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Laurence Berville, Eric Darrouzet*

IRBI, UMR CNRS 7261, University of Tours, Parc de Grandmont, 37200 Tours, France

*Corresponding author: eric.darrouzet@univ-tours.fr

Abstract:

Collective constructions are marvels of complexity, composed of networks of tunnels and chambers. However, it is difficult to study subterranean nests without using invasive techniques because the nests are built within pieces of wood and/or in the soil. Using computerized tomography scans and medical imaging software (OsiriX), we were able to observe nest creation, constructions, and architecture of two subterranean termite species. We monitor the nests' growth in three dimensions built by two *Reticulitermes* species: *R. grassei*, a species native to Europe, and *R. flavipes*, an invasive species introduced from North America, over a several-month period. Doing so, we wanted to know whether the construction of the nest could participate to the invasive success of *R. flavipes*. Although the two species displayed some similarities (*i.e.*, nest creation, chamber size, and levels of wood consumption), only *R. flavipes* built interior structures. Some of these structures changed over time and thus might play a role in the trade-off between wood consumption, colony protection, and environmental homeostasis.

Significance Statement

Nest architectures and their management is one of the triumphs of social animals such as social insects. The functional analysis of those structures composed of networks of tunnels and chambers could help to understand the biology and ecology of their builders. Excavation, consumption and nest design are important parameters in explaining a number of interesting biological traits such as the establishment of invasive species. Some recent studies have introduced X-ray tomography as a tool to analyze insect architectures. We employed this non-invasive technique to observe nest creations, construction and evolution in time, and resolve three-dimensional nest architecture. We analyzed a native and an invasive subterranean termite species. Interestingly, only the invasive species built specific interior structures which change over time. Our study leads to a hypothesis of optimal chamber number, which balances colony wood consumption against nest functionality. The constructions could play a role for environmental homeostasis that seems not necessary for the native species.

Keywords: animal architecture, tomography, *Reticulitermes flavipes*, *R. grassei*, self-organized structures, social insects

36 Introduction

37 Animals can create controlled microhabitats using self-made structures. The most impressive are the nests of
38 social insects (i.e., ants, bees, wasps, and termites). Some termite mounds rise higher than 10 meters, which is
39 more than 1,000 times the height of a worker. Even more intriguing is that such nests are produced without
40 individual workers using any sort of collective “blueprint” (Buhl et al. 2005; Deneubourg and Franks, 1995).
41 Social insects have achieved remarkable ecological success and dominance (Hölldobler and Wilson, 1990),
42 perhaps due in part to their nests, which provide benefits in terms of defense, shelter (Traniello and Leuthold,
43 2000), controlled microclimatic conditions (e.g., air composition [ants: Bollazzi et al. 2012], temperature and
44 humidity [termites: Ruelle 1964]), structure, and, in some cases, food. Indeed, certain subterranean termite
45 species also consume the wood in which they live (Grassé 1984). These species serve as excellent models for
46 studying nest development as they must trade-off between food consumption (wood) and environmental
47 homeostasis. Part of this process is controlling the number and size of both tunnels and chambers. Thus, nest
48 architecture may play an important role in regulating colony activity.

49 However, subterranean lifestyle makes it difficult to characterize nest structure. Previous studies, **which**
50 **analyzed nest architectures in several insect species**, have largely employed destructive methods such as
51 fragmentation (Bouillon 1964), step-by-step excavation (King and Spink 1969; Rabeling et al. 2007; Tschinkel
52 1999), the tracing of radioisotope-labeled foragers (Spragg and Paton 1980), or casts (i.e., latex [Garner 1953],
53 cement [Bollazzi et al. 2012; Ruelle 1964], orthodontic plaster, wax, aluminum, zinc, or lead [Tschinkel 2010,
54 2011]). However, these approaches can only yield a temporal and/or spatial snapshot of the nest. They cannot
55 provide information about structural dynamics, such as the growth of tunnel networks, nor can they be used to
56 follow nest creation, wood consumption, and/or building activity over time. More recent studies, though, have
57 begun utilizing computerized tomography (CT) scans to non-invasively visualize and characterize the
58 architecture of social insect nests (ants [Halley et al. 2005; Khuong et al. 2016; Minter et al. 2012; **Monaenkova**
59 **et al. 2015**]; bees [Greco et al. 2006]; termites [Darrouzet 2013; Eom et al. 2015; Fuchs et al. 2004; Himmi et al.
60 2014, 2016; Perna et al. 2008a,b]; wasps [Darrouzet 2013]; and hornets [Darrouzet 2013; Rome et al. 2015]).
61 Using CT scans, it is possible to reconstruct a 3D structure via a series of 2D images; the structure’s inhabitants
62 are unharmed, even after several scans. Thanks to the images’ high-contrast resolution, structural differences in
63 physical density can be distinguished (see [Sup. file Video 1](#)). The ability of structures to block the X-ray beam
64 can also be characterized. Nevertheless, despite their potential, longitudinal studies using CT scans have only
65 been carried in ants (*Linepithema humile*: 7 or 8 scans over 6 months [Halley et al. 2005] and *Lasius flavus*: 8
66 scans over 48 h [Minter et al. 2012]). Their use may be tempered by the tool’s main limitation: scanner size. It is
67 impossible to analyze large structures.

68 **An important requirement for the management of invasive species is to identify biological and**
69 **ecological factors that influence their ability to establish and spread within a new environment, and how they**
70 **present a significant advantage against native species. For example, *Reticulitermes flavipes* and *R. grassei* are**
71 **subterranean termite species which live in sympatry along the French Atlantic coast, where their colonies exhibit**
72 **important differences in their social organization, behavior, and breeding system (Perdereau et al. 2010, 2011,**
73 **2015). *R. grassei* is native to southwestern Europe (France and Spain). *R. flavipes*, in contrast, is an invasive**
74 **species that is native to the eastern United States but that has spread to South America (Aber and Fontes 1993;**
75 **Su et al. 2006) and Europe (Dronnet et al. 2006; see Evans 2013 for a review). Both species belong to the family**
76 **Rhinotermitidae and, as such, share certain traits. Some studies demonstrated that *R. flavipes* has some**
77 **advantages against *R. grassei*. For example, interspecific competition showed that *R. flavipes* was dominant over**
78 ***R. grassei*, and this invasive species foraged over a greater distance than *R. grassei* colonies (Perdereau et al,**

79 2011). Consequently, this competitive asymmetry may enable *R. flavipes* to become dominant in the
80 environment. Interestingly, the main difference between the two species concerns their capacity to produce
81 secondary neotenic reproductives. Genetic studies revealed that all French *R. flavipes* colonies produce more
82 neotenic than *R. grassei* colonies (Leniaud et al, 2011). In such conditions, its interspecific superiority, its lack
83 of intraspecific aggression, the production of numerous neotenic and large extensive colonies, seem to be some
84 of the reasons for *R. flavipes* invasive success. Among these factors, nest-building dynamics, nest shapes and
85 sizes, wood consumption dynamics are largely unknown for both species. However, information on nest
86 dynamics is essential to understand the wood consumption dynamic and to assess the efficacy of the strategies
87 used to control invasive termite infestations (Forschler 1994). For example, they both eat and live in non-
88 decomposed wood. They are initially single-piece nesters, but they eventually move to a new piece of wood
89 when their original nests have been completely consumed (Evans 2013). Both produce large subterranean
90 colonies and are therefore ideal species for studying wood consumption.

91 In this study, we sought to discover if both *Reticulitermes* species build interior structures and thereby
92 to discover the relationship between these constructions and the volume and dynamic of wood consumed. Doing
93 so, we wanted to know whether the construction of the nest could participate to the invasive success of *R.*
94 *flavipes*. As such species seems to produce larger colonies and numerous neotenic, we supposed that (1) wood
95 consumption would be greater in the invasive species (to feed numerous individuals and more particularly
96 neotenic), (2) *R. flavipes* workers would have greater capacities to build the nest, and consequently (3) nest
97 structures from both species could present some differences in size and shape. Moreover, our study leads to a
98 hypothesis of optimal chamber number, which balances colony wood consumption against nest functionality.
99 We hypothesis that a cumulative Weibull distribution or a sigmoid function will appropriately models the
100 biological process at work.

101 To test these hypotheses, we used CT scans to characterize nest creation, construction, and architecture
102 as well as wood consumption over a ten-month period in experimental fragment colonies of both species in the
103 same experimental conditions. More specifically, we exploited CT scans to investigate (1) nest construction
104 dynamics, (2) wood consumption, and (3) intercolonial variation in both.

105

106 **Materials and Methods**

107 *Colony rearing conditions*

108 *Reticulitermes* colonies were collected from wood fragments and pine tree stumps in the St. Trojan
109 forest on Oléron Island (Charente-Maritime, France). They were identified to species using cuticular
110 hydrocarbon profiles (Bagnères et al. 1990, 1991). For the duration of the experiment, colonies were kept at
111 26 °C in full darkness. We then filled plastic boxes (18 x 12 x 7 cm) with moist Fontainebleau sand (very fine,
112 homogeneous granularity); sand depth was 2 mm. We added a block of untreated pine (12 x 4 x 4 cm) as a food
113 source. The sand was moistened every day. Four fragment colonies of *R. grassei* and six fragment colonies of *R.*
114 *flavipes* were introduced into separate boxes; each contained 900 workers and was taken from a different field-
115 collected colony.

116

117 *Computerized X-ray tomography scan analysis*

118 To monitor wood consumption, each plastic box was subject to CT scans (120 keV, 180 mAs) using a
119 Phillips Brilliance CT 40 medical scanner, made available to us by the Neuroradiology Department at the Tours
120 Hospital (Tours, France). We obtained a series of virtual vertical “slices” through the box (thickness of 0.67 mm,
121 increments of 0.33 mm) (Fig. 1a and [Sup file 1](#)). The boxes were scanned before the termites were introduced

122 (T0) and then at regular intervals post introduction (Sup file 1): at 13 days (T1), 20 days (T2), 51 days (T3), 65
123 days (T4), 100 days (T5), 202 days (T6, Sup file video 1), and 258 days (T7).

124 Then, we analyzed each series of CT scans (DICOM standard; Fig. 1a, Sup file video 1) using OsiriX v.
125 4.19 image processing software (Darrouzet, 2013; Rosset et al. 2004), which is designed to allow 3D volumes to
126 be reconstructed from large sets of multidimensional and multimodality images (Fig. 1b,c and Sup file video 2).
127 Areas of wood consumption, which we called “regions of interest” (ROIs), were identified manually on each
128 image and measured (Fig. 1a). We thus obtained precise information about ROI size, shape, and boundaries. We
129 were also able to observe nest architecture over time, namely changes in tunnels (interior and exterior), chamber
130 number, wood consumption volume, and interior structures. One of the *R. grassei* colonies died between T5 and
131 T6, but we used the data available from T0 to T5.

132

133 *Statistics*

134 Our statistical analyses were performed using SigmaStat (v. 10.0.054). In all cases, data normality was
135 first assessed using Kolmogorov-Smirnov tests. In the results, means are presented with standard errors. To
136 compare the locations where the two species initially attacked the wood pieces, t-tests (normally distributed data)
137 and Mann-Whitney rank sum tests (non-normally distributed data) were used. To assess differences in chamber
138 volume and number across time for the two species, two-way repeated measures ANOVAs was used; *a*
139 *posteriori* pairwise comparisons were performed using Holm-Sidak tests.

140 To test if the data is suitable for a regression analysis we used a Kolmogorov-Smirnov test for a
141 normally distributed population and a constant variance test by computing the Spearman rank correlation
142 between the absolute values of the residuals and the observed value of the dependent variable. To gauge the
143 ability of the regression equation to predict the dependent variable, an ANOVA (F test) was performed. To
144 provide a measure of how well the number of chamber is dependent of the volume consumed, the coefficient of
145 determination (r^2) was used.

146

147 **Results**

148 *Nest creation*

149 During the nest-creation period (T1 and T2), there was no clear pattern in the way the termites attacked
150 the wood (Mann-Whitney U Statistic, $P=0.33$): they targeted both the bottom face of the block (*R. grassei*, $n=50$;
151 *R. flavipes*, $n=34$) and the exposed faces of the block (*R. grassei*, $n=50$; *R. flavipes*, $n=14$). The first exterior
152 tunnels, made of sand, wood, and fecal pellets, were observed after 51 days; these afforded protection to the
153 termites. From that point on, it was rare to observe an attack that was not associated with a sand tunnel (*R.*
154 *grassei*, $n=9$; *R. flavipes*, $n=6$), as most of the new chambers were initiated from inside the tunnels (*R. grassei*,
155 $n=215$; *R. flavipes*, $n=684$), or via the extension of other chambers (*R. grassei*, $n=188$; *R. flavipes*, $n=253$). The
156 two species did not differ in their overall approach (T3 and T7; $t= -0.994$; $P=0.359$) (Fig. 2).

157

158 *Wood consumption*

159 As they ate through the wood, the termites generated living space for themselves. We identified 1,829
160 ROIs across the 10 study colonies; their mean size was 0.43 cm^3 (range: $0.0004 - 10.25 \text{ cm}^3$). Most ROIs were
161 small ($50\% < 0.111 \text{ cm}^3$ and $90\% < 1.196 \text{ cm}^3$) (Fig. 3).

162 At each time point, the two termite species had equivalent chamber numbers (two-way repeated
163 measures ANOVA: $F=1.287$, $df=1$, $P=0.289$) after accounting for differences in the factor times ($F=0.648$, $df=7$,
164 $P=0.714$). Both species appeared to go through three phases of wood consumption (Fig. 4; $F=37,945$, $df=7$,

165 **P<0.001**): there were no differences in chamber number at T0, T1, and T2 (phase one), at T3 and T4 (phase two),
 166 and at T5, T6, and T7 (phase three). During the first phase, a small number of new chambers were made. During
 167 the second phase, chamber creation accelerated. During the third phase, chamber creation declined (Fig. 4). By
 168 the end of the experiment, both species had generated almost the same number of chambers. *R. flavipes* nests
 169 contained between 33 and 64 chambers, while *R. grassei* nests contained between 24 and 53 chambers (Fig. 4).

170 There also did not appear to be interspecific differences in wood consumption over time (T0 to T7; two-
 171 way repeated measures ANOVA: **F= 0.774**, P=0.404). At the end of the experiment (*i.e.*, after 258 days), *R.*
 172 *flavipes* colonies had eaten 15.10 – 43.66 cm³ of wood, corresponding to a mean 15.51% ± 2.068 of **total wood**
 173 **block volume** (Fig. 4). *R. grassei* had eaten 21.19 – 32.26 cm³ of wood, corresponding to a mean 14.2% ± 1.83
 174 of total wood block volume (Fig. 4). While the volume of wood consumed was equivalent between species at
 175 each time point, the mean consumption across time for species was different (two-way repeated measures
 176 ANOVA: **F=73,347**, P<0.001). Within species, all pairwise comparisons between time points were statistically
 177 significant, except during the first stage (T0 vs. T1, T0 vs. T2, and T1 vs. T2) and for T3 versus T4 (Fig. 4).
 178 Chamber number was related to the percentage of wood volume consumed in both species (*R. flavipes*: r²=0.979,
 179 F=65.07, P=0.0008; *R. grassei*: r²=0.984, F=84.27, P=0.0005; Fig. 5). The best fit for the data was a four-
 180 parameter non-linear regression model with f(x) = y₀+a/(1+exp^{(-(x-x₀)/b)}). The equations were as follows:

$$R. grassei \quad y = -2.2 + \frac{39.80}{\left(1 + e^{\left(\frac{-(x-3.85)}{1.24}\right)}\right)}$$

$$R. flavipes \quad y = -0.22 + \frac{47.98}{\left(1 - e^{\left(\frac{-(x-3.85)}{1.72}\right)}\right)}$$

181

182

183

184 with x = percentage of wood volume consumed, y= chamber number, a = the minimum value that can be
 185 obtained (*i.e.* when x=0) and b = Hill's slope of the curve. An Anova was used accordingly, demonstrating that
 186 the volumes of wood consumed by both species contribute to the prediction of the number of chamber observed
 187 in the nests.

188

189

190 *Interior structures*

191 Only *R. flavipes* seemed to have the ability to generate and modify interior architectural structures such
 192 as pillars, ceilings/floors, tunnels, and inner/outer walls (Fig. 2). The timing was colony specific: the first
 193 example of such structures occurred after 20 days in one colony (Fig. 8), but after 258 days in another colony.
 194 By the end of the experiment, a mean of 14.5 ± 7 interior structures were present in *R. flavipes* nests. Although
 195 there was marked intercolonial variability, there was a strong correlation between the number of days that had
 196 passed and the number of such structures (r²=0.992, Anova F=747.39, P=0.0001; Fig. 8). There was a significant
 197 relationship between the number of interior structures and the number of chambers (r²=0.974; F=50.45;
 198 P=0.0012; Fig. 9). The best fit for the data was a four-parameter equation (Weibull distribution), where x=the
 199 number of constructions:

$$y = 47.27 \left(1 - e^{\left(\frac{-(x + 70.4)}{22.29}\right)^{10.03}}\right)$$

200

201

202 **Discussion**

203 This study used CT scans (X-ray tomography) to provide the first detailed quantitative descriptions of
204 *Reticulitermes* nests **dynamics**. We obtained new information about nest creation, the dynamics of wood
205 consumption, the creation of chambers and tunnel networks, and shifts in interior architecture. Because initial
206 conditions were standardized (i.e., wood block size and colony size), we were able to characterize and compare
207 these features over time.

208 More specifically, we collected data on two sympatric *Reticulitermes* species, one native and one
209 invasive, which has helped enhance our understanding of their natural histories. First, we saw no interspecific
210 differences in the volume of wood consumed or in the number of chambers created. Second, we observed three
211 phases of wood consumption. Over the first 25 days of the experiment (Phase 1), wood consumption was low.
212 Then, the first exterior tunnels appeared, wood consumption accelerated, and a large number of small chambers
213 were created (Phase 2). Finally, 200 days into the experiment, the speed of wood consumption remained
214 unchanged, but a slightly smaller number of chambers were generated. However, these chambers were either
215 larger or the product of fusion.

216 By eating wood and engaging in building activity over a several-month period, termites can create
217 networks of interconnected galleries and chambers in which they live and through which they can travel (Grassé
218 1984, also see [Sup file video 1 and 2](#)). Within the nest, termites are also protected from predators and climatic
219 extremes. Nonetheless, a major challenge remains: sufficient ventilation (Ohashi et al. 2012). Air quality within
220 the nest can be compromised by colony respiration, namely the production and release of CO₂ (Risch et al.
221 2012). In general, ventilation systems are passive, driven by temperature or velocity gradients in air currents
222 (Wenzel 1990). Indeed, termites are well known for their ability to regulate nest conditions, including
223 temperature and humidity (Lüscher 1955; Korb and Linsenmair 2000; Korb 2003; see also Turner 2001 for a
224 review). The construction of different interior structures could help enhance air flow and thus improve air
225 quality. To this end, nest configurations might need to remain dynamic. The small size of most of the chambers
226 observed in this study could be the product of a trade-off between wood consumption, colony defense, and
227 environmental homeostasis. As colonies grow, old chambers can be rearranged and new chambers can be
228 created: here, we observed that some chambers increased in size or fused.

229 One of our notable results is that, although both species displayed similar levels of wood consumption
230 and created exterior tunnels, only *R. flavipes* built interior structures. These included pillars, ceilings/floors, and
231 walls (Fig. 1b). Like *Cubitermes* species (Perna et al. 2008a), *R. flavipes* could adjust interior nest architecture
232 by adding and modifying the number of interior structures, depending on chamber number or wood
233 consumption. Ceilings and floors appeared in the largest chambers, and some old entrances were sealed off.
234 Since colonies were given just one piece of wood, termites were forced to eat where they lived. Consequently,
235 for *R. flavipes*, such structures may be part of the trade-off between wood consumption, colony defense, and
236 environmental homeostasis. In our experiment, however, exterior temperature and humidity were kept constant,
237 and the termites were enclosed in boxes, preventing any air movement. Although both species were collected
238 from the same area and thus experienced the same environmental conditions, it could be that *R. grassei*, the
239 native species, did not build interior structures because laboratory conditions were acceptable. Perhaps this was
240 not the case for *R. flavipes*, the invasive species, which may have needed to manage nest ventilation. Perna et al.
241 (2008a) showed that, in *Cubitermes* species, final nest topology reflects a compromise between efficiency and
242 defense. The same could be true in *Reticulitermes* species. Furthermore, it could be that *R. flavipes* utilizes
243 interior structures to help defend against predators in its native range within North America and that this

244 behavior was conserved within its introduced range in France. However, *R. flavipes*' potential natural predators
245 remain unidentified.

246 Another hypothesis for the interspecific difference in the use of interior structures could be linked to the
247 production of neotenics. More specifically, *R. flavipes* may produce more neotenics than *R. grassei*: Leniaud et
248 al. (2011) observed that, after one year, 100% of *R. flavipes* groups (composed of workers) had produced
249 neotenics, while just 63% of *R. grassei* groups had done the same. Compared to *R. grassei*, *R. flavipes* produced
250 significantly more offspring because female numbers and productivity were both higher. Here, consequently, our
251 *R. flavipes* colonies may have been more densely populated than our *R. grassei* colonies after just a few months.
252 Higher densities could have led to higher CO₂ concentrations and thus triggered the implementation of a
253 ventilation system mediated via interior structures. It is also conceivable that *R. flavipes* neotenics are more
254 sensitive to certain environmental conditions, requiring more intensive nest management. Unfortunately, because
255 we did not assess the characteristics of our colonies at the end of the study, additional research is needed to test
256 these hypotheses.

257 In our study, there was noticeable intercolonial variation in nest configuration (Sup File 1), even though
258 nests presumably share the same functional purpose and both colony numbers and wood consumption levels
259 were similar. There were no interspecific differences in chamber organization. Nest initiation was also similar.
260 Although the colonies attacked the wood block at different locations, all their entrance cavities were excavated in
261 the springwood and the network expanded along the wood's microstructure. This pattern is evident on CT scans
262 (Fig 1c) because the degree of X-ray absorption is correlated with wood density, which is greatest for the annual
263 growth rings (Himmi et al. 2016). We found that both termite species generally excavated tunnels and chambers
264 in softer wood (Sup file 2). This particular behavior is known to be affected by wood chemistry (Abe and
265 Higashi 1991) and nutritional value (Shellman-Reeve 1994). Only a few tunnels crossed harder wood to reach
266 softer areas, which allowed nest development.

267 Taken together, our results suggest that *R. grassei* and *R. flavipes* differ in their nest-building design.
268 This study confirms that both the environment and the termites themselves can influence the expression of
269 extended phenotypes, such as nest configuration. Although substrate quality (e.g., winter/summer wood) can
270 influence nest architecture making them nest specific, while, building behavior is species specific. This study
271 also underscores the utility of CT scans as a non-invasive technique for following wood consumption and nest
272 architecture in termites over time and the expression of extended phenotypes. Indeed, the types of data it yields
273 could be used in future research efforts to model nest construction dynamics.

274

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278 Hospital.

279

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386
387

388 **Legends:**

389 **Figure 1.** Two-dimensional DICOM scans of *Reticulitermes* nests. Locations in which wood had been consumed
390 (regions of interest: ROIs) were traced manually (dotted line below). Their areas were then calculated (white
391 shapes below).

392
393 **Figure 2.** On the left is a three-dimensional image showing the exterior tunnels performed by *R. flavipes*
394 termites. In the center and on the right are two-dimensional images of interior structures (red circle: cap; orange
395 oval: wall; yellow circle: pillar) made with sand. Softer wood (i.e., of lower density) is black or gray, while
396 harder wood (i.e., of higher density) is white.

397
398 **Figure 3.** Three-dimensional images of *R. grassei* nests, where sand-based and wood-based structures can be
399 distinguished (yellow vs. brown, respectively). Here, areas in which wood is present (i.e., based on density
400 values) have been artificially removed to allow the chambers (white) to be more easily observed.

401
402 **Figure 4.** Percentage of attacks at different locations on the wood blocks for the two termite species. The
403 percentages were calculated using all the observations from all the colonies. The “outer sides” were the exposed
404 faces of the block. The “underside” was the face in contact with the ground. “Interior” indicates chambers built
405 from an earlier chamber. “After tunneling” indicates attacks initiated from within a sand tunnel that targeted the
406 sides of the block.

407
408 **Figure 5.** Species-specific distributions of ROI volume

409
410 **Figure 6.** Boxplots of chamber number (light gray bars), volume of wood consumed (dark gray bars), and the
411 percentage of the total wood consumed (dark line) over time in nests built by *R. grassei* (upper graph) and *R.*
412 *flavipes* (lower graph). Within the boxes, the solid white lines are the means and the solid black lines are the
413 medians.

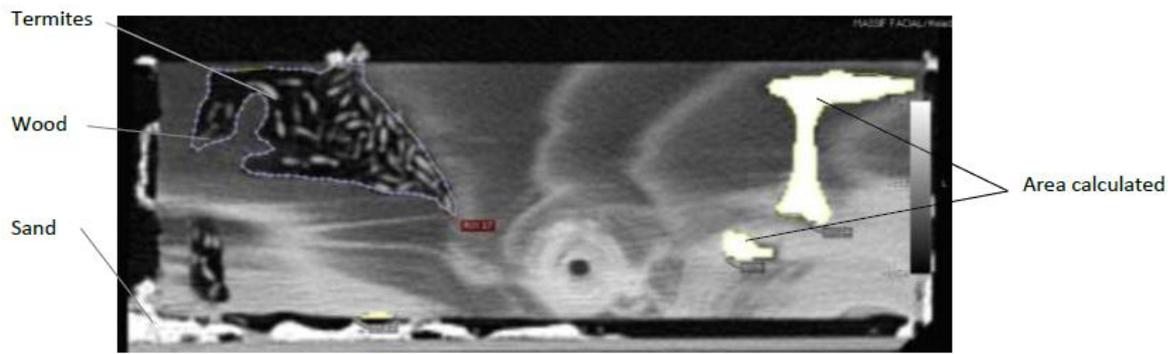
414
415 **Figure 7.** Relationship between chamber number and the percentage of wood consumed for *R. grassei* (gray)
416 and *R. flavipes* (black). The solid lines represent the best-fit regression lines, and the dotted lines indicate the
417 95% confidence intervals.

418
419 **Figure 8.** Number of interior structures built by *R. flavipes* over time (means \pm SE). The solid black line is the
420 best-fit regression line, and the dotted gray lines indicate the 95% confidence interval.

421
422 **Figure 9.** Relationship between chamber number and interior structure number for *R. flavipes* (means \pm SE). The
423 solid black line is the best-fit regression line, and the dotted gray lines indicate the 95% confidence interval.

424

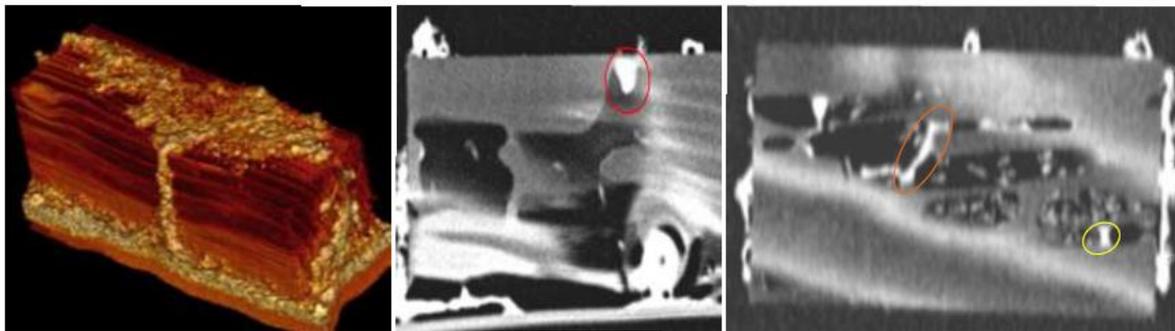
425 Figure 1



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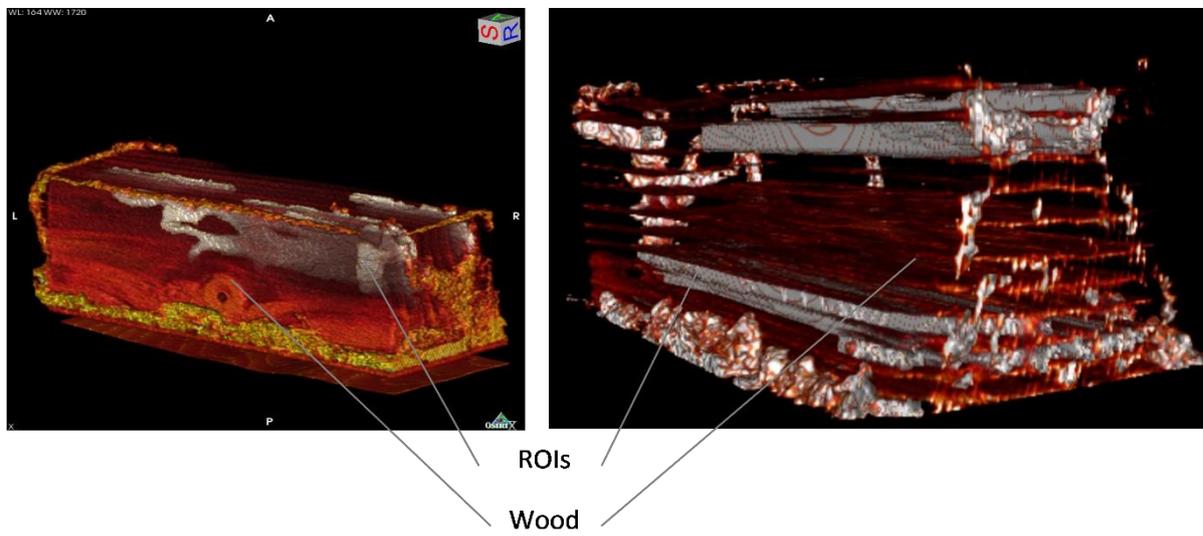
428 Figure 2



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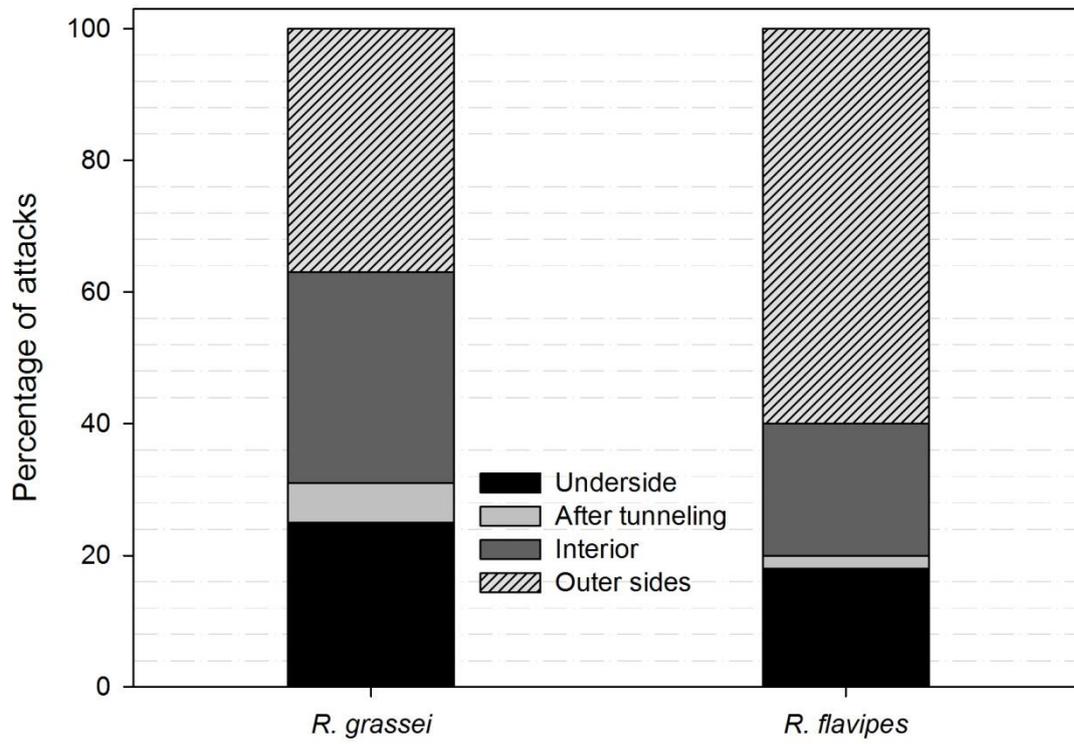
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431 Figure 3



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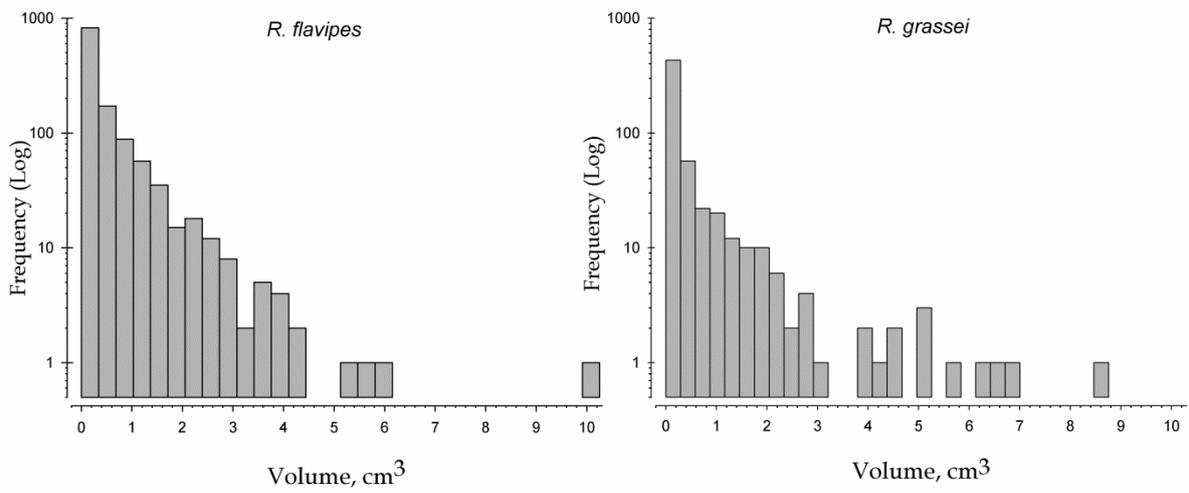


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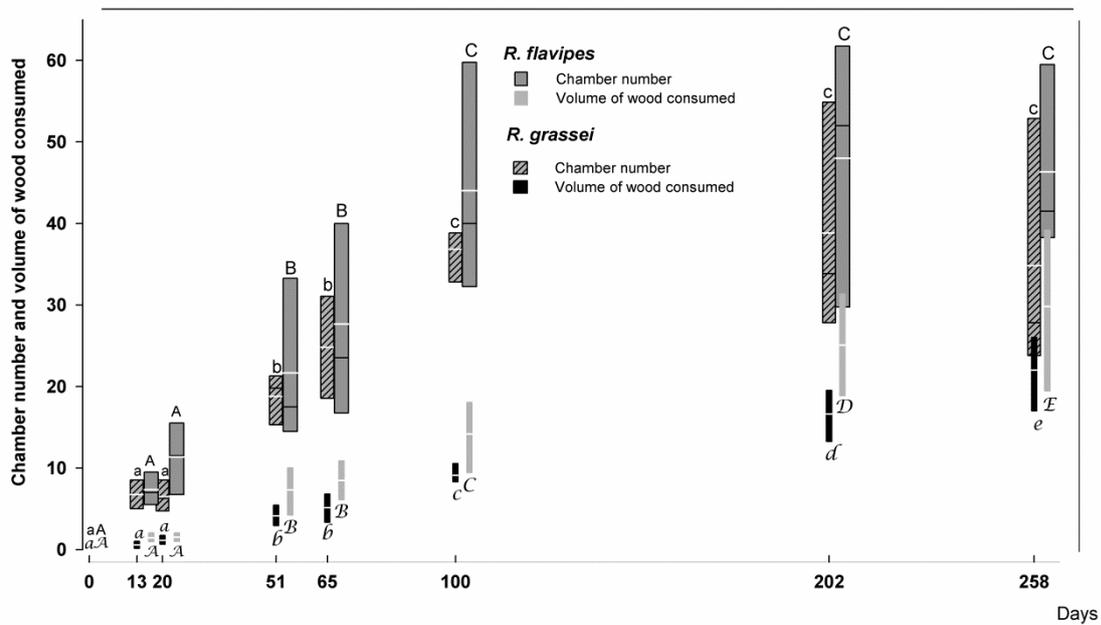
438 Figure 5



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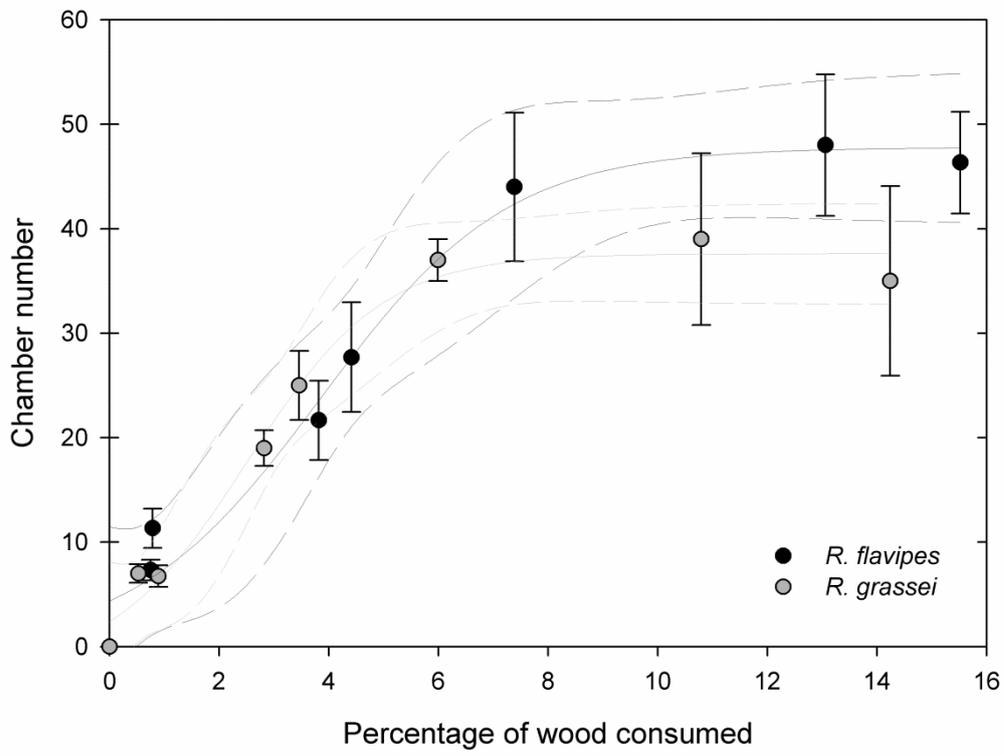
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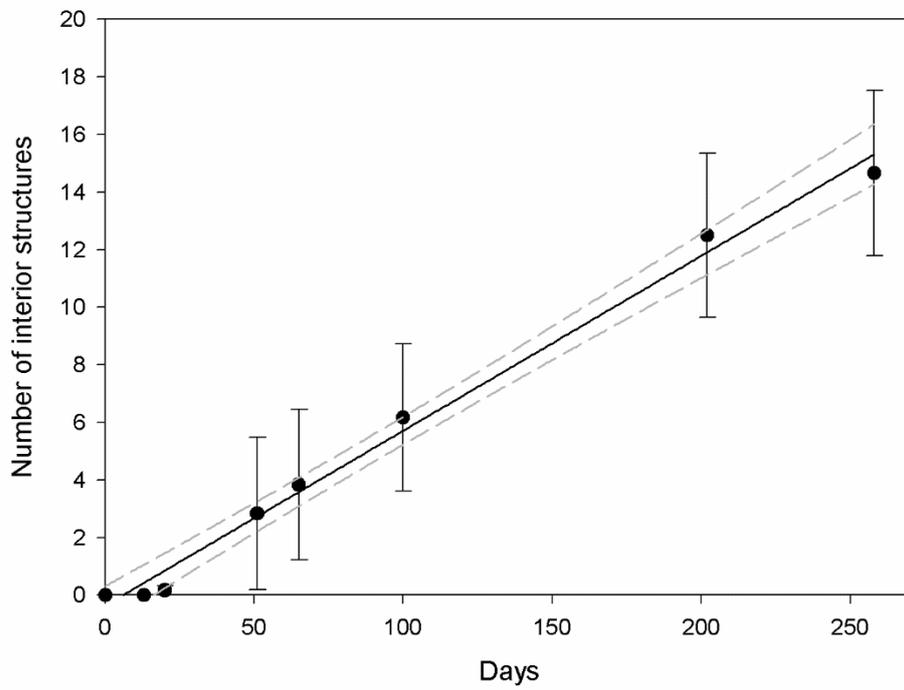
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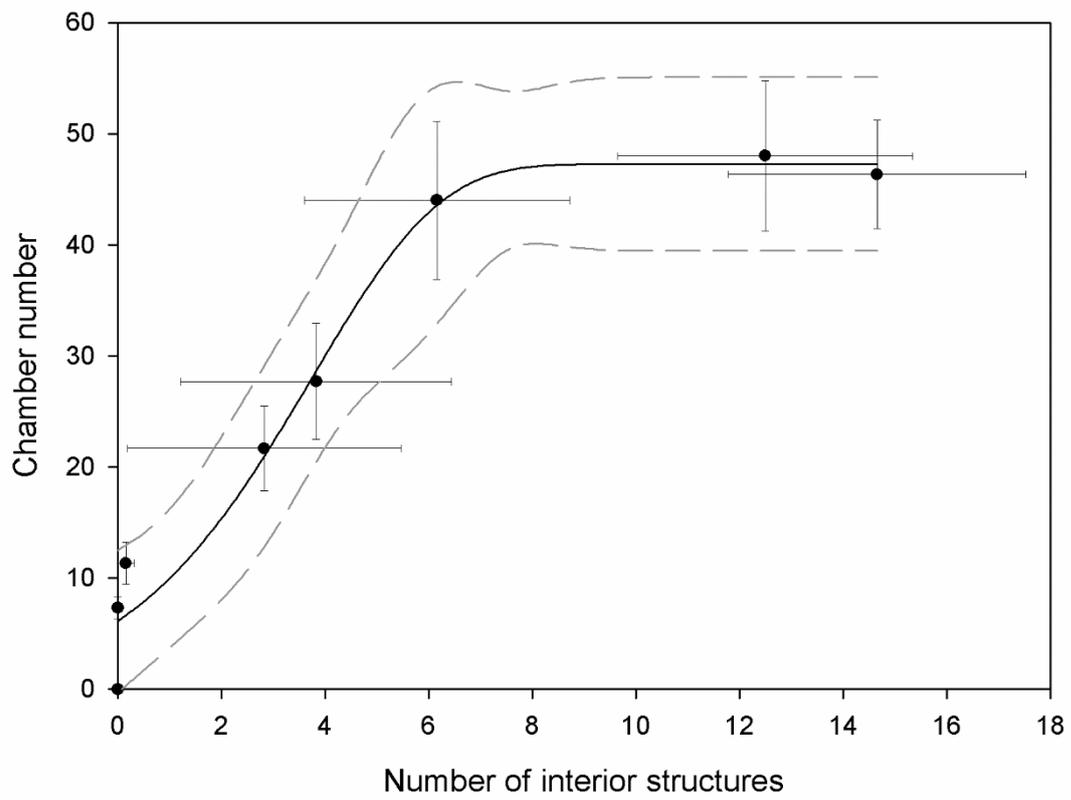


Figure 1

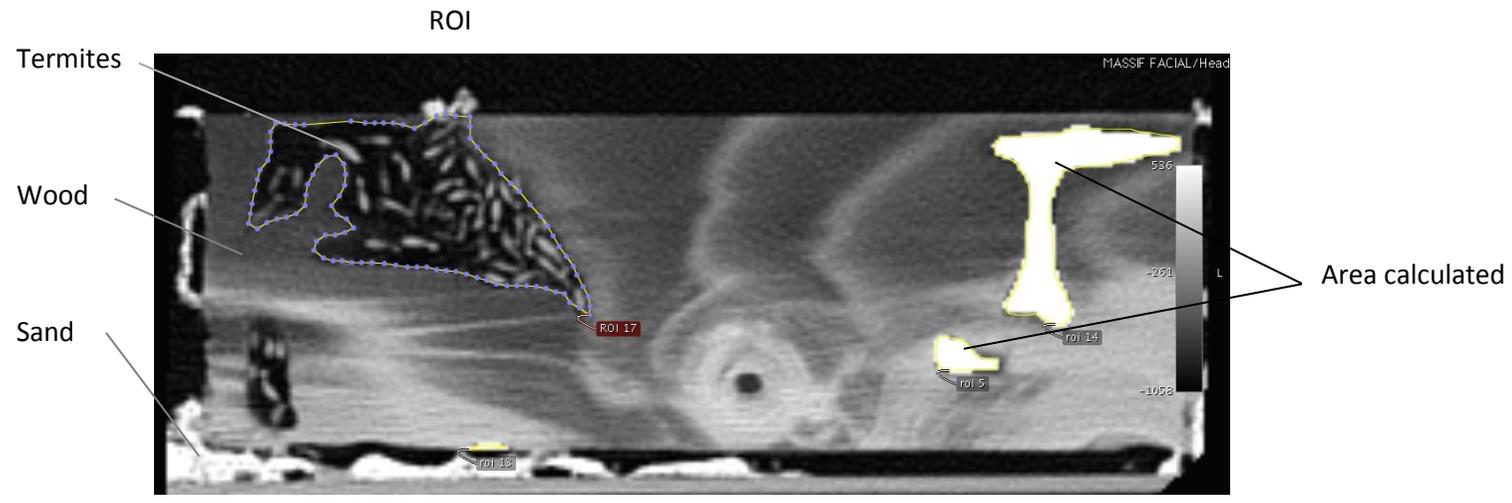


Figure 2.

