Diversity structure of the microbial communities in the guts of four neotropical termite species

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The termite gut microbiome is dominated by lignocellulose degrading microorganisms. This study describes the intestinal microbiota of Argentinian higher termite species and provides a contribution towards understanding how the diet shapes termite gut microbiomes. Four Argentinian termite species were selected based on their feeding habits: wood *Microcerotermes strunckii* (hardwood) and *Nasutitermes corniger* (softwood), organic matter from soil/grass sources (*Termes riograndensis*) and herbaceous material (*Cornitermes cumulans*). The bacterial phyla Spirochaetes, Fibrobacteres, Bacteroidetes and Firmicutes dominated in the guts from *M. strunckii*, *N. corniger* and *C. cumulans* gut samples, whereas Fibrobacteres were a relatively minor taxon in *T. riograndensis*. A single bacterial genus, *Treponema* (Spirochaetes), was dominant in all termite species. Prokaryotic α-diversity was higher in the soil/grass feeders than in the wood feeders. In addition, the β-diversity analysis of prokaryotes and fungi were highly dissimilar in strict wood- feeders, whereas that of soil- and grass- feeders grouped more closely. Ascomycota and Basidiomycota were the only fungal phyla detected in all gut samples. In summary, higher microbial diversity was evident in termites with more versatile diet preferences, suggesting diet-driven microbial community assembly in the termite gut.

1 **Diversity structure of the microbial communities in the guts of four**

2 **Neotropical Termite Species**

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24 **Abstract**

25 The termite gut microbiome is dominated by lignocellulose degrading microorganisms. This

26 study describes the intestinal microbiota of Argentinian higher termite species and provides a

27 contribution towards understanding how the diet shapes termite gut microbiomes. Four

28 Argentinian termite species were selected based on their feeding habits: wood

29 *Microcerotermes strunckii* (hardwood) and *Nasutitermes corniger* (softwood), organic matter

30 from soil/grass sources (*Termes riograndensis*) and herbaceous material (*Cornitermes*

31 *cumulans*). The bacterial phyla Spirochaetes, Fibrobacteres, Bacteroidetes and Firmicutes

32 dominated in the guts from *M. strunckii*, *N. corniger* and *C. cumulans* gut samples, whereas

33 Fibrobacteres were a relatively minor taxon in *T. riograndensis*. A single bacterial genus,

34 *Treponema* (Spirochaetes), was dominant in all termite species. Prokaryotic α-diversity was

35 higher in the soil/grass feeders than in the wood feeders. In addition, the β-diversity analysis

36 of prokaryotes and fungi were highly dissimilar in strict wood- feeders, whereas that of soil-

37 and grass- feeders grouped more closely. Ascomycota and Basidiomycota were the only fungal

38 phyla detected in all gut samples. In summary, higher microbial diversity was evident in termites

39 with more versatile diet preferences, suggesting diet-driven microbial community assembly in

40 the termite gut.

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Introduction

Termites are extremely efficient in degrading lignocellulose, and may be useful as "bioreactor

models" for the conversion of lignocellulosic biomass into biofuels and other biomaterials

(*Brune, 2014*).

Termites are broadly separated into 'lower' and 'higher' groupings, based on the presence

or absence of flagellated protistans in the hindgut. The gut microbiota of lower termites consists

of Bacteria, Archaea and Eucarya (such as flagellates and yeasts), whereas higher termites lack

flagellated protozoans (*Ni & Tokuda, 2013*). These microbial symbionts have various roles in the

digestive processes, by participating in multiple functions including carbohydrate and nitrogen

metabolism, oxygen and hydrogen consumption, N_2 fixation, modifications of aromatic polymers

and humification (*Brune, 2014; Santana et al., 2015*).

All termites feed on lignocellulose, the main component of plant cell walls. Lower

termites (families Mastotermitidae, Kalotermitidae, Termopsidae, Hodotermitidae,

Rhinotermitidae and Serritermitidae) have specific diets restricted to woody tissue, whereas

higher termites (family Termitidae) have diverse feeding habits, which include wood, grass,

fungi, lichen, litter, dung, humus and soil. Termitidae is the most diverse family of termites

(around 75% of all species). This family comprises eight subfamilies: Apicotermitinae,

Cubitermitinae, Foraminitermitinae, Macrotermitinae, Nasutitermitinae, Sphaerotermitinae,

Syntermitinae and Termitinae. To date, 80 genera and 458 species, distributed in four

subfamilies, Apicotermitinae, Nasutitermitinae, Syntermitinae and Termitinae, have been

identified in the Neotropical Region (*Krishna et al., 2013*).

The termite gut microbiome was primary believed to be determined by the host

phylogeny, with influence from the diet (*Hongoh, 2010; Rahman et al., 2015; Tai et al., 2015*).

74 More recently, *Mikaelyan et al.* (*2015*) suggested that the diet was the principal determinant of

75 the higher termite gut microbiome composition. Other reports suggest that insect gut bacterial

76 diversity is determined by environmental behavior, diet, developmental stage and host phylogeny

77 (*Hoback & Stanley, 2001; Yun et al., 2014*).

78   In this study, we used 16S rRNA gene and ITS sequencing to compare the gut microbiota

79 of four higher termite species with different feeding habits and from three different families

80 *Cornitermes cumulans* (Syntermitinae), *Microcerotermes strunckii* (Termitinae, Amitermes

81 group), *Nasutitermes corniger* (Nasutermitinae) and *Termes riograndensis* (Termitinae, Termes

82 group). *C. cumulans* builds mounds and feeds mainly on herbaceous material, predominantly

83 grasses, but its diet may include cow dung and degraded wood (*Souza et al., 2017*). The soil-

84 mound building termite *T. riograndensis* feeds mainly on soil and plant material. Finally, *M.*

85 *strunckii* and *N. corniger* are strict wood -feeders, and therefore consume dry, wet or partially

86 decayed hardwoods and softwoods (*Scheffrahn et al., 2005*). The analysis was based on 16S

87 rRNA gene and ITS sequencing for prokaryotic and fungal communities, respectively.

88

89 **Materials & Methods**

90 **Sampling site**

91 The termite species *C. cumulans*, *M. strunckii*, *N. corniger* and *T. riograndensis* are widely

92 distributed in Northeastern Argentina.

93   Individuals were field-collected in Corrientes province, Argentina. *C. cumulans* (S

94 28°04′50.2″: O 58°16′12.1″) and *T. riograndensis* (S 27°25′29.7″: O 58°38′53.7″) were

95 collected from mounds located in grasslands whose natural vegetation consists of *Andropogon*

96 *lateralis* and *Paspalum notatum*. *M. strunckii* (S 27°42′43.9″: O 59°13′35.2″) and *N. corniger* (S

97   27°27′38.3″: O 58°49′19.6″) were collected in Chaco province from live trees *Astronium*

98   *balansae* (hardwood) and *Peltrophorum dibium* (softwood), respectively. The specimens were

99   stored at -20°C until further processing.

100

101   **DNA extraction**

102   Worker caste specimens were surface sterilized with 70% ethanol and their whole guts were

103   dissected under a binocular microscope using sterile forceps. Ten dissected whole guts were put

104   in a microtube; three independent extractions were performed per termite species. Microbial

105   genomic DNA was extracted from the triplicate gut samples using the DNeasy Blood and Tissue

106   kit (Qiagen, USA) according to the manufacturer's instructions.

107   The V3 and V4 hypervariable regions of the bacterial and archaeal 16S rRNA gene were

108   amplified using the specific barcoded primers 515F (5′- GTGCCAGCMGCCGCGGTAA 3′) and

109   806R (5′-GGACTACNNGGGTATCTAAT-3′). The ITS2 region of the ITS rDNA was amplified

110   using the specific barcoded primers Forward (5′-GCATCGATGAAGAACGCAGC-3′) and

111   Reverse (5′-ATATGTAGGATGAAGAACGYAGYRAA-3′) to assess fungal diversity. The

112   samples were sequenced on an Illumina MiSeq instrument in the Molecular Research DNA (MR

113   DNA) sequencing facility (Shallowater, Texas, USA).

114

115   **Bioinformatics analysis of 16S rRNA and ITS sequences**

116   The paired end short reads were merged into single end reads and demultiplexed using the

117   barcode sequences for each sample. The analyses of the 16S rRNA and ITS sequences were

118   performed in the Qiime2 v2018.6 (https://qiime2.org) (*Caporaso et al., 2010*). Chimera

119   identification and ASV clustering were performed using DADA2 (*Callahan et al., 2016*) plugin

120   in QIIME 2. The sequences were assigned to Amplicons Sequence Variants (ASVs) using

121   SILVA 128 16S rRNA (*Quast et al., 2013*) and Unite (*Abarenkov et al., 2010*) database for

122   bacteria/archaea and fungi, respectively.

123   The resulting 16S rRNA representative sequences were aligned using MAFFT aligner

124   (*Katoh et al., 2002*) and an unrooted tree was produced using FastTree 2 (*Price et al., 2010*). The

125   tree was rooted at midpoint for the phylogenetic diversity analysis in QIIME v2. The α- and β-

126   diversity indices for the 16S rRNA were analyzed in the QIIME 2 pipeline. In addition, α-

127   diversity between the termite gut microbiome was compared using the Kruskal-Wallis test,

128   followed by false discovery rate (FDR) correction in Qiime2 pipeline. Weighted and Unweighted

129   UniFrac dissimilarities matrices were also obtained from the QIIME 2 pipeline (*Lozupone et al.,*

130   *2011*).

131

132   **Statistical Analysis**

133   The 16S rRNA ASV table was rarefied for 79,256 sequences in 4,882 ASVs (total number of

134   sequences 951,072 for 12 samples). The ITS ASV table was rarefied to 7,418 sequences. The

135   ASV table consists of 149 ASVs (66,762 sequences) for nine samples. α- and β- diversity

136   indexes were calculated and applied in the phyloseq package (*McMurdie and Holmes, 2013*) of

137   R. The test of significance for the ITS ASVs was performed by adonis function (strata =

138   location) of Vegan package (*Dixon, 2003*) in R. The analysis of significant differences based on

139   the groups (termite and diet (wood, mainly soil/grass feeders)). ITS ASV table was Hellinger

140   transformed and Bray-Curtis distances were used to produce a dissimilarity matrix. A *p* value <

141   0.05 was set as the cutoff for significance.

142

143   **Results**

144 Illumina MiSeq sequencing of 16S rRNA gene and ITS amplicons, derived from gut samples

145 from four termite species generated 1,761,565 high quality sequences. The sequences were

146 assigned to 4,882 and 172 amplicon sequence variants (ASVs) for 16S and ITS sequences,

147 respectively.

148

149 **Bacterial, archaeal and fungal taxonomy**

150 We identified 23 bacterial phyla in the termite guts of the four species tested. The dominant

151 communities in the guts of wood feeding termites (*M. strunckii* and *N. corniger*) were

152 Spirochaetes (51% to 61%), followed by Fibrobacteres (~13%). Both species also showed

153 similar relative abundances of Bacteroidetes (~8%) and Firmicutes (~8% in the case of *N.*

154 *corniger* and slightly less, ~6%, for *M. strunckii*). Only a few reads (~2%) remained as

155 unclassified bacteria (Fig. 1A). Thus, at the phylum level, gut communities of both wood-feeding

156 species shared similar profiles regarding dominant taxa.

157 For the grass-feeding termite *C. cumulans*, Spirochaetes was the dominant phylum

158 (~44%), followed by Firmicutes (~23%) and Bacteroidetes (~13%). By contrast, the dominant

159 phylum in the soil/grass-feeding species *T. riograndensis* was Firmicutes (~32%), followed by

160 Spirochaetes (~20%) and Bacteroidetes (~13%). Only 2% of the sequences remained

161 unclassified (Fig. 1A). *Treponema* was the most abundant bacterial genus, but with large

162 variations in its relative abundance among the termite species (*T. riograndensis*, 17.5%; *C.*

163 *cumulans*, 42.8%; *M. strunckii*, 59.1% and *N. corniger*, 58.3%).

164 Of the Archaea, the only two phyla detected, Euryarchaeota and Bathyarchaeota

165 accounted for less than 2% of the reads, except for *T. riograndensis*, in which they represented

166 almost 6% on average.

167   In the ITS data analysis, *C. cumulans* data were excluded from the subsequent ITS

168   sequence assessment because of the low number of reads obtained. Regarding the other three

169   termite species, the absence of matches with the available sequence data (~81%, ~93% and

170   ~99% of unclassified reads for *N. corniger*, *T. riograndensis* and *M. strunckii*, respectively) from

171   the Unite database makes the taxonomical identification of most of the fungal ASVs impossible.

172   The few fungal taxa identified were assigned to the phyla Ascomycota and Basidiomycota, with

173   Eurotiomycetes being the most abundant fungal class (Fig. 1B).

174   A rarefaction analysis performed for each gut sequence dataset retrieved rarefaction

175   curves that reached a plateau for all samples (except for *C. cumulans* ITS samples). This result

176   suggests that the sample size was large enough to represent the bacterial and fungal diversity

177   present in the communities (Fig. 2).

178

179   **Diversity of prokaryotic and fungal taxa**

180   The prokaryotic diversity of termite guts was analyzed using α- and β-diversity indices. The

181   different indexes, Shannon, Evenness and Observed, showed no significant differences regarding

182   α-diversities between all four termite species (Fig. S1). However, the α-diversity between diet

183   groups (strict wood -feeders and soil/grass -feeders) significantly differed (Fig. S2). The group of

184   soil/grass feeders displayed higher values of the α-diversity indices and this index was higher for

185   the soil/grass feeders (*T. riograndensis*) in comparison to that for the grass -feeders (*C.*

186   *Cumulans*) (Fig. 3A; Fig. S2).

187   The prokatyotic β-diversity of the termite gut microbiome was compared using the

188   Unweighted UniFrac distances. The gut microbiome composition of the polyphagous soil/grass

189   feeders was found to be similar and grouped distantly from that of the wood feeders and both

190 were separated from each other (Fig. 3B). The PERMANOVA test of Unifrac distances revealed

191 that the β-diversity was significantly different for the four termite species, with a marked

192 variation according to the feeding habit (diet group of termites) (strict wood-feeders and

193 soil/grass -feeders) ($p <0.05$) (Table 1).

194   The Shannon and Evenness α-diversity indices of fungal communities from *M. strunckii*,

195 *N. corniger* and *T. riograndensis* gut samples differed significantly (Fig. S3). However,

196 Observed-ASVs showed no significant differences in α-diversity. Fungal α-diversity according

197 to the diet group was also significantly different for the Shannon and Evenness indices but not

198 for Observed-ASVs (Fig. S4).

199   To visualize overall similarities and differences in fungal community structure, we

200 calculated Bray-Curtis distances between *M. strunckii*, *N. corniger* and *T. riograndensis*, and

201 displayed these analyses in the form of two-dimensional NMDS plots (Fig. S5). These analyses

202 revealed that the fungal community composition in the gut samples of the three termite species

203 was significantly different, whereas replicates of the same species were almost identical (Fig.

204 S5). PERMANOVA analysis confirmed significant differences in the fungal communities of the

205 different termite species and diet groups (Table 1).

206

207 **Core microbiome**

208 In total, 38 bacterial ASVs were shared across the four higher termite species; which represents

209 28.5 % of all the obtained sequences (Fig. 4A). Furthermore, the termite gut samples grouped

210 according to their feeding habits, shared 23 (wood feeders) and 38 (soil/grass feeders) ASVs

211 (Fig. 4A).

212   Of the 38 ASVs, 18 were assigned to *Treponema* sp., which represented 19% of all the

213   obtained sequences. These ASVs were present in higher relative abundances in the guts of *C.*

214   *cumulans*, *M. strunckii* and *M. corniger* than in the gut of *T. riograndensis* (Table 2).

215   We identified 11 fungal ASVs, which represented an average of 71.3% of all the

216   identified fungal sequences, shared in the gut samples of *M. strunckii*, *N. coringer* and *T.*

217   *riograndensis* (Fig. 4B). The most abundant shared ASVs were assigned as unclassified fungi in

218   all the termite guts (Table 2).

219

220   **Discussion**

221   Numerous studies of higher termite gut microbiota have been published in the last two decades

222   (*Warnecke et al., 2007; Otani et al., 2014; Mikaelyan et al., 2015; Rahman et al., 2015; Santana*

223   *et al., 2015; Su et al., 2016*). However, in a few proportions of such studies, researchers have

224   investigated the gut community composition in relation to the feeding habits of the host termite

225   species.

226   This study provides the first description of the intestinal microbiota associated with four

227   Argentinian higher termite species (*C. cumulans*, *M. strunckii*, *N. corniger* and *T. riograndensis*)

228   performed by high-throughput 16S rRNA gene and ITS amplicon sequencing analyses. These

229   termites have different diet preferences ranging from hardwood, softwood, herbaceous materials,

230   soil/grass. In contrast to softwood, the hardwood harbors high amount of carbon content

231   consisting on cellulose, hemicellulose and low proportion of lignin (*Demirbas, 2005*).

232   Herbaceous plant materials have higher nutritious contents, and lower lignin compared to the

233   wood, whereas soil contains most diverse organic content which are selectively utilized by the

234   termite species. Based on the diet preferences, we were interested in exploring the differences in

235   the gut microbiota composition of these Argentinian higher termite species.

236   Overall, Spirochaetes, followed by Fibrobacteres, Bacteroidetes and Firmicutes were the

237   dominant gut phyla in termites feeding on wood or grass (*M. strunckii*, *N. corniger* and *C.*

238   *cumulans*). By contrast, the dominant phylum in the soil/grass feeder *T. riograndensis* was

239   Firmicutes, followed by Spirochaetes and Bacteroidetes. In accordance with our study, other

240   researchers have reported Spirochaetes as one of the most abundant phylum in wood- and grass-

241   feeding higher termite guts (*Hongoh et al., 2005; Warnecke et al., 2007; Köhler et al., 2012;*

242   *Brune, 2014; Dietrich et al., 2014; Mikaelyan et al., 2015; Rahman et al., 2015*).

243   The high abundance of Spirochaetes (genus *Treponema* sp.), Fibrobateres and

244   Bacteroidetes in the wood-feeding termites may be related to the nitrogen fixation and

245   lignocellulosic processes (*Lilburn et al., 2001; Breznak, 2002; Warnecke et al., 2007; Yamada et*

246   *al., 2007; Su et al., 2016*). In addition, high abundance of phylum Firmicutes (mostly

247   Ruminococcacae) in the *T. riograndensis* was in accordance with previous reports of soil- and

248   humus- feeding termites (*He et al., 2013; Dietrich et al., 2014; Mikaelyan et al., 2015; Santana*

249   *et al., 2015*).

250   In particular, several studies have reported similar proportion of Spirochaetes which

251   constitutes approximately 50 to 60% of total prokaryotic population, in the gut microbiome of *N.*

252   *corniger* (Warnecke *et al*., 2007; He *et al*., 2013; Dietrich *et al*., 2014; Santana *et al*., 2015; Su *et*

253   *al*., 2016). However, in one report *Köhler et al*. (*2012*) observed lower proportion of

254   Spirochaetes in *N. corniger* and *N. takasagoensis*. This discrepancy could be due to variations in

255   DNA extraction methods (*Morgan et al., 2010*) and PCR oligonucleotides (different

256   oligonucleotides) performed in the studies (*Engelbrektson et al., 2010*).

257   The high relative abundance of genus the *Treponema* sp. (phylum Spirochaetes) in the

258   core of *C. cumulans*, *M. strunckii* and *N. corniger*, but not in that of *T. riograndensis*, suggests

259   that this genus have an important impact on the overall physiology and digestive processes in the

260   wood- and grass- feeding higher termites. The presence of Treponema as the principal genus of

261   gut microbiota has been described in several studies of the termite gut microbiota (*Warnecke et*

262   *al., 2007; Köhler et al., 2012; Shi et al., 2013; Benjamino & Graf, 2016*). Thus, the differences

263   in their core microbiomes might be related to the phylogeny of the termite and to their diet

264   habits.

265   A low proportion of archaea was present in the profile of higher termite gut communities.

266   The detected archaeal phyla were Euryarchaeota and Bathyarchaeota. Euryarchaeota include

267   closely related genera known for their methanogenic activity (*Rahman et al., 2015*). The

268   methanogenic archaeon *Methanimicrococcus* sp. was present in the core microbiome albeit in the

269   different proportions. This genus has been detected in the gut microbiomes of other higher

270   termites and cockroaches (*Paul et al., 2012*).

271   In the four species evaluated in this study, the diversity of the fungal community was

272   markedly lower than that of prokaryotes. The high proportion of the taxonomically unclassified

273   fungal ASVs may result from the lack of representative sequences in the SILVA database

274   (*Hongoh, 2010; Santana et al., 2015*). Although fungi are not as prevalent as bacteria in higher

275   termite guts, an important unresolved issue is to determine the role of fungi in development,

276   cellulolytic process and fitness. Some of these functions could be to provide nitrogen source,

277   degrade high molecular weight molecules and produce pheromones for mating and

278   communication (*de León et al., 2016; Zhang et al., 2018*). However, the role of fungal

279   microbiota in the digestive process of termites is not clear yet (*Brune, 2014*).

280   The fungi classes detected in the core were Eurotiomycetes and Malasseziomycetes.

281   Eurotiomycetidae are producers of secondary metabolites, fermentation agents as well as of

282   xerophile and psychrophile enzymes. They were present in the gut of the litter-feeding termite

283   *Synthermes wheeleri* (*Santana et al., 2015*). Malasseziomycetes are ecologically diverse and

284   wide spread yeast. The genus Malassezia is lipophilic yeast and has been known as a common

285   inhabitant of human skins (*Paulino et al., 2008*). A report by *Zhang et al.* (*2003*) identified this

286   yeast in the gut of beetles.

287   The presence of a common core microbiota suggests that these taxa are retained despite

288   differences in habitat, geography and food source, and regardless of host phylogeny. This core

289   composition also suggests that these taxa are important in the maintenance of key functions and

290   may serve as the basis for microbial community resistance and/or resilience (*Huse et al., 2012;*

291   *Shade & Handelsman, 2012; Benjamino & Graf, 2016*).

292   The α-diversity of gut bacterial communities in the soil/grass feeder group was

293   significantly higher than that in wood feeders, whereas the gut microbiota in *T. riograndensis*

294   (soil- and grass- feeding termites) was higher than in species that fed on grass only (*C.*

295   *cummulans*). The lower diversity indexes of gut microbiota associated with the wood- feeding

296   termites may be related to the maintenance of a specialized microbiota that is necessary for

297   performing an efficient lignocellulose metabolism, and therefore for the host survival (*Breznak*

298   *& Brune, 1994; Colman et al., 2012*). The studied species of wood- feeding termites have a very

299   limited diet, which includes complex carbohydrates (cellulose, hemicellulose and lignin), and

300   this characteristic may explain the lower α-diversity. Feeding on live trees may expose the host

301   and potentially its microbiota to tree physiological responses (*Morewood et al., 2004*), which

302   may further shape gut community dynamics of live wood- feeding termites. The high α-diversity

303 in the soil/grass feeding termites is related to the diverse range of carbon and nitrogen sources

304 available in the soil/grass diets; these more complex substrates require more complex

305 degradative capacity and therefore more complex communities. On the other hand, the host

306 habitat also may influence the relative bacterial abundances of the termite gut microbiota (*Yun et*

307 *al., 2014*).

308   An intercommunity analysis showed that the gut microbiomes of soil/grass feeders were

309 clearly separated from that of wood feeders. Soil/grass-feeding termite species grouped closely,

310 whereas the wood -feeders were spatially separated from each other. The replicates of *C.*

311 *cumulans*, *T. riograndensis* and *M. strunckii* showed little variation, whereas those of the wood

312 feeder *N. corniger* were more disperse. The difference in the β-diversity of the gut from wood

313 feeding termites could be related to the type of wood (hard and soft wood) from which they were

314 collected and, thus, this could help explain the differences in the microbiota composition. The

315 termites *M. strunckii* and *N. corniger* came from live trees of *Astronium balansae* (hardwood)

316 and *Peltrophorum dibium* (softwood), respectively. Even though the relative abundance at the

317 phylum level was similar, microbial species composition was different between them.

318   The understanding of the termite-microbiome interaction requires the exploration of the

319 composition and structure of the microbiota, as well as of the characterization of its main

320 metabolic activities in different taxonomic groups of termites with different types of diets.

321   Altogether, the results obtained in this study showed that, except for the relative

322 abundance of bacterial phyla (in the case of strict wood- feeders), no obvious pattern was

323 observed in mutual comparisons of any of the four analyzed termite species. The relationship

324 between the diet and diversity of microorganisms present in the termite digestive system warns

325   about the consequences environment changes could have on them. Indeed, this could lead to a

326   reduction in the food supply, which could result in turn in a loss of microbial gut diversity.

327

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337

338   **Author Contributions**

339   Surendra Vikram performed the experiments, analyzed the data, prepared figures and tables,

340   authored or reviewed drafts of the paper, and approved the final draft.

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342   data, authored or reviewed drafts of the paper, approved the final draft.

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347   final draft.

348   Clara Etcheverry performed the experiments, analyzed the data, reviewed drafts of the paper,

349   approved the final draft.

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351   draft.

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353   data, prepared figures and/or tables, authored and reviewed drafts of the paper, approved the

354   final draft.

355

356   **Tables and Figures titles**

357   **Table 1**: PERMANOVA analysis of bacteria/archaea and fungi.

358   **Table 2**: Relative abundance (%) of *C. cumulans*, *M. strunckii*, *N corniger* and *T. riograndensis*

359   core microbiota.

360   **Fig 1**. Relative abundance of bacteria, archaea (phyla level) and fungi (class level) in the gut of

361   Neotropical termites.

362   **Fig 2**. Rarefaction curves for the prokaryotic and fungi observed ASVs at 97% sequence identity.

363   **Fig 3**. α- and β-diversity indexes of prokaryotes.

364   **Fig 4**. Venn diagram showing the distribution of shared ASVs across the termite gut.

365

366

367   **Tables and Figures legends**

368   **Table 1**: The statistical analysis of ITS sequence data were performed based on the Hellinger

369   transformation and Bray-Curtis distance-based dissimilarity matrix.

370   **Fig 1**. (A) 16S rRNA gene (B) ITS sequence based taxonomic distribution in triplicate gut

371   samples.

372   **Fig 2**. (A) 16S rRNA gene. (B) ITS sequences.

373   **Fig 3**. (A) Shannon diversity index (B) Unweighted Unifrac distance plot. The red, blue, green

374   and orange dots represent the gut microbiome of *C. cumulans*, *M. strunckii*, *N. corniger* and *T.*

375   *riograndensis*, respectively. The circle and triangle denote the soil/grass and wood feeders,

376   respectively. The first and second principal components explain 34% (PC1) and 24% (PC2) of

377   variations, respectively.

378   **Fig 4**. (A) Shared prokaryotic ASVs between four gut microbiomes and (B) Shared fungal ASVs

379   between the three termites.

380

381   **Supporting information**

382   **Fig S1.** Prokaryotic α-diversity measures (Shannon, Evenness and Observed) for the four species

383   of termites. Comparisons were performed using the Kruskal-Wallis followed by FDR method in

384   Qiime2.

385   **Fig S2.** Prokaryotic α-diversity measures (Shannon, Evenness and Observed) according to diet

386   groups (strict wood-feeders versus soil/grass- feeders). Comparisons were performed using the

387   Kruskal-Wallis followed by FDR method in Qiime2.

388   **Fig S3**. Fungal α-diversity measures (Shannon, Observed and Evenness) in three species of

389   termites. Comparisons were performed using Kruskal-Wallis followed by FDR method in

390   Qiime2.

391 **Fig S4**. Fungal α-diversity measures (Shannon, Observed and Evenness) according to diet groups

392 (strict wood-feeders versus soil/grass- feeders). Comparisons were performed using Kruskal-

393 Wallis followed by FDR method in Qiime2.

394 **Fig S5**. NMDS plot for the three termite gut samples using Bray Curtis analysis for ITS

395 sequences.

396

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Figure 1

Relative abundance of bacteria, archaea (phyla level) and fungi (class level) in the gut of Neotropical termites

(A) 16S rRNA gene (B) ITS sequence based taxonomic distribution in triplicate gut samples.

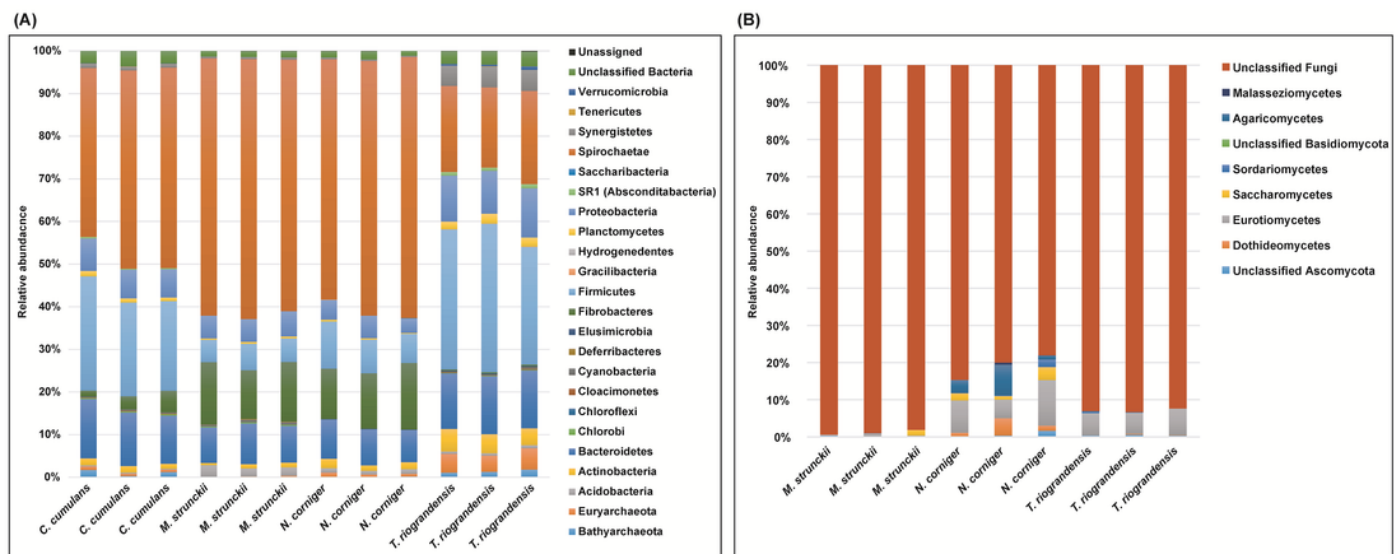



Figure 2

Rarefaction curves for the prokaryotic and fungi observed ASVs at 97% sequence identity

(A) 16S rRNA gene. (B) ITS sequences.

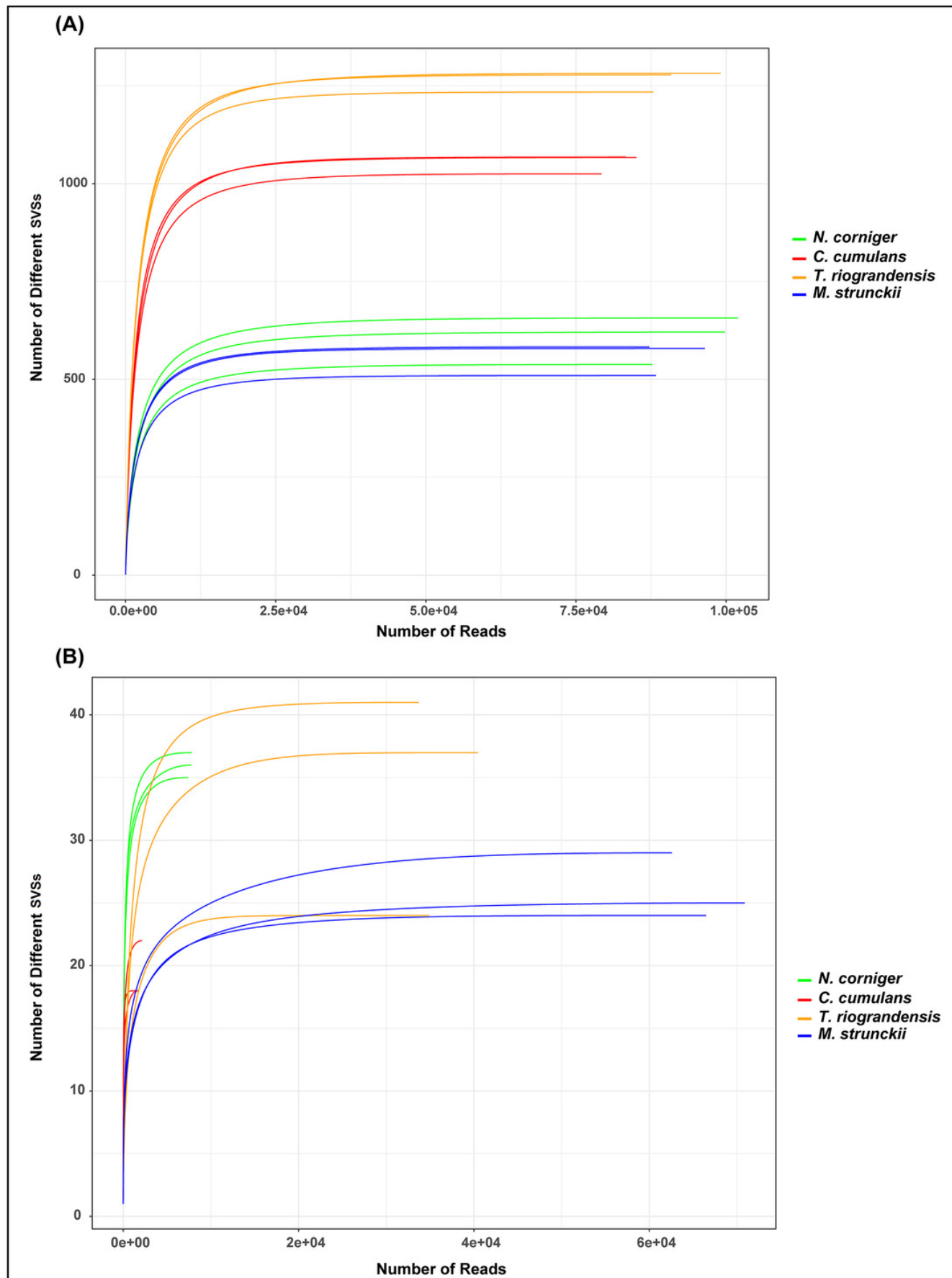



Figure 3

α- and β-diversity indexes of prokaryotes

(A) Shannon diversity index (B) Unweighted Unifrac distance plot. The red, blue, green and orange dots represent the gut microbiome of *C. cumulans*, *M. strunckii*, *N. corniger* and *T. riograndensis*, respectively. The circle and triangle denote the soil/grass and wood feeders, respectively. The first and second principal components explain 34% (PC1) and 24% (PC2) of variations, respectively.

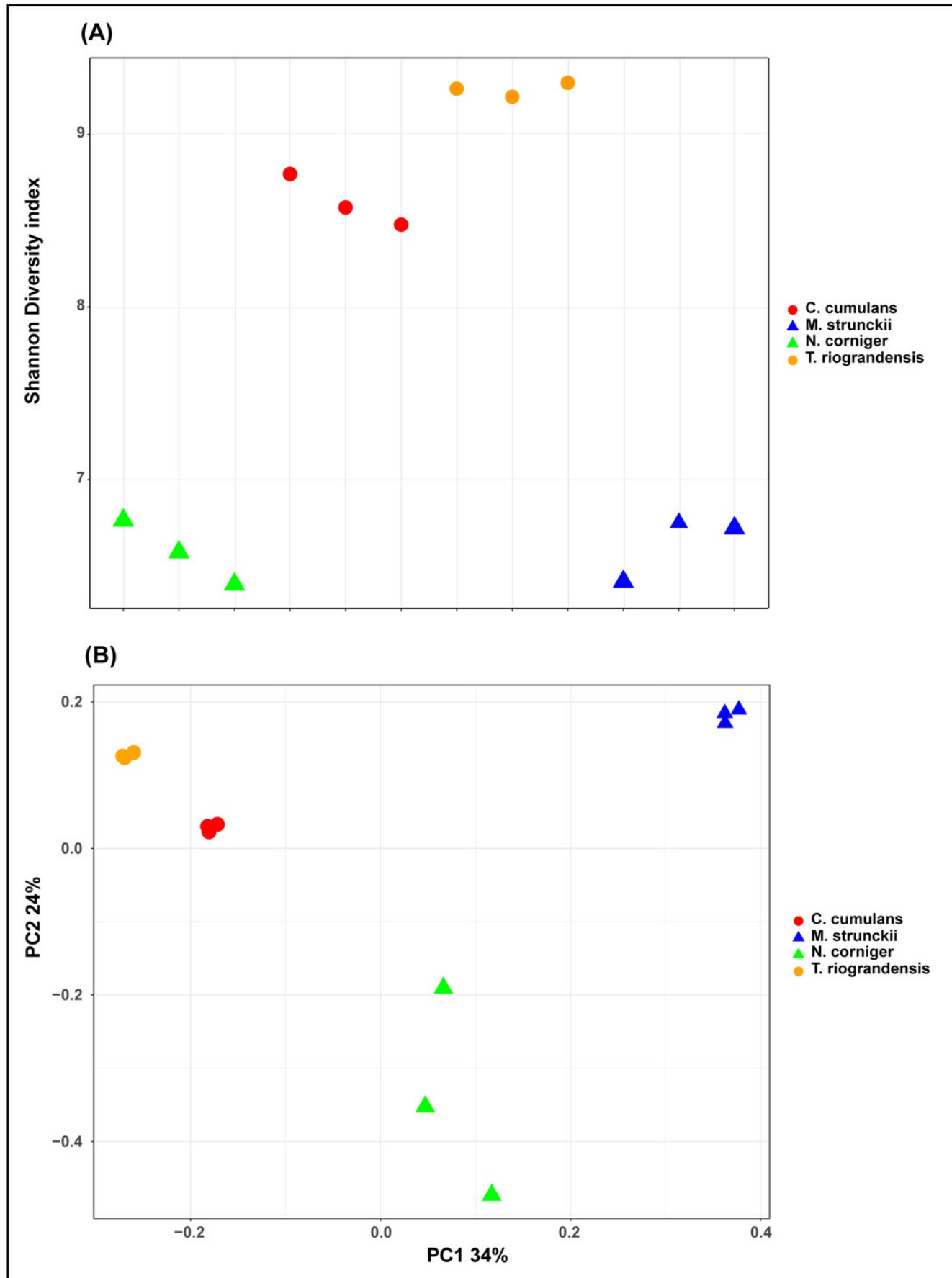
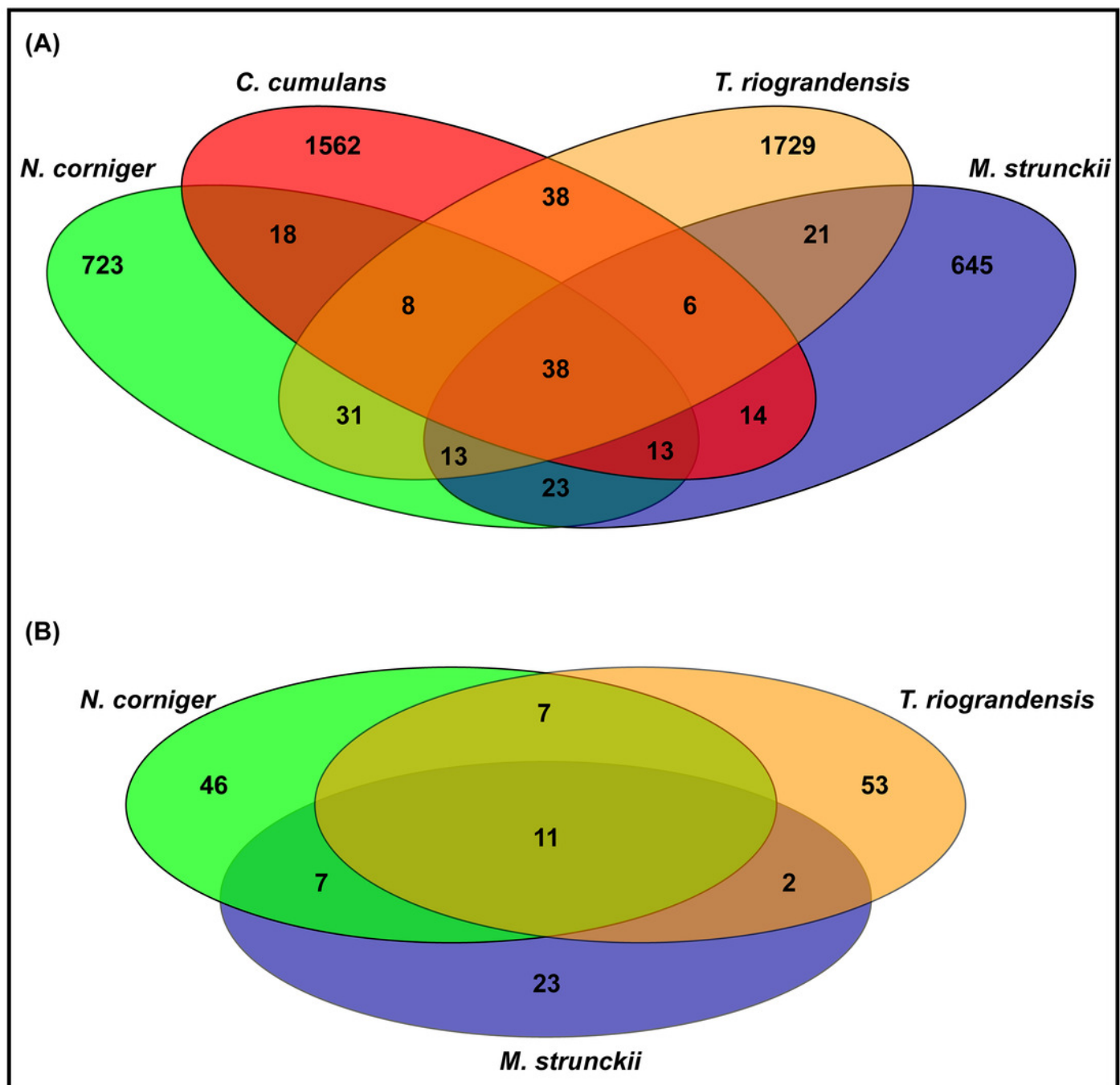



Figure 4

Venn diagram showing the distribution of shared ASVs across the termite gut

(A) Shared prokaryotic ASVs between four gut microbiomes and (B) Shared fungal ASVs between the three termites.

PERMANOVA analysis of bacteria/archaea and fungi

The statistical analysis of ITS sequence data were performed based on the Hellinger transformation and Bray-Curtis distance-based dissimilarity matrix.

16S rRNA		
	Based on termite species	
	Unweighted Unifrac	Weighted Unifrac
Pseudo F	8.5445	67.0377
p **value**	0.001	0.001
	Based on diet groups	
Pseudo F	4.5241	12.4488
p **value**	0.002	0.002
ITS (Bray-Curtis distance dissimilarity matrix)		
	Based on termite species	
Pseudo F	13.1240	
p **value**	0.005	
	Based on diet groups	
Pseudo F	3.8839	
p **value**	0.03	

Relative abundance (%) of *C. cumulans*, *M. strunckii*, *N corniger* and *T. riograndensis* core microbiota

Domain	Phylum	Class/Family	Genus or lowest classification available	Number of OTUs in taxon	Average abundance (%)	*C. cumulans* reads (%)	*M. strunckii* reads (%)	*N. corniger* reads (%)	*T. riograndensis* reads (%)
Bacteria	Spirochaetae	Spirochaetaceae	Termite Treponema cluster	15	17.70	11.35	24.90	33.79	0.58
	Spirochaetae	Spirochaetaceae	Treponema	3	1.33	0.68	1.21	3.71	0.06
	Firmicutes	Lachnospiraceae	Tyzzerella	1	0.33	0.04	1.24	0.02	0.01
	Firmicutes	Ruminococcaceae	Ruminococcaceae NK4A214 group	1	0.38	0.01	0.004	0.009	1.48
	Firmicutes	Streptococcaceae	Lactococcus	1	0.30	0.009	0.006	0.002	1.17
	Firmicutes	Enterococcaceae	Enterococcus	1	0.14	0.01	0.004	0.51	0.01
	Firmicutes	Clostridiales	uncultured Clostridiales bacterium	1	0.12	0.01	0.44	0.002	0.009
	Firmicutes	Clostridiales	Lachnospiraceae	1	0.28	0.006	0.004	0.01	1.11
	Bacteroidetes	Rikenellaceae	uncultured Bacteroidales bacterium	2	1.49	0.06	4.57	1.26	0.06
	Bacteroidetes	COB P4-1 termite group	uncultured Bacteroidales bacterium	1	0.18	0.006	0.68	0.01	0.01
	Bacteroidetes	Marinilabiaceae	uncultured Rikenellaceae bacterium	1	0.03	0.0008	0.001	0.09	0.003
	Bacteroidetes	Draconibacteriaceae		1	0.23	0.91	0.001	0.007	0.01
	Fibrobacteres	Fibrobacteraceae	uncultured Fibrobacteres bacterium	2	1.12	0.05	0.018	4.36	0.01
	Fibrobacteres	Chitinivibrionia Incertae Sedis	uncultured Chitinivibrionia bacterium	1	0.20	0.10	0.76	0.008	0.006
	Fibrobacteres	possible family 02	uncultured Chitinivibrionia bacterium	2	0.05	0.009	8.94	0.21	0.05
	Proteobacteria	Deltaproteobacteria	uncultured delta proteobacterium	1	1.29	3.50	1.60	0.024	0.02
	Actinobacteria	Micrococcales		1	0.35	0.01	0.005	0.01	1.35
Archaea	Bathyarchaeota			1	0.27	1.06	0.001	0.005	0.01
	Euryarchaeota	Methanosarcinaceae	Methanimicrococcus	1	0.45	0.005	0.001	0.003	1.79
Eukarya (Fungi)	Fungi Unclassified		Fungi	7	68.69	-	75.13	64.72	66.22
	Ascomycota		Ascomycota	1	0.05	-	0.07	0.008	0.06
	Ascomycota	Eurotiomycetes	Byssochlamys	1	0.84	-	0.004	2.50	0.004
	Ascomycota	Eurotiomycetes	Spiromastix	1	1.77	-	0.03	0.29	4.97
	Basidiomycota	Malasseziomycetes	Malassezia	1	0.01	-	0.013	0.01	0.004