MODELING WINTER FORAGING ECOLOGY OF BATS ON WORKING FOREST LANDS IN THE SOUTHEASTERN US COASTAL PLAIN

by

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(Under the Direction of Steven B. Castleberry)

ABSTRACT

Insectivorous bats in temperate zones have evolved strategies, such as migration or hibernation, to face challenges of reduced resource availability and increased energy demand during winter. In the southeastern United States Coastal Plain, many bats are year-round residents and remain active during the winter or migrate from colder areas seeking milder conditions. Southeastern Coastal Plain forests may represent important areas for remnant populations of bat species impacted by White-Nose Syndrome (WNS). Working forests represent a large proportion of the forests of the southeastern Coastal Plain, yet winter habitat use by bats in this region and how forest management practices affect habitat use remains understudied. From 2020 to 2022, my study used passive acoustic monitoring to assess winter bat activity across six forest sites, evaluating how habitat features and environmental variables shape bat foraging and occupancy patterns. In addition, I conducted DNA metabarcoding analysis of fecal samples to identify prey species and assess dietary diversity among bat species and applied structural equation modeling to disentangle the effects of forest management, temperature, and insect availability on bat activity. My study detected eleven bat species, with species-specific responses to habitat features, temperature, and prey availability. Results indicated that higher species richness was associated with areas of contiguous forest and lower basal area, suggesting that certain forest stand characteristics support overwintering bat populations. Temperature emerged as a significant predictor of bat detectability, increasing activity in warmer conditions. DNA metabarcoding revealed a diverse winter diet that included economically relevant pest species, such as *Rhyacionia frustrana*, underscoring bats' role in pest control within these ecosystems. The findings suggest that maintaining specific forest structural features, such as lower basal area and habitat connectivity, can positively impact overwintering bats and enhance their ecosystem services. By providing insights into bat foraging ecology and habitat use, this study offers valuable recommendations for forest management practices aimed at bat conservation and supports the development of sustainable forestry practices that benefit both biodiversity and forest health in the southeastern United States Coastal Plain.

INDEX WORDS: Acoustics, Bats, Coastal Plain, Foraging, Winter, Working Forests.

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INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Forests are common across the eastern United States (U.S. hereafter) and, more specifically, 86% of forests in the southeastern U.S. are privately owned (Oswalt *et al*., 2019). Forest management, primarily driven by economic incentives, plays a crucial role in conserving these landscapes by reducing the risk of forestland conversion to urban or agricultural uses (Zobrist *et al*., 2005; Wickham *et al*., 2023). One key conservation benefit of forest management is that it allows landowners to meet their objectives (e.g., timber production, recreation, wildlife management) while maintaining forested landscapes. This flexibility supports both the retention of forests and biodiversity.

In the southeastern U.S., private landowners manage a variety of forest types, conditions, and goals, with 79% of these private forests naturally regenerated (Oswalt *et al*., 2019). Although only 21% of private forests are planted, they cover a vast 45 million acres in the southeastern U.S., accounting for 71% of all planted forests nationwide (Oswalt *et al*., 2019). Pine (*Pinus* spp.) management is particularly common, with pine forests undergoing typical rotations of 20-25 years involving site preparation, seedling planting, and various management practices like herbicide application, fertilization, prescribed fire, thinning, and clearcutting. These forest practices help create and maintain structural conditions across the landscape that support wildlife species, including game and nongame species.

Historically, large-scale clearing of forests for agriculture shaped the southeastern U.S., particularly in the Coastal Plain and Piedmont regions, from the colonial period until the Civil War (Williams, 1989). Poor agricultural practices caused widespread soil erosion, leading to significant abandonment of agricultural lands between the Civil War and World War II (Fox *et al.*, 2007). The effects of these legacy practices are still evident, as lands with agricultural histories exhibit different understory species compositions compared to historically forested areas (Hedman *et al*., 2000; Dupouey *et al*., 2002; Vellend *et al*., 2007; Brudvig *et al*., 2013). However, despite the decline in agricultural land use since the 1940s due to abandonment and intensified management of remaining fields, forest cover in the southeastern U.S. has increased since the early 1900s. Today, private landowners and forest managers increasingly recognize the importance of biodiversity, with forest certification programs promoting sustainable management practices. Certification provides long-term assurances that forests will be managed sustainably, helping to maintain habitats for forest-dependent species. Retaining key structural features after harvesting further benefits species, although some may require more targeted management on a site-specific basis.

Order Chiroptera is the second largest mammalian order including over 1,465 species distributed throughout most of the planet (Simmons & Cirranello, 2023). Bats are the only mammals capable of powered flight (Altringham, 2011) and have important roles in ecosystems as they contribute to biological pest control, seed dispersal, and plant genetic diversity, among others (Kunz *et al*., 2011). Bats are important targets for conservation because they are increasingly threatened by habitat loss and modification, alteration of roosting areas, pesticides, diseases, and wind energy development (Mickleburgh *et al*., 2002; Voigt & Kingston, 2016). Compared to other mammals and birds, significantly less is known about the population status of most bat species, which makes prioritizing and planning conservation actions challenging. Over a third of bat species assessed by the International Union for Conservation of Nature (IUCN) are considered threatened or data deficient, with more than half exhibiting unknown or decreasing population trends (Frick *et al*., 2020).

In the U.S., 42 of the 45 bat species feed on insects or other arthropods. Bats contribute significantly to ecosystem services in forests, including control of phytophagous insects (Böhm *et al*., 2011; Charbonnier *et al*., 2014; Ancilotto *et al*., 2022). For example, a single little brown bat (*Myotis lucifugus*) can consume 4 to 8 g of insects each night during the active season (Anthony & Kunz, 1977; Kurta *et al*., 1989), while a colony of 150 big brown bats (*Eptesicus fuscus*) has been estimated to eat nearly 1.3 million pest insects each year, possibly contributing to the disruption of population cycles of agricultural pests (Whitaker, 1995). Given these ecological benefits, integrating bat conservation into forest management not only helps maintain biodiversity but also promotes sustainable forest management practices.

LITERATURE REVIEW

Winter Bat Ecology

In temperate zones of North America, insectivorous bats have evolved strategies such as migration (Cryan, 2003) or hibernation (Humphries *et al*., 2003) to cope with reduced resource availability and increased energy demand during winter. North American bats face significant risks during these months, such as collisions with wind turbines during migrations or the fungal disease white-nose syndrome (WNS). Bat fatalities from wind turbines occur predominantly in migratory species such as hoary (*Lasiurus cinereus*), eastern red (*L. borealis*), and silver-haired (*Lasionycteris noctivagans*) bats in late summer and early autumn (Fleming *et al*., 2003; Kunz *et al*., 2007; Cryan *et al*., 2014; Wieringa *et al*., 2021). In addition, WNS has caused extensive mortalities in cave-dwelling species, leading to widespread alterations in bat communities in the eastern U.S. (Blehert *et al*., 2009; Frick *et al*., 2015). For example, winter counts in WNS-positive regions show that populations of the most susceptible species, such as northern long-eared myotis (*Myotis septentrionalis*), little brown myotis (*M. lucifugus*), and tricolored bats (*Perimyotis subflavus*), have declined by more than 90% since WNS detection (Cheng *et al*., 2021). Unlike other regions of the eastern U.S., many bats in the southeastern Coastal Plain are year-round residents and remain active throughout the year or migrate from colder areas seeking milder winter conditions (Grider *et al*. 2016). Additionally, southeastern Coastal Plain forests may represent important areas for remnant populations of bat species impacted by WNS, such as tricolored and northern long-eared bats (Jordan, 2020; De la Cruz et al., 2022; Perea *et al*., 2022). Yet most studies in this region have been conducted during the growing season or focused on roosting ecology (e.g., Loeb & O'Keefe, 2006; Hein *et al*., 2008; Bender *et al*., 2015, 2021; Gallagher *et al*., 2021). Winter bat foraging activity and habitat use remain understudied (e.g., Grider *et al*., 2016; Kunbergen & Long, 2022).

Previous studies across North America point to temperature as a critical factor dictating winter bat activity (Schwab & Mabee, 2014; White *et al*., 2014; Grider *et al*., 2016; Johnson *et al*., 2016; Lemen *et al*., 2016; Bernard & McCracken, 2017; Parker *et al*., 2020; Jorge *et al*., 2021; Anderson *et al*., 2024). In the Coastal Plain of Texas and Louisiana, Andersen *et al*. (2022) found temperature as the most important predictor of bat activity in winter, but no evidence that landscape factors influence the distribution of any species. At a local microhabitat scale, they only detected habitat association in eastern red / Seminole bats (*Lasiurus seminolus*) for reduced clutter, large and deciduous forest microhabitats, and Brazilian free-tailed bats (*Tadarida brasiliensis*) for deciduous forest microhabitats. In contrast, Shute *et al*. (2021) demonstrated differences in bat habitat use between summer and winter in the Coastal Plain of South Carolina. During summer, habitat use by tricolored, northern yellow (*Dasypterus intermedius*), and myotis bats (genus *Myotis*) were related to characteristics that would be expected based on how bats of different morphology interact with their environment. They observed that the larger, fast-flying northern yellow bats used open areas, the more maneuverable myotis bats were associated with forests, and the edge-feeding tricolored bats used both open and closed cover types across the landscape. When resource availability, forest structure, and temperature changed during winter, they observed both northern yellow bats and tricolored bats used interior forests which provided water sources and higher temperatures than open areas, and, consequently, a potentially greater abundance of insects (Janzen & Schoener, 1968; Li *et al*., 2015). For myotis bats, although habitat use was not associated with any site characteristics, the landscape surrounding the sites was important in determining habitat use in both summer and winter.

All North American bat species are associated with forests to some extent (e.g., for roosting and/or foraging), therefore it is important to understand how bats use forests and how forest management may affect their use (Brigham, 2007; Bender *et al*., 2015; Taylor *et al*., 2020; Gallagher *et al*., 2021). A lack of understanding of how bats and other animals interact with their environment throughout the year may obscure the full scope of habitat associations and needs. This becomes especially important in regions such as the southeastern U.S., where many forests are privately owned and actively managed as working forests (Oswalt *et al*., 2019). Thus, forested landscapes provide essential habitat features that support bat diversity, including roosting sites (e.g., trees) and foraging areas (e.g., riparian zones, wetlands, or gaps in forests) (Brigham, 2007; Vindigni *et al*., 2009; Carr *et al*., 2020; Tena *et al*., 2020). Ultimately, sustainable management has the dual benefit of supporting year-round bat activity and playing a crucial role in helping remnant populations impacted by diseases such as WNS and ensuring a favorable wintering fate for migratory species, thereby improving the resilience of bat populations in the face of increasing ecological challenges. These forests will also benefit from ecological services in terms of controlling potential populations of forest pests and phytophagous insects consumed by overwintering bats (Böhm *et al*., 2011; Charbonnier *et al*., 2014; Ancilotto *et al*., 2022; Maslo *et al*., 2022).

Bat Acoustic Monitoring

The study of bat ecology is rapidly evolving due to the increasing availability of advanced techniques and analytical approaches. Currently, the development of passive acoustic recorders (PARs) and the advancement of computing infrastructure that helps researchers store and analyze large quantities of data offer a variety of cost-efficient methods (Pijanowski *et al*., 2011; Teixeira *et al*., 2024). Acoustic monitoring is a common, non-invasive technique to examine multiple facets of bat ecology (Collins & Jones, 2009), including community composition (Flaquer *et al*., 2007), habitat use (Vaughan *et al*., 1997), and activity (Russo & Jones, 2003). Bats use echolocation for navigation and foraging (Dietz & Kiefer, 2016), and different sound parameters like frequency, duration, and inter-pulse intervals can characterize those echolocation calls. Acoustic detection makes the study of individual bat species possible without handling (Russo & Jones, 2002; Loeb *et al*., 2015). Therefore, acoustic monitoring has become a proven way to explore bat richness and activity patterns in different environmental settings during the last two decades (Flaquer *et al*., 2007; Reichert *et al*., 2018).

Despite its utility, effective ecological monitoring using acoustic technology is more than just recording sound. Without proper planning and experimental design, acoustic monitoring can generate huge volumes of data that are difficult to analyze and may not be informative for conservation or management. Thus, methods and processes for implementation should align with a priori objectives and questions (Bayraktarov *et al*., 2019; Teixeira *et al*., 2024). Additionally, acoustic monitoring has other limitations that must be considered in monitoring efforts. For example, the determination of species presence can vary depending on the detector type and identification algorithms used (Adams *et al*., 2012; Russo *et al*., 2018; Perea & Tena, 2020). Echolocation calls of individual bats can vary based on habitat, presence of conspecifics, or environmental noise (Walters *et al*., 2012; Russo *et al*., 2018), influencing species detection and identification efficacy (Adams *et al*., 2012; Russo & Voigt, 2016). To overcome these limitations, studies suggest combining acoustic techniques with capture and roost search (Flaquer *et al*., 2007; Comer *et al*., 2014) and accepting the fact that not all call sequences can, or should, be definitively identified to a species using diagnostic features measurable in sound visualization/analysis software (Reichert *et al*., 2018; Russo *et al*., 2018).

Techniques are also being developed to discern specific animal species from soundscape recordings (Müller *et al*., 2012). Assisted identification software and, more recently, artificial intelligence deep learning models (e.g., convolutional neural networks) have brought about a major change in acoustic identification (e.g., Huang *et al*., 2009; Ferreira *et al*., 2020; Shivaprakash *et al*., 2022). Standard software for species identification typically require less data, are computationally simpler in design (e.g., based on MCMC vocal separators), but rely on human-guided feature engineering (i.e., "supervised machine learning"), which introduces potential subjectivity that could hinder performance, especially with diverse or noisy datasets. On the other hand, artificial intelligence deep learning models tend to be more flexible and may require fewer man-hours to generate well-performing models. However, they require large training datasets and have not yet been sufficiently investigated to date (Müller *et al*., 2023). Moreover, particularly with bats, all these methods face deviations from the expected call structure due to approach calls, cluttered environments, and call quality issues, among others. To address these limitations, researchers suggest a combination of assisted identification systems and noise filtering software with subsequent manual identification to identify calls with a minimum number of pulses and/or with good quality (e.g., Russo & Voigt, 2016; Reichert *et al*., 2018; Lopez-Baucells *et al*., 2019; Perea & Tena, 2020). Furthermore, for those species for which species-level identification is not possible by acoustics, researchers address that limitation by grouping them by genus (e.g., genera *Myotis* or *Plecotus*), or by species pairs (e.g., *Lasiurus borealis* / *L. seminolus*), and separated only when possible (e.g., Grider *et al*., 2016; Tena *et al*., 2019).

Foraging Ecology

The study of trophic resources used by species and their habitats are key aspects of addressing foraging ecology, which provides a basic understanding of relationships among consumers, resources, and the environment (Stephens & Krebs, 1986). Multiple ecologically informed management decisions rely on dietary studies, such as species trophic position and demographic regulation, based on the importance of feeding resources or species interactions (e.g., predation) on populations and communities (e.g., Alonso *et al*., 2014). In temperate zones of North America, most bats use forests at some point in their life cycle (Brigham, 2007), which provides them with foraging and roosting resources. In turn, bats provide significant ecosystem services to forests, including control of phytophagous insects (Böhm *et al*., 2011, Garin *et al*., 2019, Maslo *et al*., 2022). Therefore, knowledge about local feeding habits is also needed to make informed conservation decisions.

Previous studies of diet composition in the southeastern United States have been conducted during the bat active season (Dodd *et al*., 2014; Wilson, 2017; Weinkauf *et al*., 2018; Hughes *et al*., 2021); however, questions related to winter prey selection in relation to prey availability remain understudied (Bernard *et al*., 2021). The importance of bats in crops and working forests has already been proven in summer (Kunz *et al*., 1995; Baroja *et al*., 2019; Braun de Torrez *et al*., 2019; Garín *et al*., 2019). In North America, several studies have revealed bat predation of important pests for multiple agricultural commodities (Brown *et al*., 2015, Whitby *et al*., 2020, Hughes *et al*., 2021). For example, Boyles *et al*. (2011) valued the ecosystem services that insectivorous bats provide at $22.9 billion per year on agroecosystems across the U.S. Yet questions related to the role of winter bat communities as pest controllers in working forests of the eastern U.S. have not been explored.

Molecular techniques offer a rapid, non-invasive, cost-efficient alternative to morphological fecal analysis for the identification of predators and prey (Harper *et al*., 2020). In the last decade, DNA metabarcoding has contributed enormously to our understanding of predator-prey relationships. Even small amounts of highly degraded food residues can be effectively sequenced and often assigned to individual species (Tiede *et al*., 2016). Most studies focus only on diet and do not consider prey selection in relation to prey availability or resource limitation (Salinas-Ramos *et al*., 2020), but these molecular techniques can complement prey availability studies by providing a more complete picture of consumers' trophic preferences (Rytkönen *et al*., 2018). DNA metabarcoding has been used to reconstruct the diet of several bat species in the United States (e.g., Clare *et al*., 2014; Wilson, 2017; Weinkauf *et al*., 2018; Braun de Torrez *et al*., 2019) showing regional and temporal variations in diet for both specialist and generalist species. For example, while studies show a diet of big brown bats (Eptesicus fuscus) based mainly on Coleoptera, Clare *et al*. (2014) also highlighted that other orders such as Lepidoptera and Ephemeroptera are stable components of the diet and can be an important buffer in times of resource limitation. Similar temporal and regional variations have been observed for other species of both generalist and specialist bats in temperate regions (e.g., Dodd *et al*., 2014; Shively *et al*., 2018; Weinkauf *et al*., 2018; Braun de Torrez *et al*., 2019; O'Rourke *et al*., 2022). However, winter bat diet studies in regions with mild temperate climates have been largely overlooked (Bernard *et al*., 2021). Previous studies with European bats observed considerable differences in diet both between study sites in winter and at other times of the year (Williams *et al*., 2011; Hope *et al*., 2014).

OBJECTIVES AND GUIDE TO THE DISSERTATION

Insectivorous bats in temperate zones have evolved strategies, such as migration or hibernation, to face the challenges of reduced resource availability and increased energy demand during winter. In the southeastern U.S. Coastal Plain, many bats are year-round residents and remain active during the winter or migrate from colder areas seeking milder conditions. Southeastern Coastal Plain forests may represent important areas for migratory bats and remnant populations of species impacted by WNS. Working forests represent a large proportion of the forests of the southeastern Coastal Plain, yet winter habitat use by bats in this region and how forest management practices affect habitat use remains understudied.

Bats are elusive and challenging to study. However, the development of passive acoustic recorders (PARs) and the advancement of computing infrastructure now allow researchers to efficiently store and analyze large volumes of data, offering a cost-efficient method to explore bat richness and activity patterns in different environmental settings to aid bat conservation. By analyzing echolocation calls emitted by bats to navigate and detect prey, my first goal was to assess factors influencing winter activity and foraging habitat of bats on working forests in the southeastern U.S. Coastal Plain from late January to mid-March 2020-2022. To do so, I examined the influence of site- and landscape-level habitat characteristics on species richness, aiming to study their effects on winter bat community composition and determine species-specific winter foraging habitat occupancy responses. Additionally, I sought to enhance our understanding of bat foraging ecology in winter by identifying the diet composition of overwintering bats using DNA metabarcoding techniques. I compared the composition of prey consumed among different bat species and determined the potential role of forest bats as pest controllers. As the saying goes, "we are what we eat". Lastly, I explored the relationships among bat activity, temperature, forest structure, and nocturnal flying insect assemblages to investigate the underlying ecological dynamics.

Past research has documented diverse bat communities in working forests of the southeastern U.S. Coastal Plain, but there is limited information on how forest management practices affect bat and insect communities, particularly in winter. By providing baseline information on overwintering bat communities and foraging habitat associations, as well as answering questions about winter foraging ecology from different angles, my results will inform managers of habitat characteristics important to overwintering bats. An understanding of overwintering habitat use will help in planning forest management activities and thereby increase bat conservation opportunities within working forests. Furthermore, my results provide important information on the winter diet of bats in the southeastern U.S. Coastal Plain and their potential role in the control of economically relevant pest species and disease vectors.

This dissertation is organized into a series of manuscript-style chapters addressing questions on the winter foraging ecology of bats and the effects of forest management and landscape characteristics on working forests of the southeastern U.S. Coastal Plain.

In **Chapter 2**, I applied a multispecies spatial occupancy modeling approach that explicitly accounts for imperfect detection, spatial autocorrelation, and species correlations to examine winter bat associations on working forest lands across the southeastern United States Coastal Plain. Previous studies have observed that bat communities are shaped by landscape features at different spatial scales (Loeb & O'Keefe, 2006; Bender *et al*., 2015; Rodríguez-San Pedro & Simonetti, 2015). I focused on how site- and landscape-level habitat characteristics shape species richness and community composition, as well as species-specific foraging habitat occupancy at site- and landscape-levels.

In **Chapter 3**, recognizing the importance of bats in forest vertebrate diversity, their underexplored winter dietary preferences, and their role as controllers of arthropods (including pests of economic and health concern), I analyzed the winter diet composition of bats in private, working forests using DNA metabarcoding from fecal samples collected during winter. My objectives were to (1) identify the diet composition of overwintering bats, (2) compare prey composition among species, and (3) assess the potential role of bats as pest controllers during winter.

In **Chapter 4**, I explored the dynamics of bat and flying insect communities and how forest management practices influence bat ecology during winter. Specifically, I examined direct and indirect relationships among stand structure characteristics, temperature, insect community metrics, and winter bat activity in working forests in the southeastern U.S. Coastal Plain. My approach allowed me to disentangle the direct and indirect effects of forest management practices on bat communities, providing a holistic understanding of ecological interactions occurring within working forest landscapes to inform forest management decisions.

Finally, in **Chapter 5**, I synthesize the findings from Chapters 2, 3, and 4 to offer practical management recommendations aimed at supporting overwintering bat communities in working forest landscapes. As all bats rely on forests for roosting and/or foraging, effective forest management practices are crucial for sustaining healthy overwintering bat populations and enhancing conservation efforts across the southeastern U.S. Coastal Plain.

LITERATURE CITED

Adams, A. M., Jantzen, M. K., Hamilton, R. M., & Fenton, M. B. (2012). Do you hear what I hear? Implications of detector selection for acoustic monitoring of bats. Methods in Ecology and Evolution, 3(6), 992-998. https://doi.org/10.1111/j.2041-210X.2012.00244.x

Alonso, H., Granadeiro, J. P., Waap, S., Xavier, J., Symondson, W. O., Ramos, J. A., & Catry, P. (2014). An holistic ecological analysis of the diet of Cory's shearwaters using prey morphological characters and DNA barcoding. Molecular Ecology, 23(15), 3719-3733. https://doi.org/10.1111/mec.12785

Altringham, J. D. 2011. Bats: from evolution to conservation. Oxford University Press.

Ancillotto, L., Rummo, R., Agostinetto, G., Tommasi, N., Garonna, A. P., de Benedetta, F., ... & Russo, D. (2022). Bats as suppressors of agroforestry pests in beech forests. Forest Ecology and Management, 522, 120467. https://doi.org/10.1016/j.foreco.2022.120467

Andersen, B. R., McGuire, L. P., Wigley, T. B., Miller, D. A., & Stevens, R. D. (2022). Habitat associations of overwintering bats in managed pine forest landscapes. Forests, 13(5), 803. https://doi.org/10.3390/f13050803

Andersen, B. R., Stevens, R. D., Grimshaw, J. R., & McGuire, L. P. (2024). Interspecific variation in lower temperature thresholds of an assemblage of wintering bats. Journal of Mammalogy, 105(1), 98-106. https://doi.org/10.1093/jmammal/gyad103

Anthony, E. L., & Kunz, T. H. (1977). Feeding strategies of the little brown bat, *Myotis lucifugus*, in southern New Hampshire. Ecology, 58(4), 775-786. https://doi.org/10.2307/1936213

Bayraktarov, E., Ehmke, G., O'Connor, J., Burns, E. L., Nguyen, H. A., McRae, L., ... & Lindenmayer, D. B. (2019). Do big unstructured biodiversity data mean more knowledge? Frontiers in Ecology and Evolution, 6, 426167. https://doi.org/10.3389/fevo.2018.00239

Bender, M. J., Castleberry, S. B., Miller, D. A. & Wigley, T. B. (2015). Site occupancy of foraging bats on landscapes of managed pine forest. Forest Ecology and Management, 336, 1-10. https://doi.org/10.1016/j.foreco.2014.10.004

Bender, M. J., Perea, S., Castleberry, S. B., Miller, D. A. & Wigley, T. B. (2021). Influence of insect abundance and vegetation structure on site-occupancy of bats in managed pine forests. Forest Ecology and Management, 482, 118839. https://doi.org/10.1016/j.foreco.2020.118839

Bernard, R. F., & McCracken, G. F. (2017). Winter behavior of bats and the progression of white-nose syndrome in the southeastern United States. Ecology and evolution, 7(5), 1487-1496. https://doi.org/10.1002/ece3.2772

Blehert, D. S., Hicks, A. C., Behr, M., Meteyer, C. U., Berlowski-Zier, B. M., Buckles, E. L., ... & Stone, W. B. (2009). Bat white-nose syndrome: an emerging fungal pathogen? Science, 323(5911), 227-227. https://doi.org/10.1126/science.1163874

Böhm, S. M., Wells, K., & Kalko, E. K. (2011). Top-down control of herbivory by birds and bats in the canopy of temperate broad-leaved oaks (Quercus robur). PLoS One, 6(4), e17857. https://doi.org/10.1371/journal.pone.0017857

Braun de Torrez, E. C., Brown, V. A., McCracken, G. F., & Kunz, T. H. (2019). Sympatric bat species prey opportunistically on a major moth pest of pecans. Sustainability, 11(22), 6365. https://doi.org/10.3390/su11226365

Brigham, R.M. (2007). Bats in forests: what we know and what we need to learn. In: Lacki, M.L., Hayes, J.P., Kurta, A. (Eds.), Bats in forests: conservation and management. Johns Hopkins, Baltimore, Maryland, USA, pp. 1-15.

Brudvig, L. A., Grman, E., Habeck, C. W., Orrock, J. L., & Ledvina, J. A. (2013). Strong legacy of agricultural land use on soils and understory plant communities in longleaf pine woodlands. Forest Ecology and Management, 310, 944-955. https://doi.org/10.1016/j.foreco.2013.09.053

Charbonnier, Y., Barbaro, L., Theillout, A. & Jactel, H. (2014). Numerical and functional responses of forest bats to a major insect pest in pine plantations. PLoS ONE 10(1), e0117652. https://doi.org/10.1371/journal.pone.0117652

Clare, E. L., Fraser, E. E., Braid, H. E., Fenton, M. B., & Hebert, P. D. (2009). Species on the menu of a generalist predator, the eastern red bat (Lasiurus borealis): using a molecular approach to detect arthropod prey. Molecular Ecology, 18(11), 2532-2542. https://doi.org/10.1111/j.1365-294X.2009.04184.x

Clare, E. L., Symondson, W. O., & Fenton, M. B. (2014). An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). Molecular Ecology, 23(15), 3633-3647. https://doi.org/10.1111/mec.12519

Clare, E. L., Chain, F. J., Littlefair, J. E., & Cristescu, M. E. (2016). The effects of parameter choice on defining molecular operational taxonomic units and resulting ecological analyses of metabarcoding data. Genome, 59(11), 981-990. https://doi.org/10.1139/gen-2015-0184

Collins, J., & Jones, G. (2009). Differences in bat activity in relation to bat detector height: implications for bat surveys at proposed windfarm sites. Acta Chiropterologica, 11(2), 343-350. https://doi.org/10.3161/150811009X485576

Comer, C. E., Stuemke, L. A., Morrison, M. L., & Maxey, R. W. (2014). Comparison of systematic roost searches and acoustic detection to determine occupancy of rare forest bats. Wildlife Society Bulletin, 38(1), 103-110. https://doi.org/10.1002/wsb.375

Cryan, P.M. (2003). Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in North America. Journal of Mammalogy, 84, 579-593. https://doi.org/10.1644/1545-1542(2003)084<0579:SDOMTB>2.0.CO;2

Cryan, P. M., Gorresen, P. M., Hein, C. D., Schirmacher, M. R., Diehl, R. H., Huso, M. M., ... & Dalton, D. C. (2014). Behavior of bats at wind turbines. Proceedings of the National Academy of Sciences, 111(42), 15126-15131. https://doi.org/10.1073/pnas.140667211

De La Cruz, J. L., True, M. C., Taylor, H., Brown, D. C., & Ford, W. M. (2022). Unique land cover classification to assess day-roost habitat selection of northern long-eared bats on the Coastal Plain of North Carolina, USA. Forests, 13(5), 792. https://doi.org/10.3390/f13050792

Dodd, L. E., Lacki, M. J., Britzke, E. R., Buehler, D. A., Keyser, P. D., Larkin, J. L., ... & Rieske, L. K. (2012). Forest structure affects trophic linkages: how silvicultural disturbance impacts bats and their insect prey. Forest Ecology and Management, 267, 262-270. https://doi.org/10.1016/j.foreco.2011.12.016

Dupouey, J. L., Dambrine, É., Laffite, J. D., & Moares, C. (2002). Irreversible impact of past land use on forest soils and biodiversity. Ecology, 83(11), 2978-2984. https://doi.org/10.1890/0012-9658(2002)083[2978:IIOPLU]2.0.CO;2

Ferreira, A. I. S., Da Silva, N. F. F., Mesquita, F. N., Rosa, T. C., Monzón, V. H., & Mesquita-Neto, J. N. (2023). Automatic acoustic recognition of pollinating bee species can be highly improved by Deep Learning models accompanied by pre-training and strong data augmentation. Frontiers in Plant Science, 14, 1081050. https://doi.org/10.3389/fpls.2023.1081050

Flaquer, C., Torre, I., & Arrizabalaga, A. (2007). Comparison of sampling methods for inventory of bat communities. Journal of Mammalogy, 88(2), 526-533. https://doi.org/10.1644/06-MAMM-A-135R1.1

Fleming, T., & Eby, P. (2003). Ecology of bat migration. Bat Ecology. Chicago, Illinois: The University of Chicago Press. pp. 156-208.

Fox, T. R., Jokela, E. J., & Allen, H. L. (2007). The development of pine plantation silviculture in the southern United States. Journal of Forestry, 105(7), 337-347. https://doi.org/10.1093/jof/105.7.337

Frick, Winifred F., Sébastien J. Puechmaille, Joseph R. Hoyt, Barry A. Nickel, Kate E. Langwig, Jeffrey T. Foster, Kate E. Barlow et al. "Disease alters macroecological patterns of North American bats." Global Ecology and Biogeography 24, no. 7 (2015): 741-749. https://doi.org/10.1111/geb.12290

Frick, W. F., Kingston, T., & Flanders, J. (2020). A review of the major threats and challenges to global bat conservation. Annals of the New York Academy of Sciences, 1469(1), 5-25. https://doi.org/10.1111/nyas.14045

Gallagher, M. E., Farrell, S. L., Germain, R. H., & Rojas, V. G. (2021). Summer bat habitat use and forest characteristics in managed northeastern forests. Journal of Forestry, 119(3), 305-318. https://doi.org/10.1093/jofore/fvaa059

Garin, I., Aihartza, J., Goiti, U., Arrizabalaga-Escudero, A., Nogueras, J., & Ibáñez, C. (2019). Bats from different foraging guilds prey upon the pine processionary moth. PeerJ, 7, e7169. https://doi.org/10.7717/peerj.7169

Grider, J. F., Larsen, A. L., Homyack, J. A. & Kalcounis-Ruepell, M. C. (2016). Winter activity of Coastal Plain populations of bat species affected by White-Nose Syndrome and wind energy facilities. PLoS ONE, 11(11), e0166512. https://doi.org/10.1371/journal.pone.0166512

Harper, L. R., Watson, H. V., Donnelly, R., Hampshire, R., Sayer, C. D., Breithaupt, T., & Hänfling, B. (2020). Using DNA metabarcoding to investigate diet and niche partitioning in the native European otter (*Lutra lutra*) and invasive American mink (*Neovison vison*). Metabarcoding and Metagenomics 4: e56087. https://doi.org/10.3897/mbmg.4.56087

Hedman, C. W., Grace, S. L., & King, S. E. (2000). Vegetation composition and structure of southern coastal plain pine forests: an ecological comparison. Forest Ecology and Management, 134(1-3), 233-247. https://doi.org/10.1016/S0378-1127(99)00259-5

Hein, C. D., Castleberry, S. B., & Miller, K. V. (2008). Male Seminole bat winter roost-site selection in a managed forest. The Journal of Wildlife Management, 72(8), 1756-1764. https://doi.org/10.2193/2007-595

Hope, P. R., Bohmann, K., Gilbert, M. T. P., Zepeda-Mendoza, M. L., Razgour, O., & Jones, G. (2014). Second generation sequencing and morphological faecal analysis reveal unexpected foraging behaviour by *Myotis nattereri* (Chiroptera, Vespertilionidae) in winter. Frontiers in zoology, 11, 1-15. https://doi.org/10.1186/1742-9994-11-39

Hughes, M. J., de Torrez, E. C. B., & Ober, H. K. (2021). Big bats binge bad bugs: Variation in crop pest consumption by common bat species. Agriculture, Ecosystems & Environment, 314, 107414. https://doi.org/10.1016/j.agee.2021.107414

Humphries, M. M., Kramer, D. L., & Thomas, D. W. (2003). The role of energy availability in mammalian hibernation: an experimental test in free-ranging eastern chipmunks. Physiological and Biochemical Zoology, 76(2), 180-186. https://doi.org/10.1086/367950

Janzen, D.H., & Schoener, T. W. (1968). Differences in insect abundance and diversity between wetter and drier sites during a tropical dry season. Ecology, 49: 96-110. https://doi.org/10.2307/1933565

Jantzen, M. K., & Fenton, M. B. (2013). The depth of edge influence among insectivorous bats at forest–field interfaces. Canadian Journal of Zoology, 91(5), 287-292. https://doi.org/10.1139/cjz-2012-0282

Jordan, G. W. (2020). Status of an anomalous population of northern long-eared bats in coastal North Carolina. Journal of Fish and Wildlife Management, 11(2), 665-678. https://doi.org/10.3996/JFWM-20-018

Johnson, S. A., & Chambers, C. L. (2017). Effects of ponderosa pine forest restoration on habitat for bats. Western North American Naturalist, 77(3), 355-368. https://doi.org/10.3398/064.077.0308

Kunz, T. H., Arnett, E. B., Erickson, W. P., Hoar, A. R., Johnson, G. D., Larkin, R. P., ... & Tuttle, M. D. (2007). Ecological impacts of wind energy development on bats: questions, research needs, and hypotheses. Frontiers in Ecology and the Environment, 5(6), 315-324. https://doi.org/10.1890/1540-9295(2007)5[315:EIOWED]2.0.CO;2

Kunz, T. H., Braun de Torrez, E., Bauer, D., Lobova, T., & Fleming, T. H. (2011). Ecosystem services provided by bats. Annals of the New York Academy of Sciences, 1223(1), 1-38. https://doi.org/10.1111/j.1749-6632.2011.06004.x

Kurta, A., Bell, G. P., Nagy, K. A., & Kunz, T. H. (1989). Energetics of pregnancy and lactation in free-ranging little brown bats (*Myotis lucifugus*). Physiological zoology, 62(3), 804-818. https://doi.org/10.1086/physzool.62.3.30157928

Lemen, C. A., Freeman, P. W., & White, J. A. (2016). Acoustic evidence of bats using rock crevices in winter: a call for more research on winter roosts in North America. Transactions of the Nebraska Academy of Sciences, 36: 9-13.

Li, Y., Zhao, M., Motesharrei, S., Mu, Q., Kalnay, E., & Li, S. (2015). Local cooling and warming effects of forests based on satellite observations. Nature Communications, 6(1), 6603. https://doi.org/10.1038/ncomms7603

Loeb, S. C. (2020). Qualitative synthesis of temperate bat responses to silvicultural treatments—where do we go from here? Journal of Mammalogy, 101(6), 1513-1525. https://doi.org/10.1093/jmammal/gyaa089

López-Baucells, A., Torrent, L., Rocha, R., Bobrowiec, P. E., Palmeirim, J. M., & Meyer, C. F. (2019). Stronger together: combining automated classifiers with manual post-validation optimizes the workload vs reliability trade-off of species identification in bat acoustic surveys. Ecological Informatics, 49, 45-53. https://doi.org/10.1016/j.ecoinf.2018.11.004

Jorge, M. H., Sweeten, S. E., True, M. C., Freeze, S. R., Cherry, M. J., Garrison, E. P., ... & Ford, W. M. (2021). Fire, land cover, and temperature drivers of bat activity in winter. Fire Ecology, 17(1), 19. https://doi.org/10.1186/s42408-021-00105-4

Maslo, B., Mau, R. L., Kerwin, K., McDonough, R., McHale, E., & Foster, J. T. (2022). Bats provide a critical ecosystem service by consuming a large diversity of agricultural pest insects. Agriculture, Ecosystems & Environment, 324, 107722. https://doi.org/10.1016/j.agee.2021.107722

Mickleburgh, S. P., Hutson, A. M., & Racey, P. A. (2002). A review of the global conservation status of bats. Oryx, 36(1), 18-34. https://doi.org/10.1017/S0030605302000054

Müller, J., Mehr, M., Bässler, C., Fenton, M. B., Hothorn, T., Pretzsch, H., ... & Brandl, R. (2012). Aggregative response in bats: prey abundance versus habitat. Oecologia, 169, 673-684. https://doi.org/10.1007/s00442-011-2247-y

Müller, J., Mitesser, O., Schaefer, H. M., Seibold, S., Busse, A., Kriegel, P., ... & Buřivalová, Z. (2023). Soundscapes and deep learning enable tracking biodiversity recovery in tropical forests. Nature Communications, 14(1), 6191. https://www.nature.com/articles/s41467-023-41693-w

O’Rourke, D., Rouillard, N. P., Parise, K. L., & Foster, J. T. (2022). Spatial and temporal variation in New Hampshire bat diets. Scientific Reports, 12(1), 14334. https://doi.org/10.1038/s41598-022-17631-z

Oswalt, S.N., Smith, W. B., Miles, P. D. & Pugh, S. A. (2019). Forest resources of the United States 2017: A technical document supporting the Forest Service update of the 2020 RPA Assessment. USDA Forest Service, General Technical Support WO-97.

Parker, Jr, K. A., Li, H., & Kalcounis-Rueppell, M. C. (2020). Species-specific environmental conditions for winter bat acoustic activity in North Carolina, United States. Journal of Mammalogy, 101(6), 1502-1512, https://doi.org/10.1093/jmammal/gyaa133

Parsons, S., & Jones, G. (2000). Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. Journal of Experimental Biology, 203(17), 2641-2656. https://doi.org/10.1242/jeb.203.17.2641

Perea, S., & Tena, E. (2020). Different bat detectors and processing software… Same results? Journal of Bat Research & Conservation, 13(1): 1-5. https://doi.org/10.14709/BarbJ.13.1.2020.01

Perea, S., Yearout, J. A., Ferrall, E. A., Morris, K. M., Pynne, J. T., & Castleberry, S. B. (2022). Seven-year impact of white-nose syndrome on tri-colored bat (*Perimyotis subflavus*) populations in Georgia, USA. Endangered Species Research, 48, 99-106. https://doi.org/10.3354/esr01189

Pijanowski, B. C., Villanueva-Rivera, L. J., Dumyahn, S. L., Farina, A., Krause, B. L., Napoletano, B. M., ... & Pieretti, N. (2011). Soundscape ecology: the science of sound in the landscape. BioScience, 61(3), 203-216. https://doi.org/10.1525/bio.2011.61.3.6

Russo, D., & Jones, G. (2002). Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. Journal of Zoology, 258(1), 91-103. https://doi.org/10.1017/S0952836902001231

Russo, D., & Jones, G. (2003). Use of foraging habitats by bats in a Mediterranean area determined by acoustic surveys: conservation implications. Ecography, 26(2), 197-209. https://doi.org/10.1034/j.1600-0587.2003.03422.x

Russo, D., & Voigt, C. C. (2016). The use of automated identification of bat echolocation calls in acoustic monitoring: A cautionary note for a sound analysis. Ecological Indicators, 66, 598-602. https://doi.org/10.1016/j.ecolind.2016.02.036

Russo, D., Ancillotto, L., & Jones, G. (2018). Bats are still not birds in the digital era: echolocation call variation and why it matters for bat species identification. Canadian Journal of Zoology, 96(2), 63-78. https://doi.org/10.1139/cjz-2017-0089

Salinas-Ramos, V. B., Ancillotto, L., Bosso, L., Sánchez-Cordero, V., & Russo, D. (2020). Interspecific competition in bats: state of knowledge and research challenges. Mammal Review, 50(1), 68-81. https://doi.org/10.1111/mam.12180

Schwab, N. A. (2014). Winter acoustic activity of bats in Montana. Northwestern Naturalist, 95(1), 13-27. https://doi.org/10.1898/NWN13-03.1

Shivaprakash, K. N., Swami, N., Mysorekar, S., Arora, R., Gangadharan, A., Vohra, K., ... & Kiesecker, J. M. (2022). Potential for artificial intelligence (AI) and machine learning (ML) applications in biodiversity conservation, managing forests, and related services in India. Sustainability, 14(12), 7154. https://doi.org/10.3390/su14127154

Shute, K. E., Loeb, S. C., & Jachowski, D. S. (2021). Seasonal shifts in nocturnal habitat use by coastal bat species. The Journal of Wildlife Management, 85(5), 964-978, https://doi.org/10.1002/jwmg.22060

Simmons, N. B., & Cirranello, A. L.. (2023). Bat species of the world: a taxonomic and geographic database [online]: Available from https://batnames.org

Stephens, D. W. & Krebs, J. R. (1986). Foraging theory. Princeton University Press. 247 p.

Rydell, J., Nyman, S., Eklöf, J., Jones, G., & Russo, D. (2017). Testing the performances of automated identification of bat echolocation calls: A request for prudence. Ecological indicators, 78, 416-420. https://doi.org/10.1016/j.ecolind.2017.03.023

Rytkönen, S., Vesterinen, E. J., Westerduin, C., Leviäkangas, T., Vatka, E., Mutanen, M., ... & Orell, M. (2019). From feces to data: A metabarcoding method for analyzing consumed and available prey in a bird-insect food web. Ecology and evolution, 9(1), 631-639. https://doi.org/10.1002/ece3.4787

Teixeira, D., Roe, P., van Rensburg, B. J., Linke, S., McDonald, P. G., Tucker, D., & Fuller, S. (2024). Effective ecological monitoring using passive acoustic sensors: Recommendations for conservation practitioners. Conservation Science and Practice, e13132. https://doi.org/10.1111/csp2.13132

Tena, E., Fandos, G., de Paz, Ó., de La Peña, R., & Tellería, J. L. (2020). Size does matter: Passive sampling in urban parks of a regional bat assemblage. Urban Ecosystems, 23(2), 227-234. https://doi.org/10.1007/s11252-019-00913-2

Tena, E., Paz, O. D., Peña, R. D., Fandos, G., Redondo, M. & Tellería, J. (2020). Mind the gap: Effects of canopy clearings on temperate forest bat assemblages. Forest Ecology and Management, 474, 118341. https://doi.org/10.1016/j.foreco.2020.118341

Tiede, J., Wemheuer, B., Traugott, M., Daniel, R., Tscharntke, T., Ebeling, A., & Scherber, C. (2016). Trophic and non-trophic interactions in a biodiversity experiment assessed by next-generation sequencing. PLoS One, 11(2), e0148781. https://doi.org/10.1371/journal.pone.0148781

Vaughan, N., Jones, G., & Harris, S. (1997). Habitat use by bats (Chiroptera) assessed by means of a broad-band acoustic method. Journal of Applied Ecology, 716-730. https://www.jstor.org/stable/2404918

Vellend, M., Verheyen, K., Flinn, K.M., Jacquemyn, H., Kolb, A., Calster, H.V., Peterken, G., Graae, B.J., Bellemare, J., Honnay, O., Brunet, J., Wulf, M., Gerhardt, F., and Hermy, M. 2007. Homogenization of forest plant communities and weakening of species-environment relationships via agricultural land use. Journal of Ecology 95:565-573. https://doi.org/10.1111/j.1365-2745.2007.01233.x

Vindigni, M. A., Morris, A. D., Miller, D. A., & Kalcounis-Rueppell, M. C. (2009). Use of modified water sources by bats in a managed pine landscape. Forest Ecology and Management, 258(9), 2056-2061. https://doi.org/10.1016/j.foreco.2009.07.058

Voigt, C. C., & Kingston, T. (2016). Bats in the Anthropocene (pp. 1-9). Springer International Publishing.

Walters, C. L., Freeman, R., Collen, A., Dietz, C., Brock Fenton, M., Jones, G., ... & Jones, K. E. (2012). A continental-scale tool for acoustic identification of European bats. Journal of Applied Ecology, 49(5), 1064-1074. https://doi.org/10.1111/j.1365-2664.2012.02182.x

Weinkauf, C. J., Comer, C. E., Conway, W. C., & Farrell, C. (2018). Dietary composition of four common chiropteran species in a bottomland hardwood forest. Acta Chiropterologica, 20(1), 195-205. https://doi.org/10.3161/15081109ACC2018.20.1.015

Weller, T. J., & Zabel, C. J. (2002). Variation in bat detections due to detector orientation in a forest. Wildlife Society Bulletin, 30(3), 922-930. https://www.jstor.org/stable/3784248

Whitaker Jr, J. O. (1995). Food of the big brown bat Eptesicus fuscus from maternity colonies in Indiana and Illinois. American Midland Naturalist, 346-360. https://doi.org/10.2307/2426304

Wickham, J., Stehman, S. V., Sorenson, D. G., Gass, L. & Dewitz, J. A. (2023). Thematic accuracy assessment of the NLCD 2019 land cover for the conterminous United States. GISci. Remote Sensing, 60(1), 2181143. https://doi.org/10.1080/15481603.2023.2181143

Wieringa, J. G., Carstens, B. C., & Gibbs, H. L. (2021). Predicting migration routes for three species of migratory bats using species distribution models. PeerJ, 9, e11177. https://doi.org/10.7717/peerj.11177

Wigley, T. B., Miller, D. A. & Yarrow, G. K. (2007). Planning for bats on forest industry lands in North America. Pages 293-318 in Bats in forests: conservation and management. Baltimore, MD: Johns Hopkins.

Williams, M. (1992). Americans and their forests: a historical geography. Cambridge University Press.

White, J. A., Andersen, B. R., Otto, H. W., Lemen, C. A., & Freeman, P. W. (2014). Winter activity of bats in southeastern Nebraska: An acoustic study.

Zobrist, K. W., Hinckley, T. M., Andreu, M. G., Gehringer, K. R., Hedman, C. W., Forest, S., & Lippke, B. R. (2005). Final project report to the National Commission on Science for Sustainable Forestry (NCSSF).

CHAPTER 2

BAT WINTER FORAGING HABITAT USE IN WORKING FORESTS: A MULTISPECIES SPATIAL OCCUPANCY APPROACH[1]

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[1] Santiago Perea, Guillermo Fandos, Angela L. Larsen-Gray, Daniel U. Greene, Richard Chandler, and Steven B. Castleberry. 2024. Animal Conservation 27(4): 478-491. Reprinted here with the permission of the publisher.

ABSTRACT

Insectivorous bats in temperate zones have evolved strategies such as migration or hibernation to overcome challenges of reduced resource availability and increased energy demand during winter. In the southeastern United States Coastal Plain, bats are either year-round residents and remain active during winter or are migrants from colder areas seeking milder temperatures. Southeastern Coastal Plain forests also may represent important areas for remnant populations of species impacted by white-nose syndrome. Working pine (*Pinus* spp.) forests comprise a large proportion of southeastern Coastal Plain forests, yet winter bat habitat associations and how forest management affects bat use remain understudied. Hence, we used hierarchical multispecies spatial occupancy models to evaluate factors influencing winter bat occupancy and foraging habitat associations in working forests of the southeastern Coastal Plain. From January to March 2020–2022, we deployed Anabat Swift acoustic detectors and measured site- and landscape-level covariates on six working landscapes. We detected five species of bats and three species groups at 93% (224/240) of sites. We observed higher species richness at sites with high proportions of contiguous forest and low levels of basal area. At the species level, occupancy patterns were influenced by site and landscape covariates, which had varying effects on species with distinct foraging strategies. Temperature was an important predictor of detectability. Our findings offer new insights into the ecology of bats in working forest landscapes during winter, where we highlight positive responses in occupancy with contiguous forests and lower levels of basal area, as in previous summer work. By providing valuable information on winter community composition and foraging habitat associations, we hope to guide management decisions for forest attributes important to these species, thus increasing conservation opportunities within working forests.

INTRODUCTION

Understanding how environmental variables drive species-level patterns and shape community structure is fundamental to ecology and conservation. In temperate regions, some species, including bats, have evolved thermoregulatory adaptations (e.g., torpor) to overcome winter challenges of reduced resource availability and increased energy demand by seasonally altering their active state (Humphries & Thomas, 2003; Humphries *et al*., 2017; de Bruyn *et al*., 2021). Alternatively, some bat species cope with cold winters by migrating to warmer regions for overwintering (Cryan, 2003; Grider *et al*., 2016; Frazer *et al*., 2017). Winter activity patterns are directly related to climate conditions, particularly temperature, which influences both thermoregulatory behaviors and hibernation traits (Stawski & Geiser, 2011; Barros *et al*., 2021), as well as whether their insect prey remain active (Grider *et al*., 2016; Welti *et al*., 2022). In temperate regions at lower latitudes, coinciding with winter destinations of migratory species, torpor breaks are frequent, allowing bats to be year-round residents and rely on a combination of activity and short periods of torpor (Boyles *et al*., 2006; Barros *et al*., 2017; Mas *et al*., 2022). These species, however, are subject to changing environmental conditions and shifting insect availability, resulting in a diversity of winter activity patterns (Czenze & Willis, 2015).

Unlike regions farther north, warmer temperatures of the southeastern United States Coastal Plain favor resident bats that remain active year-round, and migratory bats that overwinter (Grider *et al*., 2016). This region is characterized by its large expanse of working pine (*Pinus* spp.) forest landscapes (approximately 15.8 million ha), about 90% of which are privately owned (Oswalt *et al*., 2019). In temperate regions, most bats are associated with forests for roosting or foraging, creating a need to understand how bat communities use forests and how forest management influences bat use (Brigham, 2007; Gallagher *et al*., 2021). Forest management practices such as thinning, clearcutting, prescribed burning, and other activities can affect habitat use by bats (Wigley *et al*., 2007; Bender *et al*., 2015; Cox *et al*., 2016; Węgiel *et al*., 2019; Taylor *et al*., 2020) by modifying forest structure, affecting distribution and abundance of living and dead trees used for roosting, and affecting the number of forest openings and edges used for foraging (Morris *et al*., 2010; Tena *et al*., 2020).

Understanding bat community- and species-level habitat use is reliant upon the ability to effectively detect bat roost and/or foraging presence. The probability of detecting bats depends on several factors, such as species behavior and physical traits, forest structure, or survey-related characteristics, making some species more detectable than others (Devarajan *et al*., 2020). Additionally, for elusive species such as bats, imperfect detection is an essential source of bias when assessing species richness among communities (Kéry & Schmidt, 2008; Dorazio *et al*., 2011). Recently, hierarchical multispecies detection/non-detection modeling was developed to address questions about habitat associations, including both community-level and species-specific responses, while addressing important complexities such as imperfect detection (MacKenzie *et al*., 2002; Dorazio & Royle, 2005; Guillera-Arroita *et al*., 2010), spatial autocorrelation (Finley *et al*., 2009; Banerjee *et al*., 2014), and residual species correlations (Ovaskainen *et al*., 2010). Controlling for other sources of bias, such as spatial autocorrelation, is key to identifying underlying processes or factors that influence observed patterns. Thus, spatially explicit models are fundamental for conservation and management decisions (Bateman *et al*., 2020).

Working forest owners and managers are increasingly committed to conserving biodiversity, as evidenced by voluntary enrollment in sustainable forestry certification programs that include biodiversity principles (Wigley *et al*., 2007). Given the geographic scale and economic and social importance of privately owned working forests (Oswalt *et al*., 2019), understanding how biodiversity can be conserved in managed landscapes is imperative (Demarais *et al*., 2017; Yeiser *et al*., 2018). However, limited data on foraging ecology and selection of foraging areas by bats in working forest landscapes, especially outside the growing season, hinders our ability to evaluate management decisions. Currently, management decisions are based largely on knowledge of bat habitat relationships during summer. If and how these relationships are consistent with relationships in other seasons is poorly understood. Thus, investigations into winter habitat use are needed to ensure that management actions provide suitable habitat conditions year-round. Hence, we used a multispecies spatial occupancy modeling approach that explicitly accounts for imperfect detection, spatial autocorrelation, and species correlations to examine winter bat associations on working forest lands across the southeastern United States Coastal Plain. Previous studies have observed that bat communities are shaped by landscape features at different spatial scales (Loeb & O'Keefe, 2006; Bender *et al*., 2015; Rodríguez-San Pedro & Simonetti, 2015). Therefore, we examined the influence of site- and landscape-level habitat characteristics on species richness to study the effect on winter bat community composition. We also determined species-specific winter foraging habitat occupancy at site- and landscape-levels.

MATERIALS AND METHODS

Study area

We conducted our study on six working forest landscapes across six states (Florida, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina) of the southeastern United States Coastal Plain during 2020–2022 (Figure 1). All study areas consisted primarily of planted loblolly pine (P. taeda) stands interspersed with streamside management zones (predominantly mature hardwood trees), roads, and wildlife openings, with other non-forest areas accounting for the remaining land area. We selected study areas >3 000 ha and comprised primarily of upland planted pine with <15% in forested wetlands. Management activities were typical of commercial forestry operations in the region, including clear-cutting at 20–35 years, mechanical and/or chemical site preparation, and planting 182–283 pine trees ha^{-1} (Gresham, 2002). Competing vegetation was temporarily suppressed through herbicide applications, prescribed fire, or mechanically, with most stands being thinned at least once.

Bat acoustic sampling

On each study area, we created a 900 × 900 m grid and used ArcGIS Pro 2.8.0 (ESRI, Redlands, California, U.S.) to randomly select grid intersections as sampling points. The grid spacing was selected to ensure that the distance between sampling points encompassed a core area that constituted much of an individual bat's foraging movements (Morris *et al*., 2011; Bender *et al*., 2015). We surveyed 40 sampling points randomly selected from the grid on each study area to ensure enough samples to adequately represent variation in stand age, stand size, and management history. We sampled all points at each study area within a 1-month period. We defined January–March as the winter sampling season as mean nightly temperatures are lowest (typically <10°C) during this time throughout most of the Coastal Plain region (NOAA

Climate.gov. https://www.climate.gov/maps-data/data-snapshots/averagetemp-monthly-1981-2010-cmb-0000-02-00?theme=Temperature).

At each sampling point, we deployed Anabat Swift acoustic detectors with omnidirectional ultrasonic microphones US-OV2 and US-OV3 (Titley Electronics, Ballina, New South Wales, Australia; Appendix S2.1. Table 1) for three consecutive nights, recording from 30 min before sunset to 30 min after sunrise (Reichert *et al*., 2018). If rain occurred during the sampling period, we left detectors out for additional nights to ensure three nights of rain-free sampling. We placed detectors on poles with microphones 3 m above the forest floor pointed in the direction of the least vegetation clutter (Weller & Zabel, 2002). We coupled each detector with a temperature logger (HOBO Pendant G Acceleration Data Logger, Onset Computer Corp., Pocasset, Massachusetts, U.S.) programmed to record hourly temperature.

Bat call analysis

We used auto-ID software and subsequent visual vetting to identify calls to species, as recommended by the North American Bat Monitoring Program (NABat; Reichert *et al*., 2018). We first used Kaleidoscope Pro 5.4.1 software (Wildlife Acoustics Inc., Maynard, Massachusetts, U.S.) to filter noise files. We selected default filter setting parameters for bat analysis specifying a signal of interest between 8 and 120 kHz, 2 to 500 ms, and at least 2 pulses per sequence. We used the batch function in Kaleidoscope Pro to split each sequence to a maximum duration of 10 s for standardization. We selected the auto classifier of Kaleidoscope Pro with a balanced sensitivity level for classification to assist the visual vetting. Subsequently, we manually analyzed all remaining files using call structure, frequency of minimum and maximum energy, characteristic frequency, duration, inter-pulse interval, and slope (O'Farrell & Gannon, 1999; Szewczak et al., 2011). We grouped bat passes into species groups for *Lasiurus borealis*/*L. seminolus*, *Eptesicus fuscus*/*Lasionycteris noctivagans*, and Myotis austroriparius/M. septentrionalis due to overlap in acoustic call characteristics between these species (Grider *et al*., 2016; Johnson & Chambers, 2017; Kunberger & Long, 2022).

Habitat and landscape metrics

We measured three components of vegetation structure at each sampling point (Appendix 2.2. Table S2). First, we used a convex spherical densiometer (Forestry Suppliers Inc., Jackson, Mississippi, U.S.) to measure percent canopy openness, which can be managed via planting density, by averaging measurements taken at the acoustic point and four additional locations in each cardinal direction 5 m from the point. Second, we characterized vegetation clutter, which can relate to forest management through mechanical, chemical, and prescribed burning practices, using methods based on Nudds (1977) and modified by Bender *et al*. (2015). To do so, we estimated average percent coverage of a 1 m^2 panel raised 4.5 m above the ground and 5 m from the acoustic point in each cardinal direction and in the direction the microphone was oriented. Third, we used a 10-factor prism (Husch *et al*., 2003) centered at the acoustic detector point to estimate basal area (m^2 ha^{-1}) of overstory trees, which again can relate to planting density, thinning, and other forest management activities.

We used ArcGIS Pro and Fragstats v4.2 (McGarigal *et al*., 2015) to calculate landscape metrics from landowner-provided and publicly available data (Appendix 2.2. Table S2). Although variables at this scale cannot be managed directly, they may be important for managers to consider when implementing landscape-scale planning. We measured proportions of forest and wetland cover types and determined total edge (m) as landscape composition metrics within a 450-m-radius circular buffer around sampling points. The 450-m buffer area represented the area that did not overlap with the buffers of neighboring sampling points. We defined edge as the boundary between any two of six cover types reclassified from the National Land Cover Database (Dewitz & U.S. Geological Survey, 2021). We grouped forest stands into growth stages (hereafter, stand age; 0–3 [early establishment], 4–7 [closing canopy], 8–13 [closed canopy, pre-thinned], 14–20 years [mid-rotation thinned], or 21+ years old [mature forest, semi-closed canopy; including streamside management zones/bottomland hardwood forests]) as it can relate to forest management activities (e.g., thinning, final harvest) and is easily interpreted by forest managers (Marshall *et al*., 2022). Lastly, we measured distance (m) from sampling points to roads and permanent water using the Near tool in ArcGIS Pro.

Multi-species modeling

We implemented the hierarchical multispecies spatial occupancy model developed by Doser *et al*. (2022). The hierarchical model, which consists of an ecological process model and an observation sub-model, accounts for residual species correlation in a joint species distribution model framework while considering imperfect detection. The model quantifies the probability of occupancy for each species by accounting for factors influencing detection (MacKenzie *et al*., 2018). This hierarchical approach, in which species-specific effects are treated as random effects arising from a common community-level distribution, allows for inference of management effects on individual species and overall communities (Zipkin *et al*., 2010). The ecological process model is $z_{i,j}$, the true state of presence or absence of species i at sites j. Similar to Tikhonov *et al*. (2020), this model uses a spatial factor model along with Nearest Neighbor Gaussian Processes (NNGP; Datta *et al*., 2016) to ensure computational efficiency of species assemblages at different spatial locations. The observational sub-model (detection sub-model hereafter) separately models imperfect detection from the latent ecological process (see Doser *et al*., 2022 for the modeling framework).

Occupancy covariates included a combination of site- (basal area, canopy openness, and vegetation clutter) and landscape-level (total forest, total wetland, total edge, distance to freshwater, distance to roads, and stand age). We expected the influence of covariates on bat species to differ depending on their foraging strategy (Appendix S1: Table S2). Detection covariates included basal area, temperature at sunset, vegetation clutter, and year. We standardized all continuous covariates for both ecological and survey processes to a mean of 0 and a standard deviation equal to 1 (Zipkin *et al*., 2009; Kéry & Royle, 2015). We tested for correlation among continuous predictor variables using Pearson's correlation coefficient to ensure that highly correlated ($r \geq |0.7|$) variables were not included in the same model.

We fit our models using Polya-Gamma data augmentation (Polson *et al*., 2013) for computational efficiency in R version 4.4.1 (R Core Team, 2020) via package spOccupancy (function sfMsPGOcc; Doser *et al*., 2022). Accommodating sources of spatial dependence among observations is key to obtaining valid inferences about species occupancy (Doser *et al*., 2022), thus we fit a spatial factor model to control for spatial correlations and residual spatial variation in species occurrence. We implemented spatial models using three replicate Markov chain Monte Carlo (MCMC) iterations to generate 10,000 samples from the posterior distribution of each model after discarding a "burn-in" of 5,000 samples, with a thinning rate of 50. We selected an exponential covariance to model spatial dependence structure among observations (Banerjee *et al*., 2014). We estimated model parameters and community summaries, setting default vague prior hyperparameter values: *hypermeans* to 0 and *hypervariances* to 2.72 (Banerjee *et al*., 2014) in Normal priors, and scale and shape parameters to 0.1 (Lunn *et al*., 2013) in inverse-Gamma priors. To control spatial autocorrelation, the spatial decay phi for each latent factor followed a uniform Unif (0, 10) distribution. We determined model convergence of Markov chains using R-hat statistic values (<1.1) for all parameters within the models (Brooks & Gelman, 1998). We used the Widely Applicable Information Criterion (WAIC; Watanabe, 2010) to compare our set of models and shortlist the best-performing models, with models with a ΔWAIC < 2 being biologically plausible and relevant. To evaluate detection covariates, we constructed models of single and all possible additive combinations of variables and compared them by including an occupancy sub-model with only the spatial structure, and no covariates. Temperature at sunset was the top-ranked detection model (Appendix S1: Table S3) and was subsequently included as the only covariate in the detection sub-model. We then developed 25 spatial models that included single and additive combinations of covariates, along with null and global models, in the occupancy sub-models and temperature at sunset in the detection sub-model (Appendix S1: Table S4). We calculated posterior mean and standard deviation of the model coefficients with 95% Bayesian credible intervals (BCI). Parameter estimates of covariates with BCI that did not cross 0 were considered important predictors of species occupancy, as this was reflective of a consistent relationship within model iterations. However, we also considered covariates as biologically meaningful if estimated 75% BCIs did not overlap zero, although the 95% BCIs overlapped zero (Cumming & Finch, 2005; Nakagawa & Cuthill, 2007; Tilker *et al*., 2020). We computed Bayesian P-values with Freeman-Tukey statistic to assess model fit, where a model with a good fit to the data had a value near 0.5, while values <0.1 or >0.9 suggested poor model fit (Gelman *et al*., 1996; Hobbs & Hooten, 2015).

RESULTS

We identified 26,650 bat passes and detected bats at 93% (224/240) of sampling points across all study areas during 738 detector nights. We detected 5 species and 3 species groups: *Dasypterus intermedius*, *L. cinereus*, *Nycticeius humeralis*, *Perimyotis subflavus*, *Tadarida brasiliensis*, *E. fuscus*/*L. noctivagans*, *L. borealis*/*L. seminolus*, and *M. austroriparius*/*M. septentrionalis*. All species were detected at all study sites except *T. brasiliensis*, which was not detected in South Carolina. The most frequently detected species was *L. cinereus* (177/240), followed by *L. borealis*/*L. seminolus* (156/240) and *E. fuscus*/*L. noctivagans* (122/240). *Dasypterus intermedius* was detected at the fewest number of sampling sites (70/240) (Table 1).

Our model selection supported a single model (Table 2). Under the supported model, basal area was an important site-level predictor of bat occupancy, with negative effects on the bat community (Figure 2, Appendix S1: Table S5) and all species except for *M. austroriparius*/*M. septentrionalis* (Figure 3, Appendix S1: Table S6). Posterior probability distributions (95% BCIs) of *L. borealis*/*L. seminolus*, *N. humeralis*, and *T. brasiliensis* did not overlap zero. We observed biologically meaningful (75% BCIs that did not include 0) influences of basal area on occupancy of *E. fuscus*/*L. noctivagans*, *L. cinereus*, *D. intermedius*, and *P. subflavus*. At the landscape level, total forest and distance to roads were biologically meaningful, positively affecting the bat community (Figure 2). Total forest also positively influenced occupancy of several species, being an important predictor for *E. fuscus*/*L. noctivagans* and biologically meaningful for *L. borealis*/*L. seminolus*, *N. humeralis*, and *P. subflavus* (Figure 3). Distance to roads was a biologically meaningful predictor of occupancy with positive relationships for *D. intermedius*, *L. cinereus*, and *M. austroriparius*/*M. septentrionalis*. Three covariates that were not influential at the community level influenced occupancy of individual species/groups (Figure 3, Appendix S1: Table S6). Distance to water had a positive influence on occupancy of *E. fuscus*/*L. noctivagans* and was biologically meaningful for *L. cinereus*. Total wetlands were a biologically meaningful predictor of *E. fuscus*/*L. noctivagans*, negatively affecting occupancy. Lastly, total edge was biologically meaningful and negatively affected occupancy of *M. austroriparius*/*M. septentrionalis*. Temperature at sunset, the only covariate included in the detection sub-model, was an important predictor of detection at both the community (Appendix S1: Table S5) and species-specific levels (Appendix S1: Table S6), positively affecting both mean species detection and individual bat species.

DISCUSSION

Our results add to the sparse literature regarding winter habitat associations for bats in working forest landscapes in the southeastern U.S. Coastal Plain. Like previous work conducted during summer, we found winter bat community richness and occupancy for most individual species/groups were positively associated with forest management practices that promote open canopy conditions. Forest thinning is a common mid-rotation management practice during which trees are selectively removed (thereby reducing basal area) to allocate resources to remaining trees (Verschuyl *et al*., 2011). Consequently, thinning promotes bat activity in mid-rotation stands by creating open space below the canopy for foraging. Furthermore, we found areas surrounded by a greater proportion of forest increased community and species occupancy. The primary objective of most working forest owners and managers is to provide a continuous, sustainable supply of wood products (Miller *et al*., 2009). As a result, working landscapes are comprised of forests of various ages and structural conditions, but with contiguous forests as the predominant land cover. Thus, consistent with results from summer studies (Loeb & O'Keefe, 2006; Brigham, 2007; Bender *et al*., 2015), our results indicate that standard management practices in working forests also provide suitable forest conditions for bats during winter.

Vegetation structure, specifically basal area, negatively influenced community richness and occupancy of most species. While information regarding the effects of vegetation structure on bat community richness in working Coastal Plain forests is limited, higher levels of basal area negatively influencing communities have previously been observed for phonic groups (i.e., categorization of species with similar ecomorphological characteristics based on similar call frequency; Beilke *et al*., 2021;

Gallagher *et al*., 2021). For example, summer occupancy probability was negatively related to increased basal area for all phonic groups on managed forest lands in the northeastern United States, regardless of their foraging strategies (Gallagher *et al*., 2021). Negative responses of bats to increased basal area typically are attributed to reduced flight ability and greater difficulty detecting insect prey (Meyer *et al*., 2004; Bender *et al*., 2021). Although insect abundance, along with vegetation characteristics, is an important predictor of bat activity during summer in Coastal Plain regions (Moore & Best, 2018; Bender *et al*., 2021), in winter, when temperatures are lower, bats may restrict foraging to areas where insects are present (Shute *et al*., 2021). In addition to seasonal changes in foraging, bat activity in forests is mainly determined by the interaction between flight accessibility and prey availability, whereas in open spaces such as forest gaps, prey availability is the primary factor driving activity (Adams *et al*., 2009; Tiago Marques *et al*., 2016; Tena *et al*., 2020; Erasmy *et al*., 2021). In contrast, the *M. austroriparius*/M. *septentrionalis* species group was not affected by higher levels of basal area. The Myotis species we documented are clutter-adapted and thus likely exhibit flexibility in their foraging strategy inside of forests (Norberg & Rayner, 1987; Henderson & Broders, 2008; Beilke *et al*., 2021).

The relationship between summer bat activity and forest composition (i.e., proportion of forest and wetlands) in the southeastern U.S. Coastal Plain is well established (Hein *et al*., 2009; Bender *et al*., 2015; Taylor *et al*., 2020). Importantly, our study demonstrated similar associations between occupancy and prevalence of forested landcover during winter, even for species in different foraging guilds. Greater species richness within larger forest patches is not surprising as all bat species in the region use forests for roosting and/or foraging (Brigham, 2007; Taylor *et al*., 2020). Consequently, we found occupancy of several species associated with higher forest cover. Positive occupancy probabilities for *L. borealis*/*L. seminolus* and *P. subflavus* at sites with higher proportions of forests were expected as these species typically forage along edges adjacent to forests or within forest gaps (Dixon, 2011; Schimpp *et al*., 2018; Beilke *et al*., 2023). Although little is known about *L. noctivagans* foraging habitat selection, the positive relationship between occupancy of *N. humeralis* and *E. fuscus* and proportion of forest we observed, supports previous studies which reported *N. humeralis* and *E. fuscus* using forested areas interspersed with edges and open areas (Duchamp *et al*., 2004; Johnson *et al*., 2008; Schimpp *et al*., 2018; Andersen *et al*., 2022). The lack of a relationship with wetlands observed for most species and lower occupancy of *E. fuscus*/*L. noctivagans* was surprising as most studies have demonstrated positive relationships (Mas *et al*., 2021). Our results may be related to the structural conditions of wetlands in our study areas, which were primarily forested wetlands as opposed to open-water wetlands. Similarly, Andersen *et al*. (2022) observed that bat activity was negatively related to forested wetlands in Coastal Plain working forests during winter. Although bats may forage along the edges, high basal area within forested wetlands likely decreases the probability of occupancy for open/edge space foragers like *E. fuscus*/*L. noctivagans*.

Distance to freshwater is often important in bat habitat selection (e.g., Ford *et al*., 2005; Rainho & Palmeirim, 2011; Janzen & Fenton, 2013), but we found no relationship with bat occupancy at the community level and few at the species level. Where we did find species-level relationships (*E. fuscus*/*L. noctivagans* and *L. cinereus*), they were contrary to our expectations and from previous studies during summer that found higher bat activity closer to water (e.g., Kalcounis-Rüppell *et al*., 2005; Ford *et al*., 2006; Ancillotto *et al*., 2019). Water availability is typically high in the southeastern Coastal Plain, due to high annual rainfall, especially during winter (Bosch *et al*., 1999), and therefore may not be a limiting factor for bats. Additionally, due to lower rates of evaporative water loss than in summer (Cryan & Wolf, 2003), frequent access to water may be less important for bats during winter. Furthermore, bats commonly feed on emergent insects over water in summer, but insect availability typically is lower during winter (Corbet, 1964). Alternatively, due to the difficulty of mapping small and often ephemeral freshwater sources, our analysis only included water sources from available spatial data layers, which could have hindered our ability to detect relationships and gave us results contrary to those expected (Bender *et al*., 2015; Perea *et al*., 2022).

Although distance to roads increased the probability of occupancy at the community level, the relationship was inconsistent among species. Unimproved roads like those in working forests likely do not represent fragmentation to bats, but often separate stands of different ages and structural characteristics. Thus, we contend that roads serve as an indicator of fragmentation despite there being continuous forest cover. Positive occupancy probabilities with distance to roads for *M. austroriparius*/*M. septentrionalis* is consistent with the well-documented preference for continuous mature stands for both foraging and roosting and avoidance of fragmented forests (*M. septentrionalis*: Henderson & Broders, 2008), typical of clutter-adapted species (Denzinger & Schnitzler, 2013; Beilke *et al*., 2021). In contrast, the observed positive response for *D. intermedius* and *L. cinereus*, two of the largest bat species in North America, is not intuitive but may be associated with their ecomorphological characteristics as open-space foraging species (Norberg & Rayner, 1987; Denzinger & Schnitzler, 2013). As open-space foragers (Veilleux *et al*., 2009; Shute *et al*., 2021; Perea *et al*., 2022), *D. intermedius* and *L. cinereus* forage above the canopy and in large canopy openings across the landscape likely without regard to roads. Thus, the observed relationship may be merely a result of greater area in interior forest compared to roads increasing the likelihood of foraging in areas farther from roads. However, further studies are needed to investigate how forest gaps and openings in mature forests are used by open-space foraging species (Loeb & O'Keefe, 2011; Tena *et al*., 2020).

Previous summer studies have generally found positive associations between bat activity and edge regardless of species' ecomorphological characteristics (Morris *et al*., 2010; Janzen & Fenton, 2013). However, in our winter study, occupancy of the community and all but one species/group was not associated with edge. Consistent with previous studies, we observed a negative relationship between occupancy probability and edge for *M. austroriparius*/*M. septentrionalis*, species that commonly forage within forest stands and avoid edges (Henderson & Broders, 2008; Morris et al., 2010). However, the varying and equivocal relationships between edge and other bat species were counter to our expectations. Based on wing morphology and echolocation call characteristics *L. borealis*/*L. seminolus*, *N. humeralis*, and *P. subflavus* are predicted to be edge foragers, which has been demonstrated in previous studies (Norberg & Rayner, 1987; Morris *et al*., 2010). Use of edges for foraging is typically attributed to avoidance of vegetation clutter and greater insect abundance (Morris *et al*., 2010). In one of the few studies that examined bat foraging habitat use in winter, Shute *et al*. (2021) observed that vegetation characteristics related to *P. subflavus* occupancy changed from summer to winter possibly in response to temporal and spatial changes in prey availability or environmental conditions. Because we also found these species/groups associated with higher forest cover, we suggest that species considered edge foragers in summer may restrict activity to areas with higher prey availability or more suitable environmental conditions during winter (Shute *et al*., 2021).

Based on results of previous summer studies, we expected canopy openness, vegetation clutter, and stand age to influence bat occupancy during winter, but none were included in our top model. Although basal area and canopy openness were not strongly correlated, we chose not to include them in the same models because forest management affects them simultaneously (e.g., thinning reduces basal area and increases canopy openness). Previous studies have demonstrated a negative relationship between bat activity and canopy cover (Ford *et al*., 2005; Froidevaux *et al*., 2016), but those studies were not in working forests and were conducted during summer. In one of the few studies conducted in working forests during winter, Andersen *et al*. (2022) found that canopy cover did not influence bat activity. Although the reason is uncertain, it appears that basal area has a greater influence on bat occupancy than canopy openness during winter. Stand age likely was not an important predictor of occupancy because bats can forage within or above all stand ages in working forests. Pre-thinned pine stands (≤13 years) are closed canopy with little uncluttered space for bats to forage. As a result, the bats we detected in those stands were foraging above the canopy. Once stands are thinned (~14 years), basal area is reduced allowing efficient foraging conditions within and below the canopy (Verschuyl *et al*., 2011). The reduction in basal area associated with thinning apparently outweighed the influence of stand age. The lack of influence of vegetation clutter was surprising as most studies have demonstrated a negative response (Loeb & O'Keefe, 2006; Loeb & Waldrop, 2008; Bender *et al*., 2015) but was likely due to management prescriptions in working forests. Following thinning, stands typically are managed with herbicides and/or prescribed fire to reduce competing trees in the midstory (Greene *et al*., 2016), which consequently reduces vegetation clutter, allowing efficient foraging conditions for bats (Verschuyl *et al*., 2011). Thus, the reduced basal area and subsequent midstory vegetation control associated with thinning in working forests appeared to diminish the influence of clutter in our study.

We found that temperature was an important factor in explaining detectability at the community and species levels, which should be considered when conducting winter bat studies. Our findings are consistent with previous studies assessing the influence of environmental conditions on bat activity (Brooks, 2009; Bender & Hartman, 2015; Parker *et al*., 2020; Barros *et al*., 2021). In temperate regions, winter temperatures can fluctuate weekly, or even daily, causing bats to enter short-term torpor bouts (Johnson *et al*., 2012; Meierhofer *et al*., 2019). Winter bat activity in the southeastern United States Coastal Plain is strongly influenced by temperature (Parker *et al*., 2020). Grider *et a*l. (2016) observed that differences in mean nighttime temperature of ~1.5°C influenced winter bat activity in North Carolina. In addition, temperature also affects availability of insect prey (Welti *et al*., 2022), as it must be warm enough for insect prey to remain active. Thus, low temperatures negatively affect the probability of detecting bats during winter, which may limit our ability to obtain unbiased occupancy estimates if temperature is not considered. However, it is important to note that although our study confirms that temperature significantly influences bat detection, other possible environmental factors (e.g., humidity or atmospheric pressure) should be considered in future work.

Our results provide new insights into bat ecology in working forest landscapes during winter, where we highlight similar responses to site and landscape covariates as previous summer work. Although bat species' richness generally does not change throughout the year, occupancy can change for some species. For example, we observed drastically higher occupancy for *L. cinereus* when compared to a similar study conducted in the Coastal Plain during summer (Bender *et al*., 2015). We consider these results of particular importance, as management decisions based on data collected during summer may positively support bat communities during winter. As previously observed during summer, promoting forests with low levels of basal area will increase occupancy at both the community and species levels. While forest thinning reduces basal area in mid-rotation stands promoting efficient foraging, the influence of low basal area was also supported in young pine stands, as we frequently recorded bats foraging over the canopy. Our study is one of the few that considers imperfect detection while controlling for spatial autocorrelation and residual correlation among bat species (Browning *et al*., 2022), which provides robust estimates of occupancy and richness of wintering bats. Our results will inform managers of vegetation characteristics important to wintering bats, thereby increasing knowledge of wintering bat foraging ecology and conservation opportunities within working forests.

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LITERATURE CITED

Adams, M. D., Law, B. S., & French, K. O. (2009). Vegetation structure influences the vertical stratification of open- and edge-space aerial-foraging bats in harvested forests. Forest Ecology and Management, 258, 2090-2100. https://doi.org/10.1016/j.foreco.2009.08.002

Ancillotto, L., Bosso, L., Salinas-Ramos, V. B., & Russo, D. (2019). The importance of ponds for the conservation of bats in urban landscapes. Landscape and Urban Planning, 190, 103607. https://doi.org/10.1016/j.landurbplan.2019.103607

Andersen, B. R., McGuire L. P., Wigley T. B., Miller D. A. & Stevens, R. D. (2022). Habitat associations of overwintering bats in managed pine forest landscapes. Forests, 13(5), 803. https://doi.org/10.3390/f13050803

Banerjee, S., Carlin, B. P. & Gelfand, A. E. (2014). Hierarchical modeling and analysis for spatial data. Boca Raton, FL: CRC press. https://doi.org/10.1201/b17115

Barros, P. A., Ribeiro C. & Cabral J. A. (2017). Winter activity of bats in Mediterranean periurban deciduous forests. Acta Chiropterologica, 19(2), 367-377. https://doi.org/10.3161/15081109ACC2017.19.2.013

Barros, P., Faria S., Pereira M., Santos J. A. & Cabral J. A. (2021). How winter prevailing weather conditions influence the bat activity patterns? Hints from a Mediterranean region. Hystrix 32(1), 27–36. https://doi.org/10.4404/hystrix-00361-2020

Bateman, B. L., Wilsey, C., Taylor, L., Wu, J., LeBaron, G. S., & Langham, G. (2020). North American birds require mitigation and adaptation to reduce vulnerability to climate change. Conservation Science and Practice, 2(8), e242.. https://doi.org/10.1111/csp2.243

Beilke, E. A., Blakey, R. V. & O'Keefe, J. M. (2021). Bats partition activity in space and time in a large, heterogeneous landscape. Ecology & Evolution 11, 6513-6526. https://doi.org/10.1002/ece3.7504

Beilke, E. A., Haulton, G. S. & O'Keefe, J. M. (2023). Foliage-roosting eastern red bats select for features associated with management in a central hardwood forest. Forest Ecology and Management, 527, 120604. https://doi.org/10.1016/j.foreco.2022.120604

Bender, M. J. & Hartman, G. D. (2015). Bat Activity Increases with barometric pressure and temperature during autumn in Central Georgia. Southeastern Naturalist, 14(2), 231-242. https://doi.org/10.1656/058.014.0203

Bender, M. J., Castleberry, S. B., Miller, D. A. & Wigley, T. B. (2015). Site occupancy of foraging bats on landscapes of managed pine forest. Forest Ecology and Management, 336, 1-10. https://doi.org/10.1016/j.foreco.2014.10.004

Bender, M. J., Perea, S., Castleberry, S. B., Miller, D. A. & Wigley, T. B. (2021). Influence of insect abundance and vegetation structure on site-occupancy of bats in managed pine forests. Forest Ecology and Management, 482, 118839. https://doi.org/10.1016/j.foreco.2020.118839

Bosch, D. D., Sheridan, J. M., & Davis, F. M. (1999). Rainfall characteristics and spatial correlation for the Georgia Coastal Plain. Transactions of the ASAE, 42(6), 1637-1644. https://doi.org/10.13031/2013.13330

Boyles, J. G., Dunbar, M. B., & Whitaker Jr, J. O. (2006). Activity following arousal in winter in North American vespertilionid bats. Mammal Review, 36(4), 267-280. https://doi.org/10.1111/j.1365-2907.2006.00095.x

Brigham, R. M. (2007). Bats in forests: what we know and what we need to learn. In: Lacki, M. L., J. P. Hayes, and A. Kurta. (Eds.), Bats in forests: conservation and management. Baltimore, MD: Johns Hopkins.

Brooks, R. T. (2009). Habitat-associated and temporal patterns of bat activity in a diverse forest landscape of southern New England, USA. Biodiversity and Conservation, 18, 529-545. https://doi.org/10.1007/s10531-008-9518-x

Brooks, S. P., & Gelman, A. (1998). General methods for monitoring convergence of iterative simulations. Journal of computational and graphical statistics, 7(4), 434-455. https://doi.org/10.2307/1390675

Browning, E., Freeman, R., Boughey, K. L., Isaac, N. J., & Jones, K. E. (2022). Accounting for spatial autocorrelation and environment are important to derive robust bat population trends from citizen science data. Ecological Indicators, 136, 108719. https://doi.org/10.1111/mam.12239

Corbet, P. S. (1964). Temporal patterns of emergence in aquatic insects. The Canadian Entomologist, 96(1-2), 264-279.

Cox, M. R., Wilcox, E. V., Keyser, P. D. & Vander Yatch, A. L. (2016). Bat response to prescribed fire and overstory thinning in hardwood forest on the Cumberland Plateau, Tennessee. Forest Ecology and Management, 359, 221–231. https://doi.org/10.1016/j.foreco.2015.09.048

Cryan, P. M., & Wolf, B. O. (2003). Sex differences in the thermoregulation and evaporative water loss of a heterothermic bat, *Lasiurus cinereus*, during its spring migration. Journal of Experimental Biology, 206(19), 3381-3390. https://doi.org/10.1242/jeb.00574

Cryan, P.M. (2009). Seasonal distribution of migratory tree bats (Lasiurus and Lasionycteris) in North America. Journal of Mammalogy, 84, 579-593. https://doi.org/10.1644/1545-1542(2003)084<0579:SDOMTB>2.0.CO;2

Cumming, G., & Finch, S. (2005). Inference by eye: confidence intervals and how to read pictures of data. American psychologist, 60(2), 170. https://doi.org/10.1037/0003-066X.60.2.170

Czenze, Z. J., & Willis, C. K. (2015). Warming up and shipping out: arousal and emergence timing in hibernating little brown bats (*Myotis lucifugus*). Journal of Comparative Physiology B, 185, 575-586. https://doi.org/10.1007/s00360-015-0900-1

Datta, A., Banerjee, S., Finley, A. O., & Gelfand, A. E. (2016). Hierarchical nearest-neighbor Gaussian process models for large geostatistical datasets. Journal of the American Statistical Association, 111(514), 800-812. https://doi.org/10.1080/01621459.2015.1044091

De Bruyn L., Gyselings, R., Kirkpatrick, L., Rachwald, A., Apoznanski, G. & Kokurewicz, T. (2021). Temperature driven hibernation site use in the Western barbastelle Barbastella barbastellus (Schreber, 1774). Scientific Reports, 11, 1464. https://doi.org/10.1038/s41598-020-80720-4

Demarais, S., Verschuyl, J. P., Roloff, G. J., Miller, D. A. & Wigley, T. B. 2017. Tamm Review: Terrestrial vertebrate biodiversity and intensive forest management in the U.S. Forest Ecology and Management. 385, 308-330. https://doi.org/10.1016/j.foreco.2016.10.006

Denzinger, A. & Schnitzler, H. U. (2013). Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. Front. Physiol. 4, 164. https://doi.org/10.3389/fphys.2013.00164

Devarajan K., Morelli, T. L. & Tenan, S. (2020). Multi-species occupancy models: review, roadmap, and recommendations. Ecography 43(11), 1612-1624. https://doi.org/10.1111/ecog.04957

Dixon, M. D. (2011). Relationship between land cover and insectivorous bat activity in an urban landscape. Urban Ecosystems 15, 683–695. https://doi.org/10.1007/s11252-011-0219-y

Dorazio, R. M., & Royle, J. A. (2005). Estimating size and composition of biological communities by modeling the occurrence of species. Journal of the American Statistical Association, 100(470), 389-398. https://doi.org/10.1198/016214505000000015

Dorazio, R., Gotelli, N. & Ellison, A. 2011. Modern methods of estimating biodiversity from presence-absence surveys. In Grillo, O., and G. Venora (Eds), Biodiversity loss in a changing planet (pp. 277–302). Rijeka, Croatia: InTech.

Doser, J. W., Finley, A. O., Kéry, M., & Zipkin, E. F. (2022). spOccupancy: An R package for single-species, multi-species, and integrated spatial occupancy models. Methods in Ecology and Evolution, 13(8), 1670-1678. https://doi.org/10.1111/2041-210X.13897

Doser, J. W., Finley, A. O. & Banerjee, S. (2022b). Joint species distribution models with imperfect detection for high-dimensional spatial data. arXiv, 2204.02707. https://doi.org/10.48550/arXiv.2204.02707

Duchamp, J. E., Sparks, D. W., & Whitaker, Jr, J. O. (2004). Foraging-habitat selection by bats at an urban–rural interface: comparison between a successful and a less successful species. Canadian Journal of Zoology, 82(7), 1157-1164. https://doi.org/10.1139/z04-095

Erasmy, M., Leuschner, C., Balkenhol, N. & Dietz, M. (2021). Three-dimensional stratification pattern in an old-growth lowland forest: How does height in canopy and season influence temperate bat activity? Ecology & Evolution, 11(23), 17273-17288. https://doi.org/10.1002/ece3.8363

Finley, A. O., Banerjee, S., & McRoberts, R. E. (2009). Hierarchical spatial models for predicting tree species assemblages across large domains. The Annals of Applied Statistics, 3(3), 1052.

Ford, W. M., Menzel, M. A., Rodrigue, J. L., Menzel, J. M. & Johnson, J. B. (2005). Relating bat species presence to simple habitat measures in a central Appalachian Forest. Biological Conservation, 126, 528–539. https://doi.org/10.1016/j.biocon.2005.07.003

Ford, W. M., Menzel, J. M., Menzel, M. A., Edwards, J. W., & Kilgo, J. C. (2006). Presence and absence of bats across habitat scales in the upper coastal plain of South Carolina. The Journal of Wildlife Management, 70(5), 1200-1209. https://doi.org/10.2193/0022-541X(2006)70[1200:PAAOBA]2.0.CO;2

Frazer, E. E., Brooks, D. & Longstafee, F. J. (2017). Stable isotope investigation of the migratory behavior of silver-haired bats (*Lasionycteris noctivagans*) in eastern North America. Journal of Mammalogy, 98(5), 1225-1235. https://doi.org/10.1093/jmammal/gyx085

Froidevaux, J. S., Zellweger, F., Bollmann, K., Jones, G., & Obrist, M. K. (2016). From field surveys to LiDAR: Shining a light on how bats respond to forest structure. Remote Sensing of Environment, 175, 242-250. https://doi.org/10.1016/j.rse.2015.12.038

Gallagher, M. E., Farrell, S. L., Germain, R. H., & Rojas, V. G. (2021). Summer bat habitat use and forest characteristics in managed northeastern forests. Journal of Forestry, 119(3), 305-318. https://doi.org/10.1093/jofore/fvaa059

Gelman, A., Meng, X. L., & Stern, H. (1996). Posterior predictive assessment of model fitness via realized discrepancies. Statistica sinica, 733-760.

Greene, R. E., Iglay, R. B., Evans, K. O., Miller, D. A., Wigley, T. B., & Riffell, S. K. (2016). A meta-analysis of biodiversity responses to management of southeastern pine forests—opportunities for open pine conservation. Forest Ecology and Management, 360, 30-39. https://doi.org/10.1016/j.foreco.2015.10.007

Gresham, C.A. (2002). Sustainability of intensive loblolly pine plantation management in the South Carolina Coastal Plain, USA. Forest Ecology and Management, 155, 69-80. https://doi.org/10.1016/S0378-1127(01)00548-5

Grider, J. F., Larsen, A. L., Homyack, J. A. & Kalcounis-Ruepell, M. C. (2016). Winter activity of Coastal Plain populations of bat species affected by White-Nose Syndrome and wind energy facilities. PLoS ONE, 11(11), e0166512. https://doi.org/10.1371/journal.pone.0166512

Guillera-Arroita, G., Ridout, M. S., & Morgan, B. J. (2010). Design of occupancy studies with imperfect detection. Methods in Ecology and Evolution, 1(2), 131-139. https://doi.org/10.1111/j.2041-210X.2010.00017.x

Hein, C. D., Castleberry, S. B. & Miller, K. V. (2009). Site-occupancy of bats in relation to forested corridors. Forest Ecology and Management, 257(4), 1200–1207. https://doi.org/10.1016/j.foreco.2008.09.054

Henderson, L. E. & Broders, H. G. (2008). Movements and resource selection of the northern long-eared myotis (*Myotis septentrionalis*) in a forest-agriculture landscape. Journal of Mammalogy, 89, 952–963. https://doi.org/10.1644/07-MAMM-A-214.1

Hobbs, N. T. & Hooten, M. B. (2015). Bayesian Models. Princeton, NJ: Princeton University Press.

Humphries, M. M., Kramer, D. L., & Thomas, D. W. (2003). The role of energy availability in mammalian hibernation: an experimental test in free-ranging eastern chipmunks. Physiological and Biochemical Zoology, 76(2), 180-186. https://doi.org/10.1086/367950

Humphries, M. M., Studd, E. K., Menzies, A. K., & Boutin, S. (2017). To everything there is a season: summer-to-winter food webs and the functional traits of keystone species. Integrative and Comparative Biology, 57(5), 961-976. https://doi.org/10.1038/s41558-020-00908-4

Husch B., Beers, T. W. & Kershaw, J. A. (2003). Forest mensuration. 4th edition. New York, NY: John Wiley.

Jantzen, M. K., & Fenton, M. B. (2013). The depth of edge influence among insectivorous bats at forest–field interfaces. Canadian Journal of Zoology, 91(5), 287-292. https://doi.org/10.1139/cjz-2012-0282

Johnson, J. B., Gates, J. E. & Ford, W. M. (2008). Distribution and activity of bats at local and landscape scales within a rural–urban gradient. Urban Ecosystems., 11, 227-242. https://doi.org/10.1007/s11252-008-0055-x

Johnson, J. S., Lacki, M. J., Thomas, S. C. & Grider, J. F. (2012). Frequent arousals from winter torpor in Rafinesque’s Big-Eared Bat (Corynorhinus rafinesquii). PloS ONE 7(11), e49754. https://doi.org/10.1371/journal.pone.0049754

Johnson, S. A., & Chambers, C. L. (2017). Effects of ponderosa pine forest restoration on habitat for bats. Western North American Naturalist, 77(3), 355-368. https://doi.org/10.3398/064.077.0308

Kalcounis-Rüppell, M. C., Psyllakis, J. M. & Brigham, R. M. (2005). Tree roost selection by bats: an empirical synthesis using meta-analysis. Wildlife Society Bulletin, 33, 1123–1132. https://doi.org/10.2193/0091-7648(2005)33[1123:TRSBBA]2.0.CO;2

Kéry, M. & Schmidt, B. R. (2008). Imperfect detection and its consequences for monitoring for conservation. Community Ecology, 9(2), 207-216. https://doi.org/10.1556/ComEc.9.2008.2.10

Kéry, M. & Royle, J. A. (2015). Applied hierarchical modeling in ecology: analysis of distribution, abundance and species richness in R and BUGS. First edition. Amsterdam, Netherlands: Academic Press.

Kunberger, J. M., & Long, A. M. (2022). The influence of forest management practices on seasonal bat species occurrence and activity at the Kisatchie National Forest in Louisiana, USA. Forest Ecology and Management, 526, 120579. https://doi.org/10.1016/j.foreco.2022.120579

Loeb, S. C., & O'keefe, J. M. (2006). Habitat use by forest bats in South Carolina in relation to local, stand, and landscape characteristics. The Journal of Wildlife Management, 70(5), 1210-1218. https://doi.org/10.2193/0022-541X(2006)70[1210:HUBFBI]2.0.CO;2

Loeb, S. C. & Waldrop, T. A. (2008). Bat activity in relation to fire and fire surrogate treatments in southern pine stands. Forest Ecology and Management, 255, 3185-3192. https://doi.org/10.1016/j.foreco.2007.10.060

Loeb, S. C. & O’Keefe, J. M. (2011). Bats and gaps: The role of early successional patches in the roosting and foraging ecology of bats. In C. Greenberg, B. Collins, and F. Thompson III (eds) Sustaining young forest communities (pp. 167–189). Springer

Lunn, D., Jackson, C., Best, N., Thomas, A. & Spiegelhalter, D. (2013). The Bugs Book: A practical introduction to Bayesian analysis. Boca Raton, FL: CRC Press.

MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S, Royle, J. A. & Langtimm, C. A. (2002). Estimating site occupancy rates when detection probabilities are less than one. Ecology, 83, 2248-2255. https://doi.org/10.1890/0012-9658(2002)083[2248:ESORWD]2.0.CO;2

MacKenzie D. I., Nichols, J. D., Royle, J. A., Pollock, K. H., Bailey L. L. & Hines, J. E. (2018). Occupancy estimation and modeling: Inferring patterns and dynamics of species occurence, 2nd edn. Elsevier Academic Press, New York

Marshall, C. D., Maerz, J. C., Larsen-Gray, A. L., Chamberlain, M. J. & Martin, J. A. (2022). Gopher tortoise (*Gopherus polyphemus*) resource selection within a private working pine (Pinus spp.) forest landscape. Forest Ecology and Management, 510, 120112. http://dx.doi.org/10.2139/ssrn.3989679

Mas, M., Flaquer, C., Rebelo, H., & López-Baucells, A. (2021). Bats and wetlands: synthesising gaps in current knowledge and future opportunities for conservation. Mammal Review, 51(3), 369-384. https://doi.org/10.1111/mam.12243

Mas, M., Flaquer, C., Puig-Montserrat, X., Porres, X., Rebelo, H., & López-Baucells, A. (2022). Winter bat activity: The role of wetlands as food and drinking reservoirs under climate change. Science of the Total Environment, 828, 154403. https://doi.org/10.1016/j.scitotenv.2022.154403

McGarigal, K., Cushman, S. A., Neel, M. C. & Ene, E. (2015). FRAGSTATS v4.2: Spatial pattern analysis program for categorical and continuous Maps. Available online: http://www.umass.edu/landeco/research/fragstats/fragstats.html.

Meierhofer, M. B., Johnson, J. S., Leivers, S. J., Pierce, B. L., Evans, J. E. & Morrison, M. L. (2019). Winter habitats of bats in Texas. PLoS ONE 14, e0220839. https://doi.org/10.1371/journal.pone.0220839

Meyer, C. F., Schwarz, C. J., & Fahr, J. (2004). Activity patterns and habitat preferences of insectivorous bats in a West African forest–savanna mosaic. Journal of Tropical Ecology, 20(4), 397-407. https://www.jstor.org/stable/4091852

Miller, D. A., Wigley, T. B., & Miller, K. V. (2009). Managed forests and conservation of terrestrial biodiversity in the southern United States. Journal of Forestry, 107(4), 197-203.

Moore, L. H. & Best, T. L. (2018). Impact of vegetation on activity of bats over wetlands in coastal South Carolina. Journal of Mammalogy, 99, 1082-1092. https://doi.org/10.1093/jmammal/gyy086

Morris, A. D., Miller, D. A., & Kalcounis-Rüppell, M. C. (2010). Use of forest edges by bats in a managed pine forest landscape. The Journal of Wildlife Management, 74(1), 26-34. https://doi.org/10.2193/2008-471

Morris, A. D., Miller, D. A., & Conner, L. M. (2011a). A comparison of ultrasonic detectors and radiotelemetry for studying bat–habitat relationships. Wildlife Society Bulletin, 35(4), 469-474. https://doi.org/10.1002/wsb.78

Morris A. D. Miller, D. A. & Conner, L. M. (2011b). Home-range size of evening bats (*Nycticeius humeralis*) in southwestern Georgia. Southeastern Naturalist, 10, 85–94. https://doi.org/10.1656/058.010.0107

Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. Biological reviews, 82(4), 591-605. https://doi.org/10.1111/j.1469-185X.2007.00027.x

Norberg, U. M., & Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 316(1179), 335-427. https://doi.org/10.1098/rstb.1987.0030

Nudds, T. D. (1977). Quantifying the vegetative structure of wildlife cover. Wildlife Society Bulletin, 113-117.

O'Farrell, M. J. & Gannon, W. L. (1999). A comparison of acoustic versus capture techniques for the inventory of bats. Journal of Mammalogy, 80, 24-30. https://doi.org/10.2307/1383204

Oswalt, S.N., Smith, W. B., Miles, P. D. & Pugh, S. A. (2019). Forest resources of the United States 2017: A technical document supporting the Forest Service update of the 2020 RPA Assessment. USDA Forest Service, General Technical Support WO-97.

Ovaskainen, O., Hottola, J. & Siitonen, J. (2010). Modeling species co-occurrence by multivariate logistic regression generates new hypotheses on fungal interactions. Ecology, 91, 2514– 2521. https://doi.org/10.1890/10-0173.1

Parker, K. A., Li, H. & Kalcounis-Rueppell, M. C. (2020). Species-specific environmental conditions for winter bat acoustic activity in North Carolina, United States. Journal of Mammalogy, 101, 1502-1512. https://doi.org/10.1093/jmammal/gyaa133

Perea, S., Morris, K.M. & Castleberry, S.B. (2022). Northern yellow bat *Dasypterus intermedius* activity and habitat selection on an Atlantic barrier island. Acta Chiropterologica, 24(2), 343-352. https://doi.org/10.3161/15081109ACC2022.24.2.005

Polson, N. G., Scott, J. G., & Windle, J. (2013). Bayesian inference for logistic models using Pólya–Gamma latent variables. Journal of the American Statistical Association, 108(504), 1339-1349. https://www.jstor.org/stable/24247065

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.Rproject.org/.

Rainho, A. & Palmeirim, J. M. (2011). The importance of distance to resources in the spatial modelling of bat foraging habitat. PLoS ONE 6(4), e19227. https://doi.org/10.1371/journal.pone.0019227

Reichert, B., Lausen, C., Loeb, S., Weller, T., Allen, R., Britzke, E., Hohoff, T., Siemers, J., Burkholder, B., Herzog, C. & Verant, M. (2018). A guide to processing bat acoustic data for the North American Bat Monitoring Program (NABat). U.S. Geological Survey Open-File Report 2018–1068.

Rodríguez-San Pedro, A. & Simonetti, J. A. (2015). The relative influence of forest loss and fragmentation on insectivorous bats: does the type of matrix matter? Landscape Ecology, 30, 1561-1572. https://doi.org/10.1007/s10980-015-0213-5

Schimpp, S.A., Li, H. & Kalcounis-Rueppell, M.C. (2018). Determining species-specific nightly bat activity in sites with varying urban intensity. Urban Ecosystems, 21, 541–550. https://doi.org/10.1007/s11252-018-0737-y

Shute, K. E., Loeb, S. C., & Jachowski, D. S. (2021). Seasonal shifts in nocturnal habitat use by coastal bat species. The Journal of Wildlife Management, 85(5), 964-978. https://doi.org/10.1002/jwmg.22060

Stawski, C., & Geiser, F. (2011). Do season and distribution affect thermal energetics of a hibernating bat endemic to the tropics and subtropics?. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 301(2), R542-R547. https://doi.org/10.1152/ajpregu.00792.2010

Szewczak, J. M., Corcoran, A. J., Kennedy, J., Ormsbee, P. C., & Weller, T. E. (2011). Echolocation call characteristics of eastern US bats. Humboldt State University Bat Lab, 1-4. http://www.sonobat.com/download/EasternUS_Acoustic_Table_Mar2011.pdf

Taylor, D. A, Perry, R. W., Miller, D. A. & Ford, W. M. (2020). Forest management and Bats. Publication of the White-nose Syndrome Response Team (www.whitenosesyndrome.org), Hadley, MA.

Tena, E., Paz, O. D., Peña, R. D., Fandos, G., Redondo, M. & Tellería, J. (2020). Mind the gap: Effects of canopy clearings on temperate forest bat assemblages. Forest Ecology and Management, 474, 118341. https://doi.org/10.1016/j.foreco.2020.118341

Tiago Marques, J., Ramos Pereira, M. J. & Palmeirim, J. M. (2016). Patterns in the use of rainforest vertical space by Neotropical aerial insectivorous bats: All the action is up in the canopy. Ecography 39, 476-486. https://doi.org/10.1111/ecog.01453

Tikhonov, G., Opedal, Ø. H., Abrego, N., Lehikoinen, A., de Jonge, M. M., Oksanen, J., & Ovaskainen, O. (2020). Joint species distribution modelling with the R-package Hmsc. Methods in Ecology and Evolution, 11(3), 442-447. https://doi.org/10.1111/2041-210X.13345

Tilker, A., Abrams, J. F., Nguyen, A. N., Hörig, L., Axtner, J., Louvrier, J., ... & Wilting, A. (2020). Identifying conservation priorities in a defaunated tropical biodiversity hotspot. Diversity and Distributions, 26(4), 426-440. https://doi.org/10.1111/ddi.13029

U.S. Geological Survey (USGS). NLCD 2019 Land Cover (CONUS). Available online: https://www.mrlc.gov/data/nlcd-2019-land-cover-conus (accessed on 1 April 2022).

Veilleux, J. P., Moosman, P. R., Reynolds, D. S., LaGory, K. E., & Walston, L. J. (2009). Observations of summer roosting and foraging behavior of a hoary bat (Lasiurus cinereus) in southern New Hampshire. Northeastern Naturalist, 16(1), 148-152. https://doi.org/10.1656/045.016.0113

Verschuyl, J., Riffell, S., Miller, D. & Wigley, T. B. (2011). Biodiversity response to intensive biomass production from forest thinning in North American forests – A meta-analysis. Forest Ecology and Management 261:221-232. https://doi.org/10.1016/j.foreco.2010.10.010

Watanabe, S., & Opper, M. (2010). Asymptotic equivalence of Bayes cross validation and widely applicable information criterion in singular learning theory. Journal of Machine Learning research, 11(12).

Węgiel, A., Grzywiński, W., Ciechanowski, M., Jaros, R., Kalcounis-Rüppell, M., Kmiecik, A., ... & Węgiel, J. (2019). The foraging activity of bats in managed pine forests of different ages. European Journal of Forest Research, 138, 383-396. https://doi.org/10.1007/s10342-019-01174-6

Weller, T. J., & Zabel, C. J. (2002). Variation in bat detections due to detector orientation in a forest. Wildlife Society Bulletin, 922-930. https://www.jstor.org/stable/3784248

Welti, E. A., Zajicek, P., Frenzel, M., Ayasse, M., Bornholdt, T., Buse, J., ... & Haase, P. (2022). Temperature drives variation in flying insect biomass across a German malaise trap network. Insect Conservation and Diversity, 15(2), 168-180. https://doi.org/10.1111/icad.12555

Wigley, T. B., Miller, D. A. & Yarrow, G. K. (2007). Planning for bats on forest industry lands in North America. Pages 293-318 in Bats in forests: conservation and management. Baltimore, MD: Johns Hopkins.

Yeiser, J. M., Morgan, J. J., Baxley, D. L., Chandler, R. B. & Martin, J. A. (2018). Private land conservation has landscape-scale benefits for wildlife in agroecosystems. Journal of Applied Ecology, 55(4), 1930-1939. https://doi.org/10.1111/1365-2664.13136

Zipkin, E. F., DeWan, A. & Royle, J. A. (2009). Impacts of forest fragmentation on species richness: a hierarchical approach to community modeling. Journal of Applied Ecology, 46, 815-822. https://doi.org/10.1111/j.1365-2664.2009.01664.x

Zipkin, E. F., Royle, J. A., Dawson, D. K. & Bates, S. (2010). Multi-species occurrence models to evaluate the effects of conservation and management actions. Biological Conservation, 143, 479-484. https://doi.org/10.1016/j.biocon.2009.11.016

Table 2.1. Summary of bat species ecomorphological characteristics, foraging strategies, and number of sampling sites (out of 240 total) where bats were detected during winter acoustic surveys in working forest landscapes of the southeastern United States Coastal Plain, 2020–2022.

Phonic group	Species	Total sites detected	Foraging strategy
Low group $F_c < 30$ kHz Duration > 5 ms	*Dasypterus intermedius*	70	Open-space aerial foragers
	Eptesicus fuscus / Lasionycteris noctivagans	122	Open and edge-space aerial foragers
	Lasiurus cinereus	177	Open-space aerial foragers
	Tadarida brasiliensis	110	Open-space aerial foragers
Mid group F_c 30-45 kHz Duration > 5 ms	*Lasiurus borealis / L. seminolus*	156	Edge-space aerial foragers
	Nycticeius humeralis	115	Edge-space aerial foragers
	Perimyotis subflavus	106	Edge-space aerial foragers
Myotis group $F_c > 40$ kHz Duration < 5 ms	*Myotis austroriparius / M. septentrionalis*	103	Narrow-space, aerial-gleaning forager

Table 2.2. Effective number of parameters (pD), Widely Applicable Information Criterion (WAIC), and difference in WAIC value between the model and the model with the lowest value (ΔWAIC) for the top 5 models of bat community occupancy (ψ) and detection probability (p) during winter 2020–2022 in working forest landscapes of the southeastern United States Coastal Plain.

Model	**pD**	**WAIC**	**ΔWAIC**
ψ (basal area + distance road + distance water + total edge + total forest + total wetland), *p* (temperature)	243.34	5597.86	0.00
ψ (basal area + clutter + distance road + distance water + total edge + total forest + total wetland), *p* (temperature)	249.98	5606.01	8.15
ψ (basal area + clutter + distance road + distance water + stand class + total edge + total forest + total wetland), *p* (temperature)	259.32	5610.25	12.39
ψ (distance water + total forest + total wetland), *p* (temperature)	242.76	5614.89	17.03
ψ (total wetland), *p* (temperature)	237.79	5616.69	18.83

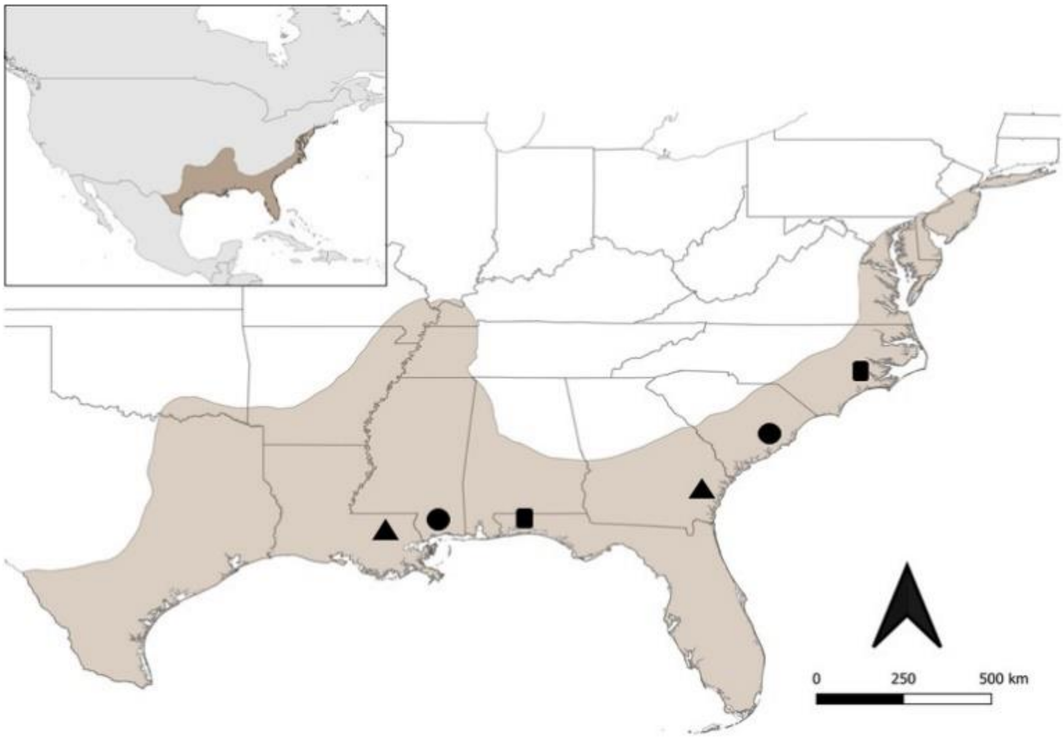

Figure 2.1. Locations of study sites in the southeastern United States Coastal Plain where bat acoustic sampling was conducted January–March 2020–2022. Sites sampled in 2020 are indicated by triangles, 2021 by circles, and 2022 by squares.

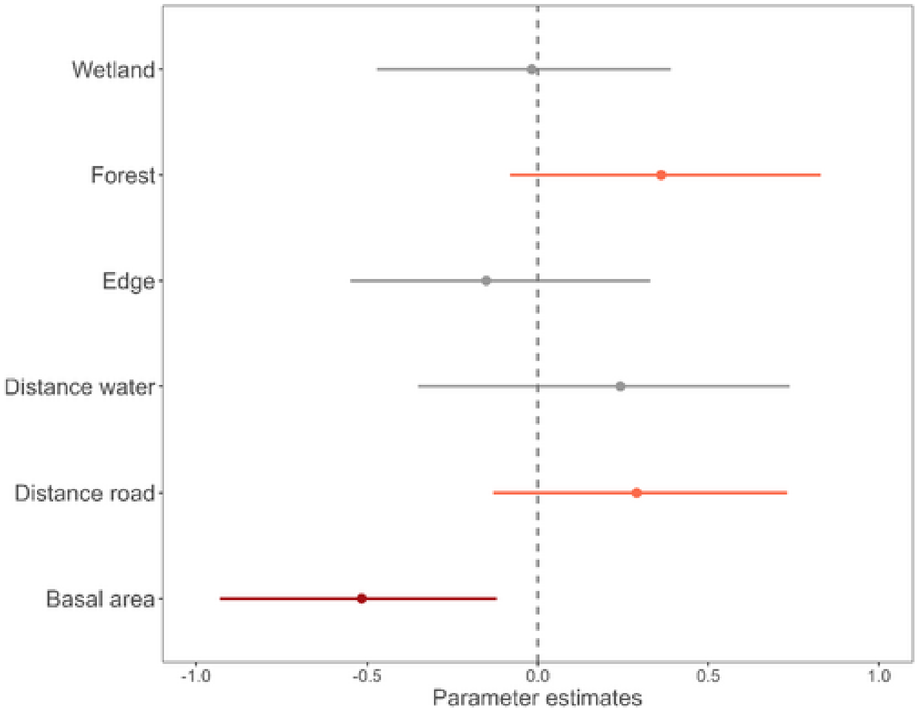

Figure 2.2. Mean beta coefficients for bat community-level occupancy in working forest landscapes of the southeastern United States Coastal Plain winter 2020–22, estimated with a spatial community occupancy model fit to acoustic detector data. Gray bars show relationships in which the 75% Bayesian credible interval (BCI) overlaps zero, orange bars indicate that the 75% BCI does not overlap zero but the 95% BCI does overlap zero, and red bars indicate that the 95% BCI does not overlap zero.

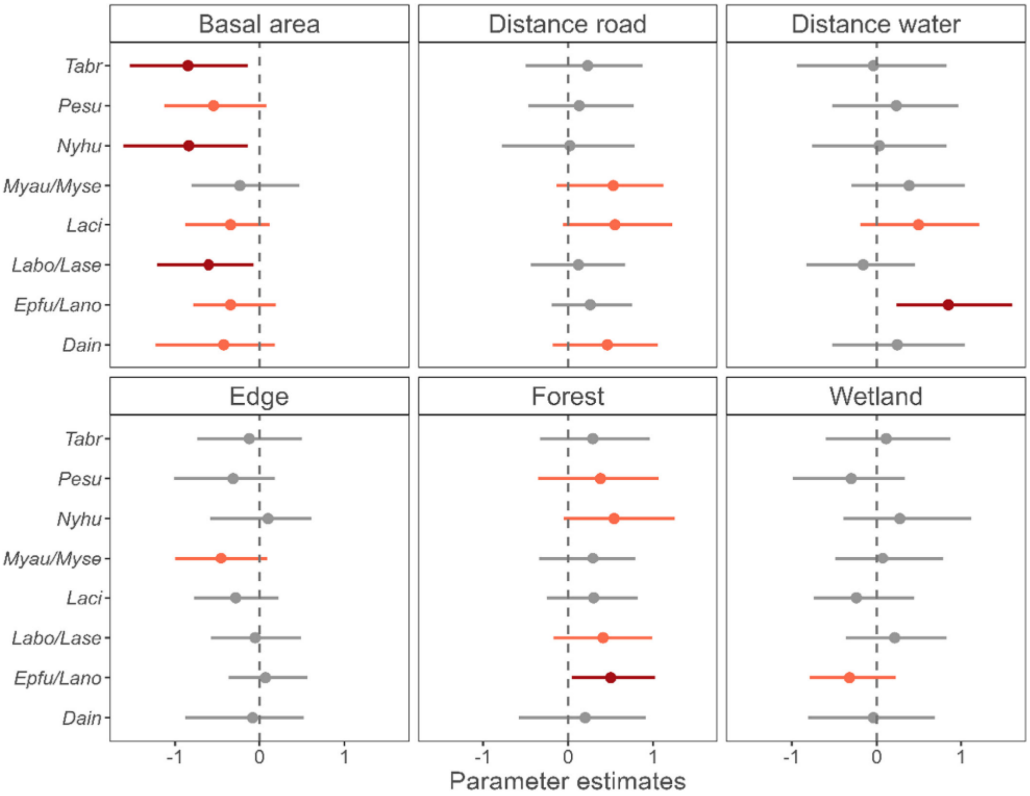

Figure 2.3. Mean beta coefficients for bat species-level occupancy in working forest landscapes of the southeastern United States Coastal Plain winter 2020–22, estimated with a spatial community occupancy model fit to acoustic detector data. Gray bars show relationships in which the 75% Bayesian credible interval (BCI) overlaps zero, orange bars indicate that the 75% BCI does not overlap zero but the 95% BCI does overlap zero, and red bars indicate that the 95% BCI does not overlap zero. Species codes*: Lasiurus cinereus* (Laci), *Dasypterus intermedius* (Dain), *Eptesicus fuscus*/*Lasionycteris noctivagans* (Epfu/Lano), *Lasiurus borealis*/*Lasiurus seminolus* (Labo/Lase), *Myotis austroriparius*/*Myotis septentrionalis* (Myau/Myse), *Nycticeius humeralis* (Nyhu), *Perimyotis subflavus* (Pesu), and *Tadarida brasiliensis* (Tabr).

CHAPTER 3

WINTER DIET OF BATS IN WORKING FORESTS OF THE SOUTHEASTERN U.S. COASTAL PLAIN[2]

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[2] Santiago Perea, Colton D. Meinecke, Angela L. Larsen-Gray, Daniel U. Greene, Caterina Villari, Kamal J. K. Gandhi, and Steven B. Castleberry. 2024. Scientific Reports 14(1), 12778. Reprinted here with permission of the publisher.

ABSTRACT

Working forests comprise a large proportion of forested landscapes in the southeastern United States and are important to the conservation of bats, which rely on forests for roosting and foraging. While relationships between bat ecology and forest management are well studied during summer, winter bat ecology remains understudied. Hence, we aimed to identify the diet composition of overwintering bats, compare the composition of prey consumed by bat species, and determine the potential role of forest bats as pest controllers in working forest landscapes of the southeastern U.S. Coastal Plain. During January to March 2021–2022, we captured 264 bats of eight species. We used DNA metabarcoding to obtain diet composition from 126 individuals of seven bat species identifying 22 orders and 174 families of arthropod prey. Although Coleoptera, Diptera, and Lepidoptera were the most consumed orders, we found that bats had a generalist diet but with significant differences among some species. We also documented the consumption of multiple insect pests (e.g., *Rhyacionia frustrana*) and disease vectors (e.g., *Culex* spp). Our results provide important information regarding the winter diet of bats in the southeastern U.S. Coastal Plain and their potential role in controlling economically relevant pest species and disease vectors.

INTRODUCTION

The study of trophic resources is a key aspect of foraging ecology, providing a basic understanding of the relationships between consumers, resources, and the environment (Stephens & Krebs, 1986; Arrizabalaga-Escudero *et al*., 2018). Insectivorous bats are important top-down regulators of arthropod populations (Böhm *et al*., 2011; Charbonnier *et al*., 2014; Beilke & O'Keefe, 2023). Many bat species are characterized by a wide range of dietary preferences and can adapt to various land cover types, which enables them to adjust to changes in food availability throughout the year (Mayne & Boyles, 2015; Alberdi *et al*., 2020). As highly mobile generalist consumers, insectivorous bats contribute to stabilizing and connecting local food webs in their ecosystem (Bartley *et al*., 2019; Brechtel *et al*., 2019). Additionally, they provide important ecosystem services by suppressing agricultural pests (Aizpurua *et al*., 2018; Kemp *et al*., 2019; Hughes *et al*., 2021; Maslo *et al*., 2022), forest pests (Charbonnier *et al*., 2014; Ancilotto *et al*., 2022), and vectors of parasites of humans (Puig-Monserrat *et al*., 2020; Hughes *et al*., 2022) and livestock (Downs & Sanderson, 2010; Ancilloto *et al*., 2017). For example, based on DNA metabarcoding of guano collected from roosts, Maslo *et al*. (2022) found that bats consumed ≥ 160 known agricultural pest species or disease vectors. Dietary studies focused on significant food resources and the effects of species interactions and communities are key to informing wildlife management decisions regarding species trophic position and population regulation (Alonso *et al*., 2014).

In most temperate zones, bats migrate or remain in torpor during winter (Cryan, 2003). However, milder climatic conditions of southern temperate latitudes, such as the Coastal Plain of the southeastern United States (U.S.), allow bats to remain active year-round or migrate from northern latitudes seeking warmer winter temperatures (Grider *et al*., 2016; Perea *et al*., 2023). This region is especially relevant because the ability of Coastal Plain populations to maintain higher activity throughout the winter could translate into lower mortality associated with white-nose syndrome (WNS), an epizootic, infectious fungal disease caused by *Pseudogymnoascus destructans* (*Pd*). WNS has become the most serious threat to North American cave-dwelling bats, affecting overwintering bats by disrupting their torpor cycles and leading to increased energy expenditure and mortality rates. The fact that these are potential areas for remnant populations of species impacted by WNS in northern regions, combined with anthropogenic factors, such as wind energy development (Kunz *et al*., 2007; Grider *et al*., 2016), underscores the importance of understanding the ecology of bats in the southeastern Coastal Plain. Forests account for an important component of the Coastal Plain landscape, with > 86% of forests being privately owned (Oswalt *et al*., 2019). Managed or working forests refer to forests that are actively maintained to achieve specific goals, such as the production of timber products, provision of recreational activities, creation of wildlife habitat, and carbon sequestration and storage. These forests are supported by economic incentives for sustainable management, which reduces the likelihood of their conversion to urban or agricultural land uses (Zobrist *et al*., 2005). Working forests provide resources for a variety of wildlife species, including foraging and roosting resources for bats (Brigham, 2007) and, in turn, bats provide essential ecosystem services to forests, such as phytophagous insect control (Mass *et al*., 2016; Garin *et al*., 2019; Maslo *et al*., 2022).

The diet of North American bat species has traditionally been identified by morphological methods which involve identifying remains of prey in fecal samples (Whitaker Jr, 1988; Carter *et al*., 2003; Fedhamer *et al*., 2009; Moosman *et al*., 2012). However, identification of remains is difficult and biased toward hard-bodied insects, such as Coleoptera, which persist through digestion less degraded (Clare *et al*., 2014). In recent years, DNA metabarcoding has contributed greatly to our understanding of predator–prey relationships, including the diet of bats in forests and agricultural systems. Metabarcoding enables elucidation of diet through simultaneous sequencing of a single DNA region from multiple constituent species of a complex sample34. Such studies have revealed predation of important pests for multiple agricultural commodities in North America (Brown *et al*., 2015; Whitby *et al*., 2020; Hughes *et al*., 2021). For example, Boyles *et al*. (2011) valued the ecosystem services that insectivorous bats provide at $22.9 billion per year on agroecosystems across the United States. In addition, these advances in molecular techniques documented the consumption of insect vectors of human diseases (Wray *et al*., 2018; Hughes *et al*., 2022), including multiple arthropod-borne viruses (arboviruses). Overall, molecular techniques provide much information on the prey consumption preferences of bats. However, much remains to be understood in terms of diet overlap, resource distribution, and differences in availability across seasons (e.g., summer vs. winter).

To date, with the exception of Bernard *et al*. (2021), who evaluated the diet of cave-dwelling bat species captured outside caves during winter in Tennessee, United States, most molecular studies in North America focused on summer diet (Clare *et al*., 2009; 2014; Wray *et al*., 2018; Hughes *et al*., 2021; O'Rourke *et al*., 2022). However, effective conservation decisions require a thorough understanding and assessment of trophic interactions among multiple species over time. Hence, it is imperative to understand the diet of bat communities throughout the year to obtain better estimates of ecological services (Boyles *et al*., 2011; Russo *et al*., 2018). Given the important representation of bats in forest vertebrate diversity, limited knowledge about dietary preferences during winter, and their roles as arthropod controllers (including pests of economic and health concern), we assessed the winter diet composition of bat communities on private, working forests of the southeastern U.S. Coastal Plain using DNA metabarcoding (Figure 1). To better understand complex diet dynamics, our objectives were to (1) identify the diet composition of overwintering bats, (2) compare the composition of prey consumed by bat species, and (3) determine the potential role of forest bats as pest controllers in winter.

MATERIALS AND METHODS

Study area

We conducted our study on private, working forest landscapes in late January through mid-March, 2021–2022 in four states (Georgia, Louisiana, Mississippi, and North Carolina) (Figure 1). Our study areas were characterized by a mosaic of forested landscapes with crop fields and areas with varying degrees of development (Wickham *et al*., 2023). We selected study areas > 3,000 ha that consisted primarily of planted loblolly pine stands interspersed with riparian management areas (predominantly mature hardwood stands), roads, and wildlife openings. Management activities were typical of commercial forestry operations in the region, including clear-cutting at 20–35 years, mechanical and/or chemical site preparation, and planting 182–283 pine trees ha^{-1} (Gresham, 2002). Competing vegetation was temporarily suppressed through herbicide applications, prescribed fire, or mechanically, with most stands being thinned at least once. We defined January–March as the winter sampling season, as mean nighttime temperatures are lowest (typically < 10 °C) during this time in most of the Coastal Plain region (Perea *et al*., 2023).

Sample collection

We captured bats using a combination of single, double, and triple high net sets (Avinet Inc., Dryden, New York, U.S.; mesh diameter: 75/2, 2.6 m high, 4-shelves, 6–12 m wide) located along forest corridors, streams, under bridges, road ruts, and small ponds. We opened mist nets 30 min before sunset and left them open for 4–5 h, checking them every 10–15 min. We placed captured bats in individual clean paper bags and held them for 25–30 min to provide time for defecation28. After holding, we identified individuals to species, recorded sex, reproductive condition, forearm length (mm), and weight (g), and released them at the capture site. We collected 3–4 fecal samples from paper bags using sterile forceps, considering fecal samples from each individual bag as a single sample. We placed them into sterile 0.5 ml

Eppendorf tubes (Eppendorf Inc., Enfield, Connecticut, U.S.) with 70% ethanol and stored them in coolers in the field and during transport to the laboratory. We stored samples at − 80 °C prior to DNA extraction.

Ethics statement

Field research followed U.S. Fish and Wildlife Service's (USFWS) WNS Decontamination Guidelines (WNS Team, 2018) and recommended strategies to reduce risk of transmission of SARS-CoV-2 from humans to bats (Kingston et al., 2021). All capture and handling techniques were approved by the University of Georgia Animal Care and Use Committee #A2019 11-017-Y3- 168 A0), in compliance with the ARRIVE guidelines, and were consistent with guidelines published by the American Society of Mammalogists (Sikes, 2016). We obtained federal (#ES60238B) and state permit collections (Georgia Scientific Collection Permit #1000598963, Mississippi Scientific Collection Permit #0210211, Louisiana Scientific Collection Permit #WDP-22-002, and North Carolina Scientific Collection Permit numbers: Endangered Species Permit #21-ES00643 and NC Wildlife Collection License #22-SC01323).

DNA extraction, library preparation, and sequencing

We ground each fecal sample in a 1.5 mL microcentrifuge tube using a micropestle to homogenize feces and increase surface area, then centrifuged. We then aspirated and discarded the ethanol, and samples were allowed to dry briefly under sterile conditions. We extracted DNA from up to 250 mg of each sample using a Qiagen QIAmp DNA Stool Mini Kit following the manufacturer's protocol (Qiagen, Germantown, Maryland, U.S.) with minor modifications. We prepared at least one blank extraction from each extraction kit and used it as a negative control in downstream analyses. We assessed quality and concentration of DNA extracts using a NanoDrop™ One microvolume UV–Vis spectrophotomter (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.) prior to library preparation. We stored all DNA extracts at − 20 °C until amplification. We selected the number of samples to be sequenced based on the quality and concentration of DNA and abundance of samples for each species, avoiding selecting multiple samples of a species from the same site and night when possible.

A segment of the cytochrome c oxidase subunit I (COI) was amplified using the ANML primer pair, LCO1490 and CO1-CFMRa (Vrijenhoek, 1994; Jusino *et al*., 2019). The ANML primers demonstrate preferential binding to arthropod COI and enhance the representation of arthropod taxa relative to mammalian and avian predator DNA (Jusino *et al*., 2019). We modified primers to contain 5′ overhang sequences required for Illumina library preparation and were synthesized by Integrated DNA Technologies (Coralville, Iowa, U.S.). The PCR reaction mixture consisted of 12.5 uL KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Cape Town, South Africa), 2.5 uL of each primer (2.0 uM), 5 uL genomic DNA, and 2.5 uL molecular-grade water, for a final volume of 25 uL. Amplification reactions began with an initial denaturation of 95 °C for 3 min, 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 5 min (Jusino *et al*. 2019). We checked all PCR products for successful amplification using gel electrophoresis, and we retained any samples that displayed at least a faint band at approximately 180 bp for further library preparation. We submitted sample amplicons to the Georgia Genomics and Bioinformatics Core (GGBC) for the remaining library preparation steps and sequencing on the Illumina NextSeq 2000 (Illumina, San Diego, California, U.S.). We generated paired-end reads (i.e., each amplicon was sequenced twice, once in each direction) at a length of 301 bp using the NextSeq 2000 P3 reagent kit (300 cycles, Illumina).

Bioinformatic analyses

We demultiplexed reads by GGBC and received in FASTQ format. We performed all DNA sequence processing using the AMPtk pipeline (Palmer *et al*., 2018). We trimmed sequences to remove low-quality (< Q20) bases and primers and merged them. We then filtered reads for overall quality, dereplicated them to identify unique sequences, sorted each by abundance, and grouped each into OTUs at a 97% identity threshold using UPARSE (Edgar, 2013; Clare *et al*., 2016). We then applied the LULU algorithm to identify and correct errors (Frøslev et al., 2017). Finally, we assigned taxonomic identities to OTUs using USEARCH (Edgar, 2010). We based taxonomic identities on the consensus agreement among three independent comparisons of sequences to the Barcode of Life Database v3 (BOLD) using global alignment, SINTAX, and UTAX algorithms (Palmer *et al*., 2018).

Statistical analyses

We conducted all analyses and visualizations in R 4.1.1 (R Core Team, 2020). We analyzed diet composition by bat species and overlap among species using OTUs with assigned taxonomy. We first identified and filtered out rare taxa, defined as those with fewer than 10 reads across all samples (Bernard *et al*., 2021), and samples with fewer than 1,000 reads using the Phyloseq package (version 1.38.0; McMurdie & Holmes, 2013). We examined diet composition using heat trees constructed with the Metacoder package, which display taxa that were identified in samples and their lineage (version 0.3.6; Foster *et al*., 2017). Specifically, we developed a single heat tree for each bat species, representing all insect families consumed by that species and individual heat trees for the three dominant insect orders consumed. Then, we used the vegan package (version 2.6.4; Oksanen, 2010) to test for variations in prey composition among bat species by Analysis of Similarity (ANOSIM) and Permutational Multivariate Analysis of Variance (PERMANOVA) tests with 999 permutations (Anderson & Walsh, 2013). Because PERMANOVA can sometimes be affected by non-homogeneity of dispersion for unbalanced sampling schemes, we also performed a permutational dispersion test (Anderson & Walsh, 2013). Lastly, we performed post-hoc pairwise multilevel comparisons using the pairwise Adonis package with Bonferroni adjustment (version 0.4.1; Martinez Arbizu, 2020) to determine differences among species.

RESULTS

We captured 264 individuals of eight bat species from late-January to mid-March 2021–2022, collecting fecal samples from 209 individuals, from which we selected samples from 195 individuals. After bioinformatics processing and quality filtering, we obtained diet composition from 126 individuals of seven species (Table 1). None of the fecal samples from the two captured *Dasypterus intermedius* passed quality control. We obtained 2703 unique Operational Taxonomic Units (OTUs), 2127 (78.69%) of which were matched to sequences in the Barcode of Life Database v3 (BOLD) reference collection after pruning. These matches belonged to 22 orders, 174 families, and 422 genera or species (Supplementary Material 1). Within analyzed fecal samples, Coleoptera (n = 610 OTUs), Diptera (n = 684 OTUs), and Lepidoptera (n = 551 OTUs) were the most consumed orders (Supplementary Material 2). These three orders were the most consumed orders by all bat species except *Lasiurus cinereus* (Figure 2, Table 1), which had a scarce representation of Coleoptera, although with a sample size of only three individual bats. For the remaining bat species, percentages varied among species such as *L. borealis* where 41.46% was based on Lepidoptera, to species such as *Eptesicus fuscus*, where 45.25% corresponded to Coleoptera, or *Perimyotis subflavus* with a preference for dipterans (49.21%) (Figure 2, Table 1).

Diet composition was dissimilar among species (Bray–Curtis: $R = 0.27$, $P < 0.001$). Additionally, we detected significant differences both in the dispersion of diet composition among species (Bray–Curtis: $F_{6,125} = 2.01$, $r^2 = 0.09$, $p = 0.001$), and when performing the permutational dispersion test (F6,125 = 4.04, Nperm = 999, $p = 0.002$). Lastly, post-hoc pairwise multilevel comparisons revealed significant differences (p adj. < 0.05) in diet composition among *E. fuscus*/*Nycticeius humeralis* (F = 2.60, p adj. = 0.02), *L. borealis*/*N. humeralis* (F = 2.61, p adj. = 0.02), *L. cinereus*/*L. seminolus* (F = 1.65, p adj. = 0.04), *L. cinereus*/*N. humeralis* (F = 2.21, p adj. = 0.02), *L. seminolus*/*Myotis austroriparius* (F = 1.80, p adj. = 0.04), *L. seminolus*/*N. humeralis* (F = 4.32, p adj. = 0.02), and *M. austroriparius*/*N. humeralis* (F = 3.14, p adj. = 0.02). All other post-hoc pairwise multilevel comparisons did not show significant differences (p adj. > 0.05).

Bats consumed agricultural and forest pest species in five orders (Coleoptera [n = 12], Diptera [n = 2], Hemiptera [n = 5], Lepidoptera [n = 27], and Trombidiformes [n = 1]). Forest pests, including *Argyrotaenia pinatubana*, *Clepsis peritana*, *Hylobius pales*, and *Rhyacionia frustrana* were consumed by multiple bat species (Table 2). As for dipteran parasite vectors, we documented five genera of mosquitoes (Family Culicidae), highlighting mosquitoes of the genus *Culex*, including *C. nigripalpus*, *C. salinarius*, and *C. territans*, widely present in the diet of all bat species except for *L. cinereus* (Figure 2, Table 2). Other known parasite vectors included two genera of black flies (Family Simuliidae), three genera of sandflies (Family Ceratopogonidae), and one genus of drain or sewer fly (Family Psychodidae) (Supplementary Material 3).

DISCUSSION

Our results show a great variability (22 arthropod orders) in diet across bat species, highlighting the consumption mainly of Coleoptera, Diptera, and Lepidoptera. As expected, diet composition differed among bat species with different foraging strategies, but surprisingly also among species in similar foraging guilds. Our findings complement previous work conducted during summer indicating that bat diets vary seasonally (Clare *et al*., 2014; O'Rourke *et al*., 2022; Aihartza *et al*., 2023), which may depend on insect phenologies and weather conditions. Specifically in winter, seasonal prey limitations may lead to shifts towards more generalist behavior in several bat species, with changes in dietary composition and diversity compared to other times of the year and life stages of bats. Further, our results confirm the role of overwintering bat communities as consumers of agricultural and forest pests and potential arthropod vectors of human and animal diseases.

Traditionally, dietary preferences of insectivorous bats have been explained based on differences in their ecomorphologies and morphometric characteristics, with larger species feeding on larger insects or insects with more resistant exoskeletons (Norberg & Rayner, 1987; Denzinger & Schnitzler, 2013). The energetic cost–benefit of feeding on smaller insects compared to larger insects or insects with more resistant exoskeletons would lead to dietary selection based on the morphological characteristics of each bat species (Freeman, 1981). For example, it is often questioned whether species, especially large-body bats, can meet energy demands consuming small soft-body insects such as flies and mosquitoes (Wetzler & Boyles, 2018). However, availability and temporal variation of prey may lead to shifts in preferences towards more generalist diets. Eptesicus fuscus, the second largest of the seven species captured, is considered a coleopteran specialist (e.g., Agosta, 2002; Feldhamer *et al*., 2009; Dodd *et al*., 2012). Recently, this assumption has been questioned, placing *E. fuscus* instead as generalist consumers in summer with preferences for Coleoptera when available (Clare *et al*., 2014; Wray *et al*., 2018; Whitby *et al*., 2020). In our study, a large portion of their diet was Coleoptera, but we found high dietary diversity, including many dipterans, possibly attributed to more dipterans in winter relative to other insect orders (Bernard *et al*., 2021). Flexible hunting strategies may allow bat species to adapt to different food availabilities by consuming prey that is abundant at the time, although of non-optimal sizes or other characteristics (Divoll *et al*., 2022). In contrast, the diet of *L. cinereus*, the largest species in our study and one of the largest species in North America, was comprised primarily of Diptera and Lepidoptera. Although our results should be interpreted with caution because of the small sample size (n = 3 individual bats), previous studies suggest that *L. cinereus* select large, soft-bodied insects (e.g., Lepidoptera and Neuroptera) and avoid small or hard-bodied insects (e.g., Coleoptera, Diptera, and Hemiptera) (Valdez & Cryan, 2009; Reimer *et al*., 2010; Perlik *et al*., 2012). Most of the dipterans we documented in the diet were large crane fly species such as *Nephrotoma ferruginea* (Table 2), which supports a preference for large, soft-bodied prey.

The remaining bat species in our study are smaller and adapted to foraging along forest edges or within forests (e.g., Loeb & O'Keefe, 2006; Bender *et al*., 2015; Perea *et al*., 2023). *Lasiurus borealis* and *L. seminolus* share similar ecomorphologies, to the point that it is difficult to separate them by the characteristics of their echolocation calls or external morphology (Laerm *et al*., 1999; Szewczak *et al*., 2011). Both species have robust dentition like other Coleoptera specialists (Freeman, 1981). However, both ours and previous dietary analyses indicate that they consume a wide range of soft-bodied prey such as Diptera, Lepidoptera, and Neuroptera (e.g., Clare *et al*., 2009; Weinkauf *et al*., 2018; Hughes *et al*., 2021). The dietary differences identified between *N. humeralis* with *L. borealis* and *L. seminolus* could be due to the partitioning of selected prey within the same spaces and slight differences in ecomorphology and general external morphologies. The morphometrics and dentition of *N. humeralis* together with previous summer dietary analyses show flexibility in its diet, which allows it to eat a wide range of arthropods, from coleopterans to soft-bodied prey (Freeman, 1981; Feldhamer *et al*., 2009; Münzer *et al*., 2016). Our results confirm similar preferences in the diet during winter, where we observed high dietary diversity, distinguishing *N. humeralis* from other species. These findings are supported by the presence of OTUs from all 22 identified orders.

Previous works indicate that *M. austroriparius* and *P. subflavus* consume primarily soft body prey (Feldhamer et al., 2009; Weinkauf *et al*., 2018). Using morphological dietary analyses, Feldhamer *et al*. (2009) found that both species consumed mainly trichopterans, suggesting a diet of soft-bodied species found predominantly above water. However, we observed numerous Coleoptera OTUs present in the diet of *M. austroriparius*, which highlights its dietary plasticity, consuming hard-bodied insects in winter. Differences between *M. austroriparius* with diets of *L. seminolus* and *N. humeralis* suggest a tendency towards a more specialized diet likely influenced by its forest-interior foraging strategies (Perea *et al*., 2023). *Perimyotis subflavus* is among the smallest bats in North America (Brigham, 1991). Previous studies have noted that *P. subflavus* shows an opportunistic approach when foraging, exhibiting one of the most diverse diets in eastern North American bat species (Dodd *et al*., 2014; Weinkauf *et al*., 2018). However, we found that *P. subflavus* consumed the second lowest number of orders, but a large proportion of dipterans, which concurs with previous research that documented frequent consumption of dipterans by *P. subflavus* in winter (Bernard *et al*., 2021). Disproportionate consumption of dipterans in winter compared to other seasons could be a consequence of a selection for small soft-bodied prey and a higher abundance of Diptera relative to other orders.

To our knowledge, our study is the first to document the consumption of agricultural and forest pests by winter bat communities in the southeastern U.S. Coastal Plain where intensive pine management and agriculture dominate the landscape. Among the most common forest pest species we documented in bat diets, *R. frustrana*, is an economically important pest of young pines, especially for loblolly pine (*Pinus taeda*), the preferred host species (Asaro *et al*., 2003). Our study coincided with the time period when *R. frustrana* typically emerges (Yates, 1981), highlighting the importance of this moth to most bat species when availability is high. Additionally, *H. pales* was also widely consumed by most bat species in our study. *Hylobius pales* causes damage to young pine seedlings and is a vector of commercially damaging Ophiostomatalean “blue-stain” fungi such as *Leptographium* spp., which discolor and degrade the value of colonized wood (Nevill & Alexander, 1992; Zanzot *et al*., 2010). Our research also reveals the consumption of various agricultural pests by bats, such as the moths *C. peritana* and *H. scabra*, which likely inhabit agricultural areas embedded within the working forest landscapes. While *H. scabra* was not the most frequently consumed pest nor found in large numbers, it was present in the winter diet of five bat species, including migratory species like *L. borealis* and *L. cinereus* (Cryan, 2003). *Hypena scabra* is a migratory moth, with most populations overwintering south of the midwestern U.S. Corn Belt (McCarville *et al*., 2010, Whitby *et al*., 2020). Although it is generally of minor economic importance, this moth is one of the most common defoliating insects in alfalfa and soybean fields (Higley & Boethel, 1994). Consumption of overwintering populations of *H. scabra* in this ecoregion may provide a yet undocumented ecosystem service in controlling populations outside of the growing season and outside the major crop-producing areas of the Corn Belt. Overall, our findings suggest that consumption of agricultural and forest pests by bats in late winter and early spring could play a crucial role in minimizing damage during the subsequent growing season, highlighting the potential significance of bats as natural pest controllers in agricultural and forested landscapes.

Finally, we identified several species of flies and mosquitoes (Diptera) in winter diets that are recognized as threats to human health. Global concern about mosquitoes (Family Culicidae) stems from their significant impact on public health, attributed to their role as disease vectors. This impact extends to the transmission of multiple diseases [e.g., West Nile virus (Hoover & Barker, 2016), malaria (Rogers & Randolph, 2000), dengue (Vicente-Santos *et al*., 2017), dog (*Canis lupus familiaris*), heartworm (Cancrini *et al*., 2003), myxomatosis (Flowerdew *et al*., 1992), or avian malaria (Atkinson *et al*., 2000)] with far-reaching consequences for human societies, wildlife, and ecosystems. Our results reveal a diverse array of mosquito vectors, including species of the genera *Aedes* and *Culex*, common vectors of diseases such as West Nile virus. In addition, we identified malaria vectors, such as *Anopheles* mosquitoes, and specific cases of non-native mosquitoes, such as *Aedes japonicus*, implicated in the transmission and/or maintenance of arboviruses, both endemic to the region (e.g., West Nile virus) and exotic (e.g., Zika, dengue, and chikungunya; Gutiérrez-López *et al*., 2022). Although little known to date (Russo *et al*., 2018), our results also demonstrated consumption of other dipterans that may pose a threat to wildlife, livestock, and poultry. For example, we confirmed consumption of Diptera such as black flies (Family Simuliidae), which are capable of transmitting pathogens, including protozoa and nematode worms to vertebrates, and are thus a veterinary concern, even if none of them cause disease in humans in North America (Adler *et al*., 2010).

Identifying diet composition in overwintering bats and recognizing differences in prey consumption among species contribute valuable insights into the ecological role of bats in working forest landscapes. As these forests are crucial for remnant populations affected by WNS and migratory species affected by wind energy development, understanding winter bat foraging ecology becomes paramount. The potential role of forest bats as pest controllers during winter underscores the importance of managing working forests in ways that support the diverse dietary needs of the bat community. Our findings have a direct connection to economics and timber quality; for example, *R. frustrana* is known to have a drastic impact on pine growth, both in tree height and diameter (Asaro *et al*., 2003). Hence, proactive forest management practices that improve bat habitat conditions (Taylor *et al*., 2020), such as retention of hardwoods, trees with exfoliating bark, and cavity trees (live and dead) also increase their economic benefits. Additionally, our results show the role of bat communities outside forest boundaries consuming agricultural pests and other potential arthropod vectors of disease. Conservation efforts thus may consider ecological services provided by bats, including their ability to contribute to control of agricultural and forest pests and potentially limit the spread of disease vectors. We also emphasize the role of private lands conservation in promoting bat habitat and their consequent ecosystem services.

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LITERATURE CITED

Adler, P. H., Cheke, R. A., & Post, R. J. (2010). Evolution, epidemiology, and population genetics of black flies (Diptera: Simuliidae). Infection, Genetics and Evolution, 10(7), 846-865. https://doi.org/10.1016/j.meegid.2010.07.003

Agosta, S. J. (2002). Habitat use, diet and roost selection by the big brown bat (*Eptesicus fuscus*) in North America: a case for conserving an abundant species. Mammal Review, 32(3), 179-198. https://doi.org/10.1046/j.1365-2907.2002.00103.x

Alberdi, A., Razgour, O., Aizpurua, O., Novella-Fernandez, R., Aihartza, J., Budinski, I., ... & Gilbert, M. T. P. (2020). DNA metabarcoding and spatial modelling link diet diversification with distribution homogeneity in European bats. Nature Communications, 11(1), 1154. https://doi.org/10.1038/s41467-020-14961-2

Alonso, H., Granadeiro, J. P., Waap, S., Xavier, J., Symondson, W. O., Ramos, J. A., & Catry, P. (2014). An holistic ecological analysis of the diet of Cory's shearwaters using prey morphological characters and DNA barcoding. Molecular Ecology, 23(15), 3719-3733. https://doi.org/10.1111/mec.12785

Ancillotto, L., Ariano, A., Nardone, V., Budinski, I., Rydell, J., & Russo, D. (2017). Effects of free-ranging cattle and landscape complexity on bat foraging: implications for bat conservation and livestock management. Agriculture, Ecosystems & Environment, 241, 54-61. https://doi.org/10.1016/j.agee.2017.03.001

Ancillotto, L., Rummo, R., Agostinetto, G., Tommasi, N., Garonna, A. P., de Benedetta, F., ... & Russo, D. (2022). Bats as suppressors of agroforestry pests in beech forests. Forest Ecology and Management, 522, 120467. https://doi.org/10.1016/j.foreco.2022.120467

Aihartza, J., Vallejo, N., Aldasoro, M., García-Mudarra, J. L., Goiti, U., Nogueras, J., & Ibáñez, C. (2023). Aerospace-foraging bats eat seasonably across varying habitats. Scientific Reports, 13(1), 19576. https://doi.org/10.1038/s41598-023-46939-7

Aizpurua, O., Budinski, I., Georgiakakis, P., Gopalakrishnan, S., Ibañez, C., Mata, V., ... & Alberdi, A. (2018). Agriculture shapes the trophic niche of a bat preying on multiple pest arthropods across Europe: Evidence from DNA metabarcoding. Molecular Ecology, 27(3), 815-825. https://doi.org/10.1111/mec.14474

Anderson, M. J., & Walsh, D. C. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecological Monographs, 83(4), 557-574. https://doi.org/10.1890/12-2010.1

Arrizabalaga-Escudero, A., Clare, E. L., Salsamendi, E., Alberdi, A., Garin, I., Aihartza, J., & Goiti, U. (2018). Assessing niche partitioning of co-occurring sibling bat species by DNA metabarcoding. Molecular Ecology, 27(5), 1273-1283. https://doi.org/10.1111/mec.14508

Asaro, C., Fettig, C. J., McCravy, K. W., Nowak, J. T., & Berisford, C. W. (2003). The Nantucket pine tip moth (Lepidoptera: Tortricidae): a literature review with management implications. Journal of Entomological Science, 38(1), 1-40. https://doi.org/10.18474/0749-8004-38.1.1

Atkinson, C. T., Dusek, R. J., Woods, K. L., & Iko, W. M. (2000). Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. Journal of wildlife diseases, 36(2), 197-201. https://doi.org/10.7589/0090-3558-36.2.197

Bartley, T. J., McCann, K. S., Bieg, C., Cazelles, K., Granados, M., Guzzo, M. M., ... & McMeans, B. C. (2019). Food web rewiring in a changing world. Nature Ecology & Evolution, 3(3), 345-354. https://doi.org/10.1038/s41559-018-0772-3

Beilke, E. A., & O'Keefe, J. M. (2023). Bats reduce insect density and defoliation in temperate forests: An exclusion experiment. Ecology, 104(2), e3903. https://doi.org/10.1002/ecy.3903

Bender, M. J., Castleberry, S. B., Miller, D. A., & Wigley, T. B. (2015). Site occupancy of foraging bats on landscapes of managed pine forest. Forest Ecology and Management, 336, 1-10. https://doi.org/10.1016/j.foreco.2014.10.004

Bernard, R. F., Willcox, E. V., Jackson, R. T., Brown, V. A., & McCracken, G. F. (2021). Feasting, not fasting: winter diets of cave hibernating bats in the United States. Frontiers in Zoology, 18, 1-13. https://doi.org/10.1186/s12983-021-00434-9

Brechtel, A., Gross, T., & Drossel, B. (2019). Far-ranging generalist top predators enhance the stability of meta-foodwebs. Scientific Reports, 9(1), 12268. https://doi.org/10.1038/s41598-019-48731-y

Barclay, R. M., & Brigham, R. M. (1991). Prey detection, dietary niche breadth, and body size in bats: why are aerial insectivorous bats so small?. The American Naturalist, 137(5), 693-703.

Brigham, R. M. (2007). Bats in forests: what we know and what we need to learn (pp. 1-15). Baltimore, Maryland: Johns Hopkins University Press.

Brown, V. A., de Torrez, E. B., & McCracken, G. F. (2015). Crop pests eaten by bats in organic pecan orchards. Crop Protection, 67, 66-71. https://doi.org/10.1016/j.cropro.2014.09.011

Böhm, S. M., Wells, K., & Kalko, E. K. (2011). Top-down control of herbivory by birds and bats in the canopy of temperate broad-leaved oaks (Quercus robur). PLoS One, 6(4), e17857. https://doi.org/10.1371/journal.pone.0017857

Boyles, J. G., Cryan, P. M., McCracken, G. F. & Kunz, T. H. (2011). Economic importance of bats in agriculture. Science 332(6025), 41–42. https://doi.org/10.1126/science.1201366

Cancrini, G., Di Regalbono, A. F., Ricci, I., Tessarin, C., Gabrielli, S., & Pietrobelli, M. (2003). Aedes albopictus is a natural vector of Dirofilaria immitis in Italy. Veterinary Parasitology, 118(3-4), 195-202. https://doi.org/10.1016/j.vetpar.2003.10.011

Carter, T. C., Menzel, M. A., Owen, S. F., Edwards, J. W., Menzel, J. M., & Ford, W. M. (2003). Food habits of seven species of bats in the Allegheny Plateau and Ridge and Valley of West Virginia. Northeastern Naturalist, 10(1), 83-88. https://doi.org/10.1656/1092-6194(2003)010[0083:FHOSSO]2.0.CO;2

Charbonnier, Y., Barbaro, L., Theillout, A. & Jactel, H. (2014). Numerical and functional responses of forest bats to a major insect pest in pine plantations. PLoS ONE 10(1), e0117652. https://doi.org/10.1371/journal.pone.0117652

Clare, E. L., Fraser, E. E., Braid, H. E., Fenton, M. B., & Hebert, P. D. (2009). Species on the menu of a generalist predator, the eastern red bat (Lasiurus borealis): using a molecular approach to detect arthropod prey. Molecular Ecology, 18(11), 2532-2542. https://doi.org/10.1111/j.1365-294X.2009.04184.x

Clare, E. L., Symondson, W. O., & Fenton, M. B. (2014). An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (Eptesicus fuscus). Molecular Ecology, 23(15), 3633-3647. https://doi.org/10.1111/mec.12519

Clare, E. L., Chain, F. J., Littlefair, J. E., & Cristescu, M. E. (2016). The effects of parameter choice on defining molecular operational taxonomic units and resulting ecological analyses of metabarcoding data. Genome, 59(11), 981-990. https://doi.org/10.1139/gen-2015-0184

Cryan, P. M. Seasonal distribution of migratory tree bats (Lasiurus and Lasionycteris) in North America. (2003). Journal of Mammalogy. 84(2), 579–593. https://doi.org/10.1644/1545-1542084%3c0579:SDOMTB%3e2.0.CO;2

Denzinger, A., & Schnitzler, H. U. (2013). Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. Frontiers in physiology, 4, 164. https://doi.org/10.3389/fphys.2013.00164

Divoll, T. J., Brown, V. A., McCracken, G. F., & O'Keefe, J. M. (2022). Prey size is more representative than prey taxa when measuring dietary overlap in sympatric forest bats. Environmental DNA, 4(6), 1407-1419. https://doi.org/10.1002/edn3.354

Dodd, L. E., Lacki, M. J., Britzke, E. R., Buehler, D. A., Keyser, P. D., Larkin, J. L., ... & Rieske, L. K. (2012). Forest structure affects trophic linkages: how silvicultural disturbance impacts bats and their insect prey. Forest Ecology and Management, 267, 262-270. https://doi.org/10.1016/j.foreco.2011.12.016

Dodd, L. E., Lacki, M. J., Cox, D. R. & Rieske, L. K. (2014) Prey consumed by bats across Central Appalachia prior to detection of white-nose syndrome. Journal of the Kentucky Academy of Science, 75(1–2), 85–93. https://doi.org/10.3101/kyac-75-01-85-93.1

Downs, N. C. & Sanderson, L. J. (2010). Do bats forage over cattle dung or over cattle? Acta Chiropterologica, 12(2), 349–358. https://doi.org/10.3161/150811010X537936

Edgar, R. (2010). Usearch. Lawrence Berkeley National Lab.(LBNL), Berkeley, CA (United States).

Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nature Methods, 10(10), 996–998. https://doi.org/10.1038/nmeth.2604

Feldhamer, G. A., Carter, T. C., & Whitaker, J. O. (2009). Prey consumed by eight species of insectivorous bats from southern Illinois. The American Midland Naturalist, 162(1), 43-51. https://doi.org/10.1674/0003-0031-162.1.43

Flowerdew, J. R., Trout, R. C., & Ross, J. (1992). Myxomatosis: population dynamics of rabbits (Oryctolagus cuniculus Linnaeus, 1758) and ecological effects in the United Kingdom. REVUE SCIENTIFIQUE ET TECHNIQUE-OFFICE INTERNATIONAL DES EPIZOOTIES, 11, 1109-1109.

Foster, Z. S., Sharpton, T. J., & Grünwald, N. J. (2017). Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. PLoS Computational Biology, 13(2), e1005404. https://doi.org/10.1371/journal.pcbi.1005404

Freeman, P. W. (1981). Correspondence of food habits and morphology in insectivorous bats. Journal of Mammalogy. 62(1), 166–173.

Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nature Communications, 8(1), 1188. https://doi.org/10.1038/s41467-017-01312-x

Garin, I., Aihartza, J., Goiti, U., Arrizabalaga-Escudero, A., Nogueras, J., & Ibáñez, C. (2019). Bats from different foraging guilds prey upon the pine processionary moth. PeerJ, 7, e7169. https://doi.org/10.7717/peerj.7169

Gresham, C. A. (2002). Sustainability of intensive loblolly pine plantation management in the South Carolina Coastal Plain, USA. Forest Ecology and Management, 155(1-3), 69-80. https://doi.org/10.1016/S0378-1127(01)00548-5

Grider, J. F., Larsen, A. L., Homyack, J. A. & Kalcounis-Rueppell, M. C. (2016). Winter activity of Coastal Plain populations of bat species affected by white-nose syndrome and wind energy facilities. PLoS ONE 11(11), e0166512. https://doi.org/10.1371/journal.pone.0166512

Gutiérrez-López, R., Logan, J., & la Puente, J. M. D. (2022). Introducing the role of mosquitoes in the transmission of pathogens to wildlife. In Ecology and Control of Vector-borne Diseases (pp. 532-533). Wageningen Academic Publishers.

Higley, L. G., & Boethel, D. J. (Eds.). (1994). Handbook of soybean insect pests (Vol. 1). Entomological Society of America.

Hoover, K. C., & Barker, C. M. (2016). West Nile virus, climate change, and circumpolar vulnerability. Wiley Interdisciplinary Reviews: Climate Change, 7(2), 283-300. https://doi.org/10.1002/wcc.382

Hughes, M. J., de Torrez, E. C. B., & Ober, H. K. (2021). Big bats binge bad bugs: Variation in crop pest consumption by common bat species. Agriculture, Ecosystems & Environment, 314, 107414. https://doi.org/10.1016/j.agee.2021.107414

Hughes, M. J., de Torrez, E. C. B., Buckner, E. A., & Ober, H. K. (2022). Consumption of endemic arbovirus mosquito vectors by bats in the southeastern United States. Journal of Vector Ecology, 47(2), 153-165. https://doi.org/10.52707/1081-1710-47.2.153

Jusino, M. A., Banik, M. T., Palmer, J. M., Wray, A. K., Xiao, L., Pelton, E., ... & Lindner, D. L. (2019). An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. Molecular Ecology Resources, 19(1), 176-190. https://doi.org/10.1111/1755-0998.12951

Kemp, J., López-Baucells, A., Rocha, R., Wangensteen, O. S., Andriatafika, Z., Nair, A., & Cabeza, M. (2019). Bats as potential suppressors of multiple agricultural pests: a case study from Madagascar. Agriculture, Ecosystems & Environment, 269, 88-96. https://doi.org/10.1016/j.agee.2018.09.027

Kingston, T., Medellin, R., Waldien, D., Newport, C., & Solari, S. (2021). IUCN SSC Bat Specialist Group. Policy.

Kunz, T. H., Arnett, E. B., Erickson, W. P., Hoar, A. R., Johnson, G. D., Larkin, R. P., ... & Tuttle, M. D. (2007). Ecological impacts of wind energy development on bats: questions, research needs, and hypotheses. Frontiers in Ecology and the Environment, 5(6), 315-324. https://doi.org/10.1890/1540-9295(2007)5[315:EIOWED]2.0.CO;2

Laerm, J., Menzel, M. A., Krishon, D. M., & Boone, J. L. (1999). Morphological discrimination between the eastern red bat, *Lasiurus borealis*, and seminole bat, *Lasiurus seminolus* (Chiroptera: Vespertilionidae), in the southeastern United States. Journal of the Elisha Mitchell Scientific Society, 131-139. https://www.jstor.org/stable/24335587

Loeb, S. C., & O'keefe, J. M. (2006). Habitat use by forest bats in South Carolina in relation to local, stand, and landscape characteristics. The Journal of Wildlife Management, 70(5), 1210-1218. https://doi.org/10.2193/0022-541X(2006)70[1210:HUBFBI]2.0.CO;2

Maas, B., Karp, D. S., Bumrungsri, S., Darras, K., Gonthier, D., Huang, J. C. C., ... & Williams-Guillén, K. (2016). Bird and bat predation services in tropical forests and agroforestry landscapes. Biological Reviews, 91(4), 1081-1101. https://doi.org/10.1111/brv.12211

Maine, J. J. & Boyles, J. G. (2015). Land cover influences dietary specialization of insectivorous bats globally. Mammal Research, 60(4), 343–351. https://doi.org/10.1007/s13364-015-0243-z

Martinez Arbizu, P. (2020). pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.4.1.

Maslo, B., Mau, R. L., Kerwin, K., McDonough, R., McHale, E., & Foster, J. T. (2022). Bats provide a critical ecosystem service by consuming a large diversity of agricultural pest insects. Agriculture, Ecosystems & Environment, 324, 107722. https://doi.org/10.1016/j.agee.2021.107722

McCarville, M., Hodgson, E., & O'Neal, M. (2010). Green cloverworms appear in soybean. https://crops.extension.iastate.edu/cropnews/2010/07/green-cloverworms-appear-soybean

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS one, 8(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Moosman, P. R. Jr., Thomas, H. H. & Veilleux, J. P. (2012). Diet of the widespread insectivorous bats *Eptesicus fuscus* and *Myotis lucifugus* relative to climate and richness of bat communities. Journal of Mammalogy, 93(2), 491–496. https://doi.org/10.1644/11-MAMM-A-274.1

Nevill, R. J. & Alexander, S. A. (1992). Transmission of *Leptographium procerum* to eastern white pine by Hylobius pales and Pissodes nemorensis (Coleoptera; Curculionidae). Plant Disease, 76(3), 307–310.

Norberg, U. M., & Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 316(1179), 335-427.

Oksanen, J. (2010). Vegan: community ecology package. http://vegan.r-forge.r-project.org/

Oswalt, S. N., Smith, W. B., Miles, P. D., & Pugh, S. A. (2019). Forest resources of the United States, 2017. General Technical Report-US Department of Agriculture, Forest Service. Forest Service. https://www.fs.usda.gov/treesearch/pubs/57903

O'Rourke, D., Rouillard, N. P., Parise, K. L., & Foster, J. T. (2022). Spatial and temporal variation in New Hampshire bat diets. Scientific Reports, 12(1), 14334. https://doi.org/10.1038/s41598-022-17631-z

Palmer, J. M., Jusino, M. A., Banik, M. T., & Lindner, D. L. (2018). Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. PeerJ, 6, e4925. https://doi.org/10.7717/peerj.4925

Perea, S., Fandos, G., Larsen-Gray, A., Greene, D. U., Chandler, R., & Castleberry, S. B. (2023). Bat winter foraging habitat use in working forests: A multispecies spatial occupancy approach. Animal Conservation. https://doi.org/10.1111/acv.12924

Perlik, M. K., McMillan, B. R., & Krenz, J. D. (2012). Food habits of the Hoary Bat in an agricultural landscape. Journal of the Minnesota Academy of Science, 75(2), 1-6.

Puig-Montserrat, X., Flaquer, C., Gómez-Aguilera, N., Burgas, A., Mas, M., Tuneu, C., ... & López-Baucells, A. (2020). Bats actively prey on mosquitoes and other deleterious insects in rice paddies: Potential impact on human health and agriculture. Pest Management Science, 76(11), 3759-3769. https://doi.org/10.1002/ps.5925

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.Rproject.org/

Reimer, J. P., Baerwald, E. F., & Barclay, R. M. (2010). Diet of hoary (*Lasiurus cinereus*) and silver-haired (*Lasionycteris noctivagans*) bats while migrating through southwestern Alberta in late summer and autumn. The American Midland Naturalist, 164(2), 230-237. https://doi.org/10.1674/0003-0031-164.2.230

Rogers, D. J. & Randolph, S. E. (2000). The global spread of malaria in a future, warmer world. Science 289, 5485. https://doi.org/10.1126/science.289.5485.1763

Russo, D., Bosso, L., & Ancillotto, L. (2018). Novel perspectives on bat insectivory highlight the value of this ecosystem service in farmland: Research frontiers and management implications. Agriculture, Ecosystems & Environment, 266, 31-38. https://doi.org/10.1016/j.agee.2018.07.024

Sikes, R. S., the Animal Care and Use Committee of the American Society of Mammalogists. (2016). Guidelines of the American Society of mammalogists for the use of wild mammals in research and education. Journal of Mammalogy, 97(3), 663–688. https://doi.org/10.1644/10-MAMM-F-355.1

Stephens, D. W. & Krebs, J. R. (1986). Foraging theory. Princeton University Press. 247 p.

Szewczak, J. M., Corcoran, A. J., Kennedy, J., Ormsbee, P. C., & Weller, T. E. (2011). Echolocation call characteristics of eastern US bats. Humboldt State University Bat Lab, 1-4. http://www.sonobat.com/download/EasternUS_Acoustic_Table_Mar2011.pdf

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular ecology, 21(8), 2045-2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Taylor, D. A., Perry, R. W., Miller, D. A. & Ford, W. M. Forest management and Bats. (2020). Publication of the White-nose Syndrome Response Team, 2020. www.whitenosesyndrome.org

Team W. D. National white-nose syndrome decontamination protocol. (2018). Version 09.13.2019, National WNS Plan.

Valdez, E. W., & Cryan, P. M. (2009). Food habits of the hoary bat (*Lasiurus cinereus*) during spring migration through New Mexico. The Southwestern Naturalist, 54(2), 195-200. https://doi.org/10.1894/PS-45.1

Vicente-Santos, A., Moreira-Soto, A., Soto-Garita, C., Chaverri, L. G., Chaves, A., Drexler, J. F., ... & Corrales-Aguilar, E. (2017). Neotropical bats that co-habit with humans function as dead-end hosts for dengue virus. PLoS Neglected Tropical Diseases, 11(5), e0005537. https://doi.org/10.1371/journal.pntd.0005537

Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biolology and Biotechnology, 3(5), 294-9.

Weinkauf, C. J., Comer, C. E., Conway, W. C., & Farrell, C. (2018). Dietary composition of four common chiropteran species in a bottomland hardwood forest. Acta Chiropterologica, 20(1), 195-205. https://doi.org/10.3161/15081109ACC2018.20.1.015

Wetzler, G. C., & Boyles, J. G. (2018). The energetics of mosquito feeding by insectivorous bats. Canadian Journal of Zoology, 96(4), 373-377. https://doi.org/10.1139/cjz-2017-0162

Weterings, R., & Umponstira, C. (2014). Bodyweight-forearm ratio, cranial morphology and call frequency relate to prey selection in insectivorous bats. Electronic Journal of Biology, 10(1), 21-27.

Whitby, M. D., Kieran, T. J., Glenn, T. C., & Allen, C. (2020). Agricultural pests consumed by common bat species in the United States corn belt: the importance of DNA primer choice. Agriculture, Ecosystems & Environment, 303, 107105. https://doi.org/10.1016/j.agee.2020.107105

Whitaker, J. O. Jr. (1988). Food habits analysis of insectivorous bats. In Ecological and Behavioral Methods for the Study of Bats (ed. Kunz, T. H.) 171–189 (Smithsonian Institution Press).

Wickham, J., Stehman, S. V., Sorenson, D. G., Gass, L. & Dewitz, J. A. (2023). Thematic accuracy assessment of the NLCD 2019 land cover for the conterminous United States. GISci. Remote Sensing, 60(1), 2181143. https://doi.org/10.1080/15481603.2023.2181143

Wray, A. K., Jusino, M. A., Banik, M. T., Palmer, J. M., Kaarakka, H., White, J. P., ... & Peery, M. Z. (2018). Incidence and taxonomic richness of mosquitoes in the diets of little brown and big brown bats. Journal of Mammalogy, 99(3), 668-674. https://doi.org/10.1371/journal.pone.0077183

Yates, H. O. (1981). Nantucket Pine Tip Moth Vol. 70 (US Department of Agriculture, Forest Service).

Zanzot, J. W., Matusick, G., & Eckhardt, L. G. (2010). Ecology of root-feeding beetles and their associated fungi on longleaf pine in Georgia. Environmental Entomology, 39(2), 415-423. https://doi.org/10.1603/EN09261

Zobrist, K. W., Hinckley, T. M., Andreu, M. G., Gehringer, K. R., Hedman, C. W., Forest, S., & Lippke, B. R. (2005). Final project report to the National Commission on Science for Sustainable Forestry (NCSSF).

Table 3.1. Bats captured, number of fecal samples collected, number of samples analyzed, number of Operational Taxonomic Units (OTUs) for each bat species within insect orders, and bat species foraging strategies in private, working forest landscapes across four states (Georgia, Louisiana, Mississippi, and North Carolina) of the southeastern U.S. Coastal Plain from late-January to mid-March 2021–2022.

Species	**Total bats captured**	**Total fecal samples**	**Number samples analyzed**	**Coleoptera**	**Diptera**	**Lepidoptera**	**Other**	**Orders**	**Foraging strategy**
Lasiurus seminolus	79	60	44	158	314	383	155	19	Edge-space aerial foragers
Nycticeius humeralis	75	54	32	281	371	132	216	22	Edge-space aerial foragers
Myotis austroriparius	41	37	14	202	186	132	68	17	Narrow-space, aerial-gleaning forager
Perimyotis subflavus	25	20	12	61	218	75	89	13	Edge-space aerial foragers
Lasiurus borealis	25	21	11	65	72	153	79	16	Edge-space aerial foragers
Eptesicus fuscus	14	12	10	200	77	92	73	16	Open and edge-space aerial foragers
Lasiurus cinereus	3	3	3	3	15	13	13	10	Open-space aerial foragers
Dasypterus intermedius	2	2	0	-	-	-	-	-	Open-space aerial foragers
Total	264	209	126	610	684	551	282	22	

Table 3.2. Prey items Operational Taxonomic Units (OTUs) consumed by seven bat species captured in private, working forest landscapes across four states (Georgia, Louisiana, Mississippi, and North Carolina) of the southeastern U.S. Coastal Plain from late-January to mid-March 2021–2022. Top ten items consumed by each bat species are highlighted in bold. Bat species codes: *Eptesicus fuscus* (EPFU), *Lasiurus borealis* (LABO), *Lasiurus cinereus* (LACI), *Lasiurus seminolus* (LASE), *Myotis austroriparius* (MYAU), *Nycticeius humeralis* (NYHU), and *Perimyotis subflavus* (PESU). The asterisk (*) denotes pest species.

Prey OTUs			**Bat species**						
Order	**Family**	**Species**	**EPFU**	**LABO**	**LACI**	**LASE**	**MYAU**	**NYHU**	**PESU**
Araneae	Salticidae	*Pelegrina montana*	2			5	4	7	1
	Theridiidae	*Robertus crosbyi*	2	1		5		**12**	
Coleoptera	Cantharidae	*Podabrus nothoides*	3	2		8	2	**22**	**4**
	Carabidae	*Oodes amaroides*	**4**			2	1	1	
	Carabidae	*Platynus cincticollis*	**5**			1	1	4	
	Carabidae	*Stenolophus ochropezus*	1					**10**	
	Curculionidae	*Hylobius pales**	**5**		**2**	3	1	1	
	Curculionidae	*Xylosandrus crassiusculus**		2		4	1	5	**3**
	Hydrophilidae	*Helocombus bifidus*	**5**	1		**19**	3	10	**5**
	Scarabaeidae	*Dyscinetus morator*		3	**1**	4		2	
	Scirtidae	*Contacyphon ochreatus*				6		**10**	2
Diptera	Chironomidae	*Chironomus df decorus*	**4**	1	1	8	**9**	**16**	2
	Chironomidae	*Chironomus harpi*	2	2	**3**	**16**	4	**18**	2
	Chironomidae	*Orthocladius oliveri*			1	1	**7**		
	Culicidae	*Culex nigripalpus*		1		14	**4**	**15**	2
	Culicidae	*Culex territans*	1	1		13	3	10	**4**
	Culicidae	*Culex salinarius*		1		**16**	2	**11**	**3**
	Limoniidae	*Erioptera caliptera*	4	1		6	1	9	**5**
	Psychodidae	*Psychoda alternata*	1			6		9	**5**
	Tipulidae	*Nephrotoma ferruginea*	3	1	**3**	5	1	4	
Hemiptera	Aphididae	*Eulacachnus rileyi*	2	3		1		1	
Hymenoptera	Apidae	*Nomada subrutila*	3	1		11	2	8	3

	Xyelidae	*Xyela spp.*	3	**3**	1	**16**		**11**	3
Lepidoptera	Erebidae	*Hypena scabra**	2	**3**	**2**	2		1	
	Erebidae	*Schrankia macula*		**3**	1	2			
	Geometridae	*Eupithecia miserulata**		**4**		4			
	Geometridae	*Orthonama obstipata**			1	1			1
	Geometridae	*Thysanopyga intractata*		**4**		**15**			
	Noctuidae	*Eupsilia vinulenta*	4	2		2	1	1	1
	Noctuidae	*Orthosia hibisci*		3	**2**	4			
	Noctuidae	*Sericaglaea signata*	2	**4**		5	2	2	2
	Plutellidae	*Plutella xylostella**			**1**				
	Tineidae	*Nemapogon interstitiella*	1			1	**5**	1	2
	Tortricidae	*Argyrotaenia pinatubana**	**7**	**7**	1	**15**	**9**	8	2
	Tortricidae	*Chimoptesis gerulae*	**5**	**6**		7	3	2	**3**
	Tortricidae	*Clepsis peritana**	3	2		**24**	1	9	3
	Tortricidae	*Rhyacionia frustrana**	**4**	**6**		**21**	**4**	**10**	**5**
Neuroptera	Chrysopidae	*Chrysoperla rufilabris*	2	**9**	**2**	**18**	3	1	1
	Hemerobiidae	*Hemerobius stigma*	4	2		2	**5**	1	
	Hemerobiidae	*Micromus posticus*	**4**	1	**2**	**20**	**9**	8	3
	Hemerobiidae	*Micromus subanticus*	2		**2**		1	1	1
Psocodea	Amphipsocidae	*Polypsocus corruptus*		1	1	6	**5**	4	**5**
Odonata	Libellullidae	*Trithemis dubia*	**8**						

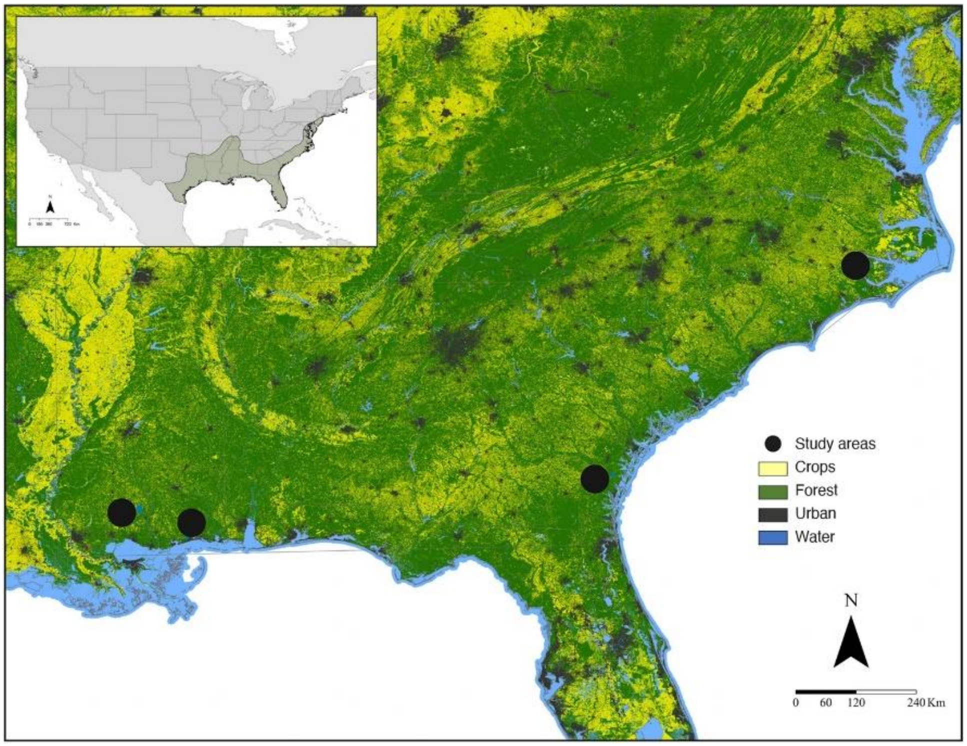
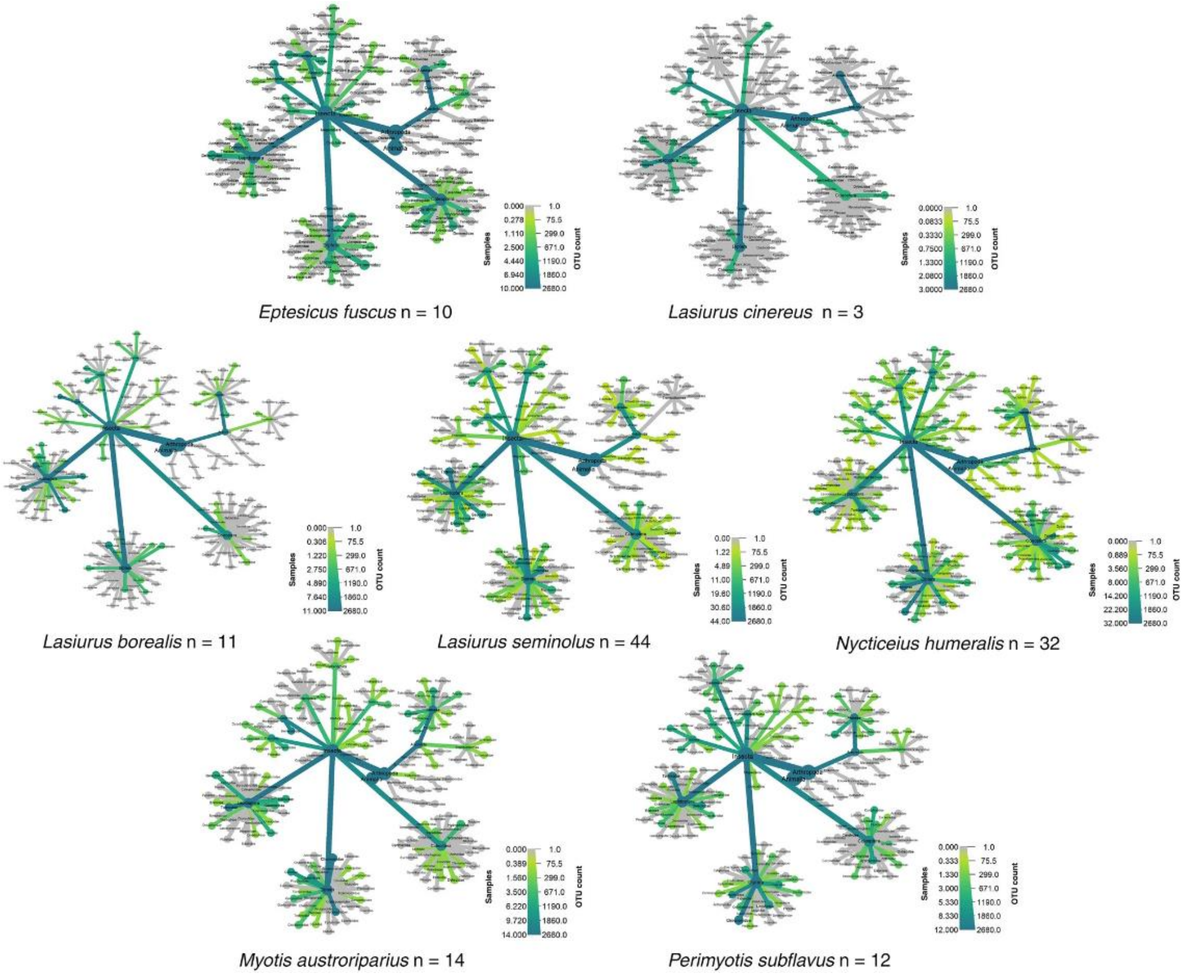


Figure 3.1. Location of study sites (circles) in the southeastern United States Coastal Plain where bat sampling was conducted from late January to mid-March 2021–2022. Landscape cover types derived from a reclassification of The National Land Cover Database 2021.



Eptesicus fuscus n = 10

Lasiurus cinereus n = 3

Lasiurus borealis n = 11

Lasiurus seminolus n = 44

Nycticeius humeralis n = 32

Myotis austroriparius n = 14

Perimyotis subflavus n = 12

Figure 3.2. Winter diet including order, family, and genus of seven bat species in private, working forests of the southeastern U.S. Coastal Plain from late-January to mid-March 2021–2022. Colors represent number of samples and width of nodes represent number of Operational Taxonomic Unit (OTU) counts for each taxonomic level.

CHAPTER 4

DISENTANGLING WINTER RELATIONSHIPS: BAT RESPONSES TO FOREST STAND STRUCTURE, ENVIRONMENTAL CONDITIONS, AND PREY COMPOSITION[3]

[3] Santiago Perea, Amanda Vicente-Santos, Angela L. Larsen-Gray, Kamal J. K. Gandhi, Daniel U. Greene, Brittany F. Barnes, and Steven B. Castleberry. Submitted to Forest Ecology and Management 08/12/2024

ABSTRACT

Private, working forests are a significant component of the landscape in the southeastern United States. Past research has documented diverse bat communities in these areas, but there is limited information on how forest management practices affect bat and insect communities, particularly in winter. To address this gap, we applied structural equation modeling to examine relationships among bat activity, temperature, forest structure, and nocturnal insect assemblages across four working pine (*Pinus* spp.) forest landscapes in the southeastern U.S. Coastal Plain during mid-January to mid-March 2021-2022. As expected, temperature directly influenced both bat activity and insect metrics. Additionally, sites with higher insect ordinal richness positively affected activity for all bat taxa except *Myotis austroriparius* / *M. septentrionalis*. Activity of most bat taxa was also directly influenced by forest structure, generally indicating preference for mid-rotation thinned and late-rotation semi-open canopied stands and a negative response to pre-thinned, closed-canopy stands. Further, forest stand structure affected several insect attributes including catches of Coleoptera, Diptera, Lepidoptera, and large-sized insects, indicating potential indirect cascading effects on bat taxa associated with specific forest insect assemblages. For instance, early establishment forest stands (0-3 years) negatively influenced lepidopteran catches, potentially indicating an indirect effect on *L. borealis* / *L. seminolus* activity. Maintaining a heterogenous forest landscape with a range of stand age and structure from early establishment to thinned and mature stands will benefit winter bat communities. Our research provides insight into overwintering bat community dynamics, offering practical guidance to forest managers to optimize conservation efforts.

INTRODUCTION

In the southeastern U.S., approximately 29.7 million ha (~86%) of forests are privately owned, with working forests accounting for much of the land area (Oswalt *et al*., 2019). Working forests are actively managed for production of forest products while also maintaining recreational opportunities, wildlife habitat, water quality and quantity, and carbon storage and sequestration. Economic incentives for forest management help maintain forests while reducing the risk of forest land conversion to urban and agricultural uses (Siry *et al*., 2005). Hence, working forests benefit biodiversity conservation by allowing landowners to achieve their objectives while conserving forest features on the landscape necessary to maintain wildlife communities (e.g., Demarais *et al*., 2017).

Forests provide foraging and roosting resources for bats while bats provide essential ecosystem services, including control of phytophagous insects (Maas *et al*., 2016, Beilke & O'Keefe, 2023). Forest management practices, such as harvest and herbicide use, affect how bats use forested landscapes by modifying structure (Bender *et al*., 2015; Carr *et al*., 2020). However, forest structure alone cannot fully explain bat species' relationships with their environment (Morrison, 2001). For insectivorous bats, habitat use predictions based on wing morphology and echolocation call characteristics may be modified by prey availability (Ford *et al*., 2005). Therefore, fully understanding how bats respond to forest management requires determining links between foraging bats and prey availability (Bender *et al*., 2021; Froidevaux *et al*., 2021).

Insects contribute significantly to forest biodiversity, biomass, and overall ecosystem functioning (Santos *et al*., 2021). In addition to serving as an important vertebrate food source (Rosenberg *et al*., 1986), many insect species influence ecosystem processes (Asquith *et al*., 1990; Westman, 1990). Forest structure and composition are among the main drivers for maintaining specific insect communities (Gossner *et al*., 2014; Leidinger *et al*., 2019). In temperate forests, there may be notable vertical stratification of insect communities with variations in abundance and species richness among feeding guilds and forest strata (Leidinger *et al*., 2019). Thus, changes in canopy cover or understory vegetation can affect insect communities (Favorito *et al*., 2023). Surrounding land use (e.g., agriculture or urbanization), can also influence insect communities, sometimes masking or altering expected relationships (Veres *et al*., 2013; Uhler *et al*., 2021). Overall, alterations in insect communities can, in turn, trigger changes in insect-dependent vertebrate populations, such as bats.

The Coastal Plain of the southeastern U.S. is characterized by mild temperatures, allowing bats to remain active year-round (Grider *et al*., 2016; Perea *et al*., 2023). Previous summer studies in working forest lands have provided valuable insights into bat responses to forest structure and insect communities. Generally, insect abundance has little influence on bat activity or presence in summer (Ford *et al*., 2006, Brooks *et al*., 2017), or where relationships with insect abundance have been detected, vegetation structure has a stronger influence (Bender *et al*., 2021). Bat prey response is also a critical question when evaluating forest management practices (Carr *et al*., 2020; Bender *et al*., 2021; Froidevaux *et al*., 2021), but there remains a gap in our understanding of how forest structure and insect community composition influence bat communities during winter. To address this information need, we aimed to better understand dynamics of bat and insect communities and how forest management practices influence bat ecology during winter. Specifically, we examined direct and indirect relationships among stand structure characteristics, temperature, insect community metrics, and bat activity in working forests in the southeastern U.S. Coastal Plain. Our approach allowed us to disentangle direct and indirect effects of forest management practices on bat communities, thus providing a holistic understanding of ecological interactions occurring within working forest landscapes to inform forest management decisions.

MATERIALS AND METHODS

Study area

We conducted our study during 2021-2022 in private, working forest landscapes (> 3,000 ha) across four states (Florida, Mississippi, North Carolina, and South Carolina) (Figure 1). Forests were composed primarily of upland planted loblolly pine (Pinus taeda) stands interspersed with riparian areas, roads, wildlife clearings, and other non-forested areas (< 15% of each area). Management activities were typical of commercial forestry operations in the region (Gresham, 2002), including clearcutting at 20-25 years, mechanical and/or chemical site preparation, and planting at 182-283 trees/ha. Competing vegetation was temporarily suppressed by selective herbicide applications, prescribed fire, or mechanical removal. Herbicide applications typically occurred during site preparation of clearcut stands, 1–4 years following stand establishment, and after thinning (Shepard *et al*. 2004). Most stands were thinned at 13-15 years. Mean area of managed stands across all study areas was 35.6 ha (SE = 0.60).

Bat acoustic sampling

In each working forest landscape, we created a 900 x 900 m acoustic sampling grid covering an area with stand types and ages representative of working forest landscapes in the region. Mean area of sampling grids was 9,894.3 ha (range 8,767.5–11,989.5 ha). We chose 900 m between grid points as a compromise among foraging areas of common bat species found on the study areas (Walters *et al*., 2007; Veilleux *et al*., 2009; Morris *et al*., 2011) and logistical considerations to minimize dependence among points (Bender *et al*., 2015). We used ArcGIS Pro 2.8.0 (ESRI, Redlands, California, U.S.) to randomly select grid intersections as sampling points. We surveyed 35 sampling points in each study area to represent variation in growth stage, stand size, and management history. We sampled all points in each study area within a one-month period. We defined mid-January to mid-March as the winter sampling season as mean nightly temperatures are lowest (typically < 10º C) during this time in the region.

At each sampling point, we deployed Anabat Swift acoustic detectors with omnidirectional ultrasonic microphones US-OV2 and US-OV3 (Titley Electronics, Ballina, New South Wales, Australia) for three consecutive nights, recording during the first four hours after sunset. If rain occurred during the sampling period, we left detectors out for additional nights to ensure three nights of rain-free sampling. We placed detectors on poles with microphones 3 m above the forest floor pointed in the direction of least vegetation clutter (Weller & Zabel, 2002). We coupled each detector with a temperature logger (HOBO Pendant G Acceleration Data Logger, Onset Computer Corp., Pocasset, Massachusetts, USA) programmed to record hourly temperature (ºC).

Bat call analyses

We used automated identification software and subsequent manual vetting to identify bat calls to species, as recommended by the North American Bat Monitoring Program (NABat; Reichert *et al*., 2018). We first filtered out noise files using Kaleidoscope Pro 5.4.1 software (Wildlife Acoustics Inc., Maynard, Massachusetts, U.S.). The default filter setting parameters for bat analysis were selected, specifying a signal of interest between 8 and 120 kHz, 2 to 500 ms, and at least three pulses per sequence (Loeb *et al*., 2015). We used the Batch function in Kaleidoscope Pro to split each sequence to a maximum duration of 10 s for standardization and the auto classifier of Kaleidoscope Pro with a balanced sensitivity level for classification to assist manual vetting. Subsequently, all non-noise files were manually analyzed using call structure, minimum and maximum energy frequency, characteristic frequency, duration, inter-pulse interval, and slope (O'Farrell *et al*., 1999; Russo & Jones, 2002). Due to overlap in acoustic characteristics of some species [i.e., eastern red bat / Seminole bat (*Lasiurus borealis* / L. *seminolus*), big brown bat / silver-haired bat (*Eptesicus fuscus* / *Lasionycteris noctivagans*), and southeastern myotis / northern long-eared bat (*Myotis austroriparius* / *M. septentrionalis*)], we grouped bat passes by these pairs (i.e., Russo & Jones, 2002; Loeb *et al*., 2015).

Insect sampling

At each sampling point, we sampled insects using light traps consisting of 12-watt, 352 nm, black fluorescent "U" tubes hung on poles 1.5 m above the ground powered by 12-volt batteries. We placed light traps 50 m from detectors within the same stand to avoid bias in bat activity resulting from insect attraction to traps (Froidevaux *et al*., 2018). We programmed light traps to turn on during the first four hours after sunset each night to coincide with bat acoustic data collection. After three days, we collected insects and froze them until identification. We identified all captured insects to taxonomic order. We measured length (mm) from the anterior of the head to the last abdominal segment and classified them into one of the following three size classes: <3 mm (small), 3-10 mm (medium), and >10 mm (large) (adapted from Bender *et al*., 2021).

Forest stand metrics

Based on landowner-provided and publicly available data, we used ArcGIS Pro to classify forest stands into growth stages (i.e., approximately 0–3 years [early establishment], 4–7 years [closing canopy], 8–13 years [closed canopy, pre-thinned], 14–20 years [mid-rotation thinned], or >20 years [semi-closed canopy]). We created a 100-m buffer around each acoustic sampling point and calculated the area (ha) in each forest stand class. We chose a 100-m buffer size as a compromise in the scale of responses to tree cover by among bat species found on the study area based on Moretto *et al*. (2019). Growth stages were revised as needed from data provided by landowners as the exact thinning year varied.

Data analyses

We used structural equation modeling (SEM; Grace, 2006) to compare relationships among winter bat activity, nocturnal insect composition metrics, temperature, and stand structure characteristics. We quantified bat activity for each species/group by calculating mean (across three nights) number of bat passes recorded at each sampling point. We calculated overall number of insects, number within size classes (small, medium, and large), and number within the most abundant orders (mean of ≥15 adult individuals; Coleoptera, Diptera, and Lepidoptera) at each sampling point. To determine nocturnal insect richness at the order level, we summed number of orders observed at each sampling point. Insect metrics were included as predictor variables along with forest stand stages and temperature.

All analyses were conducted in R 4.4.0 (R Core Team, 2024). Based on existing theory and evidence, we developed a conceptual model to guide our analyses, including all bat species/groups, insect metrics, temperature, and forest stand characteristics (Figure 2). We then developed generalized linear models (GLM) for each response variable testing null, global, single covariate, and models with biologically meaningful combinations of covariates. Shapiro-Wilks tests revealed that all response variables except insect ordinal richness were not normally distributed ($p < 0.01$) and were over-dispersed, and thus were fitted with negative binomial distributions. For richness of insect orders, we fitted our models with a Gaussian distribution. We tested for correlation between continuous predictor variables using Pearson's correlation coefficient to ensure that highly correlated variables ($r \geq |0.7|$) were not included in the same model (Supplemental Figure 1). With that information, we constructed SEMs using the most plausible GLMs for each response variable, including biologically relevant covariates. To do so, we evaluated GLMs using Akaike's Information Criterion corrected for small sample sizes and selected models with ΔAICc ≤ 1 (Supplemental Table 1).

We used the PiecewiseSEM package (Lefcheck *et al*., 2016) for structural equation modeling. We tested the hypothesized model (Fig. 2), including combinations of the most plausible models for each response variable using SEMs to determine effects of variables on bat activity. We used Shipley’s test of directed separation (Fisher’s C) to evaluate global SEM fit, where a p-value > 0.05 indicated that the model was supported by the observed data (Shipley, 2009; Lefcheck *et al*., 2016). Among models that were deemed valid ($p > 0.05$), we selected the model with the highest Fisher's C statistic as the final model. We summarized models by providing the R^2 value for each endogenous variable, standardized path coefficients, standard errors, and p-values.

To enhance clarity and visualization, we divided SEM results into open space and edge/interior foraging bat species. Northern yellow bat (*Dasypterus intermedius*), *E. fuscus* / *L. noctivagans*, hoary bat (*L. cinereus*), and Mexican free-tailed bat (*Tadarida brasiliensis*) are considered open-foraging species that emit echolocation calls with characteristic frequencies (Fc) < 30 kHz. Bats including *L. borealis* / *L. seminolus*, *M. austroriparius* / *M. septentrionalis*, evening bat (*Nycticeius humeralis*), and tricolored bat (*Perimyotis subflavus*), are adapted to foraging within forest stands or edges with calls at Fc > 30 kHz (Norberg & Rayner, 1987; Denzinger & Schnitzler, 2013).

RESULTS

We recorded 5,338 bat passes on 423 recording nights (n = 140 sites). We documented eight individual or species pairs, including *D. intermedius* (n = 87 passes), *E. fuscus* / *L. noctivagans* (n = 400 passes), *L. borealis* / *L. seminolus* (n = 1,218 passes), *L. cinereus* (n = 1,716 passes), *M. austroriparius* / *M. septentrionalis* (n = 583 passes), *N. humeralis* (n = 383 passes), *P. subflavus* (n = 336 passes), and *T. brasiliensis* (n = 615 passes). For the insect assemblage, we identified 18,882 insects in eight orders during 423 trapping nights.

Our best SEM adequately explained proposed relationships among forest stand structure characteristics, temperature, bat activity, and insect community metrics (Fisher's C = 129.14, df = 168, p-value = 0.99). Marginal R2 values ranged from 0.89 and 0.81 for *T. brasiliensis* and *M. austroriparius* / *M. septentrionalis*, respectively, to 0.53 and 0.58 for *D. intermedius* and *P. subflavus*, respectively. Marginal R2 for insect metrics also indicated that predictors explained a significant amount of variance in dependent variables ranging from 0.23 and 0.34 for richness of insect orders and Diptera, respectively, to 0.97 and 0.96 for Coleoptera and Lepidoptera, respectively (Supplemental Table 2).

Open space foragers

Temperature at sunset and richness of insects orders positively affected activity of all open-space foragers (Fig. 3, Supplemental Table 2). Additionally, *D. intermedius* was influenced by higher catches of

Diptera (estimate = 0.02, p-value = 0.01) and *E. fuscus* / *L. noctivagans* by higher and lower catches of medium-size insects (estimate = 0.04, p-value = 0.01) and Coleoptera (estimate = -0.06, p-value = 0.04), respectively. *E. fuscus* / L. noctivagans activity was also negatively influenced by closing canopy stands (estimate = -0.07, p-value = 0.02) and closed canopy, pre-thinned stands (estimate = -0.10, p-value = p < 0.01). Conversely, semi-closed canopy stands were an important variable positively influencing *D. intermedius* (estimate = 0.02, p-value = 0.03) and *T. brasiliensis* (estimate = 0.11, p-value = 0.02) activity. *Tadarida brasiliensis* activity was also positively influenced by mid-rotation thinned stands (estimate = 0.17, p-value < 0.01).

Edge/interior forest foragers

As observed in open-space foraging bats, activity of all edge/interior forest foragers increased on warmer nights (Figure 4, Supplemental Table 2). In addition, higher levels of richness of insect orders richness positively influenced activity of *L. borealis* / *L. seminolus* (estimate = 0.42, p-value < 0.01), *N. humeralis* (estimate = 0.23, p-value = 0.01) and *P. sublavus* (estimate = 0.19, p-value < 0.01). *L. borealis* / *L. seminolus* activity was positively associated with Lepidoptera catches (estimate = 0.10, p-value = 0.05) and negatively associated with Coleoptera (estimate = -0.18, p-value = 0.03) and Diptera (estimate = -0.16, p-value = 0.04) catches. *N. humeralis* activity was positively associated with Diptera catches (estimate = 0.16, p-value = 0.01). The only significant relationship with forest growth stage was between *M. austroriparius* / *M. septentrionalis* activity and semi-closed canopy stands (estimate = 0.10, p-value = p < 0.01).

Nocturnal insect metrics

High levels of coleopteran catches were associated with closed canopy, pre-thinned (estimate = 0.07, p-value = 0.04), and mid-rotation thinned stands (estimate = 0.09, p-value = 0.01). We observed lower catches of dipterans in closing canopy (estimate = -0.19, p-value = 0.03) and mid-rotation thinned stands (estimate = -0.25, p-value = 0.01). Early establishment stands negatively influenced lepidopteran catches (estimate = -0.14, p-value = 0.01). Although large-sized insect catches were not included in the top models for any bat species, they were highly correlated with other insect metrics such as Lepidoptera (Supplemental

Figure 1). Large-sized insect catches were negatively associated with early establishment stands (estimate = -0.09, p-value = 0.02) (Supplemental Table 2).

DISCUSSION

Our results underscore the complexity of factors affecting winter bat communities in private, working forests, revealing a wide range of responses among bat taxa, nocturnal flying insect metrics, and forest stand structure. We found that bat responses were distinctly species-specific, influenced either by insect metrics, forest characteristics, or a combination (Bender *et al*., 2021). Metrics related to forest stand structure emerged as informative indicators explaining activity for several bat taxa and nocturnal flying insect metrics. Furthermore, our results emphasized interconnected relationships between nocturnal flying insect catches and richness with stand structure, suggesting that vegetation structure plays an important role in shaping nocturnal insect composition and, consequently, bat foraging activity in winter. Previous research conducted in summer found little evidence of relationships between bats and insect abundance (Ford *et al*., 2006; Brooks *et al*., 2017; Bender *et al*., 2021). The contrast between our results and those from summer that bat-insect linkages may be more evident during the dormant season.

Our findings are consistent with previous winter studies assessing effects of temperature on bat activity (Parker *et al*., 2020; Barros *et al*., 2021). In temperate regions where bats remain active throughout the year, weekly or even daily temperature fluctuations can cause bats to experience short periods of torpor which influences activity (Grider *et al*., 2016; Parker *et al*., 2020; Perea *et al*., 2023). Thus, a direct relationship in which lower temperatures during winter reduced bat activity was expected (Grider *et al*., 2016; Jorge *et al*., 2021). Likewise, our results revealed an association between temperature and nocturnal flying insect abundance and diversity, consistent with existing literature (Taylor, 1963; Liu *et al*., 1995). Ectotherm behavior and ecological performance are dependent on body temperature (Huey & Kingsolver, 1989), which depends mainly on environmental conditions (Bots *et al*., 2008). For flying insects, temperatures must reach a certain threshold to maintain flight activity (Bots *et al*., 2008; Welti *et al*., 2022). Observed relationships among temperature, bat activity, and nocturnal flying insect metrics highlight the important role of temperature as a factor influencing both bat behavior and insect availability in winter.

All bat taxa detected in our study, except *M. austroriparius* / *M. septentrionalis*, displayed positive associations with nocturnal insect richness, suggesting generalist diets during winter (Bernard *et al*., 2021; Perea *et al*., 2024). Dipterans are widely consumed by bats in winter in the southeastern U.S. (Bernard *et al*., 2021; Perea *et al*., 2024), which may be a consequence of higher abundance relative to other orders. Our analyses showed positive associations of Diptera with *D. intermedius* and N. *humeralis*. Whereas *N. humeralis* exhibits great flexibility in its diet including a high abundance of dipterans (Perea *et al*., 2024), a positive association between *D. intermedius* and Diptera was unexpected. Although the diet of *D. intermedius* is poorly known, the association with dipterans may be due to a selection of large, soft-bodied insects or higher abundance of dipterans in areas with vegetation characteristics suitable for foraging (Perea *et al*., 2022). *Lasiurus borealis* / *L. seminolus* activity was positively associated with Lepidoptera and negatively associated with Coleoptera and Diptera. Although negative associations with Coleoptera and Diptera were not expected, effect sizes were small, and our results may be a consequence of combining species into a phonic group. In contrast, positive association with lepidopterans was expected considering both species consume large quantities of Lepidoptera, and *L. borealis* exhibiting a predominantly Lepidoptera-based diet (Carter *et al*., 2004; Clare *et al*., 2009; Perea *et al*., 2024). Finally, we found that *E. fuscus* / *L. noctivagans* activity was positively associated with medium-sized insects and negatively associated with Coleoptera. While positive associations between *E. fuscus* / *L. noctivagans* activity and medium-sized insects were to be expected from an ecomorphological perspective (Reimer *et al*., 2010; Denzinger & Schnitzler 2013; Clare *et al*., 2014), the negative effect with Coleoptera catches was contradictory to our expectations, especially given that E. fuscus that has been documented consuming large numbers of Coleoptera (Clare *et al*., 2014; Wray *et al*., 2018). However, *L. noctivagans* preys more commonly on small, soft-bodied insects such as Lepidoptera, Diptera, and Homoptera (Reimer *et al*., 2010). Thus, the negative relationship may be an artifact of combining the species in a single phonic group. Alternatively, we found that *E. fuscus* / *L. noctivagans* activity was negatively influenced by closed canopy pre-thinned stands and was not influenced by mid-rotation thinned stands, both of which were associated with high Coleoptera catches. Therefore, the unexpected relationship could also be related to how the structure of growth stages in managed pine forests differentially affect *E. fuscus* / *L. noctivagans* foraging ability and Coleoptera abundance. Although unlikely based on the species seasonal distribution (Cryan, 2003), it is also possible that *L. noctivagans* was more prevalent on the study areas than expected and had a greater influence on the relationship with insect metrics.

Management practices used by forest managers result in different stand structural features that can affect bat activity (e.g., Bender *et al*., 2015; Kunberger & Long 2022; Perea *et al*., 2023) and insect community structure (e.g., Joelsson *et al*., 2017; Leidinger *et al*., 2019). Our analyses identified a variety of responses to growth stages, and consequently forest stand structure, in bat activity and nocturnal flying insect metrics. Clearcutting, the predominant final harvest management method in pine stands in the southeastern U.S., is followed by reforested, early establishment stands. Although previous studies found early establishment and young, open-canopy pine stands had higher diurnal family-level insect abundance and richness (Lee *et al*., 2018), our results demonstrated lower nocturnal lepidopteran and large-sized insect catches in early establishment stands during winter. These early establishment stands allow greater light into the understory, which supports more plant resources, resulting in a positive effect on diurnal insect diversity (Taki *et al*., 2010; Favorito *et al*., 2023). However, in winter, temperature fluctuations in open stands, which may experience colder nighttime conditions, could reduce insect activity, as certain species are less active or abundant during winter. Although we found no direct associations between bat activity and early establishment stands, negative effects on nocturnal lepidopteran catches suggest indirect effects on lepidopteran-associated bat activity. In turn, the direct relationship between Lepidoptera and *L. borealis* / *L. seminolus* we observed could indicate an indirect negative effect on activity for these bats resulting from lower lepidopteran catches at sites with greater areas of early establishment stands.

In contrast, sites surrounded by closing canopy stands were negatively associated with activity of open-space foragers *E. fuscus* / *L. noctivagans* and dipteran catches. Although *E. fuscus* / *L. noctivagans* can forage above the canopy, a reduction in activity in these closing canopy stands may be explained by the fact that these sites are not particularly conducive to insect communities. The reason for a negative association between this growth stage and Diptera is unknown but may be related to a lack of important shelter and foraging resources in winter (van Hoesel *et al*., 2019). Likewise, as observed in early established stands with Lepidoptera, the negative effects on nocturnal captures of dipterans could indirectly affect activity of bats associated with dipterans, such as *D. intermedius* or *N. humeralis*.

Previous research indicates that closed canopy, pre-thinned stands are the least conducive to vertebrate biodiversity (Greene *et al*., 2019). Specifically, bats show reduced activity in such forest conditions (Bender *et al*., 2015; Blakey *et al*., 2016). Our results support previous observations of lower bat activity in closed canopy, pre-thinned forest stands for *E. fuscus* / *L. noctivagans*, large bats typically associated with foraging in open spaces (Norberg & Rayner, 1987; Beilke *et al*., 2021). Closed canopy, pre-thinned stands are characterized by greater tree densities, including more competing vegetation in the under- and mid-story, along with a closed canopy that hinders bat flight and foraging ability. As for nocturnal insect communities, previous research found species richness and abundance of multiple orders were lower in unthinned forest stands compared to thinned stands (e.g., Maleque *et al*., 2007a, b; Taki *et al*., 2010; Taniwaki *et al*., 2024). However, we found a positive association between closed canopy pre-thinned stands and catches of Coleoptera. This positive association may be attributed to a combination of more stable winter temperatures and availability of food resources compared to younger (0-7 years) and thinned stands. Although Coleoptera is widely found in the diet of most bat taxa (e.g., Clare *et al*., 2014; Bernard *et al*., 2021; Perea *et al*., 2024), structure characteristics of pre-thinned stands likely restrict bat foraging, indicating a complex interaction between habitat structure, coleopteran catches, and foraging behavior.

Thinning is commonly employed in mid-rotation pine stands (> approximately 14 years) to regulate tree density and understory structure (e.g., Thomas *et al*., 1999). In general, thinning effects on flying insect assemblages (Maleque *et al*., 2007a, b; Taki *et al*., 2010; Taniwaki *et al*., 2024) and vertebrate communities (Verschuyl *et al*., 2011; Demarais *et al*., 2017) are positive or neutral. We found a significant positive association between *T. brasiliensis* and mid-rotation thinned and semi-closed canopy stands. As an open-space forager, *T. brasiliensis* likely concentrates foraging activity within or above older forest stands with semi-closed canopies. We also observed a positive association with D. intermedius and *M. austroriparius* / *M. septentrionalis* activity at sites surrounded by greater areas of semi-closed canopy stands. Positive associations of *D. intermedius* with large patches of semi-closed canopy forest are supported by previous work showing preferences for bottomland forests during winter (Shute *et al*., 2021). Our older growth stage category contained bottomland forests in the form of streamside management zones. Conversely, as an interior forest species, high activity of *M. austroriparius* / *M. septentrionalis* in semi-closed canopy stands is consistent with preferences for contiguous older-aged stands for foraging and roosting (Shute *et al*., 2021; Perea *et al*., 2023). For nocturnal flying insects, we found positive and negative associations with Coleoptera and Diptera catches, respectively, at sites with large areas of thinned stands in mid-rotation. Associated changes in vegetation during thinning may affect insect community structure (Taki *et al*., 2010). Coleopteran diversity increases with forest structural complexity, increasing tree species diversity, and dead wood diversity (e.g., Gossner *et al*., 2016; Seibold *et al*., 2016; Joelsson *et al*., 2017). Negative association with nocturnal dipteran catches may be due to a modification of important under- and mid-story vegetation for dipterans present during winter or to preferences for areas with other characteristics such as higher availability of water sources (Allgood *et al*., 2009; van Hoesel *et al*., 2019).

Although herbicides were not a focus of our study, we recognize their influence in shaping insect communities, which in turn could affect bat communities. The relationships between conditions created by herbicides and insects are highly variable (i.e., negative, neutral, or positive), particularly regarding insect species abundance, richness, and diversity (Briggs *et al*., 2024a,b). Furthermore, insects are highly mobile. Thus, given the overall small size (<48.6 ha) and irregular shapes of stands (Sustainable Forestry Initiative, 2022), and heterogeneity in cover types (e.g., embedded streamside management zones) in working forests, insect communities are not likely influenced solely by the stands where they are detected. Moreover, effects from herbicides applied to a forest stand are typically short in duration, as herbaceous plant species composition can recover within a few years (Miller *et al*., 1999; Miller & Miller, 2004; Shepard *et al*., 2004). Consequently, insect communities, and consequently bat populations, in working forest landscapes are influenced more by the broader landscape matrix rather than by any single forest stand. Future research should examine the complex role of herbicides in shaping bat communities in working forests.

Our study provides an integrative view of overwintering bat communities, nocturnal flying insect assemblages, and factors influencing their activity in private, working forests of the southeastern U.S. Coastal Plain. Our findings highlight complex interactions and diverse responses among bat species, nocturnal flying insect metrics, and forest stand characteristics. In particular, our research reveals bat species-specific responses, as forest stand characteristics directly influence some species, while others are linked to nocturnal insect metrics. Each stage of typical pine management provides structural features necessary for the bat community. Some species depend on stands characterized by early succession and diverse understory vegetation, whereas others are generalists or show preferences for interior forest conditions. Bats in the southeastern Coastal Plain evolved in a dynamic system characterized by frequent fires and periodic disturbance from wind (e.g., hurricanes and tornados) that created a variety of stand conditions. Although forest management does not mimic natural disturbance in every way, management in southeastern Coastal Plain working forests provides a mosaic of stand conditions that provide conditions suitable foraging and roosting conditions for the bat community (Taylor *et al*., 2020). Our research provides valuable insights for bat conservation and sustainable forest management strategies, emphasizing the need for adaptive approaches that integrate bat ecology and dynamics of overwintering nocturnal insects in private, working forests.

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LITERATURE CITED

Allgood, D. W., Miller, D. A., & Kalcounis-Rueppell, M. C. (2009). Influence of intensive pine management on dipteran community structure in coastal North Carolina. Environmental Entomology, 38(3), 657-666. https://doi.org/10.1603/022.038.0317

Asquith, A., J. D. Lattin, & A. R. Moldenke. 1990. Arthropods: The invisible diversity. Northwest Environmental Journal, 6, 404-405.

Barros, P., Faria, S., Pereira, M., Santos, J. A., & Cabral, J. A. (2021). How winter prevailing weather conditions influence the bat activity patterns? Hystrix 32, 27–36. https://doi.org/10.4404/hystrix-00361-2020

Beilke, E. A., Blakey, R. V., & O’Keefe, J. M. (2021). Bats partition activity in space and time in a large, heterogeneous landscape. Ecology and Evolution, 11(11), 6513-6526. https://doi.org/10.1002/ece3.7504

Beilke, E. A., & O'Keefe, J. M. (2023). Bats reduce insect density and defoliation in temperate forests: An exclusion experiment. Ecology, 104(2), e3903. https://doi.org/10.1002/ecy.3903

Bender, M. J., Castleberry, S. B., Miller, D. A., & Wigley, T. B. (2015). Site occupancy of foraging bats on landscapes of managed pine forest. Forest Ecology and Management, 336, 1-10. https://doi.org/10.1016/j.foreco.2014.10.004

Bender, M. J., Perea, S., Castleberry, S. B., Miller, D. A., & Wigley, T. B. (2021). Influence of insect abundance and vegetation structure on site-occupancy of bats in managed pine forests. Forest Ecology and Management, 482, 118839. https://doi.org/10.1016/j.foreco.2020.118839

Bernard, R. F., Willcox, E. V., Jackson, R. T., Brown, V. A., & McCracken, G. F. (2021). Feasting, not fasting: winter diets of cave hibernating bats in the United States. Frontiers in Zoology, 18, 1-13. https://doi.org/10.1186/s12983-021-00434-9

Blakey, R. V., Law, B. S., Kingsford, R. T., Stoklosa, J., Tap, P., & Williamson, K. (2016). Bat communities respond positively to large-scale thinning of forest regrowth. Journal of Applied Ecology, 53(6), 1694-1703. https://doi.org/10.1111/1365-2664.12691

Bots, J., De Bruyn, L., Van Damme, R., & Van Gossum, H. (2008). Effects of phenotypic variation onto body temperature and flight activity in a polymorphic insect. Physiological Entomology, 33(2), 138-144. https://doi.org/10.1111/j.1365-3032.2008.00616.x

Carr, A., Weatherall, A., & Jones, G. (2020). The effects of thinning management on bats and their insect prey in temperate broadleaved woodland. Forest Ecology and Management, 457, 117682. https://doi.org/10.1016/j.foreco.2019.117682

Briggs, E. L., Greene, D. U., Clabo, D. C., & Gandhi, K. J. (2024a). Herbicides have variable effects on understory plant and insect communities in Southern United States working forests. Journal of Forestry, 122(3), 285-301. https://doi.org/10.1093/jofore/fvad060

Briggs, E. L., Greene, D. U., Fortuin, C. C., Clabo, D. C., Barnes, B. F., & Gandhi, K. J. (2024b). Wild bee community responses to forest herbicide treatments in planted loblolly pine (*Pinus taeda* L.) stands. Forest Ecology and Management, 572, 122332. https://doi.org/10.1016/j.foreco.2024.122332

Brooks, J. D., Loeb, S. C. Loeb, & Gerard, P. D. (2017). Effect of forest opening characteristics, prey abundance, and environmental factors on bat activity in the Southern Appalachians. Forest Ecology and Management, 400, 19-27. https://doi.org/10.1016/j.foreco.2017.05.045

Carter, T. C., Menzel, M. A., Chapman, B. R., & Miller, K. V. (2004). Partitioning of food resources by syntopic eastern red (*Lasiurus borealis*), Seminole (*L. seminolus*) and evening (*Nycticeius humeralis*) bats. The American Midland Naturalist, 151(1), 186-191. https://doi.org/10.1674/0003-0031(2004)151[0186:POFRBS]2.0.CO;2

Clare, E. L., Fraser, E. E., Braid, H. E., Fenton, M. B., & Hebert, P. D. (2009). Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. Molecular Ecology, 18(11), 2532-2542. https://doi.org/10.1111/j.1365-294X.2009.04184.x

Clare, E. L., Symondson, W. O., & Fenton, M. B. (2014). An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). Molecular Ecology, 23(15), 3633-3647. https://doi.org/10.1111/mec.12519

Cryan, P. M. (2003). Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in North America. Journal of Mammalogy, 84(2), 579-593. https://doi.org/10.1644/1545-1542(2003)084<0579:SDOMTB>2.0.CO;2

Denzinger, A. & Schnitzler, H-U. (2013). Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. Frontiers in Physiology 4, 164. https://doi.org/10.3389/fphys.2013.00164

Demarais, S., Verschuyl, J. P., Roloff, G. J., Miller, D. A., & Wigley, T. B. (2017). Tamm review: Terrestrial vertebrate biodiversity and intensive forest management in the US. Forest Ecology and Management, 385, 308-330. https://doi.org/10.1016/j.foreco.2016.10.006

Favorito, C. M., Barnes, B. F., Briggs, E. L., Fortuin, C. C., Greene, D. U., Larsen-Gray, A. L., ... & Gandhi, K. J. K. (2023). Stand stage affects wild bee communities in working pine forests. Forest Ecology and Management, 545, 121247. https://doi.org/10.1016/j.foreco.2023.121247

Ford, W. M., Menzel, M. A., Rodrigue, J. L., Menzel, J. M., & Johnson, J. B. (2005). Relating bat species presence to simple habitat measures in a central Appalachian forest. Biological Conservation, 126(4), 528-539. https://doi.org/10.1016/j.biocon.2005.07.003

Ford, W. M., Menzel, J. M., Menzel, M. A., Edwards, J. W., & Kilgo, J. C. (2006). Presence and absence of bats across habitat scales in the Upper Coastal Plain of South Carolina. Journal of Wildlife Management, 70(50), 1200-1209. https://doi.org/10.2193/0022-541X(2006)70[1200:PAAOBA]2.0.CO;2

Froidevaux, J. S., Fialas, P. C., & Jones, G. (2018). Catching insects while recording bats: impacts of light trapping on acoustic sampling. Remote Sensing in Ecology and Conservation, 4(3), 240-247. https://doi.org/10.1002/rse2.71

Froidevaux, J. S., Barbaro, L., Vinet, O., Larrieu, L., Bas, Y., Molina, J., ... & Brin, A. (2021). Bat responses to changes in forest composition and prey abundance depend on landscape matrix and stand structure. Scientific reports, 11(1), 10586. https://doi.org/10.1038/s41598-021-89660-z

Gossner, M. M., Weisser, W. W., Gershenzon, J., & Unsicker, S. B. (2014). Insect attraction to herbivore-induced beech volatiles under different forest management regimes. Oecologia, 176, 569-580. https://doi.org/10.1007/s00442-014-3025-4

Gossner, M. M., Wende, B., Levick, S., Schall, P., Floren, A., Linsenmair, K. E., ... & Weisser, W. W. (2016). Deadwood enrichment in European forests–Which tree species should be used to promote saproxylic beetle diversity? Biological Conservation, 201, 92-102. https://doi.org/10.1016/j.biocon.2016.06.032

Grace, J. B. (2006). Structural equation modeling and natural systems. Cambridge University Press, UK.

Greene, R. E., Iglay, R. B., & Evans, K. O. (2019). Providing open forest structural characteristics for high conservation priority wildlife species in southeastern US pine plantations. Forest Ecology and Management, 453, 117594. https://doi.org/10.1016/j.foreco.2019.117594

Gresham, C. A. (2002). Sustainability of intensive loblolly pine plantation management in the South Carolina Coastal Plain, USA. Forest Ecology and Management, 155(1-3), 69-80. https://doi.org/10.1016/S0378-1127(01)00548-5

Grider, J. F., Larsen, A. L., Homyack, J. A., & Kalcounis-Rueppell, M. C. (2016). Winter activity of coastal plain populations of bat species affected by white-nose syndrome and wind energy facilities. PLoS One, 11(11), e0166512. https://doi.org/10.1371/journal.pone.0166512

Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. Trends in Ecology & Evolution, 4(5), 131-135. https://doi.org/10.1016/0169-5347(89)90211-5

Joelsson, K., Hjältén, J., Work, T., Gibb, H., Roberge, J. M., & Löfroth, T. (2017). Uneven-aged silviculture can reduce negative effects of forest management on beetles. Forest Ecology and Management, 391, 436-445. https://doi.org/10.1016/j.foreco.2017.02.006

Jorge, M. H., Sweeten, S. E., True, M. C., Freeze, S. R., Cherry, M. J., Garrison, E. P., ... & Ford, W. M. (2021). Fire, land cover, and temperature drivers of bat activity in winter. Fire Ecology, 17(1), 19. https://doi.org/10.1186/s42408-021-00105-4

Kunberger, J. M., & Long, A. M. (2022). The influence of forest management practices on seasonal bat species occurrence and activity at the Kisatchie National Forest in Louisiana, USA. Forest Ecology and Management, 526, 120579. https://doi.org/10.1016/j.foreco.2022.120579

Lee, M. B., Campbell, J. W., Miller, D. A., & Martin, J. A. (2018). Insect community response to switchgrass intercropping and stand age of loblolly pine (*Pinus taeda*) plantations. Agricultural and Forest Entomology, 20(2), 217-227. https://doi.org/10.1111/afe.12247

Leidinger, J., Seibold, S., Weisser, W. W., Lange, M., Schall, P., Türke, M., & Gossner, M. M. (2019). Effects of forest management on herbivorous insects in temperate Europe. Forest Ecology and Management, 437, 232-245. https://doi.org/10.1016/j.foreco.2019.01.013

Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods in Ecology and Evolution, 7(5), 573-579. https://doi.org/10.1111/2041-210X.12512

Liu, S. S., Zhang, G. M., & Zhu, J. U. N. (1995). Influence of temperature variations on rate of development in insects: analysis of case studies from entomological literature. Annals of the Entomological Society of America, 88(2), 107-119. https://doi.org/10.1093/aesa/88.2.107

Loeb, S. C., Rodhouse, T. J., Ellison, L. E., Lausen, C. L., Reichard, J. D., Irvine, K. M., ... & Johnson, D. H. (2015). A plan for the North American bat monitoring program (NABat). A plan for the North American Bat Monitoring Program (NABat). General Technical Report SRS-208, U.S. Department of Agriculture Forest Service, Southern Research Station, Asheville, NC, 100 pp

Maas, B., Karp, D. S., Bumrungsri, S., Darras, K., Gonthier, D., Huang, J. C. C., ... & Williams-Guillén, K. (2016). Bird and bat predation services in tropical forests and agroforestry landscapes. Biological Reviews, 91(4), 1081-1101. https://doi.org/10.1111/brv.12211

Maleque, M. A., Ishii, H. T., Maeto, K., & Taniguchi, S. (2007a). Line thinning fosters the abundance and diversity of understory Hymenoptera (Insecta) in Japanese cedar (*Cryptomeria japonica* D. Don) plantations. Journal of Forest Research, 12(1), 14-23. https://doi.org/10.1007/s10310-006-0243-6

Maleque, M. A., Ishii, H. T., Maeto, K., & Taniguchi, S. (2007b). Line thinning enhances diversity of Coleoptera in overstocked *Cryptomeria japonica* plantations in central Japan. Arthropod-Plant Interactions, 1, 175-185. https://doi.org/10.1007/s11829-007-9016-1

Miller, J. H., Boyd, R. S., & Edwards, M. B. (1999). Floristic diversity, stand structure, and composition 11 years after herbicide site preparation. Canadian Journal of Forest Research, 29(7), 1073-1083. https://doi.org/10.1139/x99-075

Miller, K. V., & Miller, J. H. (2004). Forestry herbicide influences on biodiversity and wildlife habitat in southern forests. Wildlife Society Bulletin, 32(4), 1049-1060. https://doi.org/10.2193/0091-7648(2004)032[1049:FHIOBA]2.0.CO;2

Moretto, L., Fahrig, L., Smith, A. C., & Francis, C. M. (2019). A small-scale response of urban bat activity to tree cover. Urban Ecosystems 22, 795-805. https://doi.org/10.1007/s11252-019-00846-w

Morris, A. D., Miller, D. A., & Conner, L. M. (2011). Home-range size of evening bats (*Nycticeius humeralis*) in southwestern Georgia. Southeastern Naturalist 10(1), 85-94. https://doi.org/10.1656/058.010.0107

Morrison, M. L. (2001). A proposed research emphasis to overcome the limits of wildlife-habitat relationship studies. Journal of Wildlife Management, 65(4), 613-623. https://doi.org/10.2307/3803012

Norberg, U. M., & Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 316(1179), 335-427. https://doi.org/10.1098/rstb.1987.0030

O'Farrell, M. J., Miller, B. W., & Gannon, W. L. (1999). Qualitative identification of free-flying bats using the Anabat detector. Journal of Mammalogy, 80(1), 11-23. https://doi.org/10.2307/1383203

Oswalt, S. N., Smith, W. B., Miles, P. D., & Pugh, S. A. (2019). Forest resources of the United States, 2017. General Technical Report WO-97, US Department of Agriculture, Forest Service.

Parker, Jr, K. A., Li, H., & Kalcounis-Rueppell, M. C. (2020). Species-specific environmental conditions for winter bat acoustic activity in North Carolina, United States. Journal of Mammalogy, 101(6), 1502-1512. https://doi.org/10.1093/jmammal/gyaa133

Perea, S., Morris, K.M. & Castleberry, S.B. (2022). Northern yellow bat *Dasypterus intermedius* activity and habitat selection on an Atlantic barrier island. Acta Chiropterologica, 24(2), 343-352. https://doi.org/10.3161/15081109ACC2022.24.2.005

Perea, S., Fandos, G., Larsen-Gray, A., Greene, D. U., Chandler, R., & Castleberry, S. B. (2023). Bat winter foraging habitat use in working forests: a multispecies spatial occupancy approach. Animal Conservation. https://doi.org/10.1111/acv.12924

Perea, S., Meinecke, C. D., Larsen-Gray, A. L., Greene, D. U., Villari, C., Gandhi, K. J., & Castleberry, S. B. (2024). Winter diet of bats in working forests of the southeastern US Coastal Plain. Scientific Reports, 14(1), 12778. https://doi.org/10.1038/s41598-024-63062-3

R Core Team. (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.Rproject.org/.

Reichert, B. E., Bayless, M., Cheng, T. L., Coleman, J. T., Francis, C. M., Frick, W. F., ... & Weller, T. J. (2021). NABat: A top-down, bottom-up solution to collaborative continental-scale monitoring. Ambio, 50, 901-913. https://doi.org/10.1007/s13280-020-01411-y

Reimer, J. P., Baerwald, E. F., & Barclay, R. M. (2010). Diet of hoary (*Lasiurus cinereus*) and silver-haired (*Lasionycteris noctivagans*) bats while migrating through southwestern Alberta in late summer and autumn. The American Midland Naturalist, 164(2), 230-237. https://doi.org/10.1674/0003-0031-164.2.230

Rosenberg, D. M., Danks, H. V., & Lehmkuhl, D. M. (1986). Importance of insects in environmental impact assessment. Environmental Management, 10, 773-783. https://doi.org/10.1007/BF01867730

Russo, D., & Jones, G. (2002). Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. Journal of Zoology, 258(1), 91-103. https://doi.org/10.1017/S0952836902001231

Santos, J. C., de Almeida, W. R., & Fernandes, G. W. (2021). Arthropods: why it is so crucial to know their biodiversity? Measuring Arthropod Biodiversity: A Handbook of Sampling Methods, 3-11.

Seibold, S., Bässler, C., Brandl, R., Büche, B., Szallies, A., Thorn, S., ... & Müller, J. (2016). Microclimate and habitat heterogeneity as the major drivers of beetle diversity in dead wood. Journal of Applied Ecology, 53(3), 934-943. https://doi.org/10.1111/1365-2664.12607

Shepard, J. P., Creighton, J., & Duzan, H. (2004). Forestry herbicides in the United States: an overview. Wildlife Society Bulletin, 32(4), 1020-1027. https://doi.org/10.2193/0091-7648(2004)032[1020:FHITUS]2.0.CO;2

Shute, K. E., Loeb, S. C., & Jachowski, D. S. (2021). Seasonal shifts in nocturnal habitat use by coastal bat species. The Journal of Wildlife Management, 85(5), 964-978. https://doi.org/10.1002/jwmg.22060

Shipley, B. (2009). Confirmatory path analysis in a generalized multilevel context. Ecology, 90(2), 363-368. https://doi.org/10.1890/08-1034.1

Siry, J. P., Cubbage, F. W., & Ahmed, M. R. (2005). Sustainable forest management: global trends and opportunities. Forest Policy and Economics, 7(4), 551-561. https://doi.org/10.1016/j.forpol.2003.09.003

Sustainable Forestry Initiative. (2022). 2022 Forest management standard. https://forests.org/wp-content/uploads/2022_SFI_StandardsandRules_section2.pdf

Taniwaki, T., Tamura, A., Sashimura, N., Naruse, M., Tochigi, K., Komine, H., & Koike, S. (2024). Indirect effects of low-impact thinning on insect communities in forest floor of coniferous plantations. Journal of Forest Research, 29(4), 1-10. https://doi.org/10.1080/13416979.2024.2328463

Taki, H., Inoue, T., Tanaka, H., Makihara, H., Sueyoshi, M., Isono, M., & Okabe, K. (2010). Responses of community structure, diversity, and abundance of understory plants and insect assemblages to thinning in plantations. Forest Ecology and Management, 259(3), 607-613. https://doi.org/10.1016/j.foreco.2009.11.019

Taylor, L. R. (1963). Analysis of the effect of temperature on insects in flight. The Journal of Animal Ecology, 32(1), 99-117. https://doi.org/10.2307/2520

Taylor, D. A., Perry, R. W., Miller, D. A., & Ford, W. M. (2020). Forest management and bats. Publication of the White-nose Syndrome Response Team (www.whitenosesyndrome.org), Hadley, MA.

Thomas, S. C., Halpern, C. B., Falk, D. A., Liguori, D. A., & Austin, K. A. (1999). Plant diversity in managed forests: understory responses to thinning and fertilization. Ecological Applications, 9(3), 864-879. https://doi.org/10.1890/1051-0761(1999)009[0864:PDIMFU]2.0.CO;2

van Hoesel, W., Marzal, A., Magallanes, S., Santiago-Alarcon, D., Ibanez-Bernal, S., & Renner, S. C. (2019). Management of ecosystems alters vector dynamics and haemosporidian infections. Scientific Reports, 9(1), 8779. https://doi.org/10.1038/s41598-019-45068-4

Veilleux, J. P., Moosman, P. R., Reynolds, D. S., LaGory, K. E., & Walston, L. J. (2009). Observations of summer roosting and foraging behavior of a hoary bat (*Lasiurus cinereus*) in southern New Hampshire. Northeastern Naturalist 16(1), 148-152. https://doi.org/10.1656/045.016.0113

Veres, A., Petit, S., Conord, C., & Lavigne, C. (2013). Does landscape composition affect pest abundance and their control by natural enemies? A review. Agriculture, Ecosystems & Environment, 166, 110-117. https://doi.org/10.1016/j.agee.2011.05.027

Verschuyl, J., Riffell, S., Miller, D., & Wigley, T. B. (2011). Biodiversity response to intensive biomass production from forest thinning in North American forests–a meta-analysis. Forest Ecology and Management, 261(2), 221-232. https://doi.org/10.1016/j.foreco.2010.10.010

Weller, T. J., & Zabel, C. J. (2002). Variation in bat detections due to detector orientation in a forest. Wildlife Society Bulletin, 30(3), 922-930. https://www.jstor.org/stable/3784248

Welti, E. A., Zajicek, P., Frenzel, M., Ayasse, M., Bornholdt, T., Buse, J., ... & Haase, P. (2022). Temperature drives variation in flying insect biomass across a German malaise trap network. Insect Conservation and Diversity, 15(2), 168-180. https://doi.org/10.1111/icad.12555

Westman, W. E. (1990). Managing for biodiversity. BioScience, 40(1), 26-33. https://doi.org/10.2307/1311236

Wray, A. K., Jusino, M. A., Banik, M. T., Palmer, J. M., Kaarakka, H., White, J. P., ... & Peery, M. Z. (2018). Incidence and taxonomic richness of mosquitoes in the diets of little brown and big brown bats. Journal of Mammalogy, 99(3), 668-674. https://doi.org/10.1093/jmammal/gyy044

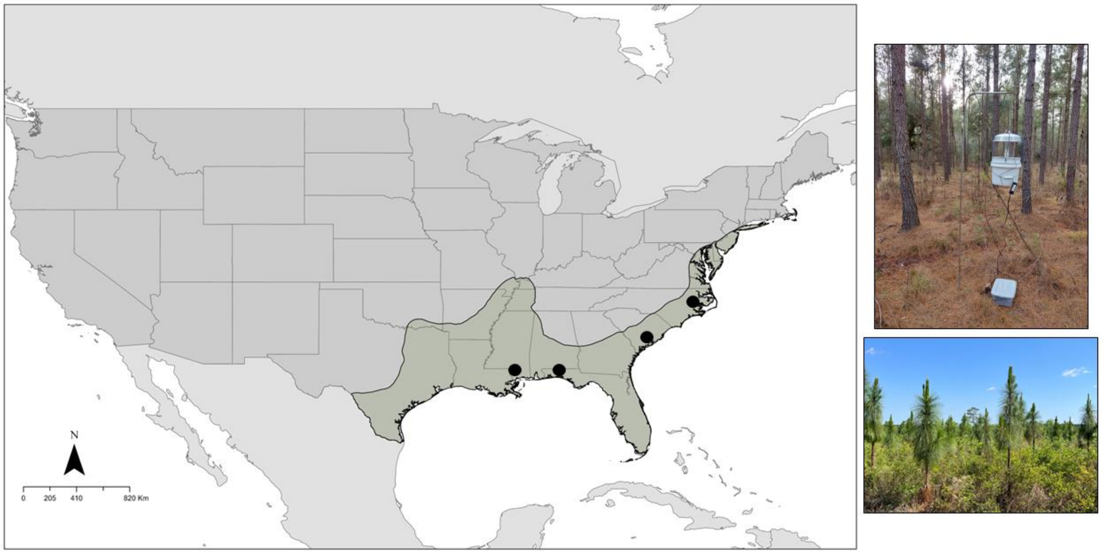


Figure 4.1. Locations of study sites in the southeastern United States Coastal Plain (green-shaded region) where we conducted bat acoustic and nocturnal insect sampling during January–March 2021–2022. Sampled sites are indicated by circles. Insets are examples of a closed canopy stand with a light trap (top right) and a young early establishment stand (bottom right).

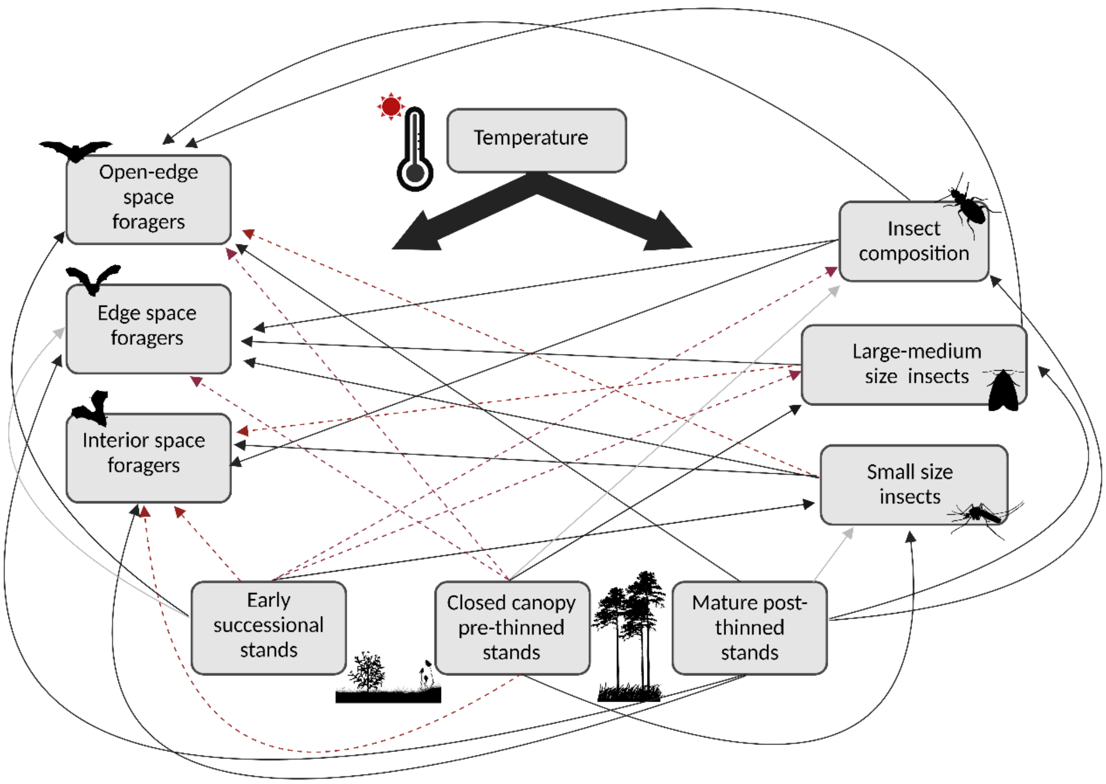


Figure 4.2. A priori hypothesized conceptual model based on previous research for winter bat communities examining relationships among winter bat activity, insect assemblage metrics, temperature, and forest stand structure characteristics in working pine forest landscapes of the southeastern U.S. Coastal Plain, mid-January to mid-March 2021-2022. Continuous arrows represent positive (solid black), negative (dashed red), or neutral (solid gray) unidirectional effects.

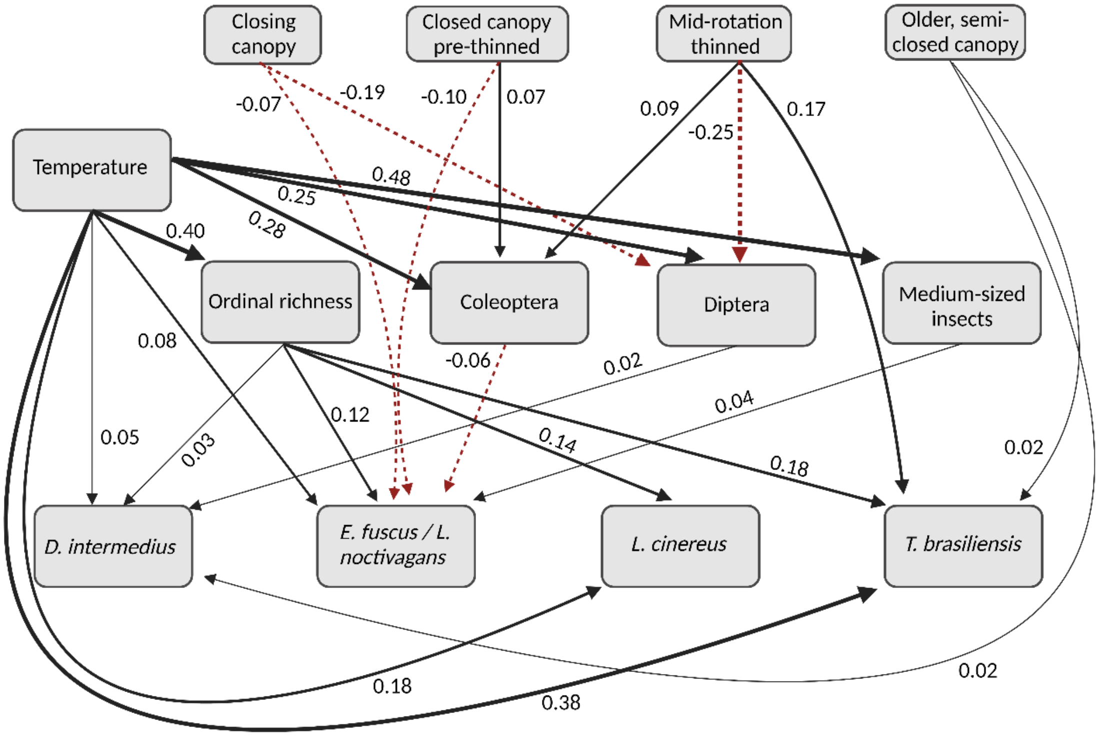


Figure 4.3. Path analysis results examining relationships among winter activity of open-edge space adapted bat species, insect assemblage metrics, temperature, forest stand structure characteristics in working pine forest landscapes of the southeastern U.S. Coastal Plain, mid-January to mid-March 2021-2022. Arrows represent significant paths, which were positive (solid black) or negative (dashed red). Arrow width represents estimate values; with stronger effects represented by wider arrows. Estimates are shown next to the arrows. All coefficients, p-values, marginal R^2, and correlated errors are provided in Appendix 1: Table 2.

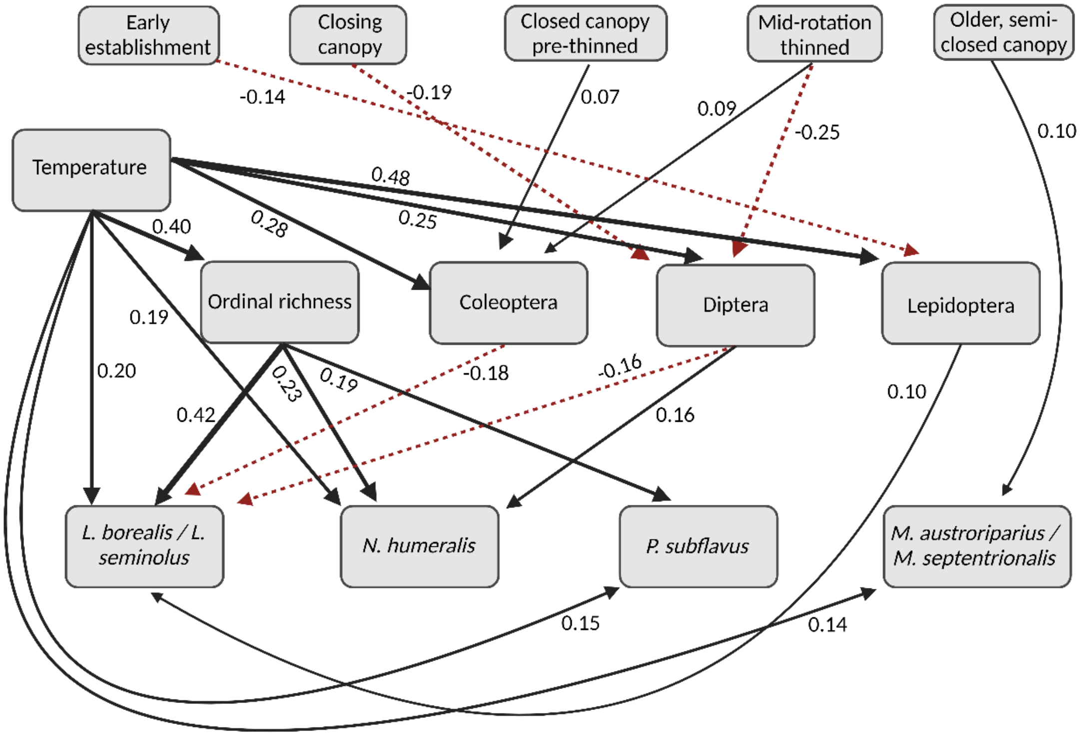


Figure 4.4. Path analysis results examining relationships among winter activity of edge and interior adapted bat species, insect assemblage metrics, temperature, forest stand structure characteristics in working pine forest landscapes of the southeastern U.S. Coastal Plain, mid-January to mid-March 2021-2022. Arrows represent significant paths, which were positive (solid black) or negative (dashed red). Arrow width represents estimate values; with stronger effects represented by wider arrows. Estimates are shown next to the arrows. All coefficients, p-values, marginal R^2, and correlated errors are provided in Appendix 4.1. Table 2.

CHAPTER 5

SUMMARY AND RESEARCH IMPLICATIONS

All North American bats are associated with forests to some extent, relying on them for essential resources such as for roosting, foraging, and drinking. The relationship between bats and forests is particularly important in regions such as the Coastal Plain of the southeastern U.S., where almost 90% of forests are privately owned and a large portion is also actively managed as working forests (Oswalt *et al*., 2019). In this region, many bats remain active during winter, either as year-round residents or as migratory species (e.g., Grider *et al*., 2016; Perea *et al*. 2023, 2024). Understanding how forest management practices, typically studied during the summer, affect bats during winter is essential for their conservation. This need is amplified by the presence of remnant populations decimated by White-Nose Syndrome (WNS) and migratory species impacted by wind energy development. Consequently, studying winter bat foraging ecology and effects of forest management is crucial to bat conservation.

Forest managers employ various strategies, such as site preparation, planting, thinning, mid-rotation management, and final harvesting, to manage pine stands sustainably. These practices influence forest structure, roost availability, and foraging habitat, with both positive and negative effects on bats (e.g., Kalcounis-Rüppell *et al*., 2005; Loeb & O'Keefe, 2006; Jung *et al*., 2012; Bender *et al*., 2015). For instance, the distribution and abundance of live and dead trees, forest clearings, and edges can either enhance or reduce availability of critical resources for different bat species. However, as previous studies have shown during summer, no single set of management recommendations benefits all species uniformly. The complexity of forest management impacts requires a multi-scale approach, as preferences often vary among site, stand, and landscape levels (Lee *et al*., 2002; Miller *et al*., 2009). My research highlights these species-specific responses, showing that overwintering bats in working forests of the southeastern U.S. Coastal Plain exhibit varied responses to management practices and forest structure across multiple scales.

Furthermore, although species richness composition does not generally change throughout the year, we observed differences in bat occupancy and activity levels and factors that influence certain species. Migratory species like the hoary bat (*Lasiurus cinereus*), highly vulnerable to wind turbines, show notably higher occupancy during winter than summer (Bender *et al*., 2015). These findings underscore the importance of considering bat conservation throughout the year, as forest management decisions made with summer data also influence bats in winter.

Foraging strategies among forest-dwelling bats are diverse, with some species foraging along forest edges, others in riparian zones, under or above the canopy forest, or in forest gaps and open stands. My research highlighted that bat occupancy is closely linked to forest heterogeneity, including features such as riparian zones, mature and late successional forest gaps, stands of various ages, edges, and corridors that facilitate movement, foraging, and roosting. Specifically, I highlight similar responses to site and landscape covariates as previous summer work (e.g., Loeb & O'Keefe, 2006, Bender *et al*. 2015, Kunberger & Long, 2022). I observed that winter bat community richness and species-specific occupancy were positively associated with management practices promoting lower basal area conditions and large contiguous forest patches. In addition, I found landscape characteristics for particular species according to their ecomorphologies such as a negative relationship between occupancy probability and edge for southeastern myotis / northern long eared myotis (*Myotis austroriparius* / *M. septentrionalis*), species that commonly forage within forest stands and avoid edges (Henderson & Broders, 2008; Morris *et al*., 2010, Perea *et al*., 2023).

Water availability is a key resource for many bat species for drinking and foraging (e.g., Ford *et al*., 2005; Rainho & Palmeirim, 2011; Janzen & Fenton, 2013). However, I found no significant relationships between water sources and bat occupancy at the community level and only a few at the species level, including some contrary results to our expectations and from previous studies during summer that found higher bat activity closer to water (e.g., Kalcounis-Rüppell *et al*., 2005; Ford *et al*., 2006; Ancillotto *et al*., 2019). One possible explanation is that water availability is typically abundant in the southeastern Coastal Plain, due to high annual rainfall, especially during winter (Bosch *et al*., 1999), and therefore may not be a limiting factor for bats. Alternatively, due to the difficulty of mapping small and often ephemeral freshwater sources, my analysis only included water sources from available spatial data layers, which could have hindered my ability to detect relationships and gave results contrary to those expected (Bender *et al*., 2015; Perea *et al*., 2022). Nonetheless, I acknowledge that water availability is crucial for bat conservation. Thus forest-management practices that eliminate or limit access to water or degrade water quality can negatively affect bats. For instance, it is well-known that several species will also arouse from torpor to drink during winter; some roost near or forage over water [e.g., southeastern myotis or tricolored bat (*Perimyotis subflavus*)] and others such as eastern red (*Lasiurus borealis*), hoary and big brown (*Eptesicus fuscus*) bats are known to use waterways for travel and foraging.

Working pine forests, characterized by a wide range of stand conditions, affect bat species differently. Practices such as clear-cutting, thinning, prescribed burning, herbicide use, and land preparation shape forest structure, influencing availability of roosting and foraging habitats. My site- and landscape-level occupancy results suggest that heterogeneous forests composed of different stand age classes promote occupancy for all bat species, with certain species-specific responses to other landscape-specific characteristics. Forest management activities alter the overall forest structure, affect the distribution and abundance of live and dead trees used for roosting, influence the vertical structure of vegetation, the number of forest clearings and edges used for foraging, and connectivity among more mature forest patches. In this context, my results evaluating bat activity among stands of different stages show effects of stand characteristics specific to different ecomorphological groups. For example, as observed in summer, activity levels of several species were generally higher in thinned stands, which have a reduced vegetation clutter that favors efficient foraging. However, I observed a gradient of responses in species adapted to open areas, such as hoary and Mexican free-tailed (*Tadarida brasiliensis*) bats, which responded to foraging characteristics above the forest canopy and in large clearings. In contrast, interior forest bats such as southeastern myotis / northern long eared myotis and smaller, more maneuverable bats such as tricolored bats tended to forage in cluttered understory vegetation and gaps in mature forests, avoiding areas composed mostly of early-stage stands.

Although my dissertation focuses primarily on the winter foraging ecology of bats, roosting is equally important for their conservation. Forest management plays a key role in ensuring a continuous supply of potential roosting trees, such as trees in various stages of decay (especially early decay), hollow trees, live trees with exfoliated bark, and old trees that may become roosting trees due to disturbances or natural processes. These roosting options benefit not only bats but also dozens of other wildlife species that depend on dead and dying trees. In working forests, late-rotational stands and hardwoods often provide more roosting opportunities for bats that roost in foliage and offer larger and more numerous roosting trees. The need for mature trees makes it essential to maintain well-distributed patches of older forests of varying size. However, even during final clearcuts, green trees, and snags can be retained, particularly in streamside management zones (SMZs), providing roosting opportunities in younger stands and resting sites between mature forest stands (Parrish *et al*. 2017, 2018). Different forest bat species show varying roosting preferences. Some roost exclusively in the foliage of living trees, while others prefer loose bark or crevices of living trees and snags. For example, bats that roost in foliage (i.e., *Lasiurus* and *Dasypterus*) typically select trees with large canopies, which offer suitable temperatures, moisture, and protection from weather and predators. Roost trees are usually located at associated hardwoods, forest edges, or in open late rotational forest stands and clearings, where they receive more solar heat and offer less obstructed flight approaches. In general, although specific roosting needs vary by species, region, and climate, many bats roosting under bark or in tree cavities, such as big brown, silver haired (*Lasionycteris noctivagans*) or evening (*Nycticeius humeralis*) bats, prefer larger snags that extend above the forest canopy, especially in early stages of decay, when the bark is loose and flaking. These preferences underscore the importance of integrative forest management strategies that support a diversity of stand ages, ensuring that enough dead and dying trees are left for the species that depend on cavities or shedding bark across the landscape.

Effective forest management must also consider arthropod communities, as all bats in the region are insectivorous. My research reveals that winter bat communities in private working forests are influenced by both forest structure and availability of nocturnal insects. Stand characteristics directly affect some bat species, while others respond more strongly to changes in prey abundance. For example, I observed that early establishment stands support lower levels of nocturnal Lepidoptera during winter, potentially reducing foraging opportunities for species such as eastern red and Seminole (*Lasiurus seminolus*) bats, which show preferences for this prey group (Carter *et al*., 2004; Clare *et al*. 2009, Hughes *et al*. 2021, Perea *et al*., 2024). Therefore, adaptive forest management strategies that integrate an understanding of bat ecology and nocturnal insect dynamics are essential to conserve overwintering bat populations. Maintaining a mosaic of stand ages that provides high availability of snags and decaying wood, applying selective thinning to increase structural complexity, thus preserving areas with high insect diversity will help ensure resilience of bat populations. By aligning bat conservation with sustainable forestry practices, land managers can provide critical winter habitats for both resident and migratory bats, even when resources are limited.

Finally, I note that sustainable practices that improve habitat quality of bat communities can benefit the economics of working pine forests. My dissertation itself integrates an extra component by discerning the diet of bats to better understand their food preferences. This led me to demonstrate that bats play an important role as pest controllers, with important economic benefits. For example, The Nantucket pine tip moths (*Rhyacionia frustrana*) and the pales weevil (*Hylobius pales*) - common forest pests that affect pine growth - are consumed by all bat species examined (Perea *et al*. 2024). Thus, managing forests to improve bat habitat can improve natural pest control and timber quality (Asaro *et al*., 2003), increasing economic incentive in these forests. In addition, we observed that bats provide ecosystem services outside forest boundaries, such as control of agricultural pests [e.g., the green clover worm (*Hypena scabra*)] and disease vectors (e.g., *Culex* mosquitoes). Private land conservation efforts that promote bat habitats can thus contribute to both biodiversity conservation and economic sustainability.

LITERATURE CITED

Ancillotto, L., Bosso, L., Salinas-Ramos, V. B., & Russo, D. (2019). The importance of ponds for the conservation of bats in urban landscapes. Landscape and Urban Planning, 190, 103607. https://doi.org/10.1016/j.landurbplan.2019.103607

Asaro, C., Fettig, C. J., McCravy, K. W., Nowak, J. T., & Berisford, C. W. (2003). The Nantucket pine tip moth (Lepidoptera: Tortricidae): a literature review with management implications. Journal of Entomological Science, 38(1), 1-40. https://doi.org/10.18474/0749-8004-38.1.1

Bender, M. J., Castleberry, S. B., Miller, D. A. & Wigley, T. B. (2015). Site occupancy of foraging bats on landscapes of managed pine forest. Forest Ecology and Management, 336, 1-10. https://doi.org/10.1016/j.foreco.2014.10.004

Carter, T. C., Menzel, M. A., Chapman, B. R., & Miller, K. V. (2004). Partitioning of food resources by syntopic eastern red (*Lasiurus borealis*), Seminole (*L. seminolus*) and evening (*Nycticeius humeralis*) bats. The American Midland Naturalist, 151(1), 186-191, https://doi.org/10.1674/0003-0031(2004)151[0186:POFRBS]2.0.CO;2

Clare, E. L., Fraser, E. E., Braid, H. E., Fenton, M. B., & Hebert, P. D. (2009). Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. Molecular Ecology, 18(11), 2532-2542. https://doi.org/10.1111/j.1365-294X.2009.04184.x

Ford, W. M., Menzel, M. A., Rodrigue, J. L., Menzel, J. M. & Johnson, J. B. (2005). Relating bat species presence to simple habitat measures in a central Appalachian Forest. Biological Conservation, 126, 528–539. https://doi.org/10.1016/j.biocon.2005.07.003

Grider, J. F., Larsen, A. L., Homyack, J. A. & Kalcounis-Ruepell, M. C. (2016). Winter activity of Coastal Plain populations of bat species affected by White-Nose Syndrome and wind energy facilities. PLoS ONE, 11(11), e0166512. https://doi.org/10.1371/journal.pone.0166512

Hughes, M. J., de Torrez, E. C. B., & Ober, H. K. (2021). Big bats binge bad bugs: Variation in crop pest consumption by common bat species. Agriculture, Ecosystems & Environment, 314, 107414. https://doi.org/10.1016/j.agee.2021.107414

Jantzen, M. K., & Fenton, M. B. (2013). The depth of edge influence among insectivorous bats at forest–field interfaces. Canadian Journal of Zoology, 91(5), 287-292. https://doi.org/10.1139/cjz-2012-0282

Jung, K., Kaiser, S., Böhm, S., Nieschulze, J. & Kalko, E. K. (2012). Moving in three dimensions: effects of structural complexity on occurrence and activity of insectivorous bats in managed forest stands. Journal of Applied Ecology, 49(2), 523-531. https://doi.org/10.1111/j.1365-2664.2012.02116.x

Kalcounis-Rüppell, M. C., Psyllakis, J. M. & Brigham, R. M. (2005). Tree roost selection by bats: an empirical synthesis using meta-analysis. Wildlife Society Bulletin, 33, 1123–1132. https://doi.org/10.2193/0091-7648(2005)33[1123:TRSBBA]2.0.CO;2

Kunberger, J. M., & Long, A. M. (2022). The influence of forest management practices on seasonal bat species occurrence and activity at the Kisatchie National Forest in Louisiana, USA. Forest Ecology and Management, 526, 120579. https://doi.org/10.1016/j.foreco.2022.120579

Loeb, S. C., & O'keefe, J. M. (2006). Habitat use by forest bats in South Carolina in relation to local, stand, and landscape characteristics. The Journal of Wildlife Management, 70(5), 1210-1218. https://doi.org/10.2193/0022-541X(2006)70[1210:HUBFBI]2.0.CO;2

Miller, D. A., Wigley, T. B., & Miller, K. V. (2009). Managed forests and conservation of terrestrial biodiversity in the southern United States. Journal of Forestry, 107(4), 197-203. https://doi.org/10.1093/jof/107.4.197

Rainho, A. & Palmeirim, J. M. (2011). The importance of distance to resources in the spatial modelling of bat foraging habitat. PLoS ONE 6(4), e19227. https://doi.org/10.1371/journal.pone.0019227

Parrish, M. C., Demarais, S., Ezell, A. W., Wigley, T. B., Jones, P. D., & Riffell, S. K. (2017). Retained vegetation density of streamside management zones and stringers in southern intensively managed pine forests. Forest Ecology and Management, 397, 89-96. https://doi.org/10.1016/j.foreco.2017.04.024

Parrish, M. C., Demarais, S., Wigley, T. B., Riffell, S. K., Ezell, A. W., & Jones, P. D. (2018). Operational green tree retention and land cover patterns in intensively managed pine forest landscapes of the southeastern United States. Forest Science, 64(5), 564-576. https://doi.org/10.1093/forsci/fxy009

Perea, S., Morris, K.M. & Castleberry, S.B. (2022). Northern yellow bat *Dasypterus intermedius* activity and habitat selection on an Atlantic barrier island. Acta Chiropterologica, 24(2), 343-352. https://doi.org/10.3161/15081109ACC2022.24.2.005

Perea, S., Fandos, G., Larsen-Gray, A., Greene, D. U., Chandler, R., & Castleberry, S. B. (2023). Bat winter foraging habitat use in working forests: A multispecies spatial occupancy approach. Animal Conservation. https://doi.org/10.1111/acv.12924

Perea, S., Meinecke, C. D., Larsen-Gray, A. L., Greene, D. U., Villari, C., Gandhi, K. J., & Castleberry, S. B. (2024). Winter diet of bats in working forests of the southeastern US Coastal Plain. Scientific Reports, 14(1), 12778. https://doi.org/10.1038/s41598-024-63062-3

APPENDICES

Appendix 2.1. Table S1. Anabat Swift acoustic detectors with omnidirectional ultrasonic microphones US-OV2 and US-OV3 (Titley Electronics, Ballina, New South Wales, Australia) settings.

Schedule	Nightly
File type	Full spectrum
Sample rate	500 k
Sensitivity	16
Minimum frequency trip	15 kHz
Maximum frequency trip	100 kHz
Minimum even time	2 ms
Recording window	2 s
Maximum file length	10 s
Analog HP filter	Off

Appendix 2.1. Table S2. Variable names, descriptions, and hypotheses (including predicted effect for each phonic group) for site- and landscape-scale habitat variables used as covariates in bat occupancy analysis during winter 2020-2022 in working forest landscapes of the southeastern United States Coastal Plain. Site variables can be directly managed via planting density, thinning, harvesting, mechanical disturbance, chemical use, and prescribed burning, while landscape variables often cannot be directly managed but can be informative for landscape-scale management planning. Fc is the characteristic frequency, i.e., the frequency (kHz) of the call at its lowest slope toward the end of the call or the lowest frequency for consistent FM sweeps. Duration is the call duration (ms) from the beginning to the end of the call. The table shows the expected positive (+), negative (-), and neutral (•) effects of each covariate for each phonic group.

Scale/Variable	**Description**	**Low group** **F_c < 30 kHz** **Duration > 5 ms**	**Medium group** **F_c 30-45 kHz** **Duration > 5 ms**	**Myotis group** **F_c > 40 kHz** **Duration < 5 ms**
Site				
Basal area	Basal area (m^2/ha) at sampling point	Foraging (-)	Foraging (-)	Foraging (+)
Vegetation clutter	Vegetation clutter (%) at sampling point	Foraging (-)	Foraging (-)	Foraging (+)
Canopy openness	Overstory canopy openness (%) at sampling point	Foraging (+)	Foraging (+)	Foraging (-)
Landscape				
Distance road	Euclidean distance (m) to the nearest road	Foraging (•), navigation (•)	Foraging (-), navigation (-)	Foraging (+), navigation (+)
Distance water	Euclidean distance (m) to the nearest permanent water	Foraging/drinking (-)	Foraging/drinking (-)	Foraging/drinking (-)
Edge	Total edge (m) within 450 m buffer	Foraging (•), navigation (+)	Foraging (+), navigation (+)	Foraging (-), navigation (•)
Forest	Total forest (m) within 450 m buffer	Roosting (+)	Roosting (+)	Foraging (+), roosting (+)
Wetland	Total wetland (m) within 450 m buffer	Roosting (+)	Roosting (+)	Foraging (+), roosting (+)
Stand ages				
0-3 years	Clearcut/early establishment stand	Foraging (+), roosting (-)	Foraging (+), roosting (-)	Foraging (-), roosting (-)
4-7 years	Closing canopy stand	Foraging (+), roosting (-)	Foraging (+), roosting (-)	Foraging (-), roosting (-)
8-13 years	Closed canopy, pre-thinned stand	Foraging (+), roosting (-)	Foraging (+), roosting (-)	Foraging (-), roosting (-)
14-20 years	Mid-rotation thinned stand	Foraging (•), roosting (-)	Foraging (+), roosting (•)	Foraging (+), roosting (-)
+21 years	Mature, semi-closed canopy stand	Foraging (-), roosting (+)	Foraging (+), roosting (+)	Foraging (+), roosting (+)

Appendix 2.1. Table S3. Confidence set of 5 best-preselected detection sub-models (*p*) with a null occupancy (ψ) term, including the effective number of parameters (pD), WAIC, and ΔWAIC during winter 2020-2022 in working forest landscapes of the southeastern United States Coastal Plain.

Model	**pD**	**WAIC**	**ΔWAIC**
ψ (.), *p* (temperature)	229.08	5626.22	0.00
ψ (.), *p* (basal area + temperature)	227.27	5631.62	5.40
ψ (.), *p* (basal area + clutter + temperature)	244.11	5640.08	13.86
ψ (.), *p* (temperature + clutter)	241.49	5666.87	40.65
ψ (.), *p* (year)	237.28	5684.91	58.69

Appendix 1: Table S4. Models evaluated to examine bat community occupancy (ψ) and detection probability (p) during winter 2020-2022 in working forest landscapes of the southeastern United States Coastal Plain. Models are ranked by WAIC value.

Model	**pD**	**WAIC**	**ΔWAIC**
ψ (basal area + distance road + distance water + total edge + total forest + total wetland), p (temperature)	243.34	5597.86	0.00
ψ (basal area + clutter + distance road + distance water + total edge + total forest + total wetland), p (temperature)	249.98	5606.01	8.15
ψ (basal area + clutter + distance road + distance water + stand class + total edge + total forest + total wetland), p (temperature)	259.32	5610.25	12.39
ψ (distance water + total forest + total wetland), p (temperature)	242.76	5614.89	17.03
ψ (total wetland), p (temperature)	237.79	5616.69	18.83
ψ (basal area + distance road + total forest + total wetland), p (temperature)	246.86	5618.45	20.59
ψ (canopy openness + clutter + distance road + distance water + total edge + total forest + total wetland), p (temperature)	249.87	5618.92	21.06
ψ (distance water), p (temperature)	239.02	5620.20	22.34
ψ (total edge), p (temperature)	230.28	5620.31	22.45
ψ (basal area), p (temperature)	236.43	5621.54	23.68
ψ (basal area + clutter + distance road + total edge + total forest + total wetland), p (temperature)	249.98	5621.87	24.01
ψ (distance water + total edge + total forest + total wetland), p (temperature)	247.77	5622.20	24.34
ψ (.), p (temperature)	229.08	5622.22	24.36
ψ (canopy openness), p (temperature)	236.08	5622.51	24.65
ψ (distance road + total edge), p (temperature)	237.13	5623.18	25.32
ψ (basal area + distance road + total edge + total forest + total wetland), p (temperature)	247.24	5624.46	26.60
ψ (basal area + total forest + total wetland), p (temperature)	248.39	5626.39	28.53
ψ (total forest + total wetland), p (temperature)	237.31	5627.14	29.31
ψ (basal area + clutter + distance road + total forest + total wetland), p (temperature)	248.43	5630.72	32.86
ψ (total forest), p (temperature)	237.14	5633.52	35.66
ψ (canopy openness + distance road + distance water + total edge + total forest + total wetland), p (temperature)	249.65	5633.84	35.98
ψ (stand class), p (temperature)	245.57	5645.50	47.64
ψ (distance road + distance water + total edge + total forest + total wetland), p (temperature)	244.88	5647.79	49.93
ψ (distance road), p (temperature)	238.38	5652.38	54.52
ψ (clutter), p (temperature)	235.10	5660.91	63.05

Appendix 2.1. Table S5. Community mean coefficient estimates (posterior means with standard deviation (SD) and 95% Bayesian credible intervals (BCI)) of the best-supported model used to predict bat occupancy (ψ) and detectability (p) in working forest landscapes of the southeastern United States Coastal Plain winter 2020-22.

Parameter	Mean	SD	95% BCI lower	95% BCI upper
Detection				
Intercept	-0.46	0.21	-0.84	-0.04
Temperature*	0.62	0.13	0.36	0.87
Occupancy				
Intercept	1.15	0.54	0.13	2.13
Basal area*	-0.52	0.22	-0.93	-0.12
Edge	-0.15	0.23	-0.55	0.33
Forest	0.36	0.23	-0.08	0.83
Wetland	-0.02	0.23	-0.47	0.39
Distance to water	0.24	0.29	-0.35	0.74
Distance to road	0.29	0.23	-0.13	0.73

*Indicates 95% BCIs do not include zero

Appendix 2.1. Table S6. Summary of species-specific parameter coefficients for occupancy (ψ) and detection (p) covariates for eight bat species / species groups detected during winter acoustic surveys in working forest landscapes of the southeastern United States Coastal Plain, 2020-2022. Parameter coefficients were extracted from the top model. Estimates include mean, standard deviation (SD) and 95% Bayesian credible intervals (BCI).

Species	Species-specific parameter	Mean	SD	95% BCI lower	95% BCI upper
Eptesicus fuscus / *Lasionycteris noctivagans*	**Detection (p)**				
	Intercept	-0.58	0.13	-0.84	-0.33
	Temperature*	0.92	0.15	0.61	1.16
	Occupancy (ψ)				
	Intercept	1.27	0.36	0.57	1.96
	Basal area	-0.34	0.24	-0.79	0.19
	Edge	0.07	0.24	-0.36	0.56
	Forest*	0.50	0.25	0.04	1.02
	Wetland	-0.32	0.27	-0.79	0.22
	Distance to water*	0.85	0.36	0.23	1.60
	Distance to road	0.26	0.26	-0.19	0.75
Lasiurus borealis / L. seminolus	**Detection (p)**				
	Intercept	-0.18	0.10	-0.41	0.00
	Temperature*	0.66	0.12	0.46	0.91
	Occupancy (ψ)				
	Intercept	2.06	0.61	0.78	3.16
	Basal area*	-0.60	0.28	-1.21	-0.07
	Edge	-0.05	0.28	-0.57	0.49
	Forest	0.41	0.30	-0.17	0.99
	Wetland	0.21	0.32	-0.36	0.83

	Distance to water	-0.16	0.32	-0.83	0.45
	Distance to road	0.12	0.29	-0.44	0.67
Lasiurus cinereus	**Detection (*p*)**				
	Intercept	0.40	0.09	0.22	0.56
	Temperature*	0.58	0.12	0.35	0.82
	Occupancy (*ψ*)				
	Intercept	2.07	0.66	0.79	3.42
	Basal area	-0.34	0.27	-0.88	0.12
	Edge	-0.28	0.27	-0.78	0.22
	Forest	0.30	0.27	-0.25	0.82
	Wetland	-0.24	0.3	-0.74	0.44
	Distance to water	0.49	0.36	-0.19	1.21
	Distance to road	0.55	0.32	-0.06	1.22
Dasypterus intermedius	**Detection (*p*)**				
	Intercept	-1.21	0.20	-1.56	-0.78
	Temperature*	0.54	0.16	0.25	0.88
	Occupancy (*ψ*)				
	Intercept	0.35	0.73	-0.94	1.61
	Basal area	-0.42	0.35	-1.23	0.18
	Edge	-0.08	0.36	-0.88	0.52
	Forest	0.20	0.36	-0.58	0.91
	Wetland	-0.04	0.40	-0.81	0.68
	Distance to water	0.24	0.42	-0.52	1.04
	Distance to road	0.46	0.33	-0.18	1.05
Myotis austroriparius / M. septentrionalis	**Detection (*p*)**				
	Intercept	-0.47	0.16	-0.74	-0.14
	Temperature*	0.77	0.17	0.46	1.07

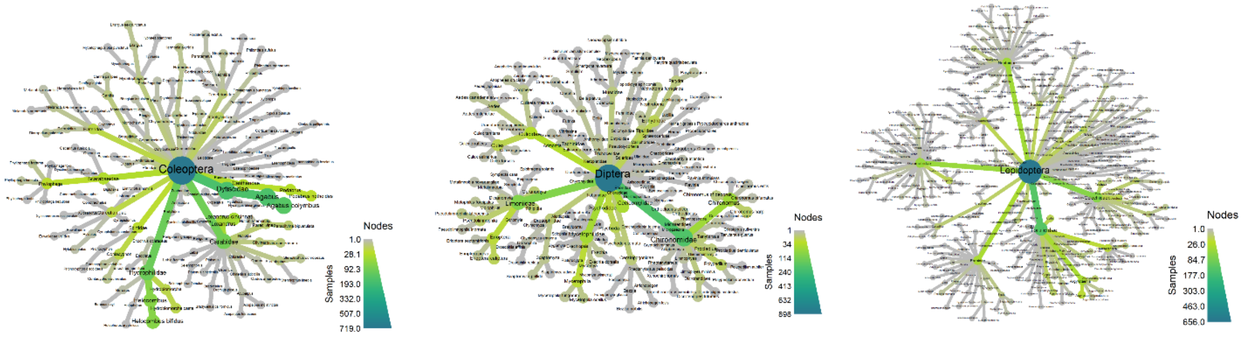
	Occupancy (*ψ*)				
	Intercept	0.71	0.60	-0.40	1.76
	Basal area	-0.23	0.33	-0.81	0.47
	Edge	-0.45	0.29	-1.00	0.09
	Forest	0.29	0.28	-0.34	0.79
	Wetland	0.07	0.32	-0.49	0.79
	Distance to water	0.38	0.35	-0.30	1.04
	Distance to road	0.53	0.33	-0.14	1.12
Nycticeius humeralis	**Detection (*p*)**				
	Intercept	-0.62	0.13	-0.85	-0.36
	Temperature*	0.53	0.13	0.29	0.78
	Occupancy (*ψ*)				
	Intercept	1.27	0.69	0.05	2.44
	Basal area*	-0.84	0.37	-1.61	-0.14
	Edge	0.10	0.33	-0.58	0.61
	Forest	0.54	0.33	-0.05	1.25
	Wetland	0.27	0.36	-0.39	1.12
	Distance to water	0.03	0.41	-0.76	0.83
	Distance to road	0.02	0.39	-0.78	0.78
Perimyotis subflavus	**Detection (*p*)**				
	Intercept	-0.46	0.15	-0.73	-0.16
	Temperature	0.22	0.12	-0.03	0.41
	Occupancy (*ψ*)				
	Intercept	0.76	0.57	-0.27	1.86
	Basal area	-0.54	0.31	-1.13	0.08
	Edge	-0.31	0.32	-1.01	0.18
	Forest	0.38	0.34	-0.35	1.06
	Wetland	-0.30	0.34	-0.99	0.33

	Distance to water	0.23	0.37	-0.52	0.97
	Distance to road	0.13	0.33	-0.47	0.77
Tadarida brasiliensis	**Detection (*p*)**				
	Intercept	-0.62	0.17	-0.93	-0.32
	Temperature*	0.73	0.17	0.41	1.06
	Occupancy (ψ)				
	Intercept	1.04	0.81	-0.55	2.73
	Basal area*	-0.85	0.36	-1.53	-0.14
	Edge	-0.12	0.32	-0.74	0.50
	Forest	0.29	0.33	-0.33	0.96
	Wetland	0.11	0.37	-0.60	0.87
	Distance to water	-0.04	0.47	-0.94	0.83
	Distance to road	0.23	0.35	-0.50	0.87

*Indicates 95% BCIs do not include zero

Appendix 3.1. Database 1. Arthropods consumed by winter bat communities, listed by order, in private, working forests of the southeastern United States from late-January to mid-March 2021-2022. https://static-content.springer.com/esm/art%3A10.1038%2Fs41598-024-63062-3/MediaObjects/41598_2024_63062_MOESM2_ESM.xlsx

Appendix 3.2. Figure 1. Family, genus, and species diversity for the three insect orders (Coleoptera, Diptera, and Lepidoptera) most abundant in the diet of wintering bat communities in private, working forests of the southeastern United States from late-January to mid-March 2021-22. Colors represent number of samples and width of the nodes represents the number of reads for each taxonomic level.



Coleoptera

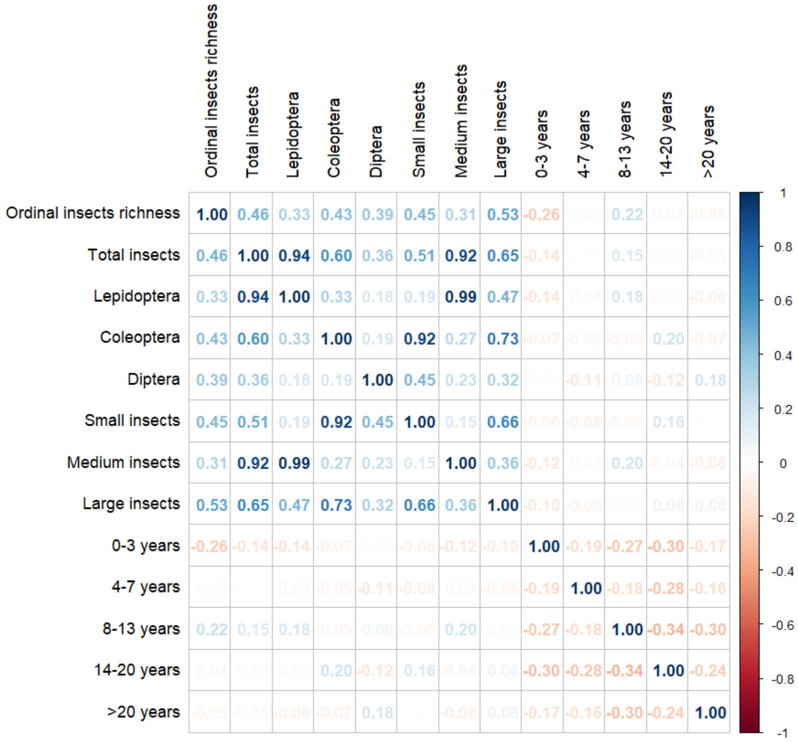
Diptera

Lepidoptera

Appendix 3.3. Database 2. List of agricultural and forest pests and arthropod disease vectors consumed by overwintering bat communities on private, working forests in the southeastern United States from late-January to mid-March 2021-2022. https://static-content.springer.com/esm/art%3A10.1038%2Fs41598-024-63062-3/MediaObjects/41598_2024_63062_MOESM4_ESM.xlsx

Appendix 3.4. Table 1. Total nights sampled, dates, and total number of bat species captured in private, working forest landscapes across four states (Georgia, Louisiana, Mississippi, and North Carolina) of the southeastern U.S. Coastal Plain from late-January to mid-March 2021-2022. Bat species codes: *Dasypterus intermedius* (DAIN), *Eptesicus fuscus* (EPFU), *Lasiurus borealis* (LABO), *Lasiurus cinereus* (LACI), *Lasiurus seminolus* (LASE), *Myotis austroriparius* (MYAU), *Nycticeius humeralis* (NYHU), and *Perimyotis subflavus* (PESU).

State	**Nights**	**Dates**	**DAIN**	**EPFU**	**LABO**	**LACI**	**LASE**	**MYAU**	**NYHU**	**PESU**	**Total**
Georgia	18	15 Feb 2024 / 07 Mar 2024	2	7	4	0	51	10	26	20	**120**
Louisiana	14	25 Jan 2024 / 11 Feb 2024	0	0	3	0	7	2	0	0	**12**
Mississippi	13	19 Feb 2023 / 13 Mar 2023	0	1	2	0	15	0	36	0	**54**
North Carolina	11	16 Feb 2024 / 07 Mar 2024	0	6	16	3	6	29	13	5	**78**



	Ordinal insects richness	Total insects	Lepidoptera	Coleoptera	Diptera	Small insects	Medium insects	Large insects	0-3 years	4-7 years	8-13 years	14-20 years	>20 years
Ordinal insects richness	1.00	0.46	0.33	0.43	0.39	0.45	0.31	0.53	-0.26	0.03	0.22	0.04	-0.05
Total insects	0.46	1.00	0.94	0.60	0.36	0.51	0.92	0.65	-0.14	-0.01	0.15	0.02	-0.05
Lepidoptera	0.33	0.94	1.00	0.33	0.18	0.19	0.99	0.47	-0.14	0.04	0.18	-0.02	-0.06
Coleoptera	0.43	0.60	0.33	1.00	0.19	0.92	0.27	0.73	-0.07	-0.05	-0.05	0.20	-0.07
Diptera	0.39	0.36	0.18	0.19	1.00	0.45	0.23	0.32	0.01	-0.11	0.06	-0.12	0.18
Small insects	0.45	0.51	0.19	0.92	0.45	1.00	0.15	0.66	-0.06	-0.08	-0.05	0.16	0.01
Medium insects	0.31	0.92	0.99	0.27	0.23	0.15	1.00	0.36	-0.12	0.03	0.20	-0.04	-0.08
Large insects	0.53	0.65	0.47	0.73	0.32	0.66	0.36	1.00	-0.10	-0.06	0.02	0.06	0.08
0-3 years	-0.26	-0.14	-0.14	-0.07	0.01	-0.06	-0.12	-0.10	1.00	-0.19	-0.27	-0.30	-0.17
4-7 years	0.03	-0.01	0.04	-0.05	-0.11	-0.08	0.03	-0.06	-0.19	1.00	-0.18	-0.28	-0.16
8-13 years	0.22	0.15	0.18	-0.05	0.06	-0.05	0.20	0.02	-0.27	-0.18	1.00	-0.34	-0.30
14-20 years	0.04	0.02	-0.02	0.20	-0.12	0.16	-0.04	0.06	-0.30	-0.28	-0.34	1.00	-0.24
>20 years	-0.05	-0.05	-0.06	-0.07	0.18	0.01	-0.08	0.08	-0.17	-0.16	-0.30	-0.24	1.00

Appendix 4.1. Figure 1. Correlation matrix among insect metrics and forest stand characteristics in private forest landscapes of the southeastern U.S. Coastal Plain during mid-January to mid-March 2021-2022. Red shows negative correlations, while blue shows positive correlations. The numbers indicate the correlation score.

Appendix 4.1. Table 1. Top generalized linear model (GLM) sets with ΔAICc ≤ 1 including number of parameters (K), corrected Akaike's Information Criterion (AICc), difference between a GLM and the GLM with the lowest AICc value (ΔAICc), and model weight (ωi) for each response variable. Models relate to bat captures and insect metrics in private forest landscapes of the southeastern U.S. Coastal Plain during mid-January to mid-March 2021-2022.

Models	**K**	**AIC_c**	**ΔAIC_c**	**W_i**
Ordinal richness				
Temperature + 0-3 years	3	499.17	0.00	0.16
Temperature + 0-3 years + 8-13 years	4	499.80	0.63	0.12
Temperature + 8-13 years	3	499.90	0.73	0.11
Coleoptera				
Temperature + 4-7 years + 8-13 years + 14-20 years	6	1061.48	0.00	0.50
Diptera				
Temperature + 4-7 years + 14-20 years	5	1248.61	0.00	0.42
Temperature + 4-7 years + 8-13 years + 14-20 years	6	1249.47	0.85	0.28
Lepidoptera				
Temperature + 0-3 years + 8-13 years	5	1371.39	0.00	0.61
Small size insects				
Temperature + 14-20 years + >20 years	5	1238.85	0.00	0.14
Temperature + 0-4 years	4	1239.67	0.82	0.09
Temperature + 8-13 years + 14-20 years + >20 years	6	1239.81	0.96	0.08
Medium size insects				
Temperature + 0-3 years + 8-13 years	5	1534.14	0.00	0.23
Temperature + 8-13 years	4	1534.19	0.05	0.23
Temperature + 8-13 years + >20 years	5	1535.02	0.88	0.15
Large size insects				
Temperature + 0-3 years	4	1074.50	0.00	0.24
Temperature + 0-3 years + 4-7 years	5	1075.25	0.75	0.16
Dasypterus intermedius				
Temperature + 14-20 years + >20 years + Ordinal richness + Coleoptera + Diptera	8	124.46	0.00	0.17
Temperature + >20 years + Ordinal richness + Coleoptera + Diptera	7	125.46	1.00	0.10
Eptesicus fuscus* / *Lasyonicteris noctivagans				
Temperature + 4-7 years + 8-13 years + Ordinal richness + Coleoptera + Lepidoptera	8	299.45	0.00	0.53

Temperature + 4-7 years + 8-13 years + Ordinal richness + Coleoptera + Medium insects	8	299.84	0.39	0.44
Lasiurus borealis* / *Lasiurus seminolus				
Temperature + 14-20 years + >20 years + Ordinal richness + Coleoptera + Diptera + Lepidoptera	9	558.41	0.00	0.37
Temperature + >20 years + Ordinal richness + Coleoptera + Diptera + Lepidoptera	8	559.30	0.89	0.24
Lasiurus cinereus				
Temperature + 14-20 years + >20 years + Ordinal richness + Medium insects	7	637.98	0.00	0.20
Temperature + 14-20 years + >20 years + Ordinal richness + Lepidoptera	7	638.35	0.37	0.17
Myotis austroriparius / Myotis septentrionalis				
Temperature + 0-3 years + >20 years + Ordinal richness + Coleoptera + Lepidoptera	8	364.57	0.00	0.52
Nycticeius humeralis				
Temperature + >20 years + Ordinal richness + Diptera	6	297.05	0.00	0.26
Perimyotis subflavus				
Temperature + 0-3 years + 4-7 years + Ordinal richness + Coleoptera + Diptera	8	305.69	0.00	0.60
Tadarida brasiliensis				
Temperature + 14-20 years + >20 years Ordinal richness + Lepidoptera	7	312.36	0.00	0.56

Appendix 4.1. Table 2. The full path model statistics including unstandardized and standardized estimated pathway coefficients, standard errors (SE), and corresponding p-values. R^2 is shown for each response variable. Last rows are correlated errors. Significant pathway p-values are bolded. Path model is visualized in Figure 2. Species code: *Dasypterus intermedius* (DAIN), *Eptesicus fuscus* / *Lasionycteris noctivagans* (EPFU / LANO), *Lasiurus borealis* / *Lasiurus seminolus* (LABO / LASE), *Lasiurus cinereus* (LACI), *Myotis austroriparius* / *Myotis septentrionalis* (MYAU / MYSE), *Nycticeius humeralis* (NYHU), *Perimyotis subflavus* (PESU), and *Tadarida brasiliensis* (TABR).

Response variable	Predictor	Standardized estimate	Estimate	SE	p-value	R^2
Insect Richness	Temperature	0.401	0.034	0.011	**0.001**	0.23
	8-13 years	0.200	0.063	0.037	0.088	
Coleoptera	Temperature	0.278	0.343	0.042	**0.000**	0.97
	4-7 years	0.038	0.224	0.205	0.277	
	8-13 years	0.071	0.321	0.154	**0.039**	
	14-20 years	0.094	0.416	0.153	**0.008**	
Diptera	Temperature	0.252	0.074	0.025	**0.003**	0.34
	4-7 years	-0.191	-0.269	0.125	**0.033**	
	14-20 years	-0.249	-0.260	0.092	**0.006**	
Lepidoptera	Temperature	0.464	0.281	0.028	**0.000**	0.96
	0-3 years	-0.136	-0.344	0.119	**0.005**	
	8-13 years	0.078	0.174	0.099	0.080	
Small Insects	Temperature	0.361	0.200	0.034	**0.000**	0.77
	14-20 years	0.064	0.126	0.117	0.286	
	> 20 years	0.061	0.151	0.146	0.303	
Medium Insects	Temperature	0.477	0.178	0.035	**0.000**	0.81
	8-13 years	0.177	0.243	0.128	0.060	
Large Insects	Temperature	0.401	0.244	0.022	**0.000**	0.86
	0-3 years	-0.090	-0.227	0.093	**0.016**	
DAIN	Temperature	0.051	0.239	0.056	**0.000**	0.53
	> 20 years	0.015	0.324	0.144	**0.027**	
	Richness	0.028	0.423	0.176	**0.018**	

	Coleoptera	-0.010	-0.002	0.002	0.350	
	Diptera	0.016	0.008	0.003	**0.011**	
EPFU / LANO	Temperature	0.069	0.145	0.052	**0.001**	0.73
	4-7 years	-0.067	-0.672	0.285	**0.020**	
	8-13 years	-0.100	-0.732	0.204	**0.001**	
	Richness	0.120	0.800	0.160	**0.000**	
	Coleoptera	-0.055	-0.001	0.002	**0.016**	
	Medium insects	0.036	0.002	0.001	**0.013**	
LABO/LASE	Temperature	0.195	0.128	0.052	**0.015**	0.80
	> 20 years	0.086	0.254	0.183	0.168	
	Richness	0.423	0.883	0.179	**0.000**	
	Coleoptera	-0.178	-0.006	0.003	**0.034**	
	Diptera	-0.158	-0.012	0.001	**0.035**	
	Lepidoptera	0.100	0.001	0.005	**0.054**	
LACI	Temperature	0.176	0.194	0.049	0.000	0.79
	4-7 years	-0.036	-0.190	0.212	**0.372**	
	8-13 years	-0.067	-0.270	0.162	**0.097**	
	Richness	0.141	0.494	0.144	**0.000**	
	Medium insects	-0.028	-0.001	0.001	0.471	
MYAU / MYSE	Temperature	0.138	0.254	0.067	**0.000**	0.81
	0-3 years	-0.070	-0.538	0.382	0.161	
	> 20 years	0.091	0.747	0.202	**0.000**	
	Richness	-0.043	-0.249	0.233	0.288	
	Coleoptera	-0.191	-0.018	0.014	0.197	
	Lepidoptera	0.042	0.002	0.001	0.178	
NYHU	Temperature	0.187	0.100	0.044	**0.026**	0.69
	> 20 years	0.119	0.285	0.152	0.062	
	Richness	0.231	0.393	0.143	**0.007**	
	Diptera	0.161	0.009	0.003	**0.005**	
PESU	Temperature	0.150	0.164	0.064	**0.012**	0.58
	0-3 years	-0.092	-0.421	0.326	0.199	
	4-7 years	-0.154	-0.811	0.438	0.067	
	Richness	0.191	0.667	0.221	**0.003**	
	Coleoptera	-0.069	-0.004	0.003	0.146	
	Diptera	-0.039	-0.004	0.005	0.369	
TABR	Temperature	0.375	0.360	0.062	**0.000**	0.89

	14-20 years	0.174	0.596	0.166	**0.005**
	> 20 years	0.112	0.481	0.200	**0.018**
	Richness	0.177	0.541	0.169	**0.002**
	Lepidoptera	-0.133	-0.003	0.002	0.133
Coleoptera	Richness	0.562	0.562	-	0.000
Diptera	Richness	0.456	0.456	-	0.000
Lepidoptera	Richness	0.501	0.501	-	0.000
Small	Richness	0.373	0.373	-	0.000
Medium	Richness	0.526	0.526	-	0.000
Large	Richness	0.510	0.510	-	0.000
Coleoptera	Lepidoptera	0.512	0.512	-	0.000
Diptera	Coleoptera	0.340	0.340	-	0.000
Small	Coleoptera	0.546	0.546	-	0.000
Medium	Coleoptera	0.514	0.514	-	0.000
Large	Coleoptera	0.563	0.563	-	0.000
Lepidoptera	Diptera	0.346	0.346	-	0.000
Small	Diptera	0.704	0.704	-	0.000
Medium	Diptera	0.581	0.581	-	0.000
Large	Diptera	0.324	0.324	-	0.000
Small	Lepidoptera	0.242	0.242	-	0.000
Medium	Lepidoptera	0.856	0.856	-	0.000
Large	Lepidoptera	0.754	0.754	-	0.000
Medium	Small	0.319	0.319	-	0.000
Large	Small	0.330	0.330	-	0.000
Medium	Large	0.546	0.546	-	0.000
EPFU LANO	LABO LASE	0.241	0.241	-	0.003
EPFU LANO	LACI	0.169	0.169	-	0.020
EPFU LANO	DAIN	0.400	0.400	-	0.000
EPFU LANO	MYAU MYSE	0.055	0.055	-	0.115
EPFU LANO	NYHU	0.123	0.123	-	0.029
EPFU LANO	PESU	0.157	0.157	-	0.134
EPFU LANO	TABR	0.307	0.307	-	0.000
LABO LASE	LACI	0.103	0.103	-	0.126
LABO LASE	DAIN	0.018	0.018	-	0.359
LABO LASE	MYAU MYSE	0.224	0.224	-	0.002
LABO LASE	NYHU	0.366	0.366	-	0.000
LABO LASE	PESU	0.566	0.566	-	0.000
LABO LASE	TABR	0.112	0.112	-	0.050
LACI	DAIN	0.167	0.167	-	0.029
LACI	MYAU MYSE	0.212	0.212	-	0.029
LACI	NYHU	0.080	0.080	-	0.057
LACI	PESU	0.060	0.060	-	0.197

LACI	TABR	0.356	0.356	-	0.000
DAIN	MYAU MYSE	0.007	0.007	-	0.361
DAIN	NYHU	-0.050	-0.050	-	0.476
DAIN	PESU	-0.078	-0.078	-	0.259
DAIN	TABR	0.278	0.278	-	0.001
MYAU MYSE	NYHU	0.007	0.007	-	0.485
MYAU MYSE	PESU	0.272	0.272	-	0.003
MYAU MYSE	TABR	0.026	0.026	-	0.120
NYHU	PESU	0.261	0.261	-	0.001
NYHU	TABR	0.197	0.197	-	0.006
PESU	TABR	0.097	0.097	-	0.274