

Forest age drives saproxylic beetle biodiversity in the southeastern United States

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ABSTRACT

Old forests are valuable for biodiversity conservation because they provide uninterrupted forest cover over time and late-successional habitat, promoting low-mobility and late-successional species. However, forest age may be less important in highly forested landscapes because dispersal limitation is reduced. We investigated how species richness and community composition of saproxylic beetles (Coleoptera) are influenced by forest age and the surrounding landscape in the southeastern United States (Georgia). Forests in this region are highly fragmented and dominated by early-successional stands. We sampled beetles in 20 old forests (mature in 1938) and 20 young forests (originating after 1938), located along a landscape forest cover (LFC) gradient. Old and young forests were structurally similar but tree communities differed along a *Quercus* (old) to *Pinus* (young) gradient. Regional diversity of saproxylic beetles was higher in old forests, and high species turnover between forest age groups suggests they both help to maintain regional biodiversity. Two models of local richness had near equal merit: one showed higher richness in old forests regardless of LFC, while the other showed higher richness in old forests at high LFC. Contrary to expectations, neither model supports old forests losing importance in highly forested landscapes. Higher richness in old forests is likely due to temporal stability and late-successional habitat, promoting low-mobility and specialist species. Because the land area of young forests exceeds that of old forests, old forests are disproportionately important for saproxylic biodiversity in the region.

1. Introduction

The southeastern United States has seen dramatic changes in forest cover and quality over the past 400 years. Forests were initially cleared by European settlers wherever agriculture was possible, but unsustainable farming made it necessary to constantly clear new lands, eventually even those with regrown forests (Oosting, 1942). By the 1930s, the region reached its lowest amount of forest ever recorded (Smith et al., 2004) and virtually all of the remaining forest had been anthropogenically altered (Oosting, 1942; Trani, 2002). Since the 1930s, region-wide agricultural abandonment occurred and forest cover increased as fields underwent natural succession (Nicholson and Monk, 1974; Ramankutty et al., 2010; Sohl et al., 2016). Today, the southeast is dominated by forest stands <100 years old (Conner and Hartsell, 2002) and the region is a leading supplier of timber (Prestemon and Abt, 2002). Despite their prevalence, these young forests may have limited benefit to species requiring uninterrupted forest cover over time (Norden et al., 2014) or late-successional habitat (Gibb et al., 2013). Additionally, in

anticipation of the forested region becoming increasingly urbanized and intensively managed (Wear, 2002; Huggett et al., 2013), studies are needed to help prioritize conservation efforts in the region while accounting for landscape factors.

Saproxylic insects are a valuable group for investigating impacts of forest change on biodiversity (Grove, 2002; Edelmann et al., 2022), but have received limited attention in the southeastern US. Saproxylic species are dependent on deadwood and associated resources for all or part of their lives and are inherently connected to forest dynamics (Stokland et al., 2012; Ulyshen and Šobotník, 2018) and decomposition pathways (Ulyshen, 2016). Between 20 and 30 % of all insect species in forests are saproxylic (Siitonen, 2001; Graf et al., 2022), representing a broad range of functional guilds and life strategies (Stokland et al., 2012). These diverse communities are used as indicators of forest health because of their sensitivity to forest management and change (Grove, 2002; Stenbacka et al., 2010). The amount and diversity of deadwood resources are key drivers of saproxylic biodiversity (Seibold and Thorn, 2018), though effects are mediated by microclimate (Vodka et al., 2009; Lassauce et al.,

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2011).

Many saproxylic species are sensitive to the spatiotemporal availability of deadwood (Sverdrup-Thygeson et al., 2014), which is especially relevant to the changing landscape of the southeastern US. Old forests are valued for conserving saproxylic communities because they provide two distinct habitat qualities: 1) the uninterrupted presence of forest over time (Nordén et al., 2014) and 2) late-successional habitat such as deadwood and tree-related microhabitats (Gibb et al., 2013). These conditions are often related, but one can occur without the other (Cateau et al., 2015). Moreover, they may have separate effects on biodiversity dependent on the surrounding landscape. For example, reliance on uninterrupted forest presence is largely driven by dispersal limitation (Nordén et al., 2014). Thus, in highly fragmented landscapes with low forest cover, forest age is more important for saproxylic richness and community composition than habitat availability (Brin et al., 2016). Conversely, in highly forested landscapes, late-successional habitat availability is more important than age (Janssen et al., 2016, 2017). This suggests that the importance of forest age is mediated by landscape context, as only the best dispersing species are able to colonize young, isolated forests (Irmeler et al., 2010; Brin et al., 2016). If the importance of age is indeed mediated by the landscape, one would expect a large disparity of species richness between old and young forests at low levels of forest cover due to dispersal limitations (e.g., Brin et al., 2016), with the disparity lessening across the gradient to become indistinct at high amounts of forest cover (e.g., Janssen et al., 2016). Additionally, if forest age influences community composition, we would expect communities in young forests to be a subset of communities found in old forests (i.e., a nested pattern, Balsega, 2010; Brin et al., 2016). However, whether nestedness is driven by the landscape remains unclear (e.g., Meyer et al., 2021). Because relative dispersal ability is related to the spatial distribution and longevity of the larval substrate (Feldhaar and Schauer, 2018; Percel et al., 2019), different responses by low- and high-mobility groups could be expected.

Here, we investigate how deadwood and saproxylic beetles are distributed in southeastern US forests varying in age and landscape context. Beetles (Coleoptera) are, by far, the most studied saproxylic insects and are commonly used as a model group because of their taxonomic and ecological diversity (Gimmel and Ferro, 2018; Ulyshen and Šobotník, 2018). Our study is specifically designed to compare

natural forests pre-dating and originating since the agricultural abandonment period (i.e., old and young) across a gradient of landscape forest cover. Because species respond to changing landscapes at various temporal and spatial scales (Sverdrup-Thygeson et al., 2014), we consider historic and current landscapes at several spatial scales. Additionally, we compare responses of functional groups that differ in dispersal ability and trophic level. We specifically ask:

1. How does deadwood in old and young forests differ in amount and composition?
2. How does regional (gamma) diversity of saproxylic beetles differ between old and young forests?
3. How does local richness (alpha diversity) of saproxylic beetles differ between old and young forests, and is the importance of forest age mediated by the landscape?
4. How does species composition (beta diversity) of saproxylic beetles differ with forest age and the landscape, and are there patterns of nestedness?

2. Methods

2.1. Selection of sampling locations and age determination

This study took place in the Piedmont region of the southeastern United States, specifically in Clarke County, Georgia, USA (Fig. 1A). This region has a humid subtropical climate with hot, humid summers and mild winters. Within the Piedmont, temperate forests are the predominant vegetation; young forests are typically dominated by *Pinus* species but are eventually replaced by hardwoods over succession (especially *Quercus* species; Oosting, 1942; Nicholson and Monk, 1974). Forty forested sites were chosen using the methods described in Traylor et al. (2022b) and will be elaborated on here. Sites fell along a gradient of landscape forest cover at multiple spatial extents using data available from the USGS National Landcover Database (Dewitz, 2019). Half of the sites ($n = 20$) were forested in 1938 and remained so since then based on historical aerial imagery maps (i.e., old; ACCUG, 2019) and the other half ($n = 20$) were reforested since 1938 (i.e., young). Age groups were mixed throughout the landscape (Fig. 1A). All sites were visited before sampling to ensure the absence of active timber management, and no

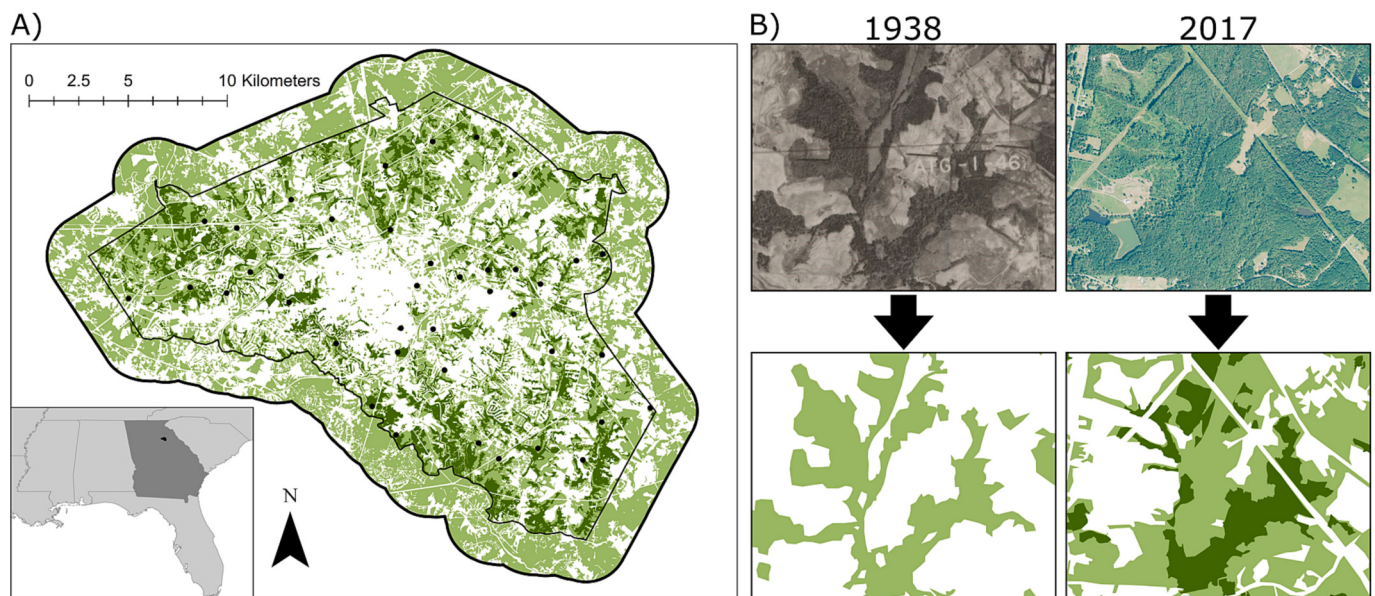


Fig. 1. A) Forest cover and sampling sites in study area (inset shows study area in the southeastern United States). Total forest cover in 2017 is shown in light green, Old forest (forest that was mature in 1938 and has persisted since; ACCUG, 2019) is shown in dark green. Sampling sites are black circles; inner boundary shows Clarke County, Georgia; outer boundary is a 2 km buffer surrounding the county. B) Process for creating forest cover from aerial imagery (dark green in 2017 is forest that has persisted since 1938).

sites are assumed to represent undisturbed, old-growth forests.

2.2. Insect sampling, identification, and saproxylic determination

Insect sampling and forest stand measurement methodology is described in detail in Traylor et al. (2022b). Briefly, at each forest site, a sampling location was chosen randomly and a single flight intercept trap was hung 5 m aboveground to collect insects from March–September 2020. Traps were suspended from branches with rope so that they were not close to trunks of trees or obscured by vegetation, meaning that collected insects are less biased by immediate surroundings (Sverdrup-Thygeson and Birkmoe, 2009). The trap consisted of two clear window panes (30.5 cm tall $\times$ 20.5 cm wide), intersected and tied over a 3.78 L white bucket, and baited with an ethanol lure. This trap design has been used to collect a wide variety of Coleoptera in forests (Ulyshen and Hanula, 2007; Ulyshen et al., 2010), and the ethanol lure increases trapping efficiency for saproxylic beetles in closed canopy forests (Bouget et al., 2009). Trap contents were emptied every two weeks during the sampling period. Additionally, we sifted leaf litter with 1.25 cm mesh to collect 1 L litter samples. These samples were taken at three time points (once in June, July, and August 2022) and insects were extracted using Berlese funnels. While these sampling techniques are unable to exhaustively document all species present (Burner et al., 2022), they are adequate for comparing richness and community composition between multiple sites with equal sampling effort.

We used the definition of saproxylic provided in Stokland et al. (2012) to determine the saproxylic status of each species collected, based on records of observed dependency on deadwood or associated resources from published literature, online resources, or museum specimen labels (references in Supplementary Table S1). All Coleoptera were identified to at least family level, and all saproxylic species were identified to species or (rarely) determined only to morphospecies within a genus. We used Gimmel and Ferro (2018) as a starting guide to distinguish families that contained saproxylic species requiring further identification. We additionally classified species with well-known natural history into three functional groups (fresh wood associated species, decayed wood and polypore fungi associated species, and predators), representing different trophic levels and dispersal abilities (Southwood, 1977; Holt, 1996; Percel et al., 2019). Our species list is limited by the lack of available natural history information for much of the North American beetle fauna. Details on species inclusion and functional group placement can be found in the Supplementary Material. Vouchers were deposited in the University of Georgia Collection of Arthropods (UGCA) located in the Georgia Museum of Natural History.

2.3. Measuring forest and deadwood characteristics

We established a 0.1 ha circular plot centered around the flight intercept trap within which we measured living trees and deadwood pieces >10 cm diameter (full details in Supplementary Material). We then calculated the median tree diameter (cm), maximum tree diameter (cm), basal area (m^2) of each tree genus, and diversity of tree genera (count) for each sampling location. We took hemispherical photos of the canopy from the trap location in order to estimate gap fraction (the proportion of open canopy) following Traylor et al. (2022b). The positioning, genus, decay class (1–5), size class (based on diameter), and volume (m^3) were determined for each deadwood piece (see Supplementary material). The total volume of deadwood and volume of each subcategory were calculated for each site. Subcategories include decay classes 1–5, large deadwood (pieces with one diameter >40 cm), small deadwood (pieces with diameters <40 cm), pooled hardwood (i.e., deciduous angiosperm tree) genera (including unknown hardwoods), and pooled conifer genera. Finally, deadwood diversity was calculated for each sampling location by counting the unique combinations of deadwood pieces according to Siitonen et al. (2000), using positioning, genera, decay class, and size class as characteristics. The DBH of snags

was recorded, and the total basal area (m^2) of snags was calculated for each sampling location.

2.4. Landscape data

All geospatial data preparation was performed in ArcMap version 10.4 (Esri, Redlands, California). Aerial imagery of Clarke County and adjacent areas was downloaded at three time points: 1938 (United States, 1938), 1980 (United States, 1980), and 2017 (US Geological Survey, 2017). The imagery was georeferenced as needed (Supplementary Material). In order to assess the landscape forest cover at each time point, we created polygon layers of all forest cover for the area of Clarke County and 2 km outside of its border in 1938, 1980, 2017 (Fig. 1B). This was done manually by outlining continuous tree cover (i.e., forest) on the imagery at a constant map scale of 1:8000. This scale allowed us to outline forest boundaries without including the details of individual tree canopies, and was kept constant to limit the detail of all time points to the time point with the worst image resolution (1938). We also obtained a dataset showing the forest that was present and has persisted since 1938 (ACCUG, 2019), which we refer to as “Old forest”. Eight concentric circles surrounding each sampling point were created at 250 m increments (250–2000 m) to allow for multiple spatial scales to be investigated. At the larger scales, concentric circles of adjacent sites overlapped. While this is not a violation of independent sampling (Zuckerberg et al., 2020), the largest scale of 2000 m was chosen to retain variability in forest cover. We calculated the proportion of landscape forest cover (= LFC) at each sampling location as the area of forest in a circle (km^2) divided by the total area (km^2) of the circle, hereafter referred to as landscape forest cover. This was done for each spatial scale and time point, including Old forest (Supplemental material).

2.5. Data analysis

2.5.1. Comparisons between old and young forests

All statistical analyses were performed in R version 4.2.0 (R Core Team, 2022). We compared the total deadwood volume, snag basal area, deadwood diversity, living tree diversity, maximum and median tree diameter, gap fraction, and stand basal area between old and young sites using two sample *t*-tests ($\alpha = 0.05$). To investigate how deadwood subcategories might differ between old and young sites, we performed a separate ANOVA for each deadwood subcategory (decay classes 1–5, large vs. small pieces, and hardwood vs. conifer) with an interaction term with forest age. Tukey's HSD was run post hoc to investigate significant differences. All continuous variables (except median tree diameter) were log (or log +1) transformed and count variables were square root transformed to meet normality assumptions. We additionally compared living tree and deadwood composition between age classes by performing separate PERMANOVA analyses using the *adonis* function in the *vegan* package (Oksanen et al., 2022). To do this, the summed basal area of each genus and total volume of each unique deadwood combination (based on positioning, genera, decay class, and size class) were calculated for all sites. Tree genera and deadwood combinations that were present in less than three sites were removed from the dataset, making it necessary to remove one site from the deadwood matrix. The PERMANOVAs were run for 9999 permutations on the dataset of 40 (tree) and 39 (deadwood) sites using a Bray-Curtis distance matrix. Because significant PERMANOVA may be caused by differences in the average distance of an observation to the group median (i.e., differences in dispersion or beta diversity), a significant PERMANOVA was followed with a PERMDISP analysis (function *betadisper*, Oksanen et al., 2022) to check for this. To see which tree species were related to differences between old and young forests, we performed non-metric multidimensional scaling (NMDS) analysis with three dimensions using the *metaMDS* function on a tree genera basal area $\times$ site matrix, followed by multiple regression of the basal area of each tree genera with the NMDS axes (*envfit* function, Oksanen et al., 2022). This

process fits environmental variables onto the NMDS ordination based on their correlation (r^2) with the ordination axes. Significance was assessed with 9999 permutations. Finally, the proportion of forest in the landscape at each time point and at several spatial scales (250 m, 1000 m, 2000 m) was compared between old and young sites using Wilcoxon rank sum tests. These spatial scales were chosen because they represent the smallest, middle, and largest scales used in latter analyses, respectively.

2.5.2. Regional diversity and indicator species analysis of saproxylic beetles

We investigated gamma (regional) diversity of saproxylic beetles by computing sample-based rarefaction curves using the package *iNEXT* (Hsieh et al., 2016). Incidence of each species was calculated for each site, then curves were produced for each age class. Significant differences between ages were determined by non-overlapping 95 % confidence intervals at 20 samples (the total sample number for each age group). This was done for the entire species dataset and separately for each functional group. Additionally, an indicator species analysis was performed to investigate statistical associations of species to forest or landscape conditions. Groups included old forest, young forest, high (>50 %) LFC, low (<50 %) LFC, old + high LFC, old + low LFC, young + high LFC, and young + low LFC. The LFC 2017 (2000 m) variable was used for the groups. This was done using the *multipatt* function in the *indicspecies* package (De Caceres and Legendre, 2009) on a species $\times$ site abundance matrix with a secondary matrix indicating the groups. This package calculates the Indicator Value of each species in reference to site groups (Dufrêne and Legendre, 1997), and significance was tested using 9999 random permutations.

2.5.3. Local diversity of saproxylic beetles

The drivers of alpha (local) species richness were investigated using generalized linear models with a Poisson distribution, or a negative binomial distribution when Poisson models were overdispersed. We first found the spatial scale at which species richness responded to landscape variables (i.e., the “scale of effect”, Holland et al., 2004). This process was done because we could not know a priori which spatial and temporal scales were the most relevant for saproxylic beetle biodiversity. To do this, we calculated Pearson's correlation between species richness and LFC for each time period (1938 and 2017) and at each spatial scale (250 m–2000 m). For each time period, the spatial scale with the largest correlation (either positive or negative) was considered to be the scale of effect and was used in the following process.

In order to investigate the effects of forest age, LFC of multiple time points, and other local habitat factors, we set up a list of four candidate models (Supplementary Table S2). Because of our interest in an interaction between forest age and LFC, the models vary in their inclusion of an interaction term, and only a single LFC was used per model due to high correlations between the landscape variables. LFC 1980 and LFC Old were not included because of very high correlations with both LFC 1938 and LFC 2017 (see Results). Thus, the four models can be considered to test historic and current LFC, with and without an interaction with forest age. All models included local habitat factors: deadwood volume (log +1 transformed), gap fraction (log transformed), and the richness of tree genera (square root transformed). These variables are considered to be local drivers of saproxylic beetle species richness (Seibold and Thorn, 2018; Lettenmaier et al., 2022; Traylor et al., 2022b) and thus are relevant here. Model comparisons were done using AICc from the *MuMIn* package (Bartoń, 2022). Models with the lowest AICc were considered the best, but models with $\Delta\text{AICc} < 2$ were also considered plausible and were reported. Finally, models were also compared to a null model (species richness ~ 1). This procedure was done for the entire species dataset and separately for each functional group. Multicollinearity of the predictor variables and spatial independence of the residuals were checked using variance inflation factors (Faraway, 2016) and Moran's I (Paradis and Schliep, 2019), respectively.

2.5.4. Community composition of saproxylic beetles

The following analyses were performed with a species $\times$ sample (site) matrix. Prior to the analysis, species occurring at fewer than three sites were removed from the dataset and each species' abundance per site was divided by its maximum abundance at any site (i.e., relativization by maximum). The removal of rare species made it necessary to remove one site from the predator matrix. We then calculated Bray-Curtis distance matrices using the *beta.pair.abund* function in the *betapart* package (Baselga et al., 2022). Using these distance matrices, PERMANOVA was used to investigate differences in community composition between old and young forests using the *adonis* function in the *vegan* package (Oksanen et al., 2022). Significant PERMANOVAs were followed with a PERMDISP analysis (function *betadis*, Oksanen et al., 2022) to compare amounts of beta diversity between the age groups.

To investigate which factors are correlated to compositional differences of saproxylic beetles between old and young forests, and to determine if landscape influences are also present, we performed NMDS analyses followed by *env.fit* (following the same method as the tree genera above). The number of axes used was three to balance acceptable levels of stress with ease of interpretation (Supplementary Fig. S1). Here, environmental variables consisted of deadwood volume, gap fraction, and tree diversity variables used in the linear modelling of species richness, the axes from the NMDS of tree basal area, and LFC 1938 and 2017 at the 250 m and 2000 m scales (i.e., the highest and lowest scales for each time point).

Finally, to investigate the relative drivers of beta diversity in our study system, we used Bray-Curtis distances and the *beta.pair.abund* function in the *betapart* package (Baselga et al., 2022) to break down beta diversity into its two components: dissimilarity attributed to species replacement (i.e., turnover) and dissimilarity attributed to richness gradients (i.e., nestedness).

3. Results

3.1. Comparisons between old and young forests

Gap fraction, total basal area, and tree diversity differed little with forest age (Supplementary Table S3), but the median tree diameter was significantly larger ($t(38) = 2.848, p = 0.007$) and the maximum tree size slightly larger in old forests ($t(38) = 1.889, p = 0.067$). Old forests had significantly higher basal area of *Quercus* ($t(38) = 3.482, p = 0.001$) while young forests had higher *Pinus* ($t(38) = -4.581, p < 0.001$). Accordingly, tree composition differed with forest age (PERMANOVA $F_{1,38} = 9.239, p < 0.001$), but this was not due to differences in amounts of dispersion (PERMDISP $F_{1,38} = 0.000, p = 0.999$). From the NMDS and *env.fit* analysis, old and young forests did show compositional overlap but also some divergence, mostly in portions of the first and third axis (Fig. 2A). Based on directionality of the *env.fit* vectors, young forests were related to the basal area of *Pinus* and *Prunus*, whereas old forests were related to the basal area of *Quercus*, *Carya*, *Liriodendron*, and *Nyssa* (Fig. 2A, Supplementary Table S4). The second axis also showed considerable explanatory power in tree composition (unrelated to age), with a gradient showing higher amounts of *Liquidambar*.

Forest plots in this study had an average deadwood volume of $2.092 \pm 0.317 \text{ m}^3$ ($=20.92 \pm 3.17 \text{ m}^3/\text{ha}$) with old forests having marginally larger volumes ($t(38) = 1.822, p = 0.076$), but deadwood diversity did not differ with forest age ($t(38) = 1.237, p = 0.224$). Volumes of the decay classes were uneven in our plots (ANOVA $F_{4,190} = 10.112, p < 0.001$; Fig. 2B), but there was no difference of decay classes with forest age (ANOVA $F_{1,190} = 1.194, p = 0.276$) nor was there an interaction with age (ANOVA $F_{4,190} = 1.205, p = 0.310$). Similarly, there was no difference in the volume of large (>40 cm) and small (<40 cm) pieces of deadwood with forest age (ANOVA $F_{1,76} = 1.610, p = 0.208$) nor was there an interaction (ANOVA $F_{1,76} = 0.010, p = 0.921$). However, the volume consisting of small pieces was higher than for large pieces

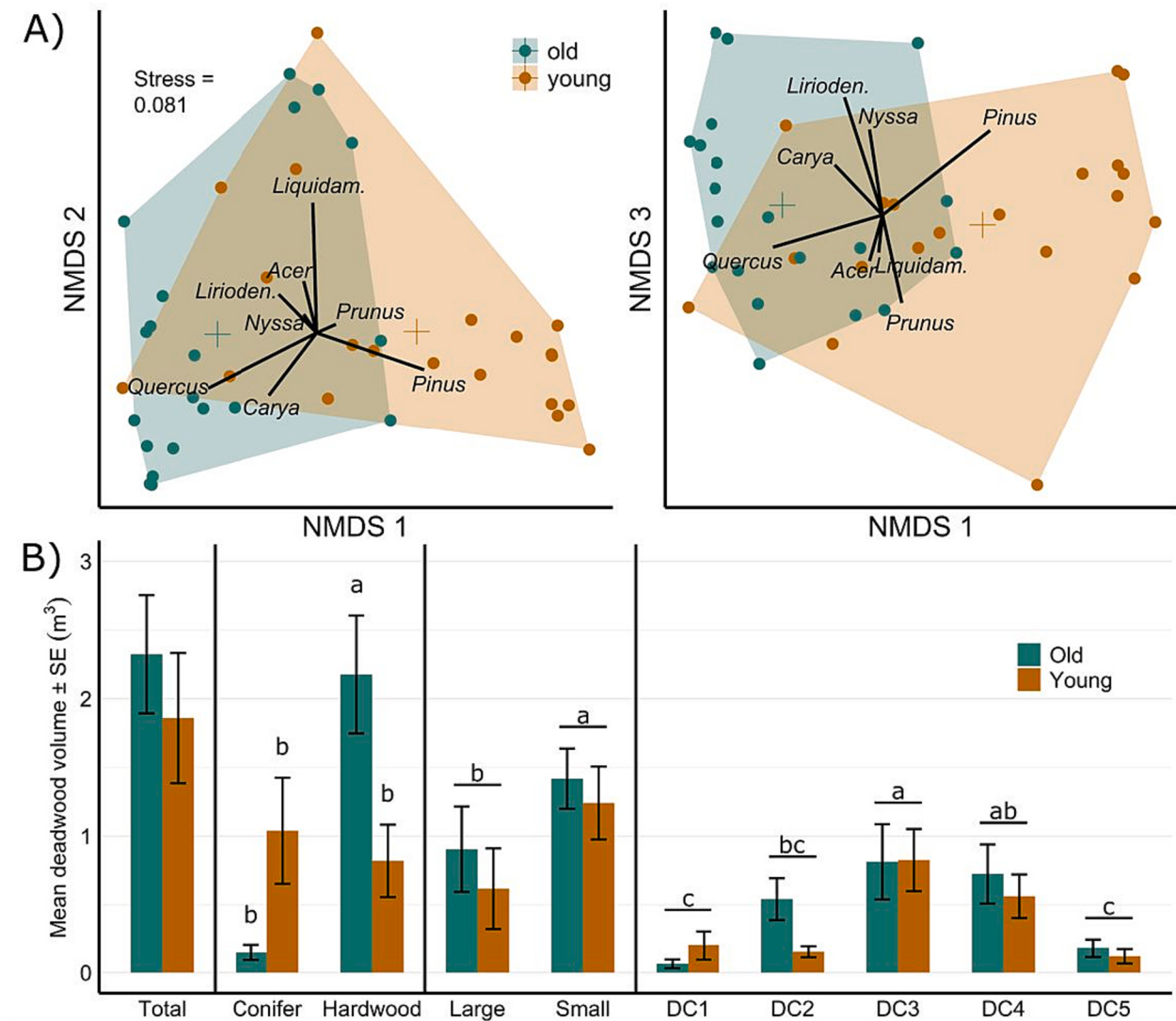


Fig. 2. Forest characteristics between age groups in the Piedmont region of the southeastern United States (Georgia). A) Results of non-metric multidimensional scaling for tree basal area data, with significant *env.fit* correlations of tree genera drawn as lines and age group centroids are shown as +. *Liquidamb.* = *Liquidambar*, *Lirioden.* = *Liriodendron*. B) Comparison of deadwood volume between old and young sites in total, and in categories based on species composition, size, and position differed significantly with forest age (PERMANOVA $F_{1,37} = 2.164$, $p = 0.001$). Post hoc PERMDISP analyses show that PERMANOVA results may be partially explained by dispersion differing with forest age (PERMDISP $F_{1,37} = 5.972$, $p = 0.019$), with deadwood composition among young forests being more variable than old (average multivariate distance to group median = 0.629 for young and 0.573 for old).

(ANOVA $F_{1,76} = 10.220$, $p = 0.002$; Fig. 2B). Additionally, there was a significant interaction between forest age and deadwood species type (hardwood vs. conifer; ANOVA $F_{1,76} = 15.660$, $p < 0.001$). Tukey's HSD suggests that old forests had more DW derived from hardwood trees than young forests, that hardwood DW is much more prevalent than conifer DW within old forests, and that volumes of hardwood DW within old forests is higher than conifer DW within young forests (Fig. 2B). Congruently and consistent with tree composition, deadwood composition based on genus, decay class, size class, and position differed significantly with forest age (PERMANOVA $F_{1,37} = 2.164$, $p = 0.001$). Post hoc PERMDISP analyses show that PERMANOVA results may be partially explained by dispersion differing with forest age (PERMDISP $F_{1,37} = 5.972$, $p = 0.019$), with deadwood composition among young forests being more variable than old (average multivariate distance to group median = 0.629 for young and 0.573 for old).

3.2. Landscape change and comparisons

Within the study area, the amount of forest cover increased drastically from 1938 (32 % of the landscape) to 1980 (56 %), then slightly declined to 2017 (53 %). Little over half of the forest present in 1938

remains today, as old forest comprises 17.6 % of Clarke County land area. The LFC surrounding our sites were highly correlated between the time points except at the smallest spatial scale: 1938 and 1980 Pearson's $r = 0.678$ – 0.911 (250 m = 0.423); 1980 and 2017 Pearson's $r = 0.737$ – 0.898 ; 1938 and 2017 Pearson's $r = 0.531$ – 0.744 (250 m = 0.104). LFC Old was highly correlated with LFC 1938 (Pearson's $r = 0.808$ – 0.926) and LFC 2017 excluding the 250 m scale (Pearson's $r = 0.628$ – 0.817 , 250 m = 0.426). LFC did not differ with forest age except at the 250 m scale for 1938 ($W = 24$, $p < 0.001$) and Old ($W = 34$, $p < 0.001$), an artefact of our study design. Other scales and time points were non-significant (Supplementary Table S5).

3.3. Regional diversity and indicator species analysis of saproxylic beetles

A total of 50,129 saproxylic beetles were collected, representing 472 species (Supplementary Table S12). Of these, 155 species (17,485 individuals) were considered as fresh wood species, 144 species (3521 individuals) as decayed wood and polypore fungi species, and 59 species (1231 individuals) as predators. Old and young forests had a total of 30,946 and 19,183 individuals, representing 402 and 342 species, respectively. Rarefactions showed that species richness differed

significantly with forest age, with old forests collectively having more species for all species and for all functional groups excluding predators (Fig. 3, Supplementary Fig. S2). Indicator species analysis showed that several species were associated with old forests, young forests, amounts of LFC, and combinations of age and LFC (Table 1).

3.4. Local diversity of saproxylic beetles

The spatial scale of effect for all species and functional groups was 250 m for LFC 1938 and 2000 m for LFC 2017 (Supplementary Table S7). An exception was predator richness, for which the scale of effect was 2000 m for both time points. For species richness, models including LFC 2017 performed better than those including LFC 1938. Additionally, models with and without an interaction between forest age and LFC 2017 showed similar AICc values (Supplementary Table S8), and we present results from both models (Fig. 4A, Table 2, Supplementary Fig. S3, Supplementary Table S9). The interaction model performed marginally better for all species, but the no-interaction model was marginally better for the functional groups.

In models without an interaction, species richness was significantly higher in old forests for all species, fresh wood species, and decayed wood and polypore species (Table 2). Richness increased with LFC 2017 for all species, fresh wood species, and predators, but the responses were weak except for predators. In models including an interaction, species richness was higher and showed larger increases across the LFC gradient in old forests. This pattern shows more similar species richness between old and young forests at low LFC, and larger disparity at high LFC (Fig. 4A, Supplementary Fig. S3). The effect of LFC 2017 was significant for old forests for all species and fresh wood species, both age groups for predators, and neither age group for decayed wood and polypore species. In both models (with and without an interaction), local habitat factors (deadwood volume, gap fraction, and tree diversity) showed positive effects, but responses differed between functional groups (Table 2).

3.5. Community composition of saproxylic beetles

Community composition of saproxylic beetles significantly differed

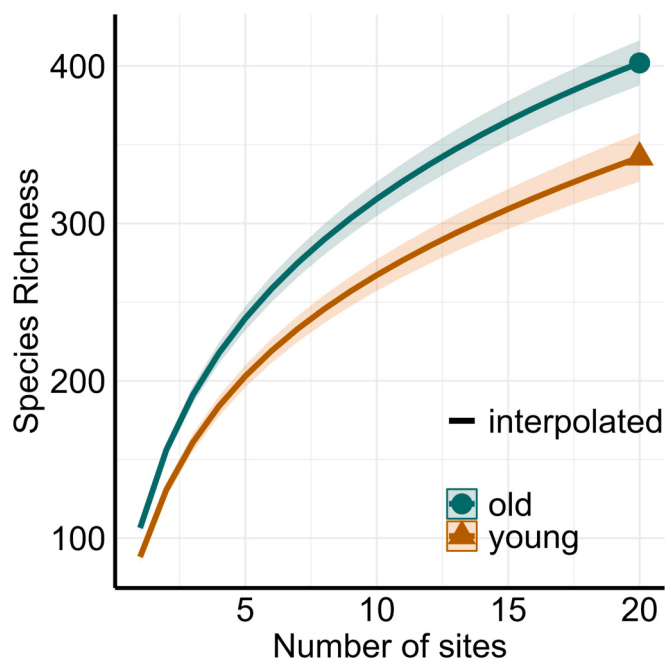


Fig. 3. Sample-based rarefaction of regional (gamma) diversity for saproxylic beetles in old and young forests in the Piedmont region of the southeastern United States (Georgia).

Table 1

Results of indicator species analysis for combinations of forest age and landscape forest cover (LFC). High LFC indicates landscapes had >50 % forest cover, low indicates <50 % cover.

Species (family/subfamily)	Group	p
<i>Monarthrum mali</i> (Scolytinae)	Old	<0.001
<i>Acmaeodera tubulus</i> (Buprestidae)	Old	0.001
<i>Hylocurus rudis</i> (Scolytinae)	Old	0.002
<i>Isorhipis obliqua</i> (Eucnemidae)	Old	0.002
<i>Acalles clavatus</i> (Curculionidae)	Old	0.016
<i>Petalium bistriatum</i> (Ptinidae)	Old	0.016
<i>Gaurotes cyanipennis</i> (Cerambycidae)	Old	0.021
<i>Anelaphus parallelus</i> (Cerambycidae)	Old	0.026
<i>Synchita parvula</i> (Zopheridae)	Old	0.027
<i>Chariessa pilosa</i> (Cleridae)	Old	0.036
<i>Bitoma quadricollis</i> (Zopheridae)	Old	0.044
<i>Bothrideres geminatus</i> (Bothrideridae)	Old + High LFC	0.005
<i>Nausibius major</i> (Silvanidae)	Old + High LFC	0.009
<i>Namunaria guttulata</i> (Zopheridae)	Old + High LFC	0.013
<i>Saperda lateralis</i> (Cerambycidae)	Old + High LFC	0.031
<i>Mordellistena tosta</i> (Mordellidae)	Young	0.029
<i>Ampedus collaris</i> (Elateridae)	Young	0.045
<i>Ampedus fuscatus</i> (Elateridae)	Young + High LFC	0.028
<i>Rhizophagus sayi</i> (Monotomidae)	Young + High LFC	0.045
<i>Pycnomerus sulcicollis</i> (Zopheridae)	Young + High LFC	0.049
<i>Mordellistena liturata</i> (Mordellidae)	High LFC	0.013
<i>Litargus sexpunctatus</i> (Mycetophagidae)	High LFC	0.020
<i>Bacanius punctiformis</i> (Histeridae)	High LFC	0.028
<i>Anelaphus villosus</i> (Cerambycidae)	High LFC	0.029
<i>Cnopus impressus</i> (Aderidae)	High LFC	0.037
<i>Mordellistena masoni</i> (Mordellidae)	High LFC	0.045
<i>Microtonus sericans</i> (Melandryidae)	Low LFC	0.035

with forest age for all species (Fig. 4B, PERMANOVA $F_{1,38} = 2.037$, $p < 0.001$), fresh wood species (PERMANOVA $F_{1,38} = 2.863$, $p \leq 0.001$) and predators (PERMANOVA $F_{1,37} = 1.893$, $p = 0.017$), but no differences were detected for decayed wood and polypore species (PERMANOVA $F_{1,38} = 1.326$, $p = 0.118$). Post hoc PERMDISP analyses show differences in beta diversity with forest age (i.e., the amount of beta diversity within an age group as measured by the average distance to the group median) for all species (PERMDISP $F_{1,38} = 6.784$, $p = 0.013$). In this case, young forests had higher amounts of beta diversity than old forests (average distance to the group median = 0.512 for young and 0.479 for old). However, this was not the case for fresh wood species (PERMDISP $F_{1,38} = 1.837$, $p = 0.183$) or predators (PERMDISP $F_{1,37} = 1.441$, $p = 0.238$). The NMDS analyses followed by *env.fit* correlations suggest that community composition of saproxylic beetles is influenced by the tree community, landscape factors, and local habitat factors, but responses for each functional group varied (Fig. 4B, Supplementary Figs. S4–6). Tree Axis 1 had the highest *env.fit* correlation with the beetle NMDS for all species and all functional groups, and its directionality suggests relation to forest age differences (full *env.fit* results in Supplementary Table S10). Finally, total beta diversity of saproxylic beetle communities across all sites in this study was considerable (Bray-Curtis dissimilarity = 0.697–0.768). The majority of observed beta diversity can be attributed to species turnover (86.6–90.4 %) rather than nestedness (9.6–13.4 %, Supplementary Table S11).

4. Discussion

Old forests represent stable, late-successional habitats known to benefit saproxylic biodiversity worldwide. However, the extent to which the importance of old forests is mediated by landscape context remains poorly resolved. The possibility that old forests are most important in highly fragmented landscapes with low forest cover (Nordén et al., 2014; Brin et al., 2016) has important implications for conservation. We found no support for this hypothesis in the current study. Instead, we found that old forests support more species and a distinct fauna regardless of surrounding forest cover. As discussed in more detail

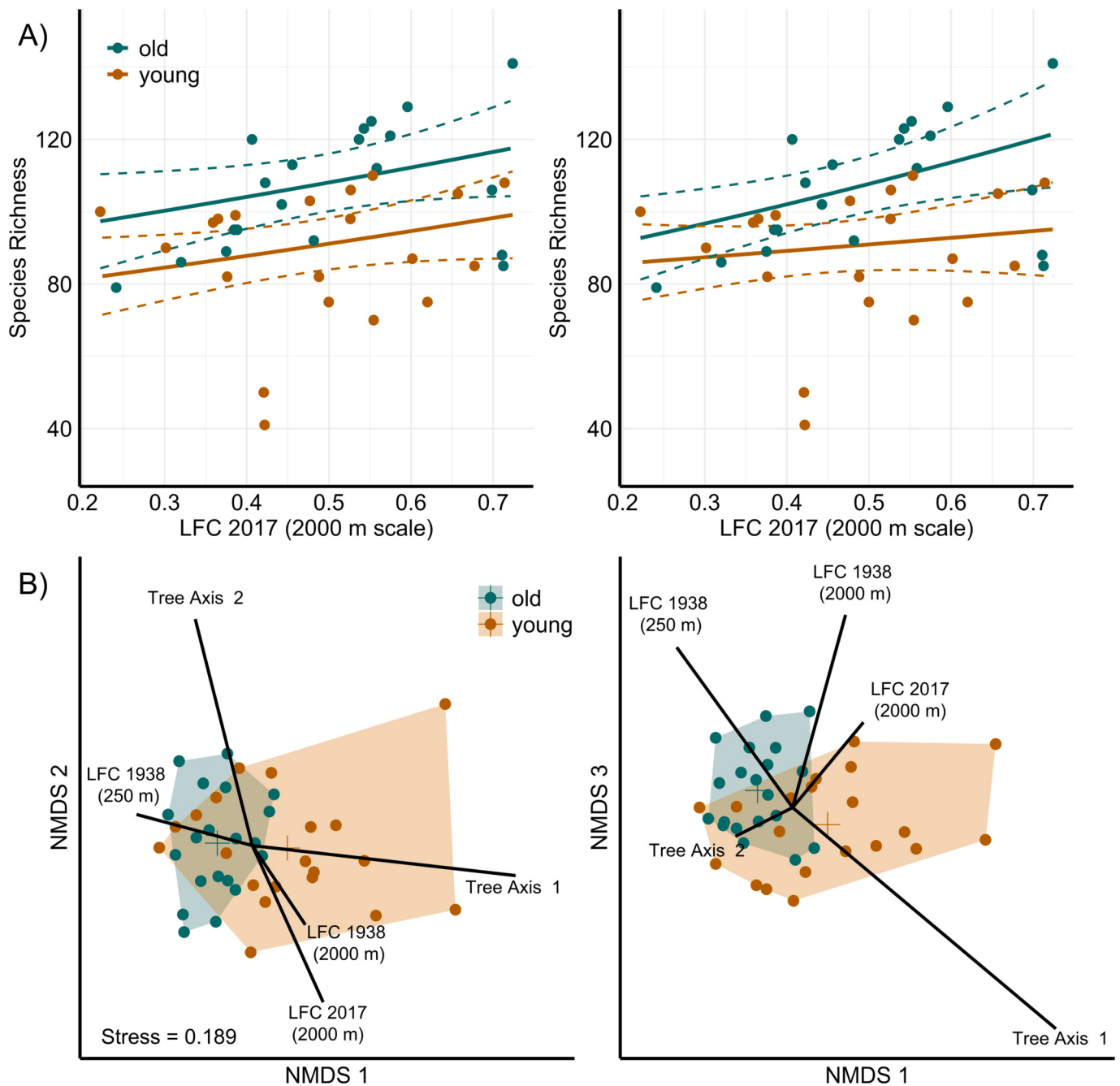


Fig. 4. Species richness and composition of saproxylic beetles in the Piedmont region of the southeastern United States (Georgia). A) Species richness (alpha diversity) results from the generalized linear model without (left) and with (right) an interaction between forest age and landscape forest cover (LFC). The interaction model has marginally better support (Table 2). Only effects of forest and LFC are shown, with other variables held at their mean. B) Community composition (beta diversity) displayed by non-metric multidimensional scaling (NMDS) with significant *env.fit* correlations overlaid. Tree Axis 1 and 2 refer to axes from the NMDS analysis of tree basal area (Fig. 2A). Age group centroids are shown as +.

below, these results suggest that preserving old forests should be a conservation priority in the region irrespective of landscape context.

4.1. Does the landscape mediate the importance of forest age?

We not only rejected the hypothesis that old forests are less important to saproxylic beetle diversity in highly forested landscapes, but we actually observed the opposite pattern. Specialist species benefit the most from increased habitat amount and connectivity (Nordén et al., 2013; Sverdrup-Thygeson et al., 2017), and specialists were more prevalent in old forests (see Section 4.3), which may explain this trend.

However, our results provide mixed evidence that the landscape mediates the importance of forest age. For example, species richness was explained nearly as well (if not slightly better, for the functional groups) by a scenario with independent influences of forest age and LFC. Thus, the expected pattern of isolation driving old forest importance is not supported, and it is unclear if the importance of forest age depends on landscape context at all. Other evidence supports that saproxylic beetle communities are influenced independently by local habitat quality and landscape habitat amount (Seibold et al., 2017; Cours et al., 2022). Additionally, we expected species in young forests to be a subset of those in old forests, and for community composition to be similarly driven by

Table 2

Summary results of generalized linear models for species richness of saproxylic beetles (coefficients and standard errors shown in Supplementary Table S9). Both models (with and without an interaction between age and landscape forest cover) are shown with their ΔAICc values. The spatial scale used for landscape forest cover was 2000 m. Significant z -values of predictors are bold. For the categorical variable Age, old was used as the reference so responses are shown for young. DW vol = deadwood volume; Gap Fr = gap fraction; LFC = landscape forest cover; Tree Div = tree diversity.

	All				Fresh wood				Decayed wood/polypore				Predator			
	No interaction ($\Delta\text{AICc} = 0.086$)		Interaction ($\Delta\text{AICc} = 0.000$)		No interaction ($\Delta\text{AICc} = 0.000$)		Interaction ($\Delta\text{AICc} = 1.629$)		No interaction ($\Delta\text{AICc} = 0.000$)		Interaction ($\Delta\text{AICc} = 0.101$)		No interaction ($\Delta\text{AICc} = 0.000$)		Interaction ($\Delta\text{AICc} = 0.308$)	
	z	p	z	p	z	p	z	p	z	p	z	p	z	p	z	p
Age (young)	-3.141	0.002			-3.732	0.000			-2.657	0.008			-1.577	0.115		
LFC 2017	1.875	0.061			1.763	0.078			0.544	0.586			2.974	0.003		
DW vol	2.050	0.040	1.827	0.068	0.176	0.861	0.043	0.966	1.879	0.060	1.680	0.093	1.625	0.104	1.559	0.119
Gap Fr	1.042	0.298	1.094	0.274	1.081	0.280	1.089	0.276	1.887	0.059	1.927	0.054	0.553	0.581	0.551	0.581
Tree Div	1.978	0.048	2.034	0.042	1.771	0.077	1.823	0.068	1.072	0.284	1.121	0.262	2.063	0.039	2.075	0.038
Age (old): LFC 2017			2.653	0.008			2.563	0.010			1.190	0.234			3.285	0.001
Age (young): LFC 2017			0.940	0.347			0.664	0.507			-0.203	0.839			2.384	0.017

LFC. We found divergent beetle community composition in old and young forests, but young forest communities were not a subset of the species found in old forests. Moreover, the local tree community showed the highest correlation to saproxylic beetle composition, and the landscape's influence could not be isolated.

Altogether, these results suggest that old forests in this region are valuable for saproxylic beetle biodiversity regardless of how much forest is in the landscape. Additionally, widespread dispersal limitation of these species is not apparent in our study landscape. As forest cover has increased since 1938, habitat amount and the related increase in connectivity may have reduced the effects of historic isolation (Nordén et al., 2014; Herrault et al., 2016). Dispersal limitation may be more notable in regions that have undergone prolonged isolation without LFC recovery (e.g., Brin et al., 2016). Additionally, in highly forested regions where younger forests have late-successional habitat, age per se may be less important (e.g., Janssen et al., 2016).

4.2. Comparing old and young forests in the Georgia Piedmont

Forest cover in the southeastern United States has increased since the 1930s (Ramankutty et al., 2010; Sohl et al., 2016), but the vast majority of stands are early-successional (Conner and Hartsell, 2002). In accordance, our data show that in the area surrounding Clarke County, GA, two-thirds of today's forest does not predate the 1930s. Yet despite the difference in age, very few structural differences exist between the old and young forests sampled. The most striking difference was in tree composition, with higher basal area of *Pinus* in young forests and *Quercus* in old forests. This compositional pattern matches closely with known successional trajectories in the Piedmont region: pines dominate early on and are gradually replaced by hardwoods at around 100 years, eventually reaching a community of oak-hickory (Oosting, 1942; Nicholson and Monk, 1974; Orwig and Abrams, 1994). It should be noted, however, that old forests in our study likely experienced occasional fires during their development, and more recent fire suppression may alter the ongoing successional patterns (Nowacki and Abrams, 2008).

4.3. Importance of forest age for saproxylic beetle richness

Old forests across the study region supported more species than young forests, and local richness was higher in old forests as well. There are two likely explanations for this which are not mutually exclusive. First, the uninterrupted presence of forest has been consistently identified as a key factor for the presence of low-mobility species (e.g., Siitonen and Saaristo, 2000; Jonsell and Nordlander, 2002; Buse, 2012; Traylor et al., 2022a). In our study, one of the flightless species collected

(*Acalles clavatus*) was associated with old forests, suggesting that forest age contributed to species richness by providing a relatively unbroken supply of habitat for low-mobility species. However, as most saproxylic insects are relatively efficient dispersers (Komonen and Müller, 2018), the importance of age for the entire community may be less apparent. By splitting our species list into functional groups differing in relative dispersal ability based on habitat stability (Southwood, 1977; Percelet et al., 2019), we were able to see how forest age may differently influence species with varying mobility. Consistent responses by species reliant on fresh deadwood (ephemeral resources = high mobility) and species dependent on decayed wood and polypore fungi (more stable resources = low mobility) suggest that older forests benefit the overall saproxylic beetle community in this region, regardless of expected dispersal ability. Thus, dispersal limitation alone cannot explain our results.

A second explanation is that old forests provided late-successional microhabitats that are disproportionately important for saproxylic species richness (Gibb et al., 2013). Occurrence of tree-related microhabitats increases as trees age, and especially as they become senescent (Larrieu et al., 2022). Oaks in particular are considered to be long-lived (Stokland et al., 2012) and develop tree-hollows as they age (Ranius et al., 2009). Moreover, as oaks grow and die, they acquire more microhabitats than other types of trees, particularly pines (Paillet et al., 2019). In Sweden, more saproxylic species (including specialist and red-listed species) develop within oaks than all other tree genera due to these microhabitats (Jonsell et al., 1998). In the southeastern United States, oak and pine support similar numbers of early-successional saproxylic beetles (Ulyshen and Hanula, 2009), but microhabitats and later decay stages have not been assessed. In our study, two known oak specialists, *Nausibius major* and *Petalium bistratum*, were associated with old forests. While pine certainly hosts specialists as well, three times more species were associated with old forests in our study ($n = 15$) than young forests ($n = 5$). This finding is consistent with the notion that old forests promote higher numbers of specialized saproxylic species (Stenbacka et al., 2010; Gibb et al., 2013; Janssen et al., 2017). The effect of forest age in our study is therefore due to the combined effects of old forests better supporting low-mobility and specialized species.

4.4. Importance of forest age for saproxylic beetle community composition

Old and young forests had distinct communities for all species, fresh wood species, and predators, but not for decayed wood and polypore species. Moreover, differences were principally driven by species replacement, or turnover. Therefore, we found no support that

saproxyllic beetle communities in young forests are a subset of old forest communities. Considering the differences in tree communities between old and young forests in our study, it is reasonable to conclude that differences in tree composition are resulting in species turnover of beetles (Pérez-Sánchez et al., 2020; Sebek et al., 2022). Deadwood of hardwoods and conifers supports different species of saproxyllic beetles (Vogel et al., 2020), but specialization tends to decrease with decomposition (Jonsell et al., 1998). Thus, it seems that old and young forests comprise different beetle communities determined by the different tree hosts available, but this difference may diminish in later decomposition stages. Therefore, both age classes are important components of regional biodiversity.

A peculiar finding from this study is that beta diversity among young forests was higher than that among old forests. Because young forests occupy twice as much land area as old forests, they may occur across a larger array of environmental conditions, leading to higher species turnover among stands. The region's history supports this, as virtually all plowable land was cleared for agriculture (Oosting, 1942). Today's old forests would then mostly occupy land that was unsuitable for cultivation, which may only encompass a fraction of the natural heterogeneity for forests of this age. Another related explanation is that more immigrant species may move through young forests due to their larger area, and this may occur somewhat stochastically. This seems plausible, given that young forests had lower species richness at the local and regional scales despite higher turnover (Sebek et al., 2022).

4.5. Study limitations

This study was performed in the Piedmont region of the southeastern United States, and care should be taken before extending these conclusions to other eco-regions, particularly where forest succession may have divergent patterns. While our insect sampling procedure underestimates the total number of saproxyllic species present (Burner et al., 2022), equal sampling effort at all sites and between forest age groups ensures that appropriate comparisons can be made. While our time frame of age is shorter than that of other studies (e.g., Ferro et al., 2012; Brin et al., 2016; Janssen et al., 2016), our distinction between forests pre-dating vs. originating after 1938 is relevant for large portions of the southeastern United States where historical forest maps are unavailable prior to 1938. Perhaps the greatest limiting factor was the lack of available natural history information for many beetle species sampled, which is the case for most saproxyllic species in North America. This knowledge gap limited our ability to classify species into functional groups, and even to determine whether they were saproxyllic or not. Documenting the life history and habitat associations of species is critical to understanding saproxyllic biodiversity in North America.

5. Conclusions

We investigated the influence of forest age and landscape forest cover on saproxyllic beetle communities in the Piedmont region of the southeastern United States. Forest age in this study was the main driver of saproxyllic beetle biodiversity, and its importance occurred regardless of the amount of forest in the landscape. Old forests, mature since at least 1938, had higher richness regionally and locally than young forests regrown since 1938. However, species turnover was higher among young forests. Moreover, due to high species turnover between age groups, both old and young forests help to maintain regional biodiversity. Because old forests occupied less than half of the land area of young forests in our study, they are disproportionately important for saproxyllic biodiversity in this area. Thus, protecting old forests and allowing young forests to mature would be beneficial to this sensitive community, particularly in regions dominated by early-successional forests.

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CRediT authorship contribution statement

Clayton R. Traylor: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Michael D. Ulyshen:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition. **Joseph V. McHugh:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

None.

Data availability

Data is available at www.zenodo.org/record/8246615.

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Appendix A. Supplementary data

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