1 *Communication*

2 3 4 Ecological drivers of species distributions and niche overlap for three subterranean termite species in the southern Appalachian Mountains, USA

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8 **Abstract:** In both managed and unmanaged forests, termites are functionally important members
9 of the dead-wood-associated (saproxylic) insect community. However, little is known about
10 regional-scale environmental drivers of geographic distributions of termite species, and how these
11 environmental factors impact co-occurrence among congeneric species. Here we focus on the
12 southern Appalachian Mountains—a well-known center of endemism for forest biota—and use
13 Ecological Niche Modeling (ENM) to examine the distributions of three species of *Reticulitermes*
14 termites (i.e., *R. flavipes*, *R. virginicus*, and *R. malletei*). To overcome deficiencies in public databases,
15 ENMs were underpinned by field-collected high-resolution occurrence records coupled with
16 molecular taxonomic species identification. Spatial overlap among areas of predicted occurrence of
17 each species was mapped, and aspects of niche similarity were quantified. We also identified
18 environmental factors that most strongly contribute to among-species differences in occupancy.
19 Overall, we found that *R. flavipes* and *R. virginicus* showed significant niche divergence, which was
20 primarily driven by dry-season precipitation. Also, all three species were most likely to co-occur in
21 the mid-latitudes of the study area (i.e., northern Alabama and Georgia, eastern Tennessee and
22 western North Carolina), which is an area of considerable topographic complexity. This work
23 provides important baseline information for follow-up studies of local-scale drivers of these
24 species' distributions. It also identifies specific geographic areas where future assessments of the
25 frequency of true syntopy vs. micro-allopatry, and associated interspecific competitive interactions,
26 should be focused.

27 **Keywords:** Biodiversity; biogeography; competitive exclusion; ecological niche model; molecular
28 taxonomic identification; PCR-RFLP; *Reticulitermes*; saproxylic; species richness

29

30 1. Introduction

31 *1.1. The southern Appalachian Mountains: a center of endemism for forest biota*

32 The southern Appalachian Mountains, extending latitudinally from northeast Alabama to
33 northwest Virginia, are some of the oldest uplands in North America. These mountains have been
34 exposed and unglaciated for over 100 million years [1]. Steep altitudinal precipitation gradients, a
35 complex heavily dissected topography, and a humid, temperate climate, have shaped southern
36 Appalachian forests into some of the most diverse environments in the eastern United States. [2].
37 While deciduous oak-hickory forests dominate much of the mid-elevation landscape [2], high
38 elevations (above 1,400 m) support spruce-fir forests [3], whereas mesic coves support hemlock, and
39 pines are commonly found at xeric low- to mid-elevations [4].
40 The southern Appalachian Mountains are incredibly rich in biodiversity [5]. The region is
41 thought to have served as a major Pleistocene refuge for numerous species. Past climatic cycles have
42 affected distributions of forest biota, resulting in major range shifts or local extinction. Following the
43 Last Glacial Maximum (ca. 21,000 years ago), recolonization is thought to have occurred relatively

44 rapidly, from 7,000-16,000 years ago [6–10]. The southern Appalachian Mountains are a well-known
45 center of endemism for salamanders and other amphibians [11,12]. However, there is increasing
46 evidence of short-range endemism in other groups, including dead wood-associated forest
47 invertebrates (e.g., millipedes [13,14], cockroaches [15,16], and centipedes [17]).

48 *1.2. Subterranean termites: functionally important ecosystem service providers in temperate forests*

49 Dead-wood-dependent (saproxylic) arthropods play critical roles in maintaining healthy,
50 productive forests by contributing to the decomposition of fallen trees and thus driving nutrient
51 cycling that affects organisms at all trophic levels [18–22]. Indeed, rotting logs may be one of the
52 most stable, thermally buffered, above-ground microhabitats that exist in forests, and the
53 decomposition process has successional stages, facilitated by wood-feeding and wood-boring
54 invertebrates [21,23]. Termites are some of the first to colonize a rotting log, and through feeding and
55 tunneling activities of the worker caste, the dead-wood substrate is modified by the creation of
56 galleries. Once established, these facilitate colonization by larger wood-feeding invertebrates [24].
57 Ultimately, the ecosystem engineering activities of termites contribute to enhancing the internal
58 heterogeneity of logs, making them habitable by a diverse array of saproxylic species.
59 Termites in the genus *Reticulitermes* (Blattodea: Rhinotermitidae) are broadly distributed across
60 the eastern United States. Morphological separation of species is notoriously difficult [25],
61 particularly given that only the worker caste can usually be readily sampled. To address this, we
62 developed an efficient molecular assay (i.e., polymerase chain reaction (PCR) amplification of a short
63 region of mitochondrial cytochrome oxidase subunit II (COII) gene, followed by screening of
64 restriction-fragment-length polymorphism (RFLP) banding profiles [26]) that can be used to
65 distinguish each of the five eastern United States species. In the southern Appalachians, several
66 *Reticulitermes* species can co-occur locally. However, true syntopy (i.e., two species co-inhabiting the
67 same rotting log) appears to be very rare, but reported instances of fine-scale sampling have been
68 limited.

69 *1.3. Ecological niche models: efficient tools for predicting organismal distributions*

70 Ecological niche models (ENMs) are broadly useful spatially explicit analytical tools that relate
71 species occurrence data with environmental variables, such as climatic temperature and
72 precipitation data [27], or topographic and land cover data. Once constructed, ENMs generate maps
73 of estimated habitat suitability, and can be used to describe the historical, current, and future climate
74 space for a given species. For example, ENMs have been used to identify areas of high conservation
75 importance [28–30], predict climate change effects on geographic ranges of species [31,32], as well as
76 determine potential threats of invasive species [33,34]. These analytical tools are becoming widely
77 used owing to the increasing accessibility of climatic data via public databases [35–37]. An important
78 assumption when using ENMs to predict historical or future distributions is niche conservatism (i.e.,
79 the stability of ecological niches over time) [38]. However, evidence suggests that niche conservatism
80 is common among closely related species [39–41], and the risks of erroneous inferences are further
81 reduced when focusing only on contemporary climate and occurrence data (i.e., when
82 reconstructing present-day ENMs).

83 *1.4. The current state of knowledge about subterranean termite distributions, and goals of this study*

84 There is a general lack of data on the natural distributions of termites in temperate forests, given
85 that most research has focused on damage that termites cause to man-made wooden structures.
86 Accordingly, occurrence records mostly come from urban areas, and they are also of low resolution
87 (e.g., presence/absence in a given county). Notwithstanding these limitations, Maynard et al. [42]
88 recently provided valuable insights into the role of climatic (temperature and precipitation)
89 variables in influencing distributions of termites in the eastern United States. Specifically, those
90 authors performed ENM for two *Reticulitermes* species (*R. flavipes* and *R. virginicus*) and the invasive
91 Formosan subterranean termite, *Coptotermes formosanus*. Furthermore, they synthesized pre-existing

92   knowledge to identify the influence on termite distributions of biotic factors, such as tree species and
93   wood traits, fungal preferences, phenology of predatory ants, and competitive asymmetries among
94   coexisting termite species. While interspecific competition may result in spatial or temporal
95   separation which could lead to niche divergence, to date, very little is known about niche
96   partitioning in subterranean termites and the environmental factors that may lead to niche
97   divergence.
98   In the present paper we aimed to generate new insights into regional-scale environmental
99   drivers of geographic distributions of termite species, and how these environmental factors impact
100   co-occurrence among congeneric species. Focusing on the southern Appalachian Mountains and
101   surrounding areas, we performed an ENM-based evaluation of niche divergence among the three
102   most common *Reticulitermes* species in the eastern United States. In addition to identifying niche
103   divergence, if present, we aimed to determine the environmental factors driving niche divergence
104   among species.

2. Materials and Methods

2.1. Termite sampling, species identification, and Ecological Niche Modeling

107   From 2012 to 2016, we collected *Reticulitermes* termites from 132 sites across the southern
108   Appalachians Mountains and surrounding areas (Table S1; Figure S1). At most sites, termite workers
109   were collected from a single rotting log at an intermediate to late stage of decay. However, at 10
110   sites, termites were also collected from additional logs within ~30 m of one another (i.e., samples
111   came from a total of 2 logs at 8 sites, 3 logs at 1 site, and 4 logs at 1 site; Table S1). Owing to the close
112   proximity of these clustered logs (i.e., at or near the typical error associated with a handheld GPS
113   unit), the same coordinates were assigned to them, but specimen collections were assigned
114   log-specific identifiers. Molecular taxonomic identifications were based on a single termite per
115   rotting log, using Garrick et al.'s [26] PCR-RFLP assay. Briefly, a short (376-bp) region of the
116   mitochondrial COII gene was amplified (using PCR primers RetCo2-F and RetCo2-R), and products
117   were then sequentially digested with three restriction enzymes (RsaI, TaqI, and MspI), which in
118   combination generate diagnostic species-specific banding patterns. Ultimately, we identified 91
119   non-redundant occurrence points for *R. flavipes*, 30 for *R. virginicus,* and 17 for *R. malletei* (Table S1).
120   ENM was conducted with the 'biomod2' package [43] in R [44] using four modeling algorithms (e.g.,
121   [45–47]). Distributions were reconstructed using mean climatological data for a period spanning
122   1960–1990, with all variables used at 1-km resolution. Nineteen bioclimatic variables [35] were
123   obtained from the WorldClim database v.1.4 (http://www.worldclim.org), and then factor analysis
124   was used to reduce the number of predictors, and the associated correlation among them (see
125   Supplementary Methods for full details of ENM methods). From the 19 bioclimatic variables, we
126   generated four environmental factors (see Supplementary Methods and Figures S2 and S3 for full
127   details of factor analysis): dry-season precipitation, wet-season precipitation, summer temperature,
128   and temperature range.

2.2. Niche occupancy, niche identity, and distributional overlap

130   Predicted niche occupancy profiles were generated for each environmental factor following
131   Evans et al. [48], implemented in the 'phyloclim' package [49]. Niche overlap for each environmental
132   factor was summarized using both Schoener's D statistic [50], and the modified Hellinger statistic, I,
133   as proposed by Warren et al. [51]. We also used the D and I statistics to determine pairwise niche
134   equivalency/identity among the three *Reticulitermes* species. The niche equivalency test asks whether
135   the ENMs of two species are more different than expected if they had been drawn from the same
136   distribution. To perform the niche equivalency test, we generated a distribution using 999
137   pseudoreplicate datasets.
138   To assess distributional overlap based on ENMs, we used maps of binary presence/absence as
139   well as continuous occurrence probabilities. We used binary predictions, because this allowed us to
140   determine which species co-occurred in areas of distributional overlap. However, since the use of

141 continuous predictions has been recommended when estimating species richness [52], we calculated
142 the sum of *Reticulitermes* species' occurrence probabilities (Figure S4), and calculated joint and
143 exclusive occurrence probabilities for each of the three species (Figure S5). For binary predictions,
144 the approach of maximizing sensitivity and specificity has consistently performed better than other
145 methods [53–55]. Thus, we used the True Skill Statistic (TSS = sensitivity + specificity – 1) [56] both as
146 a model performance metric and to identify a threshold for converting continuous occurrence
147 probabilities to binary classifications. The threshold was chosen based on maximizing the TSS,
148 without risking under-prediction of presences (i.e., selecting the lowest threshold at which TSS is
149 maximized). We used a threshold value of 0.2, where probability > 0.2 represented presence, and
150 suitability ≤ 0.2 represented absence. We merged the three species' binary maps by summing
151 re-coded maps, where absence = 0, but presence was coded depending on species: *R. flavipes* = 4, *R.*
152 *virginicus* = 2, and *R. malletei* = 1. This way, the sum of binary maps resulted in seven distinct
153 categories: single-species areas (3 categories, with aforementioned scores); areas of two-species
154 overlap (3 categories, scores of either 3, 5, or 6 depending on the identity of the species pair); and
155 areas where all three species overlap (1 category, with a score of 7).

2.3. Environmental factors and niche divergence

157 In order to determine the sources of variation in the *Reticulitermes* occurrence dataset, we
158 included the effects of spatial structure and environmental factors, and performed variance
159 partitioning using the 'varpart' function in 'vegan' [57]. To account for multiple predictors in the
160 model, we used adjusted R^2. To determine which (if any) environmental factors have significantly
161 contributed to niche divergence of *Reticulitermes* species, we first removed the effect of spatial
162 structure. We did this by performing distance-based redundancy analysis [58] using the 'capscale'
163 function. To account for spatial structure, we transformed Euclidean geographic distances to a
164 continuous rectangular vector by Principal Coordinates analysis of Neighbor Matrices (PCNM)
165 using the 'pcnm' function in 'vegan'. Only significant PCNM axes were used in partialling out
166 spatial structure. Significance of the environmental and spatial predictors was assessed using
167 multivariate F-statistics with 9999 permutations.

3. Results

3.1. Niche occupancy, niche identity, and distributional overlap

170 Predicted niche occupancy profiles for the three *Reticulitermes* species (Figure 1) showed
171 differences in peak values across all four environmental factors. The two temperature factors,
172 summer temperature and temperature range, showed differences in peaks between *R. flavipes* and *R.*
173 *virginicus*, whereas *R. malletei* was intermediate. Similarly, the two precipitation factors, dry-season
174 precipitation and wet-season precipitation, showed more marked differences between *R. flavipes* and
175 *R. virginicus* than for any of the other pairwise species comparisons. The bimodality of wet-season
176 precipitation is a result of occurrence of *Reticulitermes* species in two areas with pronounced
177 differences in wet-season precipitation (see Figure S3). Statistics that characterize the extent of niche
178 overlap showed the least amount of niche overlap was between *R. flavipes* and *R. virginicus* (D =
179 0.582, I = 0.843; Table 1). Furthermore, the niche identity test between these two species showed
180 significant differentiation (P < 0.001; Table 1). *R. malletei* was more similar to *R. flavipes* in terms of
181 temperature range (D = 0.889) and dry-season precipitation (D = 0.872), but showed more overlap
182 with *R. virginicus* for summer temperature (D = 0.894) and wet-season precipitation (D = 0.848). *R.*
183 *virginicus* showed the least overlap with *R. flavipes*, across all four environmental factors (Table 2).
184 The predicted distribution of *R. flavipes* spanned a larger area in the northern portion of the
185 southern Appalachians than that of the other two species, even excluding them in some areas
186 (Figure 2; Figure S5). *R. flavipes* overlapped with *R. malletei*, to the exclusion of *R. virginicus*, in an area
187 including Kentucky, Virginia, and West Virginia (Figure 2; Figure S5). The overlap between *R.*
188 *flavipes* and *R. virginicus,* excluding *R. malletei*, spanned a smaller area, with lower probability (Figure

189   S5). Predicted distributions of all three species overlapped in eastern Tennessee, western North
190   Carolina, northern Alabama and Georgia (Figure 2; Figures S4 and S5).
191
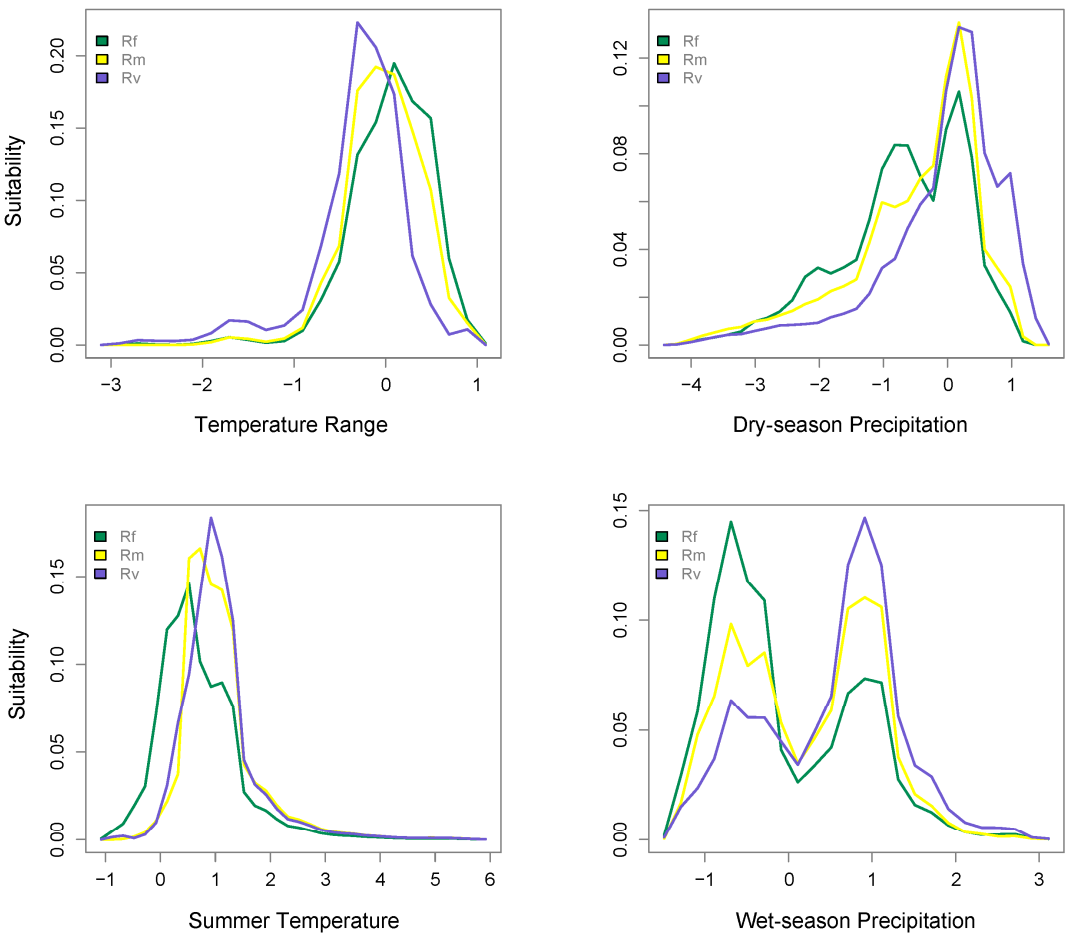
192
193   **Figure 1.** *Predicted niche occupancy.* Four environmental factors were used to estimate niche
194   occupancy of *R. flavipes* (Rf), *R. malletei* (Rm), and *R. virginicus* (Rv): left two panels: temperature
195   range and summer temperature; right two panels: dry- and wet-season precipitation. The y-axis
196   represents niche occupancy, or suitability, and the area under the curves sums to 1, the total
197   suitability.

198   **Table 1.** *Niche identity test.* The upper off-diagonal shows Schoener's D statistic, and the lower
199   off-diagonals shows the modified Hellinger statistic, I. Significant niche divergence is reported in
200   bold text with red highlighting. The more dissimilar of the other two niche comparisons is
201   highlighted in pink. Abbreviations used for *R. flavipes*, *R. malletei*, and *R. virginicus* are Rf, Rm, and
202   Rv, respectively.

	Rf	Rm	Rv
Rf	-	D = 0.744 P = 0.280	**D = 0.582** **P < 0.001**
Rm	I = 0.935 P = 0.239	-	D = 0.788 P = 0.630
Rv	**I = 0.843** **P < 0.001**	I = 0.961 P = 0.750	-

203
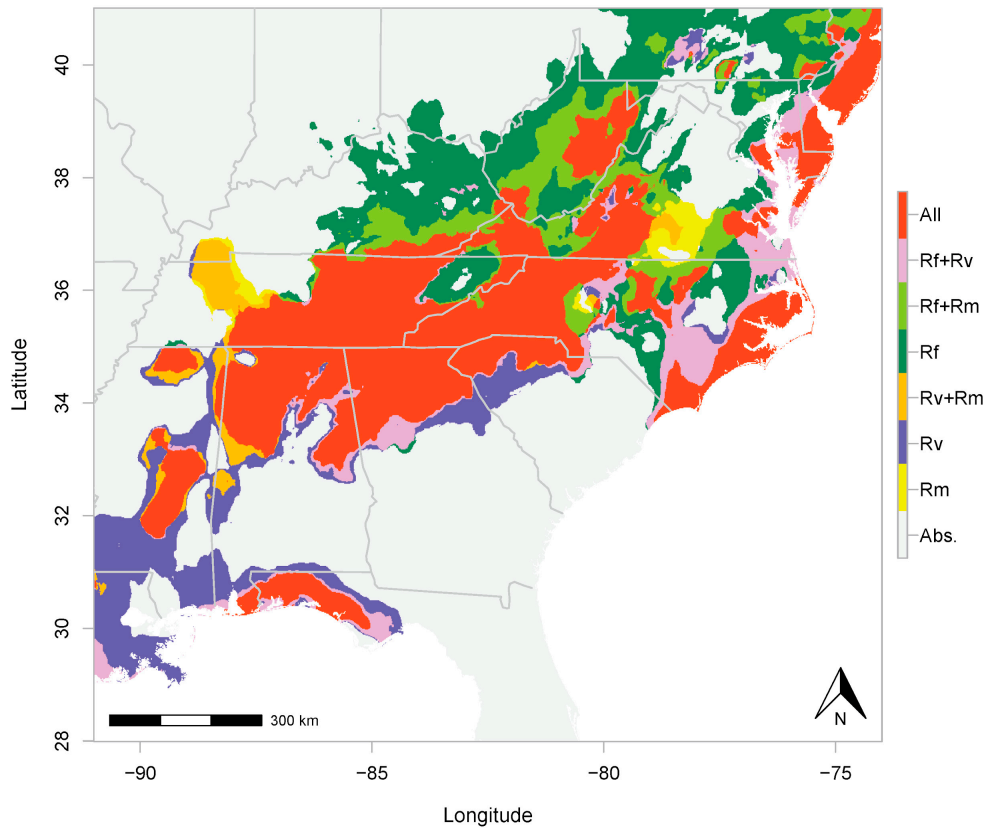
204 **Figure 2.** *Distributional overlap of* R. flavipes *(Rf),* R. malletei *(Rm), and* R. virginicus *(Rv).*   Overlap is
205 color coded based on the number of species. "All" is where occurrence of all three species is
206 predicted. Areas of two-species overlap are shown in the legend as "Rf+Rv," "Rf+Rm," and
207 "Rv+Rm." Absence of all three species is shown in grey and referred to in the legend as "Abs.".

208 *3.2. Environmental factors and niche divergence*

209 Distance-based redundancy analysis (Figure 3) showed that only the dry-season precipitation
210 factor contributed significantly ($F_{1, 127}$ = 8.673, P = 0.001) to differences in occurrence among the three
211 *Reticulitermes* species. After accounting for spatial structure by partialling out six significant spatial
212 components (PCNM axes 1, 4, 6, 17, 43, and 58), dry-season precipitation remained significant ($F_{1, 121}$
213 = 5.622, P = 0.003). The six significant spatial components along with dry-season precipitation
214 accounted for 18.5% of the observed variation in the occurrence data. Spatial structure alone
215 explained 9.6% of the variation, environmental factors accounted for 3.3%, and the interaction
216 between the two explained an additional 5.6% of the variation.

217 Following the removal of spatial structure effects, the highest correlation coefficient between
218 environmental factors and ordination axes of the distance-based redundancy analysis was recorded
219 for dry-season precipitation (r = 0.730) and axis 1. This axis captured the divergence of *R. virginicus*
220 from the other two species (Figure 3). Thus, dry-season precipitation contributed significantly to *R.*
221 *virginicus* divergence. While not significant, temperature range (r = -0.383) and wet-quarter
222 precipitation (r = 0.376) were correlated with axis 2, which captured the divergence of *R. malletei*
223 (Figure 3).

224

225 **Table 2.** *Pairwise niche overlap among* Reticulitermes *species for each of four environmental factors.* The top
226 three rows show Schoener's D statistic, and the bottom three rows show the modified Hellinger
227 statistic, I. The four environmental factors are: temperature range (TR), dry-season precipitation
228 (DP), summer temperature (ST), and wet-season precipitation (WP). Niche overlap is highest in
229 green and lowest in red. *R. flavipes*, *R. malletei*, and *R. virginicus* are abbreviated as Rf, Rm, and Rv,
230 respectively.

231

232

		TR	DP	ST	WP
D	Rf/Rm	0.889	0.872	0.693	0.820
	Rf/Rv	0.683	0.707	0.680	0.680
	Rm/Rv	0.791	0.809	0.894	0.848
I	Rf/Rm	0.991	0.990	0.919	0.982
	Rf/Rv	0.917	0.928	0.926	0.942
	Rm/Rv	0.952	0.961	0.990	0.984

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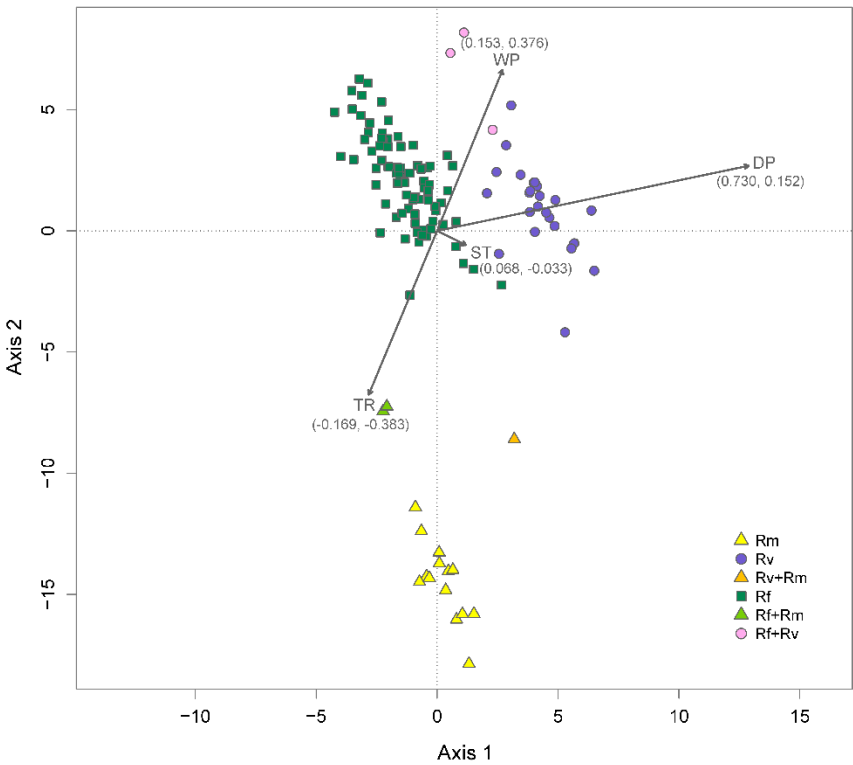
Figure 3. *Distance-based redundancy analysis.* The plot shows a constrained ordination of 132 sampling
sites, color coded based on the number of species present. Sites where only *R. flavipes, R. virginicus,* or
R. malletei were sampled are referred to in the legend as "Rf," "Rv," and "Rm," respectively.
Two-species sites are shown in the legend as "Rf+Rv," "Rf+Rm," and "Rv+Rm." The ordination is
conditional on six significant spatial components (PCNM axes 1, 4, 6, 17, 43, and 58) and constrained
by four environmental factors: dry-season precipitation (DP); wet-season precipitation (WP);
summer temperature (ST); temperature range (TR). Arrows show strength of correlation (coefficients
in parentheses) of environmental factors with ordination axes 1 and 2.

244

4. Discussion

This study provides insights into the ecology of subterranean termites with regard to geographic distributions and niche partitioning among three broadly co-distributed *Reticulitermes* species in the southern Appalachians Mountains and surrounding areas. This region is a biogeographically significant center of endemism, yet the ecology of its resident invertebrate fauna—particularly saproxylic insects—is poorly known. Our ENMs suggest that an area in the mid-latitudes of the southern Appalachians, characterized by complex topography and an array of six ecoregions, provides suitable habitat to support all three *Reticulitermes* species. Our study also highlights the roles that temperature and precipitation play in driving niche divergence among *Reticulitermes* species. To our knowledge, this work represents the first evidence of significant regional-scale niche divergence between *R. flavipes* and *R. virginicus*. Below, we consider the broader context of these findings, as well as caveats and future directions for follow-up studies that build on the information presented here.

4.1. Reticulitermes *distributions and climatic drivers of niche divergence among species*

Our analyses predicted extensive co-occurrence of all three *Reticulitermes* species in the mid-latitudes of the southern Appalachians (Figure 2; Figures S4 and S5). Based on paleoclimatic [59], biogeographic [60] and comparative phylogeographic [61] data, the southern Appalachians remained free from Pleistocene ice sheets and served as a major refuge for many species during glacial periods, consequently maintaining higher levels of biodiversity. Indeed, the present-day complexity of this mid-latitude region harbors many different niches, which could facilitate long-term coexistence of closely related species. However, in addition to predicted co-occurrence of *Reticulitermes* species in the montane regions of the southern Appalachians, our ENMs also identified areas of two- and three-species co-occurrence along the Gulf coast of western Florida, and the Atlantic coast from North Carolina to New Jersey and New York. To empirically confirm the co-occurrence of subterranean termites in these coastal areas, future studies should include these regions in their sampling efforts. In the case of another forest-dependent invertebrate, the millipede *Narceus americanus*, the Florida Gulf coast has been identified as an important refuge during the Last Glacial Maximum [62]. Indeed, the paleoclimatic history of areas to the south and east of the southern Appalachian Mountains are increasingly being recognized as reservoirs of forest invertebrate biodiversity during past periods of environment change. The incidence of high termite species diversity—even though only assessed here for one genus—is therefore not unexpected.

In addition to co-occurrence of *Reticulitermes* species, our study provides novel insights into climatic drivers of niche divergence. Consistent with the findings of Maynard et al. [42], we determined that *R. virginicus* is more restricted to the south, whereas *R. flavipes* has a broad latitudinal range. Furthermore, we determined that *R. flavipes* occurs further north than the other two species, even excluding other *Reticulitermes* (Figure S5), potentially because it tolerates lower amounts of precipitation (both dry- and wet-season; Figure 1). . Maynard et al.'s [42] ENMs showed that temperature variables were the most important predictors of termite distributions. Based on our formal assessment of niche overlap between *R. flavipes* and *R. virginicus*, we determined that both temperature and precipitation seasonality (as represented by temperature range, summer temperature, and dry- and wet-season precipitation) play non-negligible roles in the significant niche divergence between *R. flavipes* and *R. virginicus.* Furthermore, using distance-based redundancy analysis, we identified dry-season precipitation as a major driver of this divergence. In the mid-latitudes of the southern Appalachians, where dry-season precipitation is high (Figure S3), all three *Reticulitermes* species co-occur (Figure 2; Figures S4 and S5), but further north, where dry- and wet-season precipitation is low (Figure S3), *R. flavipes* is more competitive.

4.2. Potential explanations for lack of empirical evidence for local-scale coexistence of Reticulitermes *species*

Interestingly, despite the significant niche divergence between *R. flavipes* and *R. virginicus*, we collected both of these species from the same rotting log at one sampling site (i.e., #37 located near

294 the Georgia/Southern Carolina state border; Table S1). To our knowledge, this is the first record of
295 true syntopy between *Reticulitermes* species. The apparent rarity of syntopy and general lack of
296 coexistence of *Reticulitermes* species at local scales could be explained by competitive exclusion.
297 Colony size and soldier number are important features for termite competitive ability. Termite
298 species with small colonies have been observed to relinquish resources and be eliminated by
299 dominant interspecific competitors with large colonies [63]. Through avoidance of dominant
300 competitors, interspecific competition may result in spatial separation [64], but also temporal
301 separation (i.e., phenological differences). Termites may be able to avoid other related species using
302 vibrational cues. Indeed, vibrational cues are important for termite sensory perception and
303 communication, as these signals can travel over long distances [65,66]. For instance, the drywood
304 termite *Cryptotermes secundus* can distinguish conspecifics from the dominant competitor in the
305 environment, the subterranean termite *Coptotermes acinaciformis* [65]. Furthermore, *Coptotermes
306 acinaciformis* detects its major predator, the ant *Iridomyrmex purpureus*, using vibrational cues only
307 [66]. Overall, given these highly tuned sensory capabilities, it stands to reason that competitive
308 exclusion, or competitor avoidance, could be important factors in preventing local co-occurrence
309 among *Reticulitermes* species. Alternatively, the dominant competitor may ultimately outcompete
310 the other species. For instance, *R. flavipes* has a broad distribution and occurs further north than the
311 other two species, possibly due to a competitive advantage stemming from the fact that it tolerates
312 conditions of lower dry- and wet-season precipitation. Furthermore, interspecific aggression
313 coupled with low levels of intraspecific agonism (even colony fusion) [67,68], may make *R. flavipes*
314 the dominant competitor.

315 *4.3. Caveats and future directions*

316   While our sampling suggests that true syntopy and local co-occurrence of different species at
317 the same site is very rare, our detection of only one species in all but one rotting log, and at the
318 majority of sampling sites (i.e., 126 out of 132), may actually be a consequence of the sampling
319 strategy that was employed (see Methods). Briefly, we simply aimed to collect termites from each
320 site, rather than provide a complete assessment of termite diversity at each site. Indeed, variance
321 partitioning reflects this, showing that most (81.5%) of the variance in the occurrence data did not
322 stem from spatial structure (9.6%), or environmental differences (3.3%), or interaction between the
323 two (5.6%). Accordingly, while competitive exclusion is a plausible explanation for apparent rare
324 local-scale co-occurrence (i.e., micro-allopatry) among *Reticulitermes* species, a dedicated sampling
325 approach would be required to formally test this idea. For example, exhaustively sampling multiple
326 logs per site, at a series of sites arranged along a transect traversing a region where two or more
327 species occur in close proximity would be a productive approach. Fortunately, the present study
328 identified specific geographic areas where future assessments of the frequency of true syntopy vs.
329 micro-allopatry, and associated interspecific competitive interactions, should be focused (Table S1;
330 Figure S1).
331   Although we have shown separation in niche space between species, particularly *R. flavipes* and
332 *R. virginicus*, these inferences were underpinned by regional-scale environmental variables, and so
333 they do not take into account local-scale drivers of niche divergence such as differences in
334 microhabitat preference, phenology, or diet. Indeed, Maynard et al. [42] highlighted that biotic and
335 soil characteristics play a role in termite distribution and abundance. Thus, our assessment of niche
336 divergence is necessarily incomplete. While it does provide important baseline information,
337 follow-up studies of local-scale drivers of species' distributions could examine aspects of the
338 microhabitat (e.g., humidity and temperature of soil and rotting logs), timing of nuptial flights along
339 latitudinal and altitudinal clines, and/or use stable isotopes to determine decomposition stage of
340 ingested wood and the importance of microbial biomass in termite diets at a given location [69].

341 **Supplementary Materials:** The following are available online, Supplementary Methods: Environmental
342 variables and Ecological Niche Modeling methods; Table S1: Sampling sites with number of species occurrences
343 at each site and number of logs per site; Figure S1: Map of Reticulitermes sampling depicting occurrences of one
344 or more species at each site; Figure S2: Factor analysis; Figure S3: Environmental factors and bioclimatic

345   variables; Figure S4: Distributional overlap of *Reticulitermes* species; Figure S5: Probability of joint and exclusive
346   occurrence of *Reticulitermes* species.

359   **References**

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