussion

In alignment with other previous studies conducted by Ranius and Jansson, 2008 and Pilskog et al. 2016, there was a higher abundance of tree cavities associated with the larger trees (Figure 11). The two trees present within this investigation with a diameter of 1.9m and 1.5m had a reported four and five cavities between them, significantly higher than the smaller veteran trees with an average of one to two cavities. These results match the common understanding that tree size, which is related to age, significantly impacts the abundance of microhabitats, with the ancient pedunculate oak tree in this study having the highest presence of cavities (Pilskog et al., 2016). A previous investigation into the formation of cavities in pedunculate oaks provides further evidence to these claims, with every recorded tree over 400 years old showing signs of cavities, in comparison to 50% within trees aged 200-300 and 1% in trees under 100 years (Ranius et al., 2009). With larger trees generally being

older, ancient trees offer more time for cavity formation, which is a slow natural process (Sebek *et al.*, 2013; Whitford and Williams, 2002).

Like previous studies, the tree size did impact the diversity and species composition of fungi colonizing the tree trunk (Figure 8B). Due to this investigation being conducted in the winter, many of the fruiting bodies and mycelia were not visible on the tree trunk, making it challenging to assess species composition and diameter preferences between veteran and mature trees (Ranius *et al.*, 2000). However, studies looking at diameter preferences by Norden *et al.* 2004 indicate that ascomycetes tend to prefer smaller trees, with 75% of the recorded species being found exclusively on those substrates and only 2% preferring to colonize larger trees. The 2% of fungi likely consist of heart-rot fungal species like the candle-snuff fungus (*Xylaria hypoxylon*), a wood-inhabiting fungal species found inhabiting the ancient tree within this study. Although the relative importance of diameter preferences for the candle-snuff fungus and other saproxylic species is uncertain (Stokland *et al.*, 2012), it is believed that the larger, ancient trees benefit these species through an increased colonization period and by being less susceptible to droughts, retaining more stable levels of moisture compared to the smaller trees (Ranius *et al.*, 2008; Ranius *et al.*, 2009; Heilmann- Clausen and Christensen, 2004).

In addition, ancient trees are likely to have more pathways or exposures to the heartwood due to their extreme age, which is essential for heart-rot fungi colonization (Ranius *et al.*, 2008). Uncertainties on the relative importance also derive from the variations within basidiomycetes, where Norden *et al.* 2004 indicates that basidiomycetes are distributed evenly between small and large diameter units. Basidiomycetes species like the common bonnet mushroom (*Mycena galericulata*) could be more affected by other tree characteristics, such as tree host species or the presence of moss. This is because common bonnet mushrooms were found exclusively colonizing the trunks of pedunculate oaks within this study. Still, with the limited sample size, this investigation cannot say with certainty.

It was also observed that the number of microhabitats positively influences the species richness of fungi (Figure 8A). Although there are currently limited studies on the relationship between fungi and tree cavities (Jankowiak *et al.*, 2019), tree cavities are likely important features for wood-inhabiting fungal development (Jusino *et al.*, 2015). These microhabitats are particularly beneficial for saproxylic species, with the cavities and wood mould providing a stable microclimate and a continuous supply of deadwood for fungi (Sebek *et al.*, 2013). It is expected for fungal communities to significantly differ between veteran, ancient and mature trees due to the variations in gradients, abiotic factors and availability of deadwood and size (Sebek *et al.*, 2013; Ranius *et al.*, 2009; Stokland *et al.*, 2012). Despite this, no evidence in the ordination (Figure 6) suggests that fungal communities were affected by the host tree's characteristics. This is likely due to the limitations within this investigation regarding the sampling period, lack of visible fruiting bodies, small sampling sizes and issues with the identification (Stokland *et al.*, 2012).

Regarding epiphyte species richness and tree size, there is a weak relationship between the two, with results from the linear model suggesting that epiphyte species richness increases with the diameter at breast height (Figure 9B). However, these results are heavily affected by the ancient tree, an outlier with an epiphyte species richness score of seven, compared to the mean of three. As discovered in previous studies by Ódor *et al.* 2006 and Ranius *et al.* 2008, epiphyte communities are more

diverse on the larger ancient tree. This is due to an increased colonisation period and an increased volume of the woody substrate providing more space and resources for competing species (Flores- Palacios and Garcia-Franco; Ranius *et al.*, 2008). The variation within this linear model is also highly affected by the three random categorical variables accounting for 89% of the total variation caused by the diameter. Although most lichen species seem to be present on many trees, the composition and species richness of epiphytic bryophytes is heavily affected by the variations in the locations, tree host species and type, which would provide different climate conditions impacting epiphyte colonisation.

For the veteran and ancient trees, size could be more critical for epiphytes in terms of its influence on the number of microhabitats, with this investigation discovering that epiphyte species richness is higher on trees with a more significant presence of tree cavities (Figure 9A). Like fungi, many epiphyte species likely colonize inside the tree hollows of veteran and ancient trees due to the stable levels of temperature, moisture, and pH as well as the enriched levels of nutrients provided by the wood mould (Schauer *et al.*, 2017; Ceballos *et al.*, 2016; Kirby and Watkins, 2015). In addition, a study by (Tatsumi *et al.*, 2017) suggests that the tree cavities increase invertebrate species richness, promoting ecological interactions like breeding, pruning and defecation between the two groups, which can indirectly affect epiphytes. However, as previously mentioned, this test was likely impacted by the ancient tree outlier, supporting a much more extensive range of epiphytes due to the diverse range of abiotic conditions that the veteran or mature trees cannot fully replicate (Zhao *et al.*, 2015; Ancient Tree Forum, 2023). Furthermore, studies indicate that ancient trees also support unique communities of epiphytes with their increased number, diversity, and complexity of tree cavities, increasing habitat heterogeneity (Woods *et al.*, 2015). However, despite the research from past studies, no evidence was observed regarding the impact of microhabitats and tree size on epiphyte communities. This could result from only having thirteen recorded trees, many of which share a similar size or number of microhabitats, making it challenging to compare epiphyte species richness and composition between tree types. In addition, most of the recorded species are predominantly made up of lichen species that colonized most trees present in this investigation.

According to the results in Figure 7B, tree size has no significant effect on the invertebrate species richness. This conclusion is contrary compared to multiple studies, suggesting that beetle species richness is higher for larger trees (Pilskog *et al.*, 2016; Ranius and Jansson, 2000). One explanation could be that the increased surface area would promote the co-existence of competing species (Stokland *et al.*, 2012). However, Pilskog also discovered that tree size indirectly influenced the species richness of beetles by increasing the abundance and quality of tree cavities (Pilskog *et al.*, 2016). There are two outliers within this investigation, the first being the ancient tree with a species richness of five. As previously mentioned, this is expected, with ancient trees offering a more stable, heterogenous environment for invertebrates compared to the smaller trees due to their structurally more complex cavities (Pilskog *et al.*, 2016; Wetherbee *et al.*, 2022; Campos *et al.*, 2006). The other outlier with a species richness value of five was a small mature common beech tree with a diameter of 0.9m. This is also likely a result of the limitations within this investigation, with pitfall traps only partially representative of the tree (Leather, 2005). There should be some careful consideration before using this data since it is

likely that pitfall traps were influenced by other factors that have not been accounted for within this investigation.

Although previous studies have found that the number and quality of tree cavities positively influence the richness of beetles (Pilskog *et al.*, 2016), no evidence observed suggested that the number of microhabitats significantly impacted the invertebrate diversity within this investigation (Figure 7A). This was unexpected since microhabitats such as tree hollows are regarded as keystone vegetation features (Remm and Löhmus, 2011), providing specialised communities of invertebrates with stable temperatures, moisture, and pH levels (Müller *et al.*, 2014; Sebek *et al.*, 2013). In addition, wood mould within these cavities will likely benefit invertebrates acting as the predominant substrate and contributing towards a nitrogen-rich environment and stable microclimate (Schauer *et al.*, 2017; Kirby and Watkins, 2015). Previous studies have also shown cavity mould as biodiversity hotspots, supporting not only obligative groups, including species of beetles (Coleoptera) and flies (Diptera), but also facultative species such as spiders (Araneae), springtails (Collembola), mites (Acari) and millipedes (Diplopoda) groups that were all present within this study (Cuff *et al.*, 2020; Kirby and Watkins, 2015; Stokland *et al.*, 2012). Even though it was expected that tree type and microhabitats would increase invertebrate species richness, the results were likely a result of the limitations within this investigation. With this investigation being conducted during the winter, this data is very likely underestimating the value of these microhabitats compared to other studies. These limitations, alongside the low sample size and variations of tree size and microhabitats within this study, make it difficult to compare the relative importance of tree characteristics for invertebrates between the mature, veteran, and ancient trees. This could explain the lack of evidence suggesting that these characteristics between the two tree types have impacted the species composition for invertebrates, despite research suggesting that veteran and ancient trees provide resources not available in mature trees (Wetherbee *et al.*, 2020; Müller *et al.*, 2014).

Results indicate that the presence of fungi does not positively influence the species richness of invertebrates within this investigation (Figure 10A). These results were unexpected, with previous studies acknowledging that the presence of fungi, especially in larger trees, improved the overall availability, quality and diversity of resources utilized by saproxylic beetles (Ranius *et al.*, 2000). It is currently believed that these beetles will visit the perennial fruiting bodies often known as sporocarps when they're sporulating to breed, locate potential prey and feed (Hagvar, 1999). However, no relationship between fungi and invertebrate species richness was found, likely due to the limited number of visible fruiting bodies recorded in this study. Although the relationship between fungi and facultative species is limited, it is still likely that these conditions created by the veteran and ancient trees will benefit other invertebrate groups, including springtails (Collembola) and mites (Acari). Evidence has been seen in previous studies (Graves, 1960) showing that species-rich assemblages of mites and springtails, which are secondary fungivores, occur in all stages of development within sporocarps. Although abundance was not accounted for within this investigation, it was still noted that the presence of springtails was high for the ancient tree, as stated by (Graves, 1960) and in these species are dominant in wet rotting environments, which is provided by the larger ancient trees which can retain moisture more effectively (Ranius *et al.*, 2008; Heilmann- Clausen and Christensen, 2004).

In this investigation, no evidence was provided in (Figure 10B) that a more extensive diversity of epiphyte species increased the species richness for invertebrates. Unlike fungi, interactions between epiphytes and invertebrates seem minor in terrestrial environments. However, it is challenging to say with certainty because a limited number of studies focused on the relationship between invertebrates and epiphytic bryophytes. As previously mentioned by Tatsumi *et al.*, 2017, they found that invertebrates interact with epiphytes through breeding, pruning and defecation, which would benefit both groups. Although there is limited evidence between epiphytes and invertebrate species richness, some studies suggest that the genetic structure of epiphyte communities could influence the overall species community through bottom-up effects. For example, plant genotypes can influence predation and mutualistic interactions impacting herbivorous invertebrates like aphids. However, the relative importance is unknown (Lamit *et al.*, 2015; Johnson, 2008).

Conclusion for conservation

The value of hollow trees for the invertebrate species cannot be disputed; however, with these habitats experiencing a sharp decline due to past management and their short lifespan, management should focus on maintaining and generating new veteran and ancient trees either through natural regeneration or planting and monitoring (Woodland Trust, 2009; Ranius *et al.*, 2009). However, with the formation of tree hollows taking a long time, it is recommended that artificial habitats that mimic the environmental conditions of a tree cavity using the wood mould and wooden boxes are utilized. These habitats have successfully supported endangered populations; however, these solutions are short-term and can only hold small populations at a time (Jansson, 2009; Sebek *et al.*, 2013). With these practices either taking a long time or not being able to support the same population levels as our current veteran and ancient trees, current silvicultural practices like coppicing should be reconsidered since it has been shown to reduce the availability of deadwood and cavities for fungi, epiphytes, and invertebrates (Kirby and Watkins, 2015; Sebek *et al.*, 2013).

Limitations of study

During winter, many invertebrate species go into diapause and are likely to spend their time inside the tree (Royal Park, 2020). With this investigation taking a non-destructive approach making it difficult to study the inactive invertebrates, the data regarding the importance of tree type and microhabitats on invertebrate species richness will likely be underestimated. As a result, hand-searching proved ineffective in showing significant differences between veteran and mature trees, with most species, predominantly spiders, colonising both trees consistently. Although the invertebrate species richness score and composition were similar between the two types of trees, it was discovered in the field that species likely preferred veteran trees over mature trees, with veteran trees containing a higher abundance of spiders, many of which utilised the tree cavities (Corral *et al.*, 2021).

Although the pitfall traps and hand searches were successful in capturing many of the important epigeal invertebrates, like the Carabidae beetles that are active on the soil surface around the selected trees, they were limited in their ability to quantitatively sample flighted species of invertebrates that benefit from these trees like the group Diptera. In addition, with the pitfall traps placed underneath the tree canopy rather than inside the tree cavities, species collected from the pitfall traps may not be associated with the microhabitats (Kirby and Watkins, 2015; Leather,

2005). Finally, with pitfall traps only being collected once after 48 hours during December, insect activity is lower, meaning the data is very likely underestimated and will only partially portray the value of these habitats on the invertebrate species.

Due to the complexity of identifying specific groups of invertebrates, fungi and lichen, there is potential for misidentification. In addition, the differing levels of taxonomic classification and lack of saproxylic and epixylic groups within this study make it difficult to assess how the characteristics and habitats associated with veteran and ancient trees influence the species richness and composition of these obligative groups compared to the mature trees.

This investigation aimed to assess the impact of management techniques such as coppicing and pollarding on the tree's characteristics and species richness. However, with the planning permission for Saltram not being finalised, this investigation could not analyse the relative importance of management techniques such as coppicing and pollarding on species richness and community structure due to insufficient data. According to the Ancient Tree Inventory, Saltram is abundant in veteran and ancient trees, which would have significantly increased the sample sizes of variables like invertebrate species richness and tree form, which were less than desirable (Woodland Trust, 2023).

Many fungal and invertebrate species associated with veteran and ancient trees were not visible at a surface level during the winter (Ranius and Jansson, 2000). As a result, data analysis on the species richness for these groups is likely to be underestimated and will not accurately represent the relationship of tree characteristics on these species.

Conclusion

This study has contributed towards the current understanding that older larger trees, measured as the diameter at breast height, provide a wider range of microhabitats in the form of tree cavities, essential for a variety of epiphyte, fungal and invertebrate species. This relationship was observed in the report, with tree size accounting for 68% of the variation in microhabitats between the recorded trees. Although tree characteristics seem important for the colonization of epiphytes and fungal species, no evidence was observed suggesting that invertebrate species richness or composition is significantly affected by the tree size or the number of microhabitats. This is due to the statistical analysis proving non-significant, despite the veteran and ancient trees hosting a higher diversity of invertebrates compared to the mature trees. Therefore, this investigation indicates that further research is still required to determine the relative importance of these characteristics, especially for the less observed taxa such as spiders, springtails and mites using species data. This data should be collected using various sampling methods, including pitfall traps buried within the tree cavity wood mould and planted malaise traps outside the cavities to account for flighted and ground-dwelling invertebrates (Kirby and Watkins, 2015). Sampling should be conducted over a two-three-month period during the warmer months, when insects are not in diapause and activity is highest to get sufficient species richness data. This would allow the investigation to fully analyse whether the presence of tree cavities increases the overall species richness. Although not accounted for, empirical evidence suggests that abundance should be incorporated into further studies assessing the importance of veteran and ancient trees for invertebrates, especially during the winter months, which need to be studied more.

Further research is also required on the impact of forest management regimes like coppicing and pollarding to assess their impact on the availability of dead-wood habitats for species. These studies could shape future woodland management techniques to preserve and create veteran and ancient tree habitats necessary to sustain threatened endemic populations of invertebrates.

Future Research

Data collection was not supposed to occur at the end of November; however, this was due to complications with planning permission and the formation of a non-destructive invertebrate sampling method. Invertebrate sampling could be performed over two to three months between September and early November, when invertebrate activity will be the most active on the tree trunk, to better represent the importance of veteran trees and their characteristics. With the selected trees being deciduous, leaf litter will also likely contribute to the wood mould within the cavity, meaning the investigation will accurately assess the value of the tree cavities for the studied facultative species, which are currently understudied (Corral *et al.*, 2021). Pitfall traps would also be buried within the wood mould inside the cavities, making the traps more representative in attempting to quantify the relationship between tree cavities and invertebrate species richness. Finally, as recommended by (Kirby and Watkins, 2015), malaise traps would be placed outside of the cavity, capturing, and measuring the activity of flighted species such as Diptera and other wood-boring insects, allowing this investigation to account for a more extensive range of invertebrate species (Leather, 2005; Lamarre *et al.*, 2012). With these methods likely to improve the sampling size and robustness of the invertebrate richness data, the relationship between invertebrate richness and tree distance can be undertaken to account for the variations in habitat connectivity, which was initially planned for this investigation. There is still a gap in literature on the importance of these characteristics on invertebrates during the winter period, however as shown by this investigation, measuring species richness is unlikely to provide any significant data. Future studies conducted during the winter should potentially focus on quantifying species abundance rather than species richness. Future sampling would also be conducted in managed woodlands like Saltram to collect sufficient data on the importance of management techniques like coppicing and pollarding to analyse how management impacts tree size and habitat availability, which would affect species richness and composition.

Acknowledgments

The completion of this investigation would not be possible without the expertise and guidance of my project advisor Dr Sophie Fauset. I would also like to thank Richard Hartley for providing me with the equipment necessary for my study and supporting me with the Trimble® Geo 7X Global Navigation Satellite System. A debt of gratitude is also owed to the specialist Jane Akerman for providing me with the skills and lab induction required for identifying invertebrates.

I would also like to thank my friends: Henry Leonard, Izzy Haines, Katie Etherington and Alex Sutton, for their support and encouragement in the library.

Finally, I would like to give thanks to my family for their help, encouragement, and interest in my academic achievements.

References

- An Australian Government Initiative (2012) Two Major Groups. Available at: <https://www.anbg.gov.au/fungi/two-groups.html> (Accessed: 25 April 2023).
- Ancient Tree Forum (2017) Valuing and recording. Available at: <https://www.ancienttreeforum.org.uk/ancient-trees/protecting-ancient-and-veteran-trees/value-and-record-ancient-and-veteran-trees/#:~:text=The%20Ancient%20Tree%20Inventory%20is,cherishing%20and%20caring%20for%20them.> (Accessed: 31 March 2023).
- Ancient Tree Forum (2020) Veteran Trees Initiative. Available at: https://www.ancienttreeforum.co.uk/wp-content/uploads/2020/06/SSM_HandBook.pdf (Accessed: 21 April 2023).
- Ancient Tree Forum (2023) Epiphytes and ancient trees. Available at: <https://www.ancienttreeforum.org.uk/ancient-trees/ancient-tree-ecology-wildlife/epiphytes/> (Accessed: 16 April 2023).
- Belcik, M., Goczal, J. and Ciach, M. (2018) 'Large- scale habitat model reveals a key role of large trees and protected areas in the metapopulation survival of the saproxylic specialist *Cucujus cinnaberinus*', *Biodiversity and Conservation*, 28, 3851-3871.
- British Geological Survey (2023) Geology Viewer Available at: https://geologyviewer.bgs.ac.uk/?_ga=2.1015670.619683194.1675959525-25818539.1675959525 (Accessed: 9 February 2023).
- Buczacki, S., Shields, C. and Ovenden, D. (2012) *Collins Fungi Guide: The most complete field guide to the mushrooms and toadstools of Britain & Ireland*. 1st edn. HarperCollins Publishers, London, UK.
- Campos, R., Vasconcelos, H., Ribeiro, S., Neves, F. and Soares, J. (2006) 'Relationship between tree size and insect assemblages associated with *Anadenanthera macrocarpa*', *Ecography*, 29, 442-450.
- Ceballos, S., Chacoff, N. and Malizia, A. (2016) 'Interaction network of vascular epiphytes and trees in a subtropical forest', *Acta Oecologica*, 77, 152-159.
- Coding Club (n.d.) Introduction to Ordination. Available at: <https://ourcodingclub.github.io/tutorials/ordination/> (Accessed: 10 April 2023).
- Corral, J.H., López, A.G., Ferrández, M.G., Ángel, M. and Estefanía. (2021) 'Physical and biotic factors driving the diversity of spider assemblages in tree hollows of Mediterranean *Quercus* forests', *Insect Conservation and Diversity*, 14, 515-526.
- Cuff, J., Müller, C., Gilmartin, E., Boddy, L. and Jones, T. (2020) 'Home is where the heart rot is: violet click beetle, *Limoniscus violaceus* (Müller, 1821), habitat attributes and volatiles', *Insect Conservation and Diversity*, 14, 155-162.
- Flores-Palacios, A. and García-Franco, J.G. (2006) 'The relationship between tree size and epiphyte species richness: testing four different hypotheses', *Journal of Biogeography*, 33, 323-330.

- Gibbons, P., Lindenmayer, D.B., Fischer, J., Manning, A.D., Weinberg, A., Seddon, J., Ryan, P. and Barret, G. (2008) 'The Future of Scattered Trees in Agricultural Landscapes', *Conservation Biology*, 22, 1309-1319.
- Graves, R.C. (1960). 'Ecological observations of the insects and other inhabitants of woody shelf fungi (Basidiomycetes: Polyporaceae) in the Chicago area', *Annals of the Entomological Society of America*, 53, 61-78.
- Hagvar, S. (1999) 'Saproxylic beetles visiting living sporocarps of *Fomitopsis pinicola* and *Fomes fomentarius*', *Norwegian Journal of Entomology*, 46, 25-32.
- Hajduk, G. (2022) *Introduction to Linear Mixed Models*. Available at: <https://ourcodingclub.github.io/tutorials/mixed-models/> (Accessed: 4 March 2023).
- Heilmann-Clausen, J. and Christensen, M (2004) 'Does size matter?: On the importance of various dead wood fractions for fungal diversity in Danish beech forests', *Forest Ecology and Management*, 201, 105-117.
- Henttonen, H.M., Nojd, P., Suvanto, S., Heikkinen, J. and Mäkinen, H. (2019) 'Large trees have increased greatly in Finland during 1921-2013, but recent observations on old trees tell a different story', *Ecological Indicators*, 99, 118-129.
- Jacobsen, R., Birkemoe, T., Evju, M, Skarpass, O. and Sverdrup-Thygeson, A. (2023) 'Veteran trees in decline: Stratified national monitoring of oaks in Norway', *Forest ecology and management*, 527, p.120624.
- Jankowiak, R., Ciach, M., Bilański, P. and Linnakoski, R (2019) 'Diversity of wood-inhabiting fungi in woodpecker nest cavities in southern Poland', *Acta Mycologica*, 54, [no pagination].
- Johnson, M.T.J. (2008) 'Bottom-Up Effects of Plant Genotype on Aphids, Ants and Predators', *Ecology | Ecological Society of America*, 89, 145-154.
- Jusino, M.A, Lindner, D.L, Banik, M.T. and Walters, J.R. (2015) 'Heart rot hotel: fungal communities in red-cockaded woodpecker excavations', *Fungal Ecology*, 14, 33-43.
- Kirby, K.J. and Watkins, C. (2015) *Europe's changing woods and forests: from wildwood to managed landscapes*. Edition: 1. Croydon: CABI.
- Lamarre, G.P., Molto, Q., Fine, P.V. and Baraloto, C. (2012) 'A comparison of two common flight interception traps to survey tropical arthropods', *ZooKeys*, 216, 43-45.
- Lamit, L.J., Busby, P.E., Lau, M.K., Compson, Z.G., Wojtowicz, T., Keith, A.R., Zinkgraf, M.S., Schweitzer, J.A., Shuster, S.M., Gehring, C.A. and Whitham, T.G. (2015) 'Tree genotype mediates covariance among communities from microbes to lichens and arthropods', 103, 840-850.
- Lassauce, A., Paillet, Y., Jactel, H. and Bouget, C. (2011) 'Deadwood as a surrogate for forest biodiversity: Meta-analysis of correlations between deadwood volume and species richness of saproxylic organisms', *Ecological Indicators*, 11, 1027-1039.

Leather, S. (2005) *Insect Sampling in Forest Ecosystems*. 1st edn. Oxford: Blackwell Publishing.

Magrath, M. (2018) 2018 Veteran and Ancient Tree Survey Sheep's Green and Coe Fen, Cambridge. Available at: <https://www.cambridge.gov.uk/media/7446/180831-12-sg-veteran-tree-report-reduced.pdf> (Accessed: 13 February 2023).

Müller, J., Müller, J., Bussler, H. and Gossner, M. (2014) 'Hollow beech trees identified as keystone structures for saproxylic beetles by analyses of functional and phylogenetic diversity', *Animal conservation*, 17, 154-162.

Nieto, A. and Alexander, K.N.A. (2010) *European Red List of Saproxylic Beetles*. Luxembourg: Publications Office of the European Union.

Nolan, V., Reader, T., Gilbert, F. and Atkinson, N. (2020) 'The Ancient Tree Inventory: a summary of the results of a 15 year citizen science project recording ancient, veteran and notable trees across the UK', *Biodiversity and Conservation*, 29(11-12), 3103-3129.

Norden, B., Ryberg, M., Gotmark, F. and Olausson, B. (2004) 'Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests', *Biological Conservation*, 117, 1-10.

Ódor, P., Heilmann-Clausen, J., Christensen, M., Aude, E., van Dort, K.W., Piltaver, A., Siller, I., Veerkamp, M.T., Walley, R., Standovár, T., van Hees, A.F.M., Kosec, J., Matočec, N., Kraigher, H. and Grebenc, T. (2006) 'Diversity of dead wood inhabiting fungi and bryophytes in semi-natural', *Biological Conservation*, 131, 58-71.

PictureThis (n.d.) *Plant Identifier App | Plant Identification Online*. Available at: <https://www.picturethisai.com/> (Accessed: 1 April 2023).

Pilskog, H.E., Birkemoe, T., Framstad, E. and Sverdrup-Thygeson, A. (2016) 'Effect of Habitat Size, Quality, and Isolation on Functional Groups of Beetles in Hollow Oaks', *Journal of Insect Science*, 16, 26.

Plymouth City Council (n.d.a) *Beaumont Park*. Available at: <https://www.plymouth.gov.uk/beaumont-park> (Accessed: 20 April 2023).

Plymouth City Council (n.d.b) *Ham Woods (LNR)*. Available at: <https://www.plymouth.gov.uk/ham-woods-?Inr#:~:text=We%20own%20and%20manage%20the,enjoy%20and%20learn%20about%20nature>. (Accessed: 9 February 2023).

Ranius, T. and Jansson, N. (2000) 'The influence of forest regrowth, original canopy cover and tree size on saproxylic beetles associated with old oaks', *Biological Conservation*, 95, 85-94.

Ranius, T., Johansson, P., Berg, N. and Niklasson, M. (2008) 'The influence of tree age and microhabitat quality on the occurrence of crustose lichens associated with old oaks', *Journal of vegetation science*, 19, 653-662.

Ranius, T., Niklasson, M. and Berg, N (2009) 'Development of tree hollows in pedunculate oak (*Quercus robur*)', *Forest Ecology and Management*, 257, 303-310.

Remm, J. and Lõhmus, A. (2011) 'Tree cavities in forests- The broad distribution pattern of a keystone structure for biodiversity', *Forest Ecology and Management*, 262, 579-585.

Schauer, B., Steinbauer, M.J., Vailshery, L.S., Müller, J., Feldhaar, H. and Obermaier, E. (2017) 'Influence of tree hollow characteristics on saproxylic beetle diversity in a managed forest ', *Biodiversity Conservation*, 27, 853-869.

Sebek, P., Altman, J., Platek, M. and Cizek, L. (2013) 'Is Active Management the Key to the Conservation of Saproxylic Biodiversity? Pollarding Promotes the Formation of Tree Hollows', *PLOS | One*, 8, e60456.

Stokland, J.N., Siitonen, J. and Jonsson, B.G. (2012) *Biodiversity in Dead Wood*. 1st edn. New York: Cambridge University Press.

Tatsumi, S., Ohgue, T., Azuma, W., Tuovinen, V., Imada, Y., Mori, A.S., Thor, G. and Ranlund, A (2017) 'Tree hollows can affect epiphyte species composition', *Ecological Research*, 32, 503-509.

The Royal Parks (2020) Where do invertebrates go in winter. Available at: [https://www.royalparks.org.uk/whats-on/blog/where-do-invertebrates-go-in-winter#:~:text=While%20most%20invertebrates%20don't,state%20known%20as%20'torpor'](https://www.royalparks.org.uk/whats-on/blog/where-do-invertebrates-go-in-winter#:~:text=While%20most%20invertebrates%20don't,state%20known%20as%20'torpor'.). (Accessed: 24 April 2023).

Thygeson, S., Skarpaas, A., Blumentrath, O., Birkemoe, S. and Evju, T. (2017) 'Habitat connectivity affects specialist species richness more than generalists in veteran trees', *Forest ecology and management* 403, 96-102.

Webster, C.R. and Jenkins, M.A. (2005) 'Coarse woody debris dynamics in the southern Appalachians as affected by topographic position and anthropogenic disturbance history', *Forest Ecology and Management*, 217, 319-330.

Wetherbee, R., Birkemoe, T., Asplund, J., Renčo, M. and Thygeson, A. (2022) 'It takes a community to maintain a tree hollow: Food web complexity enhances decomposition and wood mould production', *Functional Ecology*, 36, 2215-2226.

Whitford, K.R. and Williams, M.R. (2002) 'Hollows in jarrah (*Eucalyptus marginata*) and marri (*Corymbialcalophylla*) trees: II. Selecting trees to retain for hollow dependent fauna', *Forest ecology and management*, 160, 215-232.

Woodland Trust (2008) What are ancient, veteran and other trees of special interest? Available at: <https://www.woodlandtrust.org.uk/media/1836/what-are-ancient-trees.pdf> (Accessed: 15 April 2023).

Woodland Trust (2009) Ancient trees for the future. Available at: <https://www.woodlandtrust.org.uk/media/4727/ancient-trees-for-the-future.pdf> (Accessed: 20 April 2023).

Woodland Trust (2023) Ancient Tree Inventory. Available at: <https://ati.woodlandtrust.org.uk/tree-search/?v=2223719&ml=map&z=13&nwLat=50.396078582327746&nwLng=->

4.241824424072256&seLat=50.35590071075028&seLng=-4.0313675759277245
(Accessed: 24 April 2023).

Woods, C.L., Cardelus, C. and DeWalt, S.J. (2015) 'Microhabitat associations of vascular epiphytes in a wet tropical forest canopy', *Journal of Ecology*, 103, 421-430.

Zhao, M., Geekiyanage, N., Xu, J., Khin, M.M., Nurdiana, D.R., Paudel, E. and Harrison, R.D. (2015) 'Structure of the Epiphyte Community in a Tropical Montane Forest in SW China', *PloS one*, 4, p. e0122210.

Appendices are provided separately as supplementary files (see additional downloads for this article).