

Persistence of Functional Microbiota Composition Across Generations

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1 **Persistence of functional microbiota composition across**
2 **generations**

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17

18 **Abstract**

19 **Background:** Holobionts are defined as a host and its microbiota, and there is not a consensus
20 about their status as a unit of selection. The “it’s the song, not the singer” theory proposes that
21 functional traits, instead of taxonomical composition, could be preserved across generations if
22 interspecies interaction patterns perpetuate themselves. We used a novel combination of community
23 level analysis on the functional composition of microbiota-communities to test this theory by using
24 empirical and simulated data. We tested the conservation of functional composition across
25 generations using mosquito and plant datasets. Then, we tested if there is a change of functional
26 composition over time within a generation in human datasets. Finally, we simulated microbiota
27 communities with different amounts of pairwise interspecies interactions and initial configurations
28 to investigate if the interactions can lead to multiple stable community compositions.

29 **Results:** Our results suggest that the vertically transmitted microbiota starts a predictable change of
30 functions performed by the microbiota over time (i.e. an ecological succession) whose robustness
31 depends on the arrival of diverse migrants. This succession culminates in a stable functional
32 composition state. The pairwise interactions between species of the community are not sufficient to
33 explain the stability of the final community and the existence of alternative stable states, which
34 suggests that the host-microbiota interaction and non-pairwise interactions in general have an
35 important contribution to the robustness of the final community.

36 **Conclusions:** If the proposed mechanism proves to be valid for a diverse array of host species, this
37 would support the concept of holobionts being used as units of selection, suggesting this has a wider
38 applicability, including animal breeding.

39

40

41 **Background**

42 A holobiont is defined as the combination of an organism (also called host) with its microbiota [1, 2]. The
43 microbiota affects the phenotype of a host, and that effect is different from the host genotype or its
44 environment [3]. The microbiota composition is at least partially transmitted across generations, which
45 makes it a non-genetic form of inheritance [4]. Non-genetic forms of inheritance are not yet incorporated
46 into breeding strategies [4]. Hence, to understand how the microbiota is assembled within a host, and then
47 inherited across generations, could increase the progress of animal breeding programs.

48 The mechanisms of symbiont microbiota inheritance and persistence vary across species of hosts and
49 symbionts [5]. This phenomena is observed throughout evolution, in the animal kingdom for instance, squid
50 (*Euprymna scolopes*) parents release the bacteria *Vibrio fischeri* to open water and by that way transmitting
51 the bacteria [6]; Juvenile koalas get tannin degrading bacteria eating enriched maternal feces called 'pap'
52 [7]; In chickens, bacteria from the cloaca and oviduct colonize the egg shell, egg white and from there, the
53 embryo [8, 9]; In livestock, newborns are initially colonized through physical contact when passing through
54 the birth canal [4]; within the plant kingdom, some plants transmit specific bacteria and/or fungi via
55 vegetative reproduction [10]. Such transmission systems can be classified as vertical, horizontal, or a
56 combination of both [11].

57 Besides the arrival of a specific symbiont to a host, its extinction or survival depends on the interactions
58 with the host and the resident microbiota, and whether or not the host environment acts as a filter [12].

59 The host regulates the microbiome composition through a constant crosstalk that involves its innate and
60 adaptive immune system as well as the epithelial cells in direct contact with symbionts [13]. The
61 interactions or lack thereof between symbionts are typically described with competition models borrowed
62 from ecology, that incorporate or omit time structure [14]. For instance, the generalized Lotka-Volterra
63 models use a matrix to represent the interactions between every pair of species in a community (pairwise
64 species interactions) and describe the population dynamics over time of each species with a differential

65 equation [14]. The self-organized instability (SOI) model also describes interspecies interactions using a
66 matrix, but the population dynamics are stochastic and represented with a mechanistic set of rules that
67 allows the simulation of a community [15]. Such descriptions do not capture higher order interactions nor
68 account for the migration between hosts [12]. The process of maturation of a community within a host
69 sometimes displays clear sequences, as the transition from facultative to strict anaerobic bacteria in
70 humans [16]; this process is analogous to an ecological succession, which in this case leads to a stable
71 community [17, 18].

72 Whether or not a holobiont can be considered as an evolutionary unit of selection is still debated [2, 3, 11,
73 19–21]. Given the diverse array of symbionts within a host, selection at the holobiont level cannot account
74 for all the host-symbiont interactions, nor the interactions between symbionts, as the requirements for
75 partner fidelity are unlikely to be met for every interaction [20]. Yet, there are developmental,
76 physiological, anatomical, and immunological traits that rely on host-symbiont interactions [11]. Doolittle &
77 Booth [19] proposed that interspecies interaction patterns can perpetuate themselves over time through
78 “recurrence” (as opposed to replication), regardless of the specific species that form part of the interaction.
79 The mechanism of mutual perpetuation between the interaction patterns and the specific traits of the
80 interacting species is called the “It’s the song, not the singer” (ITSNTS) theory [22].

81 The ITSNTS theory applied to holobionts [19] assumes that the interaction patterns within a single
82 generation remain constant across time, but this does not need to be the case. Chang et al. [23] and Shaw
83 et al. [24] showed that if we consider microbiota as a dynamical system, then the communities display
84 metastability: there are several possible discrete stable configurations, that correspond to alternative late
85 succession communities. For instance, coral reefs can display bistability [25] and semiarid/arid communities
86 are two configurations of a bistable system that depends on the aridity level [26]. The coexistence of
87 alternative late successional states is also displayed by ecological systems with asymmetrical competition
88 [27] and by metacommunities far from equilibrium (steady state) or with variation in local community

89 quality or species traits [28]. Given that the attractor (i.e. a stable discrete configuration) reached by a
90 community depends on the initial state, as shown e.g. for coral reefs [25], metastability combined with the
91 aforementioned ecological succession could account for the conservation of functional composition (the
92 biochemical functions performed by the symbionts) across generations predicted by Doolittle & Booth [19].
93 The vertically inherited fraction of the community could define a starting configuration, from which the
94 community converges to a specific attractor in a functional space, regardless of the taxa of the migrants.
95 Therefore, our objectives were to investigate to what extent functional composition is preserved across
96 generations in experimental lab conditions (1), and to propose a mechanism for this preservation based on
97 the ITSNTS theory. For the latter, we tested if there is an ecological succession functional space, and if it
98 depends on the initial community composition (2); we then searched for metastability in the functional
99 composition of communities (3); and tested if pairwise interactions can account for that metastability (4).

100 These objectives were addressed by investigating different publicly available datasets that matched the
101 requirements for one or more of those objectives. For (1), we compared the functional composition of
102 mosquito and plant microbiota across generations. For (2), we compared the functional composition of
103 monthly fecal samples from babies born either vaginally or with caesarian section. All datasets can display
104 metastability and are used for (3). We simulated communities based on stochastic mechanistic models for
105 (4). Whereas (1) tests directly the ITSNTS theory applied to holobionts, (2) and (3) together test a possible
106 mechanism of transmission across generations and (4) narrows down the set of conditions needed by that
107 mechanism.

108

109 **Methods**

110 **Datasets**

111 To test functional persistence across generations, we used the mosquito dataset from [29] and the
112 plant dataset from [10] (Table 1). The first one comprises generations 0, 5 and 10 of two isolated
113 mosquito populations bred with lab or field water, originally used to test if the microbiota is
114 preserved or not under different lab breeding conditions (with the different water sources). The
115 second one comprises generations 0, 1 and 2 of plants cloned via stolons used to infer a core set of
116 taxa transmitted vertically common to 10 representative ecotypes of the species. Hence, horizontal
117 transmission was completely inhibited and both roots and stolons were sampled.

118 To test the ecological succession on the functional landscape, we used the human datasets of [30]
119 and [31]. The first one was originally used to describe how different perturbations (antibiotics, birth
120 mode, diet) affect the early community development and the second one is part of the
121 DIABIMMUNE project, aimed at testing the role of the microbiota in the development of
122 autoimmune diseases. These two human datasets comprise the fecal microbiota of approximately 40
123 and 200 human babies (Table 1) repeatedly sampled from birth and up to three years, born either
124 naturally or with a caesarian section.

125 **Data processing and analysis**

126 All the raw samples were processed using the DADA2 pipeline [32]. The human samples from [30]
127 were truncated at 150bp, the forward and reverse mosquito samples were truncated at 250 and
128 200bp respectively and both forward and reverse plant samples were truncated at 250bp; Every
129 other filter and trimming parameter was kept at the standard configuration for all samples (maxN =
130 0, maxEE = 2, truncQ = 2, rm.phix = TRUE). The unique sequences were inferred without pooling
131 across samples. The corresponding operational taxonomic unit (OTU) tables were functionally

132 annotated using Tax4Fun2 using the default reference database and parameters [33]. The second
133 human dataset was already processed through the MGnify pipeline [34] version 4.1 [35] (Study
134 identifier MGYS00003619, project identifier PRJEB26925), and the Gene Ontology (GO)
135 composition of each community was used directly.

136 **Landscape analysis of the functional composition**

137 The functional predictions were then used to run the landscape model [23]. The frequencies of the
138 predicted functions were used to calculate the Jensen Shannon divergence between communities,
139 instead of the frequencies of OTUs. The resulting dissimilarity matrices were then used to run a
140 Principal Coordinate Analysis (PCoA). The two first Principal Coordinates (PCos) were used as the
141 filter functions for the Mapper algorithm [36], with the following hyperparameters for all datasets:
142 Number of intervals for rank = 15 for both PCos, % overlap = 70% and number of bins = 10.
143 Finally, the mapper output was transformed in a directed graph to find the attractors and
144 corresponding basins [23].

145 **Simulations of the Hubbell and SOI model**

146 Simulations were run to test if pairwise interactions can account for metastability in the taxonomical
147 community composition. The communities were simulated using either the SOI model [15] or the
148 Hubbell model [37] using the R-package seqtime [14]. While the Hubbell model simulates
149 communities without pairwise interactions, the SOI model includes the fraction of all the possible
150 pairwise interactions as a parameter (connectivity). The chosen connectivity values were 0 (the
151 Hubbell model), 0.01 (as used in the simulations of [14]), and 0.1 (as used in [15]). In addition, a
152 fraction of individuals from the initial composition was fixed: every simulation started with a
153 randomly chosen and a fixed group of individuals.

154 Either 0, 100, or 200 of all 500 simulated individuals were fixed. 450 replicates were simulated for
155 every parameter combination. Every replica from a given parameter combination shares the
156 interaction matrix, the initial fixed individuals, and the migration probabilities. When generating the
157 interaction matrices, the positive edge percentage (the percentage of non 0 values of the interaction
158 matrix that are greater than 0) was kept at most at 30%, switching randomly the signs of the matrix
159 elements [14]. All communities were simulated for 600 timesteps. For all the communities the
160 metacommunity species number was kept at 50, the migration probability per species was drawn
161 from a standard uniform distribution.

162 The dissimilarity between pairs of communities with the same parameters was measured using the
163 Morisita index (which increases with higher beta diversity and approaches 0 for completely
164 different communities) [38], and each community was used only once, resulting in 225 pairs. The
165 effect of the connectivity, the number of fixed individuals and the interaction between both on the
166 Morisita index was tested using a generalized linear model with an inverse link and a gamma
167 distribution:

$$168 \quad y_{ijk} = g^{-1}(\beta_0 + \beta_1 c_i + \beta_2 x_j + \beta_3 c x_{ij} + e_{ijk})$$

169 Where c_i is the connectivity, x_j is the number of individuals fixed at the start of the simulation, $c x_{ij}$
170 is the interaction term and y_{ijk} is the Morisita index. The main and nested models were ranked
171 according to their Akaike information criterion corrected for small samples (AICc), using the
172 MuMIn package [39].

173

174

175 **Results**

176 **Persistence of functional traits across generations**

177 Both the mosquito and plant datasets show metastability (Figure 1 C and D). Additionally, both
178 populations show an increase in the diversity of occupied attractors across generations (Figure 1 E
179 and F) even when accounting for the plant tissue or the water type, respectively (Figure 1 A, B, E
180 and F). The lab water-bred mosquitoes belong to a single attractor, or do not belong to an attractor
181 at all (Figure 1 B and D). There are fewer counts on generation 5 of lab water mosquitoes and not a
182 single count from generation 10 mosquitoes, because every corresponding vertex is a singleton (has
183 a single sample) and thus is removed from the mapper plot. The increase of diversity of occupied
184 attractors and the increase of singletons imply that the functional composition is not preserved
185 across generations.

186 **Ecological succession in the functional space**

187 After comparing the functional composition across generations, we tested if there is a successional
188 pattern in the human datasets within a generation. The mapper algorithm also splits the mapper
189 network into attractors for both datasets (Figure 2B and Figure 3B); but, given that the samples only
190 span the first three years of life of every subject, those attractors are only transiently occupied. All
191 the samples converge to the attractors, regardless of the source of the microbiota (vaginal or
192 caesarean section) (Figure 2 A, B and Figure 3 A, B). There are clear trajectories in the functional
193 space across time that are independent of the source of the microbiota (Figure 2 C, D and Figure 3
194 C, D).

195 **Intraspecies interactions as a source of metastability**

196 Having found evidence suggesting metastability in the plant and mosquito datasets, we simulated
197 communities to investigate if pairwise interspecies interactions can account for it. As the amount of

198 interactions increases, the differing starting conditions could either lead to convergence or
199 divergence of community composition between pairs of communities. This would be reflected as a
200 change in the Morisita index explained by the interaction between the two parameters (connectivity
201 and initial fixed individuals). Most Morisita index values lie between 0 and 0.75 (Figure 4), and all
202 are smaller than 1. Hence, the alpha diversity is higher than the beta diversity for all simulations, i.e.
203 the diversity within hosts is higher than the diversity between hosts. The best model only includes
204 connectivity (Table 2) and the complete model only appears in third place. As there is not a
205 significant difference between the best and complete models (Deviance = 1.2672, df = 2, P =
206 0.2853), there is no evidence that the interaction effect predicts the beta diversity. Therefore, the
207 results of the simulations do not support metastability. In other words, the different initial
208 conditions do not diverge to different community states over time, even when there is high
209 connectivity.

210

211

212 **Discussion**

213 In this research we tested if there is conservation of microbiota functional composition across
214 generations using plants and mosquitoes as model organisms. Then we explored possible
215 mechanisms to explain this conservation across generations. Two human datasets were used to
216 investigate if the functional composition can be understood as an ecological succession; in other
217 words, if there is a robust change of functional composition over time, common to every host.
218 Finally, we simulated communities to observe if pairwise interactions between bacteria can generate
219 the metastability needed to explain the succession. This section starts with discussing the
220 implications of the results of each of those individual analyses. Thereafter, we make a synthesis of
221 our results, which allows to propose a mechanism that could explain the inheritance of the
222 functional composition of microbiota across generations. We end with a brief discussion of possible
223 implications for animal breeding.

224 **Conservation of functional composition across generations**

225 Neither plants nor mosquitoes showed conservation of functional composition across generations.
226 This could imply that the hypothesis from Doolittle & Booth [19] only works when there is a wide
227 enough metacommunity to draw migrants from, as both mosquitoes and plants were bred under
228 conditions with decreased or non-existent horizontal transmission. Similarly, plant monocultures
229 show decreased taxonomical diversity [40], and decreased vertical and horizontal transmission is
230 leading to cumulative microbial extinctions in humans, affecting the phenotype and development of
231 the immune system [41]. In neutral metacommunities, as the migration rates decrease, beta diversity
232 increases at the cost of alpha diversity [42]. That increase in beta diversity could explain the
233 functional divergence if stochastic processes can override deterministic processes in both mosquito
234 and plant populations. Alternatively, it could be that only a core subset of functional traits gets

235 preserved across generations. Assuming competition between functionally similar bacteria, Jiang et
236 al. [43] showed that there is a subset of genes that shapes the structure of a community, denoted as
237 community structure and shaping genes. Given that those genes are carried by a minority of the
238 community [43], the communities across different generations would not belong to the same
239 attractors. Finally, (microbial) gene composition and community structure do not define function on
240 their own [44]. There could be conservation at a metabolome or gene expression level instead of
241 higher biological levels (KEGG and GO). Thus, the actual proteins and or metabolites produced by
242 the microbiota in a host could be preserved even if the gene composition or the predicted functions
243 of the genes change. This could be tested by using metabolome or gene expression datasets (instead
244 of the KEGG or GO annotations used here) to build the dissimilarity matrices used to find the
245 attractors.

246 **Ecological succession in the functional space**

247 The human datasets showed a robust ecological succession across the functional phase space, in
248 agreement with the bacterial traits based succession from [45]. This succession can be understood
249 as an extension of the development of the host [44]. This succession was not disrupted by the
250 differing modes of delivery, suggesting that it is robust for a wide array of initial community
251 compositions, which is also true for most traits in the succession from [45]. Similarly, the phylum
252 level composition of the gut microbiota during the first weeks has a trajectory over time that is
253 independent of the delivery mode and is at least partially driven by interphylum interactions [46].
254 Given that breastfeeding affects microbiota alpha and beta diversity [30], as well as functional
255 composition [47], it could be argued that it contributes to the stability of the ecological succession.
256 Since both datasets used here contain breastfed and non-breastfed individuals and all samples still
257 converge to a single attractor at the end, this contribution is not the only stabilizing mechanism.
258 Additionally, the crosstalk between innate immune system, the epithelial layer, and the microbiota

259 control the community composition [13]. In other words, both the resilience and resistance of the
260 community [44] and host mediated mechanisms could contribute to the stability of the ecological
261 succession.

262 **Pairwise interactions as a cause of metastability**

263 The SOI simulations did not display metastability, which suggests that pairwise interactions alone
264 do not account for community metastability. The generalized Lotka-Volterra (gLV) models also
265 emphasize first order interspecies interactions [14], so the analytical results of gLVs should
266 coincide with the results of the SOI model. It has been shown that as community richness increases,
267 the probability of gLVs of having fixed points (community configurations that remain constant over
268 time) decreases exponentially (citar 46). Moreover, the probability of stability of the fixed points
269 increases asymptotically to one [48], which means that the communities will be able to reach those
270 points. If instead of drawing parameters for the species within a community they are drawn for a
271 pool of species from a metacommunity, the communities display metastability for specific regions
272 of the parameter space, particularly for large metacommunity species pools [49]. Furthermore, those
273 attractors are non-fixed points, and the community composition is history dependent and can be
274 changed with perturbations [49]. Nevertheless, gLV models can fail to capture the qualitative
275 dynamics of microbiota community when the mechanisms of interaction cannot be represented by
276 additive pairwise effects in the fitness of the populations [50]. Goyal et al. [51] developed a model
277 that only assumes that each species will only consume one resource at a time and prefers some
278 nutrients over others, and it also displays metastability and transition between local attractors.
279 Experimentally, the fruit fly (*Drosophila melanogaster*) gut microbiota displays metastability,
280 caused by the different colonization strategies of each symbiont (e.g. the preferential attachment to
281 a tissue) and stochasticity [52]. Therefore, metastability and history dependence can arise from

282 pairwise interactions if there is a large metacommunity species pool or with non-pairwise
283 interactions, as with nutrient preference or preferential attachment to a tissue.

284 **A proposed mechanism for the ITSNTS theory applied to holobionts**

285 Taking all the results together, we propose the following mechanism (Figure 5) that extends the
286 ITSNTS theory [22]. Given a population of holobionts and a rich metacommunity, the vertically
287 inherited fraction of the microbiota defines the initial composition of a host. From that starting
288 composition, there is a robust trajectory in the functional space analogous to an ecological
289 succession, showed in the human datasets. This succession requires horizontal transmission and
290 relies on the (high) richness of the metacommunity, as suggested by the mosquito and plant datasets
291 that lacked those and did not show succession. In our simulations the pairwise interactions alone
292 were insufficient to achieve stability of the trajectory and the mature community, suggesting that
293 this additionally requires interactions between symbionts and with the host. If the trajectory always
294 converges to the same attractor in the functional space, there would be conservation of functional
295 composition across generations. Further experiments are needed to test the complete mechanism,
296 particularly if the predicted gene functions are the adequate level of resolution to look for functional
297 convergence and if there is a robust trajectory in the functional space leading to that convergence
298 for other organisms. The robustness of the functional succession and the final composition need to
299 be tested experimentally. Moreover, there is not yet a model that explains how and when the host
300 could induce or promote the community level metastability and robustness. From an ecological
301 perspective, it remains to be seen if there is a characteristic function-abundance distribution, and if
302 there is, how it could be influenced by stochastic and deterministic assembly processes. From an
303 evolutionary perspective, that function-abundance distribution and the (functional) composition
304 could also be affected by demographic (host population growth and decrease), microevolutionary

305 (host selection, drift, migration and mutation) and holobiont specific (symbiont mutation, horizontal
306 gene transfer, horizontal transmission) processes.

307 **Implications for animal breeding**

308 The holobiont concept has practical implications for animal breeding [3]. If the functional (instead
309 of the taxonomical) composition is preserved across generations, then the mixed models used in
310 animal breeding could be adapted to account for that, hence potentially increasing the explained
311 phenotypic variance. A similarity matrix between individuals has been calculated based on the
312 taxonomical composition [4, 53–55], therefore, individuals with different bacteria that perform
313 similar functions will still have a high dissimilarity. Nevertheless, if the functional composition is
314 vertically inherited according to the proposed mechanism, then using similarity calculated based on
315 the functional composition could increase the progress over time. Finally, if the community of a
316 given holobiont follows a clear ecological succession, then the optimal moment to sample
317 microbiota composition would be when this equilibrium is reached. The trait turnover in the
318 succession from Guittar et al. [45] decreased before the taxonomical turnover, suggesting that the
319 functional composition could also reach a mature state earlier than the taxonomical composition.

320

321 **Conclusions**

322 The main limitation of considering holobionts as units of selection is that most of the microbiota
323 could not be actively selected for, which gave rise to the idea that the functions performed by the
324 symbionts could self-perpetuate over host generations regardless of who is performing the function.
325 In this study we propose that vertically inherited symbionts start an ecological succession that
326 reconstructs the final functional composition of the community, which requires the arrival of
327 horizontally transmitted migrants to be robust. This cannot be explained with pairwise interspecies

328 interactions only, hence host-microbiota interactions and non-pairwise interactions are required to
329 explain the stability of the final community. To establish whether the concept of holobionts as units
330 of selection have a wider applicability, requires investigating this mechanism for a wide array of
331 host species.

332

333 **List of abbreviations**

334 gLV: generalized Lotka Volterra

335 GO: Gene Ontology

336 ITSNTS: It's the song, not the singer (theory)

337 KEGG: Kyoto Encyclopedia of Genes and Genomes

338 OTU: Operational Taxonomic Unit

339 PCoA: Principal Coordinates Analysis

340 SOI: Self-Organized Instability

341

342 **Declarations**

343 **Ethics approval and consent to participate**

344 Not applicable.

345 **Consent for publication**

346 Not applicable.

347 **Availability of data and materials**

348 All the code for the landscape analysis is available on Github ([https://github.com/Christian-Ramos-](https://github.com/Christian-Ramos-Uria/Holobiont-Landscape/Landscape)

349 [Uria/Holobiont-Landscape/Landscape](https://github.com/Christian-Ramos-Uria/Holobiont-Landscape/Landscape)). All the code for the community simulations is available on

350 Github (<https://github.com/Christian-Ramos-Uria/Holobiont-Landscape/Beta-diversity>).

351 The datasets analysed during the current study are available in the datadryad and ENA repositories,
352 <https://datadryad.org/stash/dataset/doi:10.5061/dryad.98jj7gk> [29],
353 <https://www.ebi.ac.uk/ena/data/search?query=PRJEB20603> [10],
354 <https://www.ebi.ac.uk/ena/data/search?query=PRJEB14529> [30],
355 <https://www.ebi.ac.uk/ena/data/search?query=PRJNA290380> [31].

356 **Competing interests**

357 The authors declare that they have no competing interests.

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359 Not applicable

360 **Authors' contributions**

361 CR and DS acquired the microbiota data, subsequently CR analyzed the data with guidance of MC
362 and DS. CR wrote the manuscript, with guidance of MC and DS. All authors read and approved the
363 final manuscript.

364

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368 **Author's information**

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370

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501

502

503 **Figures**

504 **Figure 1 Persistence of the functional composition of plant and mosquito microbiota across**
505 **generations.**

506 A) Mapper representation of root and stolon plant samples. The colour represents the fraction of
507 root samples per node. B) Mapper representation of the mosquito samples. The colour represents
508 the fraction of samples from mosquito communities bred in field water as opposed to lab water. C)
509 Partition of the plant mapper network into metastable states. Each colour represents a different
510 attractor. D) Partition of the mosquito mapper network into metastable states. Each colour
511 represents a different attractor. E) Count of plant samples that belong to each attractor, per
512 generation and tissue. R = root; S = Stolon. F) Count of mosquito samples that belong to each
513 attractor, per generation and treatment.

514

515 **Figure 2 Ecological succession of human microbiota communities in a functional phase space,**
516 **using the KEGG annotation.**

517 A) Mapper representation of the samples. The colour represents the fraction of samples from
518 individuals born vaginally, instead of caesarean section. B) Partition of the mapper network into
519 metastable states. Each colour corresponds to an attractor. C) and D) Attractor occupied per
520 individual and month. Each row corresponds to a single individual. The colours correspond to the
521 attractors in B. C) Individuals born vaginally. D) Individuals born via caesarean section.

522

523 **Figure 3 Ecological succession of human microbiota communities in a functional phase space,**
524 **using the GO annotation.**

525 A) Mapper representation of the samples. The colour represents the fraction of samples from
526 individuals born vaginally, instead of caesarean section. B) Partition of the mapper network into
527 metastable states. Each colour corresponds to an attractor. C) and D) Attractor occupied per
528 individual and month. Each row corresponds to a single individual. The colours correspond to the
529 attractors in B. C) Individuals born vaginally. D) Individuals born via caesarean section.

530

531 **Figure 4 Histograms of the Morisita dissimilarity index for pairs of simulated communities.**

532 Rows: fraction of the connectivity matrix filled with non-zero values (see methods). Columns:

533 Number of fixed shared individuals (out of a total of 500) at the start of each simulation.

534

535 **Figure 5 A mechanism for the “It’s the song, not the singer” theory applied to holobionts,**
536 **created with biorender (<https://biorender.com/>).**

537 The vertically transmitted microbiota initiates an ecological succession in the functional space,

538 which requires the arrival of horizontally transmitted symbionts. The final stable state reached is the

539 same as in the previous generation, which implies the conservation of the functional composition

540 across generations.

Figures

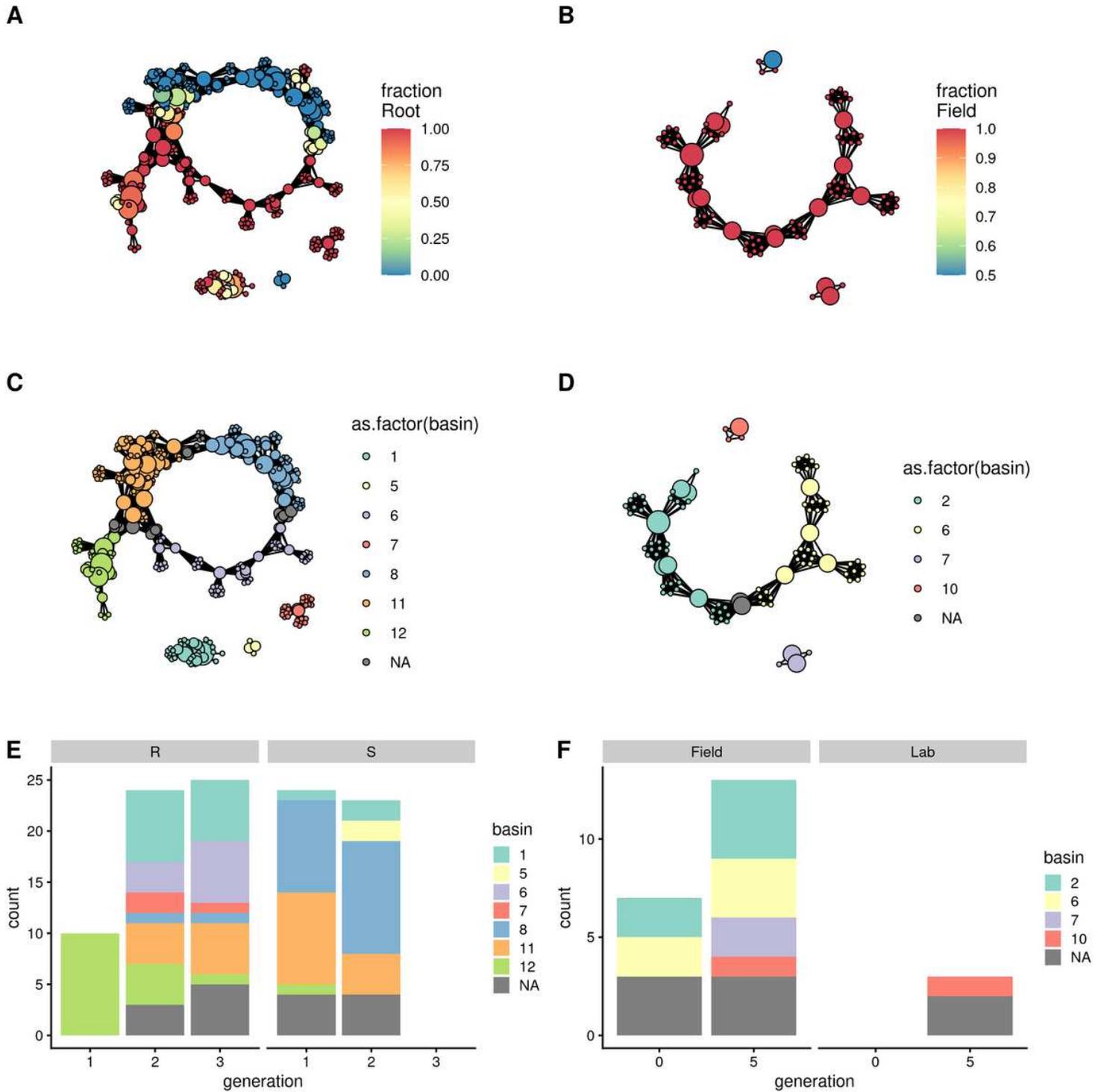


Figure 1

Persistence of the functional composition of plant and mosquito microbiota across generations. A) Mapper representation of root and stolon plant samples. The colour represents the fraction of root samples per node. B) Mapper representation of the mosquito samples. The colour represents the fraction of samples from mosquito communities bred in field water as opposed to lab water. C) Partition of the

plant mapper network into metastable states. Each colour represents a different attractor. D) Partition of the mosquito mapper network into metastable states. Each colour represents a different attractor. E) Count of plant samples that belong to each attractor, per generation and tissue. R = root; S = Stolon. F) Count of mosquito samples that belong to each attractor, per generation and treatment.

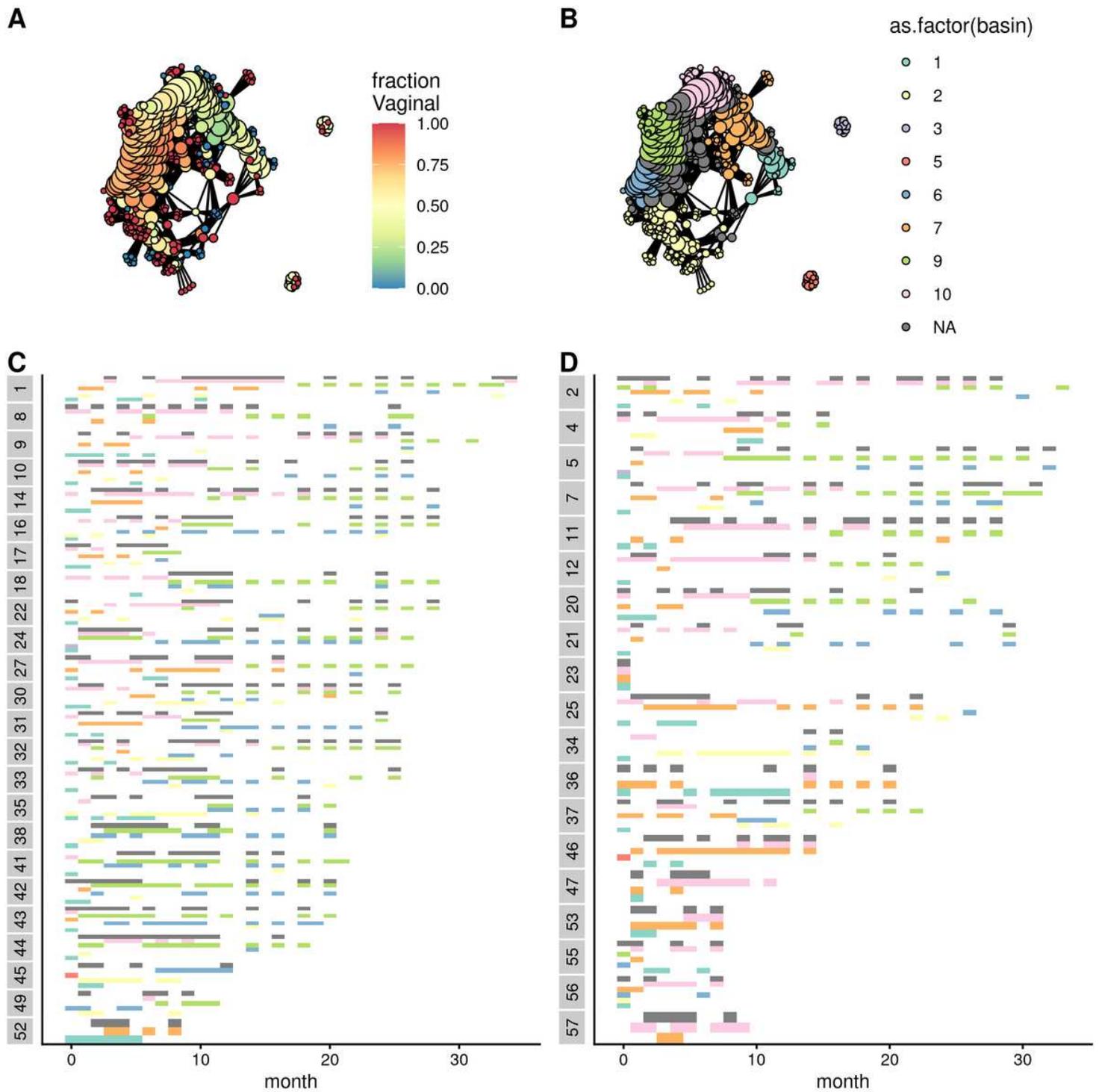


Figure 2

Ecological succession of human microbiota communities in a functional phase space, using the KEGG annotation. A) Mapper representation of the samples. The colour represents the fraction of samples from

individuals born vaginally, instead of caesarean section. B) Partition of the mapper network into metastable states. Each colour corresponds to an attractor. C) and D) Attractor occupied per individual and month. Each row corresponds to a single individual. The colours correspond to the attractors in B. C) Individuals born vaginally. D) Individuals born via caesarean section.

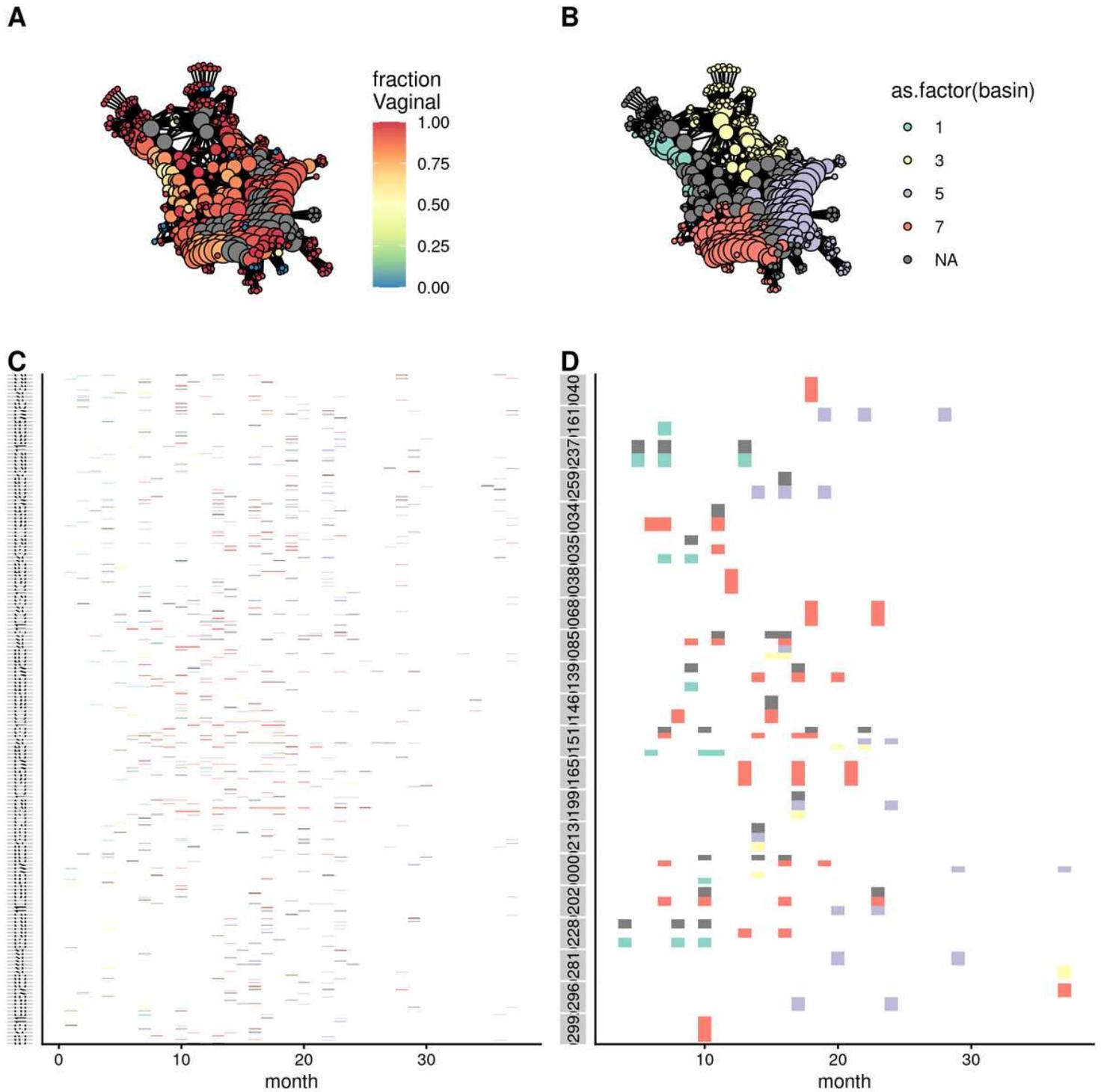


Figure 3

Ecological succession of human microbiota communities in a functional phase space, using the GO annotation. A) Mapper representation of the samples. The colour represents the fraction of samples from

individuals born vaginally, instead of caesarean section. B) Partition of the mapper network into metastable states. Each colour corresponds to an attractor. C) and D) Attractor occupied per individual and month. Each row corresponds to a single individual. The colours correspond to the attractors in B. C) Individuals born vaginally. D) Individuals born via caesarean section.

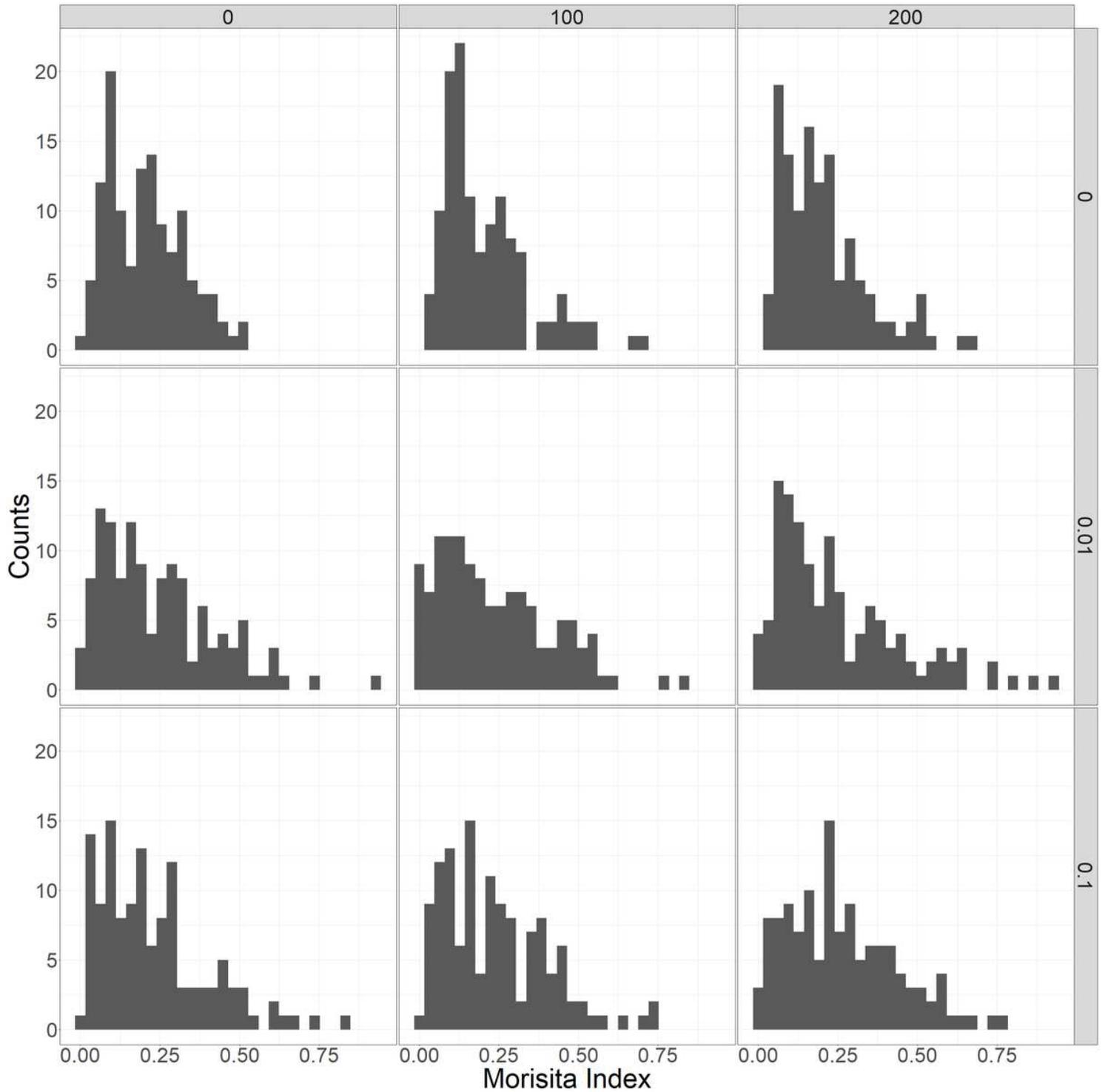


Figure 4

Histograms of the Morisita dissimilarity index for pairs of simulated communities. Rows: fraction of the connectivity matrix filled with non-zero values (see methods). Columns: Number of fixed shared

individuals (out of a total of 500) at the start of each simulation.