

Nutrition in extreme food specialists: an illustration using termites

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19 Abstract

20

21 1. Recent nutritional ecology theories predict that an organism feeding on a single,
22 highly predictable food should lack the typical active regulation of nutrient balance
23 observed in all other organisms studied so far. It could instead limit itself to
24 controlling the amount of food eaten alone. Such an animal would however be
25 strongly affected by nutrient imbalances.

26 2. Termites are an ideal model animal to test those predictions.

27 3. We investigated how the nutritional content of food affected termites' intake and
28 performance by constraining groups of *Nasutitermes exitiosus* to artificial diets
29 varying in their macronutrient ratios.

30 4. We showed that (1) termites, contrary to other insects, did not compensate for
31 nutrient imbalance by adjusting food collection (2) longevity in workers was strongly
32 influenced by carbohydrate intake, while in soldiers it depended almost entirely on
33 the number of workers remaining to feed them (3) tunnelling activity increased with
34 the quantity of food collected and (4) intake had very little influence on lipid and
35 protein termite body contents.

36 5. We provide evidence that extreme food specialists might have lost the ability to
37 regulate macronutrient intake.

38

39 *Keywords:* caste, longevity, macronutrients, nutrition, termites, tunnelling.

40 1. Introduction

41

42 All living organisms need a nutrient intake, which accurately matches their needs to
43 sustain their metabolism, growth, reproductive performance, and immune system. Hence a
44 large number of studies and approaches have been developed to understand how living
45 organisms might regulate their intake. The Geometric Framework (GF) is an approach which
46 has recently brought new insights in the field of nutrition and foraging ecology, and has also
47 been applied to a wide range of biological questions involving diverse taxa and
48 fields.(Simpson and Raubenheimer, 2012). The GF allows the relationship between any life
49 history trait and nutrition to be mapped, providing the basis for integrative models of
50 nutritional biology. It is a state-space modelling platform in which the food intake and the
51 nutritional state of individuals are represented in a nutritional space where each axis
52 represents key food components such as macronutrients. The GF provides a means to
53 quantify whether and how individuals regulate the amount and ratios of specific nutrients
54 they ingest to reach areas in the nutrient space where their fitness is maximised (the "intake
55 target"). When faced with imbalanced nutritional environments that prevent the intake target
56 to be reached, individuals must balance consuming excesses and deficits of different
57 nutrients, with associated fitness costs (the so-called "rules of compromise"). These rules of
58 compromise depend on the nutritional ecology of the organisms being studied. For example,
59 a specialist feeder which usually restricted to a narrow range of nutrient compositions will be
60 less willing to overeat from an imbalanced food than a generalist because the chance that it
61 will encounter a food with a complementary imbalance later on is relatively low
62 (Raubenheimer and Simpson 2002; Behmer, 2009; Simpson and Raubenheimer, 2012).

63

64 In recent years collective nutrition has emerged as a new field of research, within the
65 broader field of nutritional ecology (Raubenheimer et al., 2009). Numerous studies have

66 investigated how insect colonies maintain the balanced supply of nutrients at both a
67 collective and an individual level (review in Feldhaar, 2014). In an insect colony, only a small
68 proportion of the individuals collect the food for the entire colony. Hence the regulation of
69 nutrition is more complex than for a solitary individual, as the foragers do not possess a
70 global knowledge of the colony's nutritional status or food stores. Despite the challenge,
71 bees and ants offered a choice of foods varying in nutrient composition have been shown to
72 regulate their intake and collection of protein and carbohydrate to reach the intake target for
73 the colony ants (Cook et al., 2010; Dussutour and Simpson, 2009), honeybees (Altaye et al.,
74 2010), bumblebees (Vaudo et al., 2016a; Vaudo et al., 2016b). Notably, foragers can
75 respond to the varying nutritional needs of the colony by increasing their collection of protein
76 to meet the needs of developing larvae for example (ants: Dussutour & Simpson, 2009).

77 Termites differ in several important respects from the more intensively studied
78 hymenopteran social insects, and offer new opportunities to test predictions arising from
79 nutritional ecology and social foraging theory. According to nutritional ecology theory, the
80 extent to which species have evolved the capacity to regulate intake of multiple nutrients
81 depends upon three factors: 1) the degree to which specific nutrients are correlated in their
82 concentrations within foods; 2) the extent of heterogeneity among foods in nutritional
83 composition, and 3) the extent to which nutritional requirements change qualitatively (*i.e.* in
84 target ratio) over time (Raubenheimer et al., 2009; Simpson and Raubenheimer, 2012).
85 Nutrients for which specific appetite systems have evolved are expected to be those that are
86 not reliably positively correlated with one another within foods (such that **regulating the**
87 **intake of only one nutrient does not** ensure a balanced intake of the others), and which vary
88 in their ratio between different foods (such that different foods need to be mixed to attain a
89 balanced complement of nutrients). For example, where there are separate protein-rich and
90 carbohydrate-rich foods, an animal must mix its intake of the two food types to satisfy its
91 requirements for protein and carbohydrate, a process known as complementary feeding. To
92 balance nutrient intake through complementary feeding requires that the animal has a mean
93 of assessing the composition of different foods and of relating this to current requirements

94 for protein and carbohydrate (either its own needs, or the colony's). When faced with a
95 single suboptimal food, this animal will reduce its intake if the food contains nutrients that
96 can become harmful if ingested in high volumes, or in the opposite it will overeat foods with
97 low nutritional values to reach a minimum quantity of essential nutrients. If an organism
98 specialises on a single food of invariant composition, it needs only to evolve the capacity to
99 find, select and process that type of food and then simply to regulate the volume ingested to
100 attain a balanced intake of all required nutrients. Such a species would be predicted not to
101 have evolved specific appetites for different nutrients.

102 Termites offer a rare example which closely matches such a nutritional ecology.
103 Whereas Hymenoptera alternate between foods that vary in their macronutrient composition
104 to reach a balanced diet (e.g. prey vs honeydew in ants), termites feed mainly on a single
105 type of food, wood, which is relatively invariant in its macronutrient composition (Pettersen,
106 1984). We chose the Australian termite *Nasutitermes exitiosus* as our model species
107 because they are specialists of Eucalyptus trees. Like all trees, Eucalyptus are mostly
108 composed of carbohydrates, and their composition in carbohydrate, protein and lipid is
109 stable (Etvugin and Neto, 2007). Therefore, we expect *N. exitiosus* foragers to regulate
110 only the amount of food they collect to meet their macronutrient requirements.

111 Another reason to expect that termites may not have evolved specific appetites for
112 macronutrients is that they are hemimetabolous. Juvenile and adult stages differ less in the
113 qualitative nutritional requirements than is the case for larval and adult forms of
114 holometabolous insects such as Hymenoptera, where larval and adult forms have very
115 different intake targets (Sorensen and Vinson, 1981; Cassill and Tschinkel, 1999; ; Weeks Jr
116 et al., 2004; Dussutour and Simpson, 2009). However termites are able to moult and change
117 caste, which comes with a cost (Bernays, 1986), and different castes might display variation
118 in their macronutrient requirements. A reason why animals might have evolved specific
119 appetite systems, even when feeding on a relatively nutritionally invariant food type, is where
120 nutritional requirements change markedly over time, for example, across larval and adult life-
121 stages in holometabolous species, necessitating that consumption is adjusted to respond to

122 the currently most limiting nutrient (Raubenheimer et al., 2009). Given their hemimetabolous
123 development and their high degree of food specialisation, we therefore predicted that termite
124 foragers, in contrast to Hymenoptera, would be less able to regulate colony nutrition by
125 adjusting foraging behaviour in response to experimental manipulation of their diet. In this
126 paper, we used the GF approach to address this prediction, by investigating whether
127 termites adjusted their food consumption when faced with foods varying in their
128 macronutrient ratios, and how their longevity, physiology and behaviour were impacted by
129 the diet composition.

130 2. Material and Method

131

132 a) Species studied and rearing conditions

133

134 Four colonies of *Nasutitermes exitiosus* of similar sizes were collected in Adelaide
135 (South Australia) in late spring, between the 22nd of November and the 2nd of December 2016,
136 on a hillcrest, where Eucalyptus trees were the main vegetation. The mother colonies were
137 kept in the lab for at least 2 weeks with ad libitum wood, insect vitamins (Vanderzant vitamin
138 mixture for insects - Sigma) and salt mixture W (MP biomedical) prior to the experiment to
139 reduce eventual variability in colonies initial nutritional state. From these mother colonies, 76
140 experimental colonies of 100 individuals were constructed.

141

142 Each experimental colony consisted of 70 large workers (stage 2 to 5, see (McMahan
143 and Watson, 1975)) and 30 minor soldiers. We used castes that are sterile so we could
144 study the effects of nutritional challenges, independent of reproductive effort. Termites were
145 housed in a 10*10 cm Petri dish. Two third of the Petri dish was filled with 4% agar gel, to
146 provide humidity and allow tunnelling. This type of husbandry has been used previously in
147 this species (Eutick et al., 1978). To prevent fungus infections, a solution of fungicide (10
148 drops of Zaleton per litre) was sprayed on the agar and allowed to dry before the termites
149 were introduced. Each experimental colony was transferred to a new nest every 6 days to
150 prevent infections and dryness. The nests were kept at room temperature (27^oC) under
151 complete darkness.

152 b) Synthetic diets

153

154 In the field, termites feed almost exclusively on wood which they explore building
155 tunnels. Wood only varies slightly in its composition of macronutrients (Carbohydrate 65-
156 75%, Nitrogen :0.03-0.1%, lipids: 0.50-0.90%; Bertaud & Holmbom 2004). For the
157 experiment we used synthetic foods varying in their ratio of protein, lipid and digestible
158 carbohydrate. Wood is typically poor in nitrogen and lipid content and varying those two
159 nutrients in our diet was important to explore whether termites might specifically regulate
160 their intakes. Cellulose is the main energy source used by termites. The protein content of all
161 the foods consisted of a mixture of whey (90%, Myopure) and egg white (10%, Myopure),
162 lipids were added as 50% phytosterol (Bulk Supplements) and 50% ergosterol (Sigma), and
163 cellulose (Sigma) was used as a digestible carbohydrate source. Each food contained 0.5%
164 of vitamins (Vanderzant vitamin mixture for insects, Sigma) and 0.5% of mineral salts (salt
165 mixture W, MP biomedical). The foods were presented to the termites as a powder (see
166 Table 1 below) in 2 mL Eppendorf tubes, which they had access through a small plastic
167 tube. We confined 76 experimental colonies to one of 11 diets differing in their ratio of
168 protein (P), lipids (L) and carbohydrates (C). The P:C:L ratios used are indicated in Table 1.
169 For each treatment, we used 6 to 7 experimental colonies originating from four different
170 mother colonies. As our experimental setups and artificial diets had never been tested
171 before, we also confined 7 experimental colonies to wood, their natural food, using the same
172 experimental conditions as artificial diet groups, and measured mortality. We used two
173 species of Eucalyptus found locally on the Waite campus, Adelaide (*E. cneoriflora*, *E.*
174 *kruseana*). The wood was cut in pieces of approximately 4 cm long (one of each
175 species), and placed untreated in the nest. We did not observe fungus growth on the wood.

176 • Table 1: Proportion and ratio of macronutrient in the artificial diets (C=Carbohydrate,
 177 P=protein, L=lipid). p1, p2, p3 and p4 are diets presenting more protein than lipids
 178 with increasing concentrations in proteins from p1 to p4. l1, l2, l3 and l4 are diets
 179 presenting more lipids than proteins with increasing concentrations in lipids from l1 to
 180 l4. s1 and s2 are diets presenting an equal balance of lipids and proteins with
 181 increasing concentration in carbohydrates. ce is a diet composed of carbohydrate
 182 only and micronutrients.

183

Diet name	C	P	L	Ratio P:C	Ratio L:C	Ratio P:L
p1	0.99	0.007	0.003	0.007	0.003	2.333
p2	0.95	0.048	0.002	0.05	0.002	24
p3	0.9	0.09	0.01	0.1	0.011	9
p4	0.8	0.16	0.04	0.2	0.05	4
l1	0.99	0.003	0.007	0.003	0.007	0.428
l2	0.95	0.002	0.048	0.002	0.05	0.042
l3	0.9	0.01	0.09	0.011	0.1	0.111
l4	0.8	0.04	0.16	0.05	0.2	0.25
s1	0.99	0.005	0.005	0.005	0.005	1
s2	0.9	0.05	0.05	0.055	0.055	1
ce	1	0	0	0	0	NA

184

185

186 c) Experiment

187

188 In this experiment, we investigated the link between nutrition and performance in
189 terms of food collection, longevity, tunnelling activity and body composition in termites.

190

191 Food collection

192 All experimental colonies had ad libitum access to food that was replenished every 3 days.
193 Colonies never collected all the food offered before it was renewed. In order to evaluate the
194 colony's intake the food was dried at 40⁰C and weighed, before it was placed in the nest,
195 and again after they were removed. We divided the colony intake by the number of termites
196 in each colony **at the time the food was offered**, to take into account differences in mortality
197 between colonies.

198

199 Survival

200 To assess mortality in all experiments, the number of dead termites within each experimental
201 colony was counted every day for the first 2 weeks and then every 3 days until all termites
202 had died. Corpses were removed from the colony and kept in Eppendorf tubes at -14⁰C (for
203 body composition analysis). Pictures of the nest were also taken whenever the nest was
204 changed to check that mortality was accurately recorded and that no dead bodies were
205 missed (by counting live termites).

206

207 Tunnelling activity

208 During the whole experiment, we took a picture of the nest every six days (SI, picture 1), just
209 before each colony transfer to a new nest. Tunnelling activity was quantified by measuring
210 the total tunnel length using imageJ. A total of 562 pictures were analysed.

211

212 Body composition

213 We measured the body composition of termites found dead in the nest during the
214 experiment. **Studies conducted in ants showed that the effects of diet composition on energy stores**
215 **are still observable on dead individuals (Bazazi et al. 2016, Arganda et al. 2017).** First, we used a
216 chloroform extraction protocol modified from (Marden, 1987) to extract whole-body lipids.
217 Groups of 5 termites (5 workers or 5 soldiers) were dried for 24h at 50⁰C (to attain a stable
218 dry weight), weighed to nearest 0.01mg and placed in an Eppendorf. Next, we added 0.3 mL
219 of chloroform to each Eppendorf. After 24h we aspirated the chloroform and added fresh
220 chloroform. A total of three chloroform soaks were completed. After these extractions, we
221 allowed the termites to dry completely at 50⁰C and we reweighed each group of 5 termites.
222 The difference in weight of termite bodies before and after lipid extraction gave us the weight
223 of lipids in the samples.

224 Second, we used a protein extraction protocol described in Rho and Lee (2014). The
225 remaining bodies from the lipid extraction were used, and a similar procedure was followed,
226 using 0.35M sodium hydroxide solution instead of chloroform. The difference in weight of
227 termites' bodies before and after protein extraction gave us the weight of proteins in the
228 samples. A total of 469 groups of workers and 264 groups of soldiers were analysed.

229

230 **Statistics**

231 **To compare food collection on each diet, we used a Kruskal-Wallis test. All generalized**
232 **linear mixed models (using the function fit glme, with Distribution = normal, link function=**
233 **identity) and surface regression (using the function fit lme) were done in Matlab, with colony**
234 **as a random factor. All consumptions were standardized ((value-mean)/standard deviation).**
235 **This procedure reduces the covariation between linear variables and their interaction terms**
236 **(Aiken 1991). All statistical tests were performed using Matlab.**

237

238 **3. Results**

239 **a) Food collection**

240

241 First, we measured food collection of experimental colonies of termites forced to feed
242 from a single diet, which varied in its macronutrient composition. Thus, termites were
243 confronted with the situation wherein there is a potential conflict between meeting their
244 requirements for protein, lipids and carbohydrates. Termites did not modify food collection
245 according to the proportion of macronutrient (SI, Fig. S1) but maintained the amount of food
246 collected constant on all diets (Fig. 1, $\chi^2=14.14$, DF=10, P=0.167, SI, table S1).

247

248 **b) Effect of macronutrient collection on lifespan**

249

250 We then investigated whether there was a ratio and quantity of protein, lipid and
251 carbohydrate collected by workers that maximized worker and soldier lifespan. Experimental
252 colonies confined to the control diet (wood) survived well (Mean colony half-life \pm CI 95%=
253 57.25 \pm 12.9 days) and similarly to what was reported in the literature (Cookson, 1987). This
254 indicates that our experimental nests were appropriate for the study.

255 Taken together, our 11 diets allowed us to generate maps of the macronutrient intake
256 space, on which lifespan could be represented and regressed (Fig. 2). Worker mortality was
257 significantly affected by macronutrient composition, and was mostly influenced by the
258 quantity of carbohydrate collected (P<0.001, t=4.72, DF= 68, SI, table S2). Termites lived
259 longest when the daily collection of carbohydrate was around 0.03mg per individual (Fig. 2a
260 and 2b). Survival also depended on protein collection, falling when it increased (P=0.002, t=-

261 3.2, DF= 68, SI, table S2). Survival also decreased when the ratio L:C increased (P=0.002,
262 t=-3.3, DF= 68, SI, table S2).

263 Soldier lifespan decreased slightly when the L:C ratio increased (P=0.009, t=-2.67,
264 DF=68, SI, table S3). If we included the worker lifespan as an explanatory variable in the
265 equation, it became the most significant factor affecting the soldier survival (Adjusted
266 R²=0.20 vs 0.39, SI, tables S3 and S4). In short, worker survival depended on food
267 collection, while soldiers survival was mainly affected by the number of workers remaining in
268 the colony to feed them (Fig. 2, R² for the soldiers' surface regression were very low,
269 contrary to workers', SI, tables S5 to S10).

270

271 c) Effect of macronutrient collection on tunnelling activity

272

273 We also measured how tunnelling activity was affected by food collection (Fig. 3).
274 The tunnel lengths were positively correlated with carbohydrate (P<0.001, t=8.97, DF=554,
275 SI, table 11) and protein collection (P<0.001, t=4.87, DF=554, SI, table 11), but not with lipid
276 collection (P=0.066, t=1.84, DF=554, SI, table 11).

277

278 d) Effect of macronutrient collection on body composition

279

280 We then examined the effect of macronutrient collection on lipid and protein body
281 contents. Body lipid content was very low in workers (mean lipid proportion \pm 95% CI = 0.73
282 \pm 0.13), while it was relatively high in soldiers (20.9 \pm 1.19) (Fig. 4a). However, when the
283 soldier bodies were analysed without their heads, their lipid content was comparable to the
284 ones of full worker bodies. Thus, heads alone accounted for the higher lipid content of

285 soldier bodies (Fig. 4b). Protein content was also higher in soldiers than in workers (mean
286 protein proportion \pm 95% C = 82.5 \pm 3.2 vs 62.7 \pm 3.6, respectively).

287 In workers, both lipid and protein body content decreased with time (P=0.002, t=-
288 3.12, DF=452, SI, table S15; P<0.001, t=8.01, DF=452, SI, table S16 respectively) meaning
289 that workers became leaner during the experiment. This effect was not observed in soldiers
290 (P=0.098, t=1.65, DF=246, SI, table S17; P=0.155, t=-1.42, DF=246, SI, table S18
291 respectively).

292 Body composition was slightly affected by macronutrient collection in workers but not
293 in soldiers (Fig. S2 and S3, SI, tables S15 to 18). Lipid content increased with lipid collection
294 (P= 0.037, t=2.09, DF=452, SI, table S15) and protein content increased with protein
295 collection (P<0.001, t=3.9, DF=452, SI, table S16).

296

297

298 4. Discussion

299 Nutritional ecology theory (Raubenheimer et al., 2009; Simpson and Raubenheimer,
300 2012) predicts that there would be no need for organisms to evolve (or retain) mechanisms
301 to regulate their intake of separate nutrients (e.g. macronutrients) in the case of extreme
302 food specialisation. All that would be required to ensure nutritional balance would be to
303 control the volume eaten (or collected in the case of a social forager). In this study, we have
304 demonstrated that, in agreement with these predictions, termites kept food collection
305 constant across a wide range of P: C: L ratios. Contrary to termites, other social insects such
306 as ants have a pronounced ability to compensate for carbohydrate deficiency or amino acids
307 excess by adjusting the amount of food collected as a function of the imbalance. For
308 example, they collect more food on low carbohydrate diet (Cook et al., 2010; Dussutour and
309 Simpson, 2009), and less food on high amino acids diet (Arganda et al., 2017). The species

310 of ants used in these studies were generalists from a nutritional point of view, while
311 *Nasutitermes exitiosus* a dietary specialist.

312 Nutrient regulation has been shown to differ between specialist and generalist
313 feeders in non-social insects such as caterpillars and locusts (Lee *et al.* 2004, 2006;
314 Raubenheimer and Simpson 2003; Simpson *et al.* 2002, reviewed in (Behmer, 2009)).
315 Specialists experience a lower range of food compositions and lower nutritional variability
316 than a generalist and are typically less likely to overconsume nutritionally imbalanced foods
317 than are generalists. It has been hypothesised that generalists effectively mortgage the
318 short-term costs of ingesting excess nutrients on an imbalanced food against the higher
319 likelihood that they will encounter a nutritionally complementary food in the future, thereby
320 balancing the previous excess (Simpson and Raubenheimer, 2012). However, none of the
321 specialist species that have been studied to date is as limited in the range of foods eaten as
322 are termites, and they have all evolved the capacity to regulate both protein and
323 carbohydrate intake (reviewed in Simpson and Raubenheimer, 2012). Here, we are
324 proposing that termites, because of their extreme specialism, have lacked the need for fine
325 macronutrient regulatory mechanisms and hence do not respond to variation in the
326 macronutrient content of their food. We postulate that they might have lost this regulatory
327 ability secondarily, as they evolved from generalist cockroach-like ancestors (Hunt and
328 Nalepa, 1994), and cockroaches are known to regulate their intake of macronutrient
329 accurately (Jones and Raubenheimer, 2001; Raubenheimer and Jones, 2006). An additional
330 reason why termites may not have developed the ability to increase consumption in the face
331 of nutritional imbalance in food is the physically challenging nature of their food, where the
332 energy lost in processing more food might outweigh the gain of nutrients (Hunt and Nalepa,
333 1994). The impact of diet hardness is striking in *Blattella germanica*, where the growth of
334 individuals raised on hard diets is delayed by 43% compared to those fed a crushed diet
335 (Cooper and Schal, 1992).

336 While termites seem unable to regulate macronutrient intake, they are able to control
337 precisely their intake of micronutrients (Judd *et al.*, 2017, Botch *et al.* (2010)). This

338 seemingly contradictory result is easily explained by the fact that termite gain their
339 micronutrients from the soil (Janzow et al., 2015; Seymour et al., 2014). Unlike wood, soil
340 composition in micronutrients is variable, and the correlation between individual elements is
341 low (Heuvelink and Webster, 2001; Yavitt et al., 2009) – conditions which are hypothesised
342 to lead to the evolution of regulatory feeding abilities. Indeed, previous studies from Judd *et*
343 *al.* (2017) and have provided evidence of such an active regulation of micronutrients by
344 termites. Other insects have been shown to balance their intake of minerals orthogonally to
345 macronutrients by food selection (Simpson and Raubenheimer, 2015; Trumper and
346 Simpson, 1993). Thus termite might behave as food specialists for macronutrients, but as
347 generalists toward micronutrients.

348 Because it is predicted that extreme specialists will not actively regulate their intake
349 of macronutrients, it follows that their performance (*e.g.* life-history responses) will be
350 sensitive to experimentally imposed changes in food macronutrient composition. Termites
351 were indeed strongly affected by such a variation in the present study. The key determinant
352 of the relationship between diet and longevity in termites was the quantity of carbohydrate
353 and protein collected, and to a far lesser extent to the quantity of lipid collected. Termite
354 workers survived best when they collected a daily amount of carbohydrate comprised
355 between 0.02 and 0.04mg of per individual and a low quantity of protein (0-0.001mg) and
356 lipid (0-0.003mg). This optimum in term of lifespan is relatively narrow in comparison to other
357 insects studied (Arganda et al., 2017; Dussutour and Simpson, 2012; Lee et al., 2008;
358 Maklakov et al., 2008).

359 That the optimal macronutrient balance was carbohydrate biased reflects the
360 nutritional composition of wood. Termites have evolved to rely on their gut bacteria to
361 provide the necessary nitrogen from their low-protein food (review in (Hongoh, 2011)) and
362 can even survive on a pure cellulose diet. For example, *Reticulitermes flavipes* lived for
363 more than 4 months on cellulose (Cleveland, 1923). This ability relies on their association
364 with a large community of gut symbionts, which help break down plant tissues. In addition,
365 termites rely on the biosynthetic capacities of these symbionts as a nutritional resource. For

366 example, wood-feeding termites such as *N. exitiosus*, can take up nitrogen from the
367 atmosphere with the aid of N₂-fixing gut bacteria to balance the low nitrogen content in their
368 food (Hongoh, 2011). In our experiment termites could also survive on pure cellulose for a
369 certain time but survived better on *Eucalyptus* wood, their natural diet (Fig. 5). A similar
370 difference between wood and cellulose substrate was also recorded in *Coptotermes*
371 *formosanus* (Su et al., 1985). **The lower survival on cellulose and other artificial diets**
372 **suggests that termites** lacked some essential nutrients that can be found in *Eucalyptus* wood
373 such as **additional** sugars (xylose, mannose, galactose, rhamnose, and arabinose), proteins,
374 lipids as well as micronutrients (Evtugin and Neto, 2007) which might not be provided by
375 gut symbionts.

376 In our experiments, using artificial diets varying in P:C:L, lifespan was reduced when
377 there was a lack or excess of carbohydrates and/or an elevated intake of protein. High
378 protein intake and carbohydrate excess have been shown to shorten lifespan in many
379 animals from insects to mammals due to protein toxicity and various metabolic disorders
380 (e.g. in ants: Arganda et al., 2017; Dussutour and Simpson, 2012; *Drosophila* : Lee et al.,
381 2008, mice : Solon-Biet et al., 2014). Interestingly, the survival of soldiers depended mostly
382 on worker survival and was only marginally affected by macronutrient collection. Workers
383 and soldiers have distinct eating habits, and the results obtained here may reflect this
384 difference. Soldiers are unable to feed themselves and receive trophallaxis from workers
385 (Grassé, 1984). Thus, our results suggest that secondary feeding by trophallaxis appears to
386 protect the soldiers from the detrimental effects of nutritional imbalance. Soldiers represent a
387 strong investment for the colony for several reasons: 1) they must be nutritionally supported,
388 2) they provide no energetic input into colony growth and 3) they are the sink of most energy
389 brought back to the nest, as suggested by their high content in lipid and protein. However,
390 these energy draining, sterile, and dependant individuals defend the colony against
391 predators and they require energy to synthesize **the sticky lipid secretions** (terpenoids) they
392 store in their head capsule as a defence mechanism (Prestwich, G and Collins, 1981;
393 Prestwich, 1979). We found that lipid content in soldiers was indeed mostly localised in the

394 head capsule. Thus, our results suggest that the colony, to protect its investment, may
395 overcome the deleterious effects of macronutrient imbalance in soldiers by getting the
396 workers to process the diet for them. This type of communal nutrition has been observed in
397 ants where workers avoid the negative effects of macronutrient excess by getting the larvae
398 to digest them (Dussutour and Simpson, 2009).

399 Food intake per se is only part of the complex and fully integrated feeding behaviour
400 of termites. Foraging is an important component related to food intake that can also be
401 regulated. Termites such as *N.exitiosus* explore and exploit their environment by tunnelling
402 through soil and wood. Total tunnel length therefore gives an indication of general foraging
403 activity (Mackay et al., 1985). Digging tunnels is an activity that is energetically costly
404 (Mackay et al., 1985). Tunnelling is related to food discovery (Campora and Grace, 2004)
405 and is stimulated under food deprivation (Gallagher and Jones, 2005; Hedlund and
406 Henderson, 1999). Interestingly, in our experiment, where termites had unlimited access to
407 food, tunnelling activity increased with carbohydrate and protein collection. The most likely
408 explanation is that termites increased their foraging activity to maximize exploration and
409 therefore chances of finding a better food. Elevated foraging activity under adverse
410 nutritional conditions, such as food deprivation or confinement to imbalanced food, has been
411 reported in many animals (e.g. in rodents: Pirke et al., 1993; in flies : Fanson et al., 2013;
412 Isabel et al., 2005; Knoppien et al., 2000; Lee and Park, 2004; Meunier et al., 2007; Yang et
413 al., 2015; in ants : Dussutour and Simpson, 2012; Dussutour et al., 2016) and has been
414 suggested to be the best way to find scarce or complementary food. An alternative or
415 additional explanation could be that termites increased their foraging activity as a means to
416 burn off excess nutrients (protein or carbohydrate) to rebalance their nutrient intake. By
417 keeping their food collection constant on all diets, termites on imbalanced diets acquire
418 certain nutrients in excess while other nutrients remain limiting. Were they to selectively
419 metabolise the excess nutrient to fuel increased tunnelling, they could rebalance the ratio of
420 macronutrients post-ingestively (e.g. Clissold et al., 2010; Woodring et al., 2009; Zanotto et
421 al., 1993).

422 In summary, our experiment showed that termites did not actively compensate for
423 experimentally imposed changes in the macronutrient content of foods by adjusting food
424 intake, nor did they avoid the detrimental effect of macronutrient imbalances. We postulate
425 that in species such as termites with highly specialised diets, the need to finely regulate
426 macronutrient intakes is lost: regulating the amount of food ingested alone will be sufficient
427 to attain nutrient balance when the composition of foods is invariant. An additional capacity
428 to rely upon communities of gut symbionts has allowed termites to exploit wood, an
429 otherwise nutritionally unpromising substrate.

430

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434

435 **Contribution**

- 436 • L.A.P. conceived and performed all the experiments and statistical analyses and
437 wrote the first draft of the manuscript
- 438 • S.A. assisted with the statistical analyses
- 439 • A.D. and J.B. co-supervised the work of L.A.P., and designed the study
- 440 • J.B. funded the experiments
- 441 • All authors contributed critically to the drafts and gave final approval for publication.

442 ***Data available from the Dryad Digital Repository***

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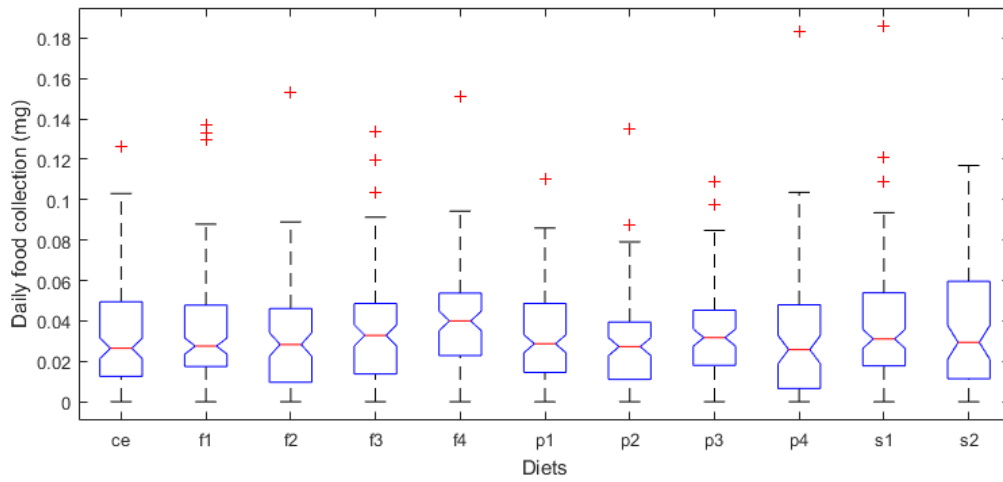
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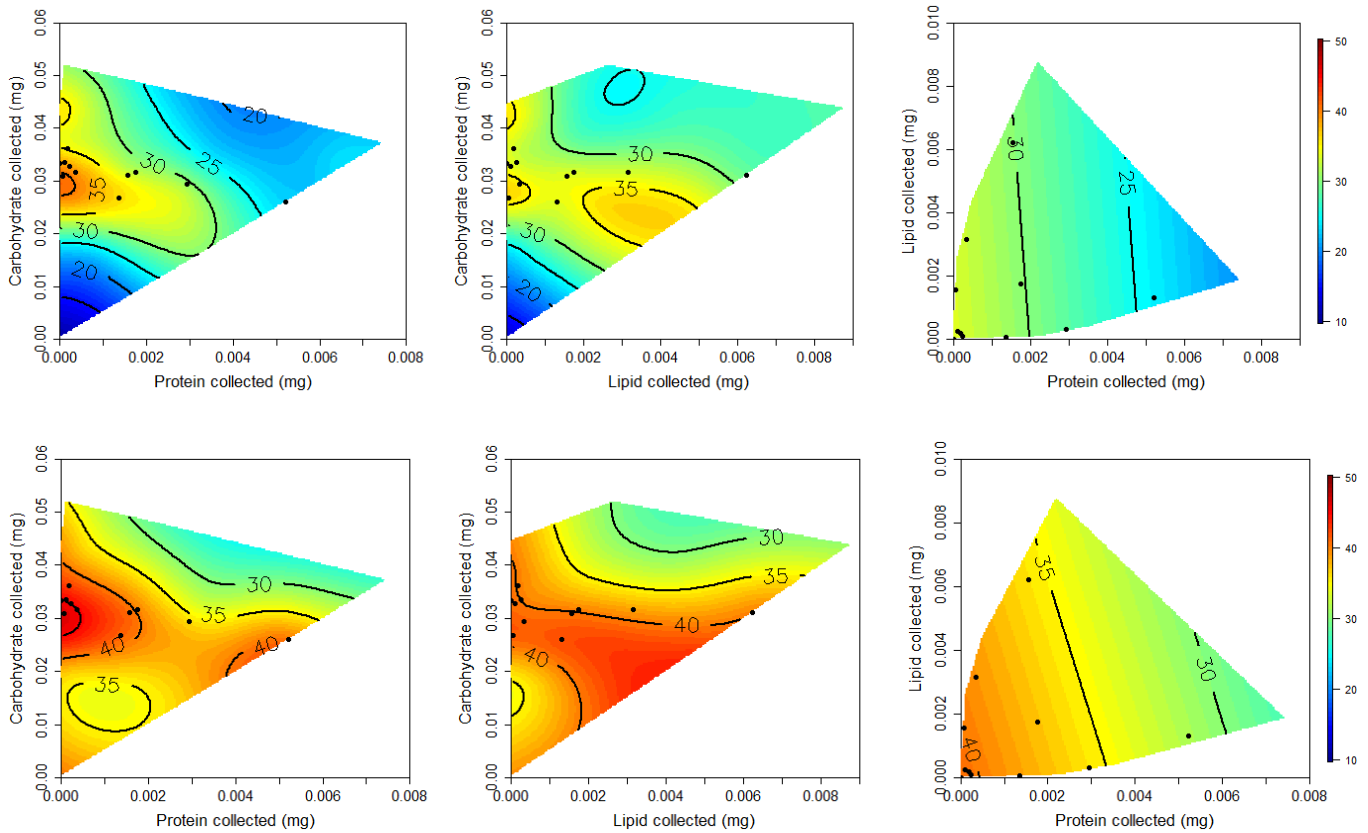
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591 Fig. 1: Macronutrient collection: Notched boxplot presenting the average daily intake per individual
592 (mg) when experimental colonies of termites were confined on one of 11 diets varying in
593 macronutrient content and composition (76 experimental colonies in total, N=7 per diet, except for diet
594 S2, N=6). The red central mark is the median, the edges of the box are the 25th and 75th percentiles,
595 the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted
596 individually. Refer to table 1 for the definition of each diet.

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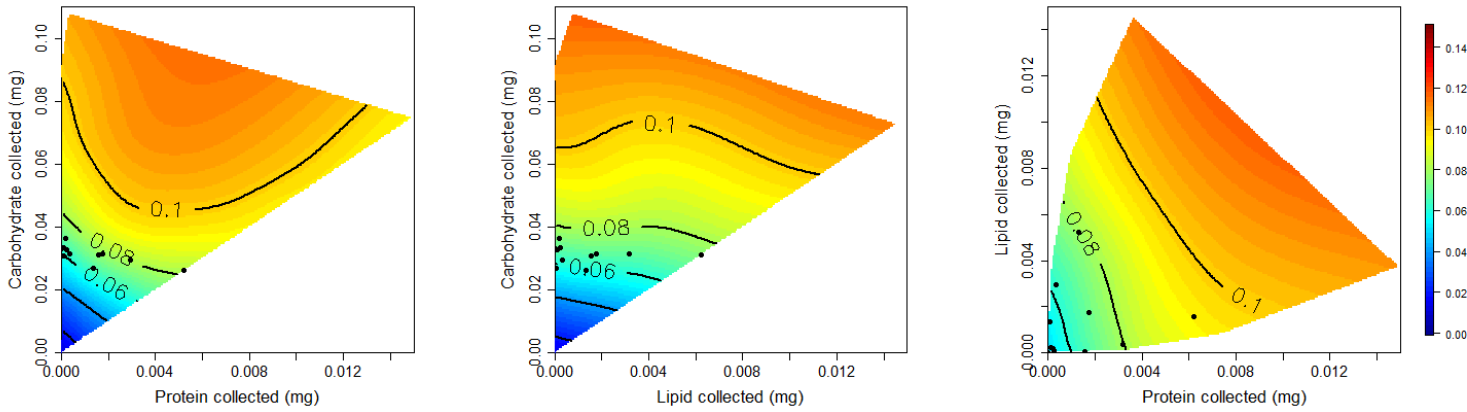
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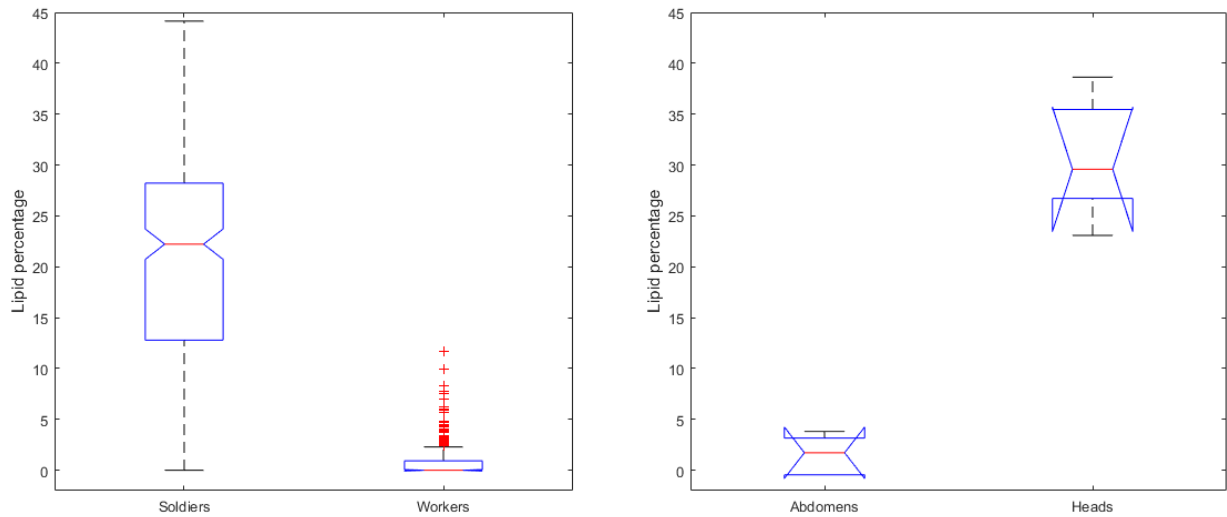
Fig. 2: Effects of nutrient collection on termite survival. Data were recorded for each experimental colonies of termites (70 workers and 30 soldiers in each colony) confined for the whole duration of the experiment to one of 11 diets varying in macronutrient content and composition. Response surfaces were visualized using the function *tps* in the package *fields* in the statistical software R. Red indicates the highest values for the lifespan, while blue regions are associated with the lowest values. Black circles indicate the mean intake per individual on each of the diet. (a) Effects of nutrient intake on workers survival (Mean lifespan for each experimental colony). (b) Effects of nutrient intake on soldiers survival (Mean lifespan for each experimental colony). We adjusted intake to the number of termites still alive in each colony, to take into account differences in mortality between colonies. Tested from left to right as a function of protein and carbohydrate, lipid and carbohydrate, and protein and lipid. Adjusted R^2 of surface regression of lifespan as a function of nutrient collected are respectively 0.67, 0.64 and 0.35 for panel a), and 0.08, 0.1 and 0.1 for panel b) (ESM tables S5 to S10).

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620 Fig. 3: Effects of nutrient collection on tunnelling activity. Data were recorded for each experimental
621 colonies of termites (70 workers and 30 soldiers in each colony) confined for the whole duration of the
622 experiment to one of 11 diets varying in macronutrient content and composition. Response surfaces
623 were visualized using the function *tps* in the package *fields* in the statistical software R. Red indicates
624 the highest values for tunnelling activity, while blue regions are associated with the lowest values. The
625 tunnel length was measured for each colony every six days until the end of the experiment, and
626 associated with the consumption of food over those 6 days (N=562). We adjusted intake to the
627 number of termites still alive, and tunnelling activity (cm dug per day) to the number of workers still
628 alive in each colony (as soldiers do not dig), to take into account differences in mortality between
629 colonies. Adjusted R^2 of surface regression of tunnelling as a function of nutrient collected are
630 respectively 0.3, 0.28 and 0.11 (ESM, tables S12 to S14).

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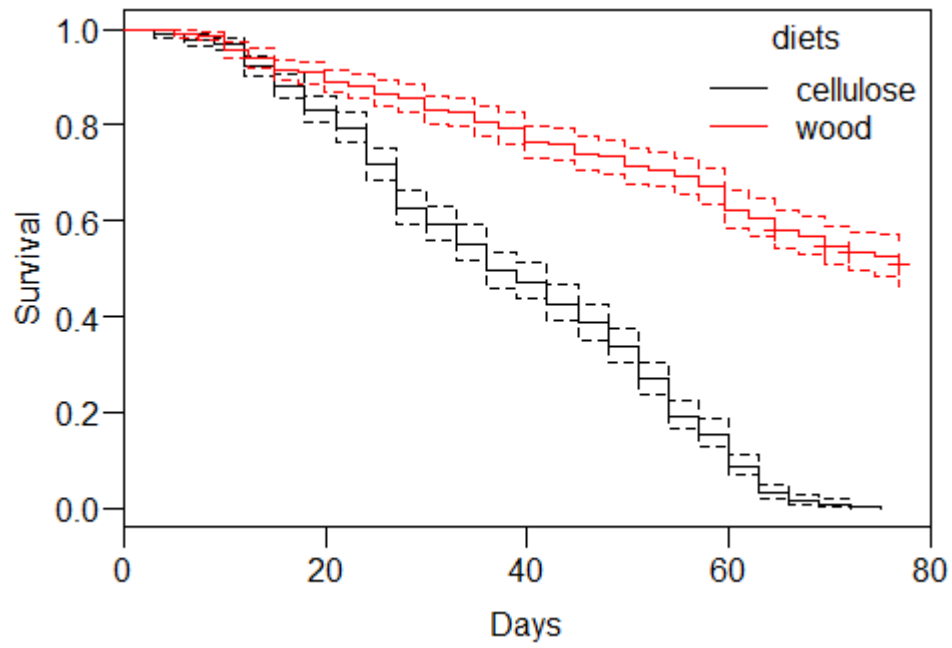


634 Fig. 4: Body composition of termites. Notched boxplot of the body lipid percentage of a) dead soldiers
 635 (N=264 groups of 5) and dead workers (N=469 groups of 5) from colonies confined from the start of
 636 the experiment until their natural death to one of 11 diets varying in macronutrient content and
 637 composition, and b) lipid percentage in the heads and the abdomens of soldiers sacrificed before
 638 starting the experiment (N=5 groups of 5). The red central mark is the median, the edges of the box
 639 are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered
 640 outliers, and outliers are plotted individually.

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646 Fig. 5: Survival curves of workers kept on a wood diet vs a pure cellulose diet. The dashed lines are

647 the 95% intervals.