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1 **Network analysis of gut microbiota literature: an overview of the research landscape in non-human**  
2 **animal studies**

3

4 **Running title: Animal gut microbiota**

5

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26

27 **Abstract**

28 A wealth of human studies have demonstrated the importance of gut microbiota to health. Research on non-  
29 human animal gut microbiota is now increasing, but what insight does it provide? We reviewed 650  
30 publications from this burgeoning field (2009-2016) and determined that animals driving this research were  
31 predominantly domestic (48.2%), followed by model (37.5%), with least studies on wild (14.3%)  
32 animals. Domestic studies largely experimentally perturbed microbiota (81.8%) and studied mammals  
33 (47.9%), often to improve animal productivity. Perturbation was also frequently applied to model animals  
34 (87.7%), mainly mammals (88.1%), for forward translation of outcomes to human health. In contrast, wild  
35 animals largely characterised natural, unperturbed microbiota (79.6%), particularly in pest or pathogen  
36 vectoring insects (42.5%). We used network analyses to compare the research foci of each animal group:  
37 diet was the main focus in all three, but to different ends: to enhance animal production (domestic), to study  
38 non-infectious diseases (model), or to understand microbiota composition (wild). Network metrics quantified  
39 model animal studies as the most interdisciplinary, while wild animals incorporated the fewest disciplines.  
40 Overall, animal studies, especially model and domestic, cover a broad array of research. Wild animals,  
41 however, are the least investigated, but offer under-exploited opportunities to study real-life microbiota.

42

43 **Key-words** microbial ecology, microbial diversity, microbiome, network theory, wildlife

44 **The dawn of modern microbiota research**

45 Technological advances in multi-omic platforms such as metataxonomics and metagenomics, have helped  
46 fuel the recent expansion of microbiota research (Marchesi and Ravel, 2015), especially on humans, as  
47 exemplified by large-scale efforts such as The Human Microbiome Project, started in 2007 (Peterson *et al.*,  
48 2009). Research on microbiota from non-human habitats has followed: in 2010 the Earth Microbiome Project  
49 ([www.earthmicrobiome.org](http://www.earthmicrobiome.org)) was initiated to document microbial diversity across multiple biomes (Gilbert *et*  
50 *al.*, 2014). Studies focusing on microbiota of the gut have especially captivated scientific interest; it is the  
51 most dense and diverse microbial community of the body, is influenced by a range of intrinsic and extrinsic  
52 variables including diet, genetics and environmental factors (Khachatryan *et al.*, 2008; Phillips, 2009;  
53 Claesson *et al.*, 2012; Bright and Bulgheresi, 2010), and is vital to health and development (Round and  
54 Mazmanian, 2009; Lozupone *et al.*, 2012). In recent years non-human animal gut microbiota studies have  
55 started to appear, for example, characterising the microbiota of giant pandas, *Ailuropoda melanoleuca*, to  
56 make microbial comparisons across age groups (Tun *et al.*, 2014), or of the European honey bee, *Apis*  
57 *mellifera*, to understand the role of bacteria in nutrition (Engel *et al.*, 2012). But, what other species have  
58 been studied, and why? Given this field is burgeoning, it is timely to take stock of the non-human animal gut  
59 microbiota literature and examine the research landscape thus far.

60

61 Here, we ask what drives research in animal gut microbiota? by quantifying the subject of each study as a  
62 domestic, model or wild animal. Within these three animal groups we determine whether data collection is  
63 purely observational or instead, the result of experimentation; which animal taxa are used, and which  
64 research questions are addressed. In addition, we use network analyses to determine unique and overlapping  
65 research foci for each animal group. Finally, we determine the extent that animal groups consider microbiota-  
66 host-environment interactions, by calculating the interdisciplinarity of studies within each group.

67

68 **Data-mining the literature**

69 A search for peer-reviewed articles on non-human gut microbiota published between the years 1911 and  
70 2016 was performed in Web of Science<sup>Æ</sup> and PubMed. Search terms were microbi\* AND gut OR other  
71 gut-related terms ( anal OR anus OR caec\* OR cec\* OR cloac\* OR colon OR duoden\* OR faec\*

72 OR fec\* OR gastro\* OR ile\* OR intest\* OR jejun\* OR rect\* OR rum\* OR stomach ). The search  
73 excluded common irrelevant terms ( ferment\* , microbiol\* , reactor\* , review\* , vitro ), and those  
74 related to humans ( child\* , human\* , infan\* , men , paedi\* , patient\* ). All abstracts of the resulting 3  
75 095 articles were reviewed manually and 1 419 were found to characterise the microbiota of the non-human  
76 animal gut (either the entire digestive tract, one or more sections, and/or faeces). A sub-set of 650 studies  
77 (November 2009 – July 2016) were randomly selected for analysis based on corresponding randomly  
78 generated numbers from all studies (Figure 1, Supplementary Information 1). Firstly, we categorised each  
79 study as focussing on animal species that were: domestic (livestock and companion animals), model  
80 (studied to provide insight into the microbiota of other organisms), or wild (free-living or undomesticated  
81 animal species studied in their natural habitat or captivity). For each publication we noted whether data were  
82 observational , i.e., purely descriptive, or the result of a perturbation , i.e., a treatment was applied, such as  
83 a probiotic. We categorised the focal taxon for each study as mammal, bird, fish, reptile, amphibian, insect or  
84 non-insect invertebrate. Finally, 36 broad lines of enquiry ( research questions ) were identified and  
85 quantified within each of the three animal groups (Figure 1, Supplementary Information 1).

86

87 <Figure 1 here>

88

### 89 **What is driving animal microbiota studies?**

90 The 650 publications reviewed here were dominated by studies on domestic animals (48.2%) followed by  
91 model animals (37.5%), while wild animal studies were comparatively few (14.3%; Table 1). Perturbation is  
92 crucial to understand how a system functions, as exemplified by classic ecological experiments (Paine,  
93 1966), and we found that it was used heavily, as opposed to observational data, in domestic studies (81.1%;  
94 Table 1). Likewise, perturbation was frequent in model studies (87.7%), but was rarely used in wild animals  
95 (20.4%), where instead observational data (79.6%) were favoured. All of the reviewed studies focussed on  
96 the bacterial communities of the microbiota, and of these, 12.5% studies also characterised at least one other  
97 microbial community: archaea (8.8%), fungi (4.3%), protozoa (2.8%) and/or viruses (0.6%; Supplementary  
98 Information 1). Just over half (54.3%) of studies that investigated the non-bacterial microbiota used

99 perturbation, the remaining half being observational; in addition, about half investigated domestic animals  
100 (53.1%), followed by wild (32.1%) and model (14.8%) organisms.

101

102 In domestic animals, perturbation was used with the aim of improving animal productivity (29.7%), for  
103 example by administering probiotics (16.3%, e.g., Ahmed *et al.*, 2014) or prebiotics (6.4%, e.g., Hoseinifar *et*  
104 *al.*, 2014; Figure 2A). In model animals perturbation was used to determine interactions between gut  
105 microbiota and host health, e.g., the role of microbiota in eliciting an immune response (immunity; 36.6%;  
106 e.g., Brinkman *et al.*, 2011) for forward translation to humans. For model animals, perturbation also included  
107 therapeutics, such as antibiotics (13.5%; e.g., Carvalho *et al.*, 2012), and more rarely, organ transplants  
108 (1.2%; Li *et al.*, 2011) and other surgical procedures (0.8%; Devine *et al.*, 2013, Figure 2B). The few wild  
109 animal studies to use perturbation did so to understand system functions, e.g., by examining the effect of  
110 dietary treatments on microbiota of wild-caught giraffes, *Giraffa camelopardalis*, as a means to understand  
111 microbial symbioses (Roggenbuck *et al.*, 2014). Instead, observational data were the norm for wild animals  
112 in order to characterise natural microbiota structure and function, especially community composition  
113 (41.9%; Figure 2C).

114

115 Although perturbation, under controlled conditions, is more straightforward in domestic and model animals,  
116 thus facilitating treatment comparisons and reducing confounding factors such as genetic variation and diet,  
117 the complex combination of factors that influence microbiota are unlikely to be understood by looking at  
118 laboratory animals alone (McGuire *et al.*, 2008; Amato, 2013). Standardisation may appear logical to obtain  
119 less noisy data, but it does not reflect the human condition, where such identical factors are not experienced  
120 throughout life nor between individuals, and risks, what Ronald Fisher stated as *(supplying) direct*  
121 *information only in respect of the narrow range of conditions achieved by standardisation* (Fisher, 1937). It  
122 would appear that wild animals could provide an opportunity not only to examine natural gut microbiota  
123 function, but to extend observations to incorporate understanding of complex multidirectional microbiota-  
124 host-environment interactions that they are subject to. Already, other areas of traditionally animal-model  
125 dominated research, such as immunology, study and sometimes perturb wild model systems, giving rise to  
126 wild immunology (Pedersen and Babayan, 2011), and it could be timely for microbiota research to follow

127 suit. Consequently, the obvious progression of wild studies is to understand how natural microbiota  
128 responds to perturbation as a model for humans and other species, and to determine directionality of  
129 microbiota-host-environment interactions (Gordon, 2012). However, difficulties in doing so may be imposed  
130 by legislation relating to scientific procedures on wild animals in any given country. In the UK, for example,  
131 the Animals Scientific Procedures Act 1986, must be complied with under Home Office regulations. In  
132 addition, species may be afforded protection from perturbation due to their international conservation status,  
133 for example, those appearing on the International Union for Conservation of Nature (IUCN) red list.  
134 Movement of samples between collaborators working on protected species may also be complex due to  
135 Convention on International Trade in Endangered Species (CITES) regulations; and permits are required for  
136 the translocation of samples from given species between countries. In a compromise between studying wild  
137 animals and meeting legal and logistical requirements, 40.9% of wild studies examined here used wild-  
138 caught (captured for purposes of study) or captive wild animals (e.g., from a zoo or research facility), with  
139 the remaining 59.1% investigating free-living, or a combination of free-living and captive animals. Even this  
140 level of compromise may significantly alter research outcomes, as it has consistently been found that wild  
141 animals exhibit a loss of natural microbes following captivity (Xenoulis *et al.*, 2010; Nelson *et al.*, 2013;  
142 Kohl and Dearing, 2014).

143

144 <Table 1 here>

145 <Figure 2A-2C here>

146

#### 147 **How taxonomically diverse are animal microbiota studies?**

148 Domestic and model studies were composed of similar taxonomic groups (predominantly vertebrates, i.e.  
149 mammals, birds and fish, in 97.1% and 93.0% of studies respectively), but the opposite was true of wild  
150 studies, which predominantly focussed on invertebrates (52.2%; Figure 3). Domestic animals that have large  
151 farmed populations in economically developed regions were most studied; i.e., pigs, cattle (49.7% and 28.7%  
152 of mammals respectively), and chickens (80.5% of birds; Figure 3). Species from all six taxonomic  
153 categories have been exploited as models, but model studies mostly focused on laboratory mice (70.2%

154 mammals) or rats (23.3% mammals; Figure 3), in part because the dominant bacterial phyla in the rodent and  
155 human gut are similar - *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Spor *et al.*, 2011).  
156  
157 Laboratory model rodent studies have been fundamental for progressing our understanding of microbiota  
158 function and modulation, for example rats have demonstrated microbiota may be used as a biomarker to  
159 predict liver transplant rejection (Ren *et al.*, 2013). However, extrapolating data from laboratory animals to  
160 other species (including humans) has limitations, e.g., similarities in microbiota between rodents and humans  
161 are reduced beyond the phyla level (Spor *et al.*, 2011; Nguyen *et al.*, 2015). In addition, laboratory animals  
162 have a highly inbred genetic background (Hufeldt *et al.*, 2010), and are exposed to very different conditions  
163 to those experienced by humans and wild animals, but which influence microbiota, e.g., captive rearing  
164 (Zeng *et al.*, 2012), and constant extrinsic factors such as diet and housing conditions (Le Floch *et al.*,  
165 2014). Indeed, the disparity between laboratory animals and humans is believed to be a major contributing  
166 factor towards attrition, whereby drug trials are successful in laboratory animals but later fail in human  
167 trials (Garner, 2014), and this same lack of successful forward translation is also likely to occur in microbiota  
168 research. As such, there appears to be a niche for utilising wild rodents as model organisms, which are  
169 physiologically and genetically similar to those already used and understood in the laboratory (Pedersen and  
170 Babayan, 2011), but host an intact and diverse gut microbiota (Amato, 2013). However, microbiota studies  
171 on wild mammals are currently relatively uncommon (30.6%) and include species not related to those  
172 traditionally used as model organisms e.g., arctic ground squirrels (*Urocitellus parryii*) have been studied to  
173 monitor temporal changes in microbiota composition (Stevenson *et al.*, 2014). Instead, wild studies focussed  
174 on insects (42.5%), and although wild insects such as *Drosophila*, whose simple microbiota has provided  
175 insight into host-microbe interactions, could be developed as a model system (Chandler *et al.*, 2011), studies  
176 were instead driven by the potential for microbiota manipulation to be used in biocontrol. As such, wild  
177 insect studies were mainly focussed on agricultural pests and vectors of pathogens e.g., bee (23.4%), termite  
178 (22.1%) and mosquito species (13.0%; Figure 3). These, and similar studies, have suggested that removal of  
179 important symbiotic bacteria responsible for lignocellulose digestion could be used to control crop pests  
180 (Schloss *et al.*, 2006), and probiotics may be used to control vector-borne pathogens such as *Plasmodium*



181 (malaria) in mosquitoes, since bacteria can stimulate an up-regulation of immunity genes that reduce  
182 *Plasmodium* acquisition (Dong *et al.*, 2009; BoissiÈre *et al.*, 2012).

183

184 <Figure 3 here>

185

### 186 **Using network analyses to visualise and quantify the research landscape**

187 To visualise research foci and interdisciplinarity, network graphs were constructed for domestic, model and  
188 wild animal studies based on research questions. A network graph consists of nodes linked by edges; in this  
189 case, a node represented one of the 36 research questions identified, and the edges the co-occurrence of those  
190 questions within a scientific paper(s). Each network was constructed from an  $n$  by  $n$  symmetrical adjacency  
191 matrix; composed of a corresponding row and column for every node, where entries indicated links between  
192 two nodes ( $i, j$ ). Edges were non-directed, i.e., a link between the nodes  $i, j$  had the same value as  $j, i$ . Node  
193 size ( $s$ ) was weighted according to the total number of studies addressing that question, and edge width was  
194 weighted by the number of studies in which the two research questions co-occurred (Figure 2A-C).

195

### 196 **What are the research foci of animal microbiota?**

197 To quantify and compare the foci of research questions between animal groups, we calculated a series of  
198 network metrics. Node size ( $s$ ), or the number of studies investigating any given question depicts how  
199 common a question is; node degree ( $k$ ) represents the number of edges connected to a question, thus its  
200 importance in forging links between disciplines; and node strength ( $NS$ ) is the sum of weighted connections  
201 to a question, hence how core the question is to the research.

202

203 Diet was consistently a question of focus in all three animal groups (Table 1), but its research associations  
204 differed. In domestic animals Diet was most commonly studied ( $s=158$ ), created the most links to other  
205 questions ( $k=20$ ) and did so frequently ( $NS=175$ , Table 1). Thus, diet was fundamental and at the core of this  
206 research; often as a means to manipulate animal health via the microbiota, particularly to increase animal  
207 production (38.0% domestic diet studies; Figure 2A). Diet was also most frequently studied in model  
208 animals ( $s=95$ ), but with respect to host health and disease: 34.7% of such studies used diet specifically to

209 treat or simulate non-infectious diseases such as obesity (Esposito et al., 2015) and diabetes (Prajapati *et al.*,  
210 2015; Figure 2B). Despite its popularity diet was not the most integrated or interdisciplinary question in the  
211 network, but immunity was ( $k=23$  and  $NS=164$ ; Table 1), highlighting the importance of the shared  
212 relationship between microbiota and immunity, and how it consequently affects many other aspects of health  
213 (Round and Mazmanian 2009). In contrast community composition was most studied ( $k=13$ ) and embedded  
214 ( $NS=41$ ) within wild studies, but diet was key to creating research links between questions ( $s=39$ , Table 1).  
215 This link results from the fact that wild studies focus on microbiota structure (e.g., Delsuc et al. 2014), and  
216 suggests we are currently acquiring more basal knowledge on wild animal microbiota. In addition, only  
217 25.9% of wild animal diet studies used perturbations, with the remaining 74.1% observing microbiota  
218 composition under a natural diet (33.3%; Figure 2C). Given that 72% of emerging zoonotic pathogens are  
219 transmitted to humans from wildlife (Jones et al. 2008), and microbiota and immunity are strongly  
220 interlinked (Round and Mazmanian 2009), determining how microbiota interacts with host immunity and/or  
221 infectious disease (currently only 17.9% and 9.3% in domestic animals which have frequent contact with  
222 humans, and 3.2% and 10.8% of wild studies, respectively) deserves further consideration.

223

#### 224 **Do animal microbiota studies take an interdisciplinary approach?**

225 Animal microbiota studies with a single research focus have provided important basal knowledge on  
226 microbial composition and function e.g., in-depth analyses of microbiota community composition in  
227 laboratory mice has revealed that the intestinal crypts, which harbour gut stem cells, also accommodate a  
228 niche microbial community (PÈdron *et al.*, 2012). Likewise, there is also great value in an interdisciplinary  
229 approach in which multiple factors are studied simultaneously, and can aid in progressing knowledge and  
230 teasing apart complex and multidirectional host-microbiota-environment interactions (Gordon, 2012). We  
231 quantified the interdisciplinarity of each group by measuring the mean betweenness centrality ( $BC$ ) of  
232 each network:  $BC$  indicates how closely associated all questions are in relation to each other, and is the  
233 number of shortest paths required to pass through each question to connect it to all other questions; larger  
234 values indicate questions are more closely associated (Leydesdorff, 2007). Network density ( $D$ ), indicates the  
235 level at which interdisciplinarity has been exploited in each group, calculated as a proportion of the total  
236 number of possible connections, whereby 0 = no connections present and 1 = all possible connections are

237 present and maximum interdisciplinarity has been reached. Network analyses were conducted using the  
238 igraph package in R v. 3.6.3 (Csardi and Nepusz, 2006).

239

240 Model studies exploited the an interdisciplinary approach the most, with the highest proportion of possible  
241 links between questions ( $D=0.23$ ), followed by domestic ( $D=0.17$ ) and wild ( $D=0.08$ ) studies (Table 1). In  
242 addition, research questions in model studies were more closely associated, directly or indirectly, with one  
243 another, (mean  $BC=19.09 \pm 3.99$ ), than in domestic ( $BC=15.99 \pm 3.41$ ) or wild ( $BC=12.19 \pm 3.41$ ) studies  
244 (Table 1). The comparatively high interdisciplinarity of model studies reflects the large range of questions  
245 addressed ( $N=34$ ), compared to the domestic ( $N=27$ ) and wild ( $N=22$ ) groups, and the motivation of many  
246 model studies to improve medical treatments which often requires an interdisciplinary approach to monitor  
247 the range of subsequent effects on health (e.g., to investigate the associations between organ transplantation,  
248 non-infectious disease, immunity and microbiota; Xie *et al.*, 2014). Conversely, wild studies were the least  
249 integrated and interdisciplinary, and more questions were addressed independently of one another. However,  
250 this group did address a unique research question: phylogeny and how phylogeny is driven across species  
251 by gut microbiota and diet, and *vice versa*; for example, myrmecophagous mammals from different  
252 evolutionary lineages exhibit striking convergence with respect to gut microbial composition, driven by  
253 dietary adaptations (Delsuc *et al.*, 2014).

254

255 While the more focussed approach of wild animal research has allowed us to assemble fundamental  
256 microbiota knowledge, it has been argued that an interdisciplinary approach is necessary to progress research  
257 on basic and applied gut microbiota (Gordon, 2012). We predict that the interdisciplinarity of wild animal  
258 studies will increase as they are adopted in microbiota research, particularly if done so as model organisms.  
259 Indeed the first interdisciplinary microbiota studies using wild populations provide interesting insight into  
260 the interactions between host, microbiota and environment. For example, parasitic helminths infecting the  
261 gut have up- and down-stream effects on microbiota composition (Kreisinger *et al.*, 2015; Maurice *et al.*,  
262 2015) and seasonal variation in wild rodent microbiota is largely driven by changes in food availability  
263 (Maurice *et al.*, 2015).

264

265 **Conclusion and outlooks**

266 Although more than 10% of studies investigated the microbial community of non-bacterial species in  
267 addition to the bacterial component of the microbiota, of these only 0.6% studies investigated the virome,  
268 despite evidence that viruses bestow a number of functional traits to bacteria (Ogilvie and Jones, 2015).  
269 Complementary studies that simultaneously investigate multiple components of the gut biome are likely to  
270 shed light on microbiota composition and functionality (see for example, Glendinning *et al.*, 2014). We  
271 demonstrate that most animal gut microbiota studies are driven by economic (domestic animals) or human  
272 health (model animals) issues, although more microbiota studies on immunity and/or infectious disease in  
273 domestic animals could benefit both livestock and humans in close proximity to them. There are, however,  
274 well-founded concerns regarding the limitations of laboratory animals as model organisms, as highlighted by  
275 attrition (Fisher, 1937; Garner, 2014). In 2013 the former director of the NIH, Prof. Elias Zerhouni, stated  
276 that *We have moved away from studying human disease in humans* (NIH Record: <http://bit.ly/2f5UpII>),  
277 arguing that we should *.refocus and adapt new methodologies for use in humans to understand disease*  
278 *biology in humans* ; raising interesting issues about the use of animal models, including in microbiota  
279 research, and whether it is scientifically legitimate to forward translate our findings to humans. This does not  
280 mean that we should not use animal models, but rather that we should consider changing the way in which  
281 we study them, so that they may more accurately represent human inter-individuality. The intact gut biomes  
282 of wild species that experience inter-individual and environmental variation more similar to humans than  
283 their laboratory counterparts, rendering the results more realistic , could form the basis of more relevant  
284 models to study microbiota. However, field experiments would need to be carefully designed to provide  
285 statistical power in the face of extensive variation (e.g., controlling for genetic background, diet, sex, etc.).  
286 Under some circumstances, manipulation of microbiota in wildlife is not possible (e.g., for rare, elusive or  
287 protected species). In these cases, development of mathematical and/or statistical models to assign  
288 directionality to observational data could be beneficial. Examples of applications in other fields include,  
289 identifying interactions between immune components using network theory (Thakar *et al.*, 2012), and  
290 determining interspecific interactions among an unperturbed community of gut parasites, using generalised  
291 linear mixed models (Fenton *et al.*, 2010). Studies on wild animals are currently comparatively few, and

292 generally aim to characterise natural microbiota, combining few disciplines. However, we expect  
293 interdisciplinarity to increase in wild animals should they be developed as model systems.

294

295 Supplementary information is available at ISME Journal s website.

296

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303

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### Figure legends

305 **Figure 1:** Work flow for categorising gut microbiota studies on non-human animals following searches in  
306 Web of Science<sup>Æ</sup> and PubMed. Of the 1 419 relevant articles identified, 650 recently published studies  
307 (2009-2016) were categorised into one of three animal groups (domestic, model or wild animals). Data  
308 collection method, animal taxon and research question(s) addressed were determined for each study.

309

310 **Table 1:** The number of studies categorised into three animal study groups: domestic, model or wild, from  
311 650 non-human animal gut microbiota studies, showing data collection methods (observation or  
312 perturbation) and network indices of three network graphs investigating research question interdisciplinarity  
313 and overlap.

314

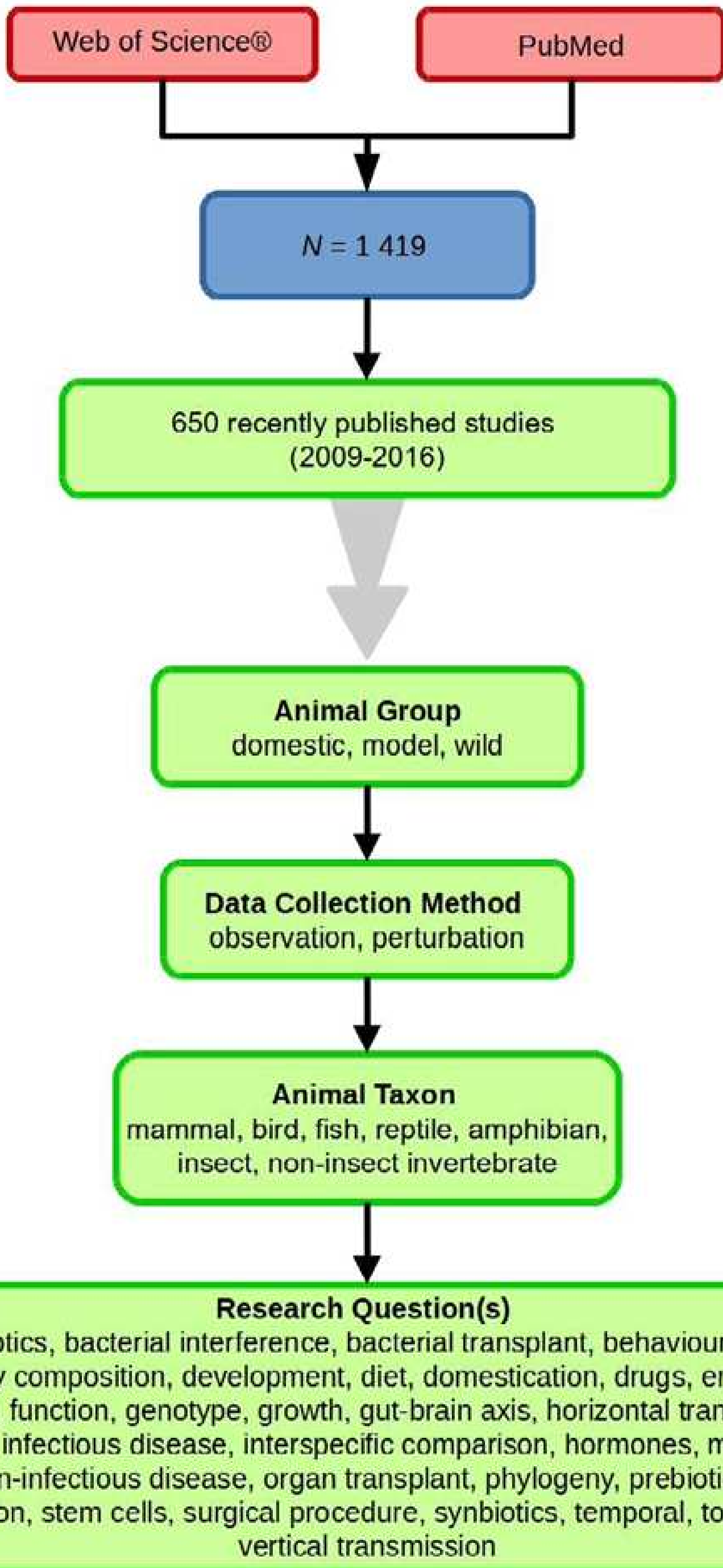


315 **Figure 2A-C:** Network graphs illustrating the frequency of 36 research questions addressed by gut  
316 microbiota studies on a) domestic b) model and c) wild animals, and how frequently these questions co-occur  
317 within the 650 studies. Each node (circle) represents a research question, with diameter weighted by the  
318 number of studies. Edges (lines) connecting each node represent the co-occurrence of different research  
319 questions, with width weighted by the total number of co-occurrences.

320

321 **Figure 3:** The percentage of gut microbiota studies within three animal groups: domestic (black), model  
322 (grey) or wild (white), investigating different animal taxa. For each animal group the combined percentage of  
323 studies across all taxa equates to 100% of studies for that group.

FIGURE 1



**Table 1**

| Animal group               | Data collection method |             | Number of nodes<br>( <i>N</i> ) | Maximum node size<br>( <i>s</i> ) | Maximum node degree*<br>( <i>k</i> ) | Maximum node strength<br>( <i>NS</i> ) | Network density $\beta$<br>( <i>D</i> ) | Mean betweenness centrality $\S$ ( $\pm$ SEM)<br>( <i>BC</i> ) |
|----------------------------|------------------------|-------------|---------------------------------|-----------------------------------|--------------------------------------|--|---|--|
|                            | Perturbation           | Observation |                                 |                                   |                                      |  |   |  |
| <b>Domestic</b><br>(48.2%) | 256 (81.8%)            | 57 (18.2%)  | 27                              | Diet<br>(158)                     | Diet<br>(20)                         | Diet<br>(175)                          | 0.17                                    | 15.99 ( $\pm$ 3.41)  |
| <b>Model</b><br>(37.5%)    | 214 (87.7%)            | 30 (12.3%)  | 34                              | Diet<br>(95)                      | Immunity<br>(23)                     | Immunity (164)                         | 0.23                                    | 19.09 ( $\pm$ 3.99)  |
| <b>Wild</b><br>(14.3%)     | 19 (20.4%)             | 74 (79.6%)  | 22                              | Community<br>composition (39)     | Diet<br>(13)                         | Community<br>composition (41)          | 0.08                                    | 12.19 ( $\pm$ 3.41)  |

\* Node degree (*k*): The number of edges connected to a node, i.e. the number of research questions that co-occur.

Node strength (*NS*): The sum of the weighted edges connected to a node, i.e. the total number of separate co-occurrences of a research question and all others that it is connected to.

$\beta$ Network density (*D*): The connections present in a network as a proportion of the total number of possible connections.

$\S$ Mean betweenness centrality (*BC*): The mean shortest number of paths required to pass through each research question in the network, i.e. how well connected research questions are and thus interdisciplinarity of the whole network.

# FIGURE 2A-C

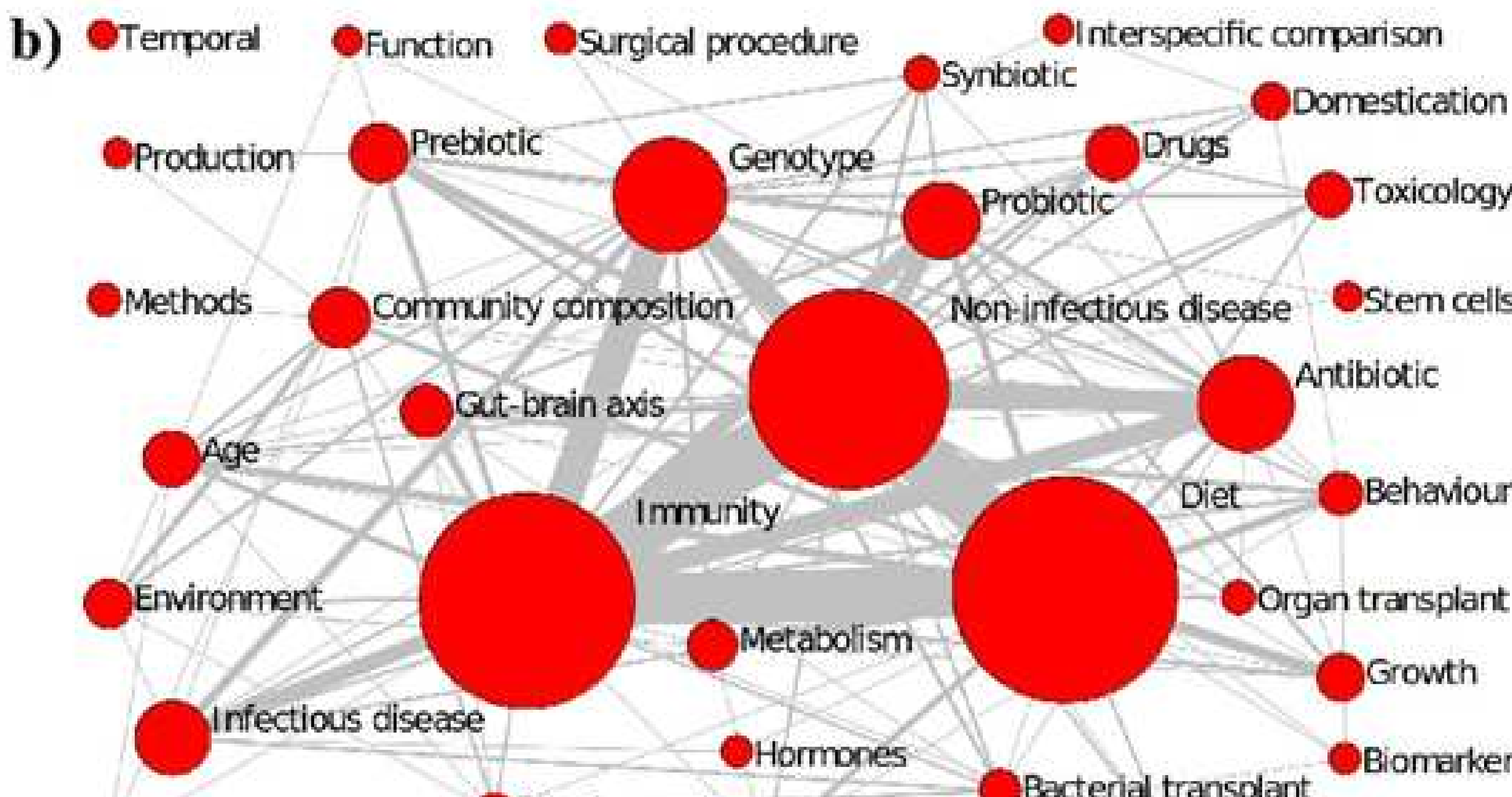
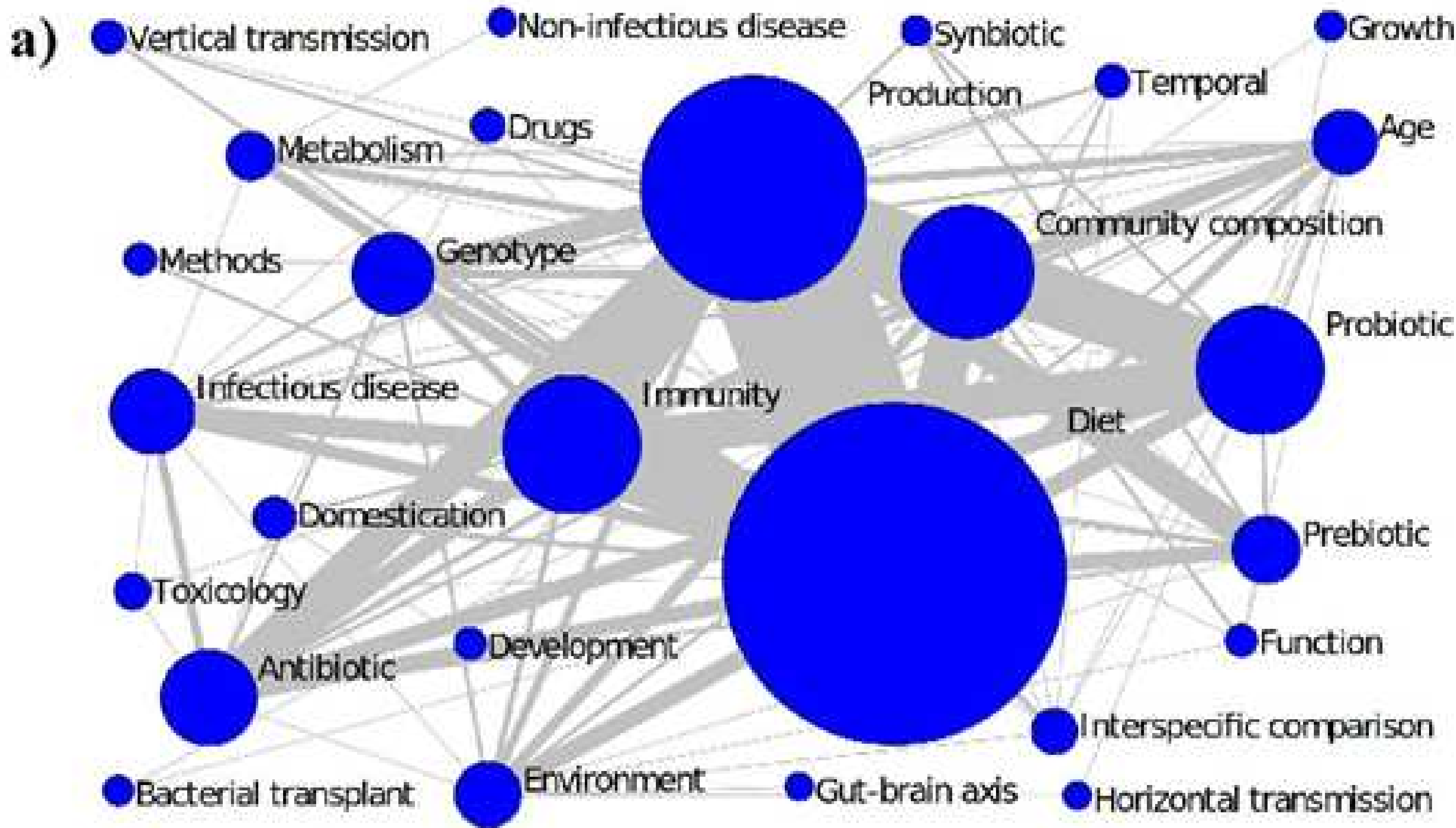


Figure 3

