

1 **Microbial symbionts of honeybees: a promising tool to improve honeybee health**

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19 **Highlights:**

20 - Different biotic and abiotic stressors are affecting honeybees and large losses have been reported  
21 worldwide impacting economically agriculture.

22 - Microbial symbionts are emerging as modulators of the innate immune system and, more in  
23 general, of the insect health.

24 - Due to their crucial involvement in insect physiology, microbial symbionts could represent a  
25 powerful tool to preserve and improve insect health through the application of Microbial Resource  
26 Management (MRM) concept.

27 **Abstract**

28 Among pollinators, honeybees are the most important ones and exert the essential key ecosystem  
29 service of pollination for many crops, fruit and wild plants. Indeed, several crops are strictly  
30 dependent on honeybee pollination. Since few decades, honeybees are facing large scale losses  
31 worldwide, the causes of which are found in the interaction of several biotic and abiotic factors,  
32 such as the use of pesticides, the habitat loss, the spread of pathogens and parasites, and the  
33 occurrence of climate changes. Insect symbionts are emerging as a potential tool to protect  
34 beneficial insects, ameliorating the innate immune homeostasis and contributing to the general  
35 insect wellbeing. A review about the microbial symbionts associated to honeybees is here  
36 presented. The importance of the honeybee microbial commensals for the maintenance and  
37 improvement of honeybee health is discussed. Several stressors like infestations of *Varroa* mites  
38 and the use of pesticides can contribute to the occurrence of dysbiosis phenomena, resulting in a  
39 perturbation of the microbiocenosis established in the honeybee body.

40

41 **Keywords**42 Honeybee; microbiota; dysbiosis; *Varroa*; pesticides; honeybee homeostasis

43

## 43 **Introduction**

44 Non-conventional habitats, among which extreme environments (like hot or cold deserts, inland or  
45 coastal saline systems), polluted sites and animal gut, have been less explored in terms of  
46 biodiversity, richness and functionality as compared to other well-studied conventional habitats,  
47 such as soil- and water-associated matrices. Nonetheless, they represent a considerable source of  
48 compounds and microorganisms with interesting biological and biotechnological potential [1-2].

49 Growing attention has been recently directed to the study of these niches and, among these various  
50 non-conventional habitats, to the animal gut or, in general, body intended as niches in which  
51 microorganisms survive and flourish [3].

52 All metazoans hosting a gut microbiota, including arthropods, establish with their microbes  
53 complex and dynamic symbiotic interactions, which recently have been shown to go beyond a mere  
54 nutritional complementation of the host diet, embracing a wide set of aspects related to the host  
55 physiology, behavior, reproduction, evolution and immunity [3-4]. Insects are the most diverse  
56 animal group on earth and during their evolutionary history they adapted to feed on a variety of  
57 substrates and matrices, ranging from wood or phloem sap to blood. These nutritionally unbalanced  
58 diets are exploited and/or complemented through insect microbiota [see the review 5].  
59 Microorganisms also played a major role in insect adaptation and evolution [6].

60 Among insects, honeybees are of great importance worldwide due to their pollination activity for  
61 crops, fruit and wild plants. They offer a key ecosystem service, essential for a sustainable  
62 productive agriculture and for the maintenance of the non-agricultural ecosystem. Pollination  
63 services are mandatory for the production of crops like fruits, nuts and fibers, whereas the results of  
64 many other agricultural crops are significantly improved by pollination. It has been estimated that  
65 without pollinators a decrease by more than 90% of the yields of some fruit, seed and nut crops  
66 could occur [7]. In the case that wild bees do not exert their pollination service in a specific  
67 agricultural crop, managed honeybees, which are versatile, cheap and convenient, represent the only  
68 solution to ensure pollination [8]. The dependence of worldwide crops on pollinators is extremely

69 deep and during 2005 the global economic value of insect pollination was estimated to be € 153  
70 billion a year, which corresponds to 9.5% of the total economic value of agricultural crops for  
71 human consumption [9].

72 Since few years, concerns are rising over honeybee health and, consequently, over its impact on  
73 economy [10]. Large-scale losses have been reported worldwide and related to several causes, i.e.  
74 the habitat loss of pollinators, the increasing use of agrochemicals, the outbreak of diseases, the  
75 attacks of parasites, the alarm related to climate change, the introduction of alien species and the  
76 interaction among all of these factors [10]. Managed honeybees are facing increasing threats of  
77 diseases, pests, and reluctance among younger generations to learn the skills of beekeeping. In the  
78 last years, to define and to calculate the vulnerability of world agriculture pollinator decline have  
79 become a primary point of action [8-9-11-12]. Recently, Colony Collapse Disorder [CCD] has  
80 attracted the attention of academic and public opinion, but this poorly understood syndrome is just  
81 one cause of the colony losses. Recent studies suggest that several factors are involved in CCD, as  
82 parasites, pathogens, pesticides (and other environmental stressors) and, above all, the interactions  
83 among them [13-14].

84 Honeybee symbionts could be exploited in order to actively counteract bee pathogens and parasites  
85 or to enhance bee immunity, and thus indirectly to increase the protection of honeybees' health.  
86 Probiotic bacteria, such as lactic acid bacteria have been administered in laboratory conditions to  
87 honeybees, resulting in the stimulation of the innate immune system and the prevention of attacks  
88 by pathogen [15]. Recent studies in the insect model *Drosophila* emphasize how complex, intimate  
89 and multifaceted is the relation subsisting between the host and the microbiota, which, if well  
90 balanced, leads to the optimal insect wellness [4].

91 In this review, we present the current understanding of the importance of honeybee symbionts for  
92 the maintenance and improvement of the insect health. In particular, it is discussed the microbiota  
93 involvement in the stimulation of the insect immune system and body homeostasis - with a special

94 focus on the gut dysbiosis - and how this may be related to the use of pesticides, the spread of  
95 viruses and the occurrence of parasites.

96

### 97 **Microbial community associated to the honeybee *Apis mellifera***

98 Cultivation dependent and independent approaches have been long used to define the composition  
99 and the structure of the honeybee microbiota, analyzing different honeybee developmental stages,  
100 as larvae, pupae, newly emerging adults and adults; different genders, as females and drones; and  
101 different social individuals, as queens, nurses or foragers [16]. Six phylogenetic groups, i.e.  $\alpha$ -,  $\beta$ -  
102 and  $\gamma$ -Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria have been found as the major  
103 bacterial taxa of the honeybee bacterial community, representing moreover the bacterial core  
104 maintained in honeybees worldwide [16].

105 The recent technological innovations in the genomics and metagenomics fields revolutionized the  
106 potential of applications and the throughput of the analyzed data, allowing DNA sequencing of high  
107 numbers of nucleotides with low costs and high accuracy. The microbial composition and structure  
108 of a specific community can be evaluated with high sensitivity, low cost, and short times, thanks to  
109 new sequencing technologies and the multiplexing approach [17-18]. Also honeybee microbiota has  
110 been evaluated by the use of these techniques [19-20-21-22, Tab. 1]. Interestingly, eight bacterial  
111 phylotypes have been retrieved as major constituents of honeybee bacterial community, i.e. Alpha-  
112 1, Alpha-2, Beta, Gamma-1, Gamma-2, Firm-4, Firm-5, and Bifido, which correspond to the six  
113 phylogenetic groups mentioned above.

114 The metagenomic survey on honeybees from CCD-affected and not affected hives performed by  
115 Cox-Foster and colleagues [19] revealed that in non-affected honeybees Firmicutes and  $\alpha$ -  
116 Proteobacteria are more abundant than in CCD colonies. Similarly, in the work by Cornman et al.  
117 [20], deep sequencing on honeybees showed a high proportion of Alpha-1, Alpha-2 and Bifido  
118 phylotypes in individuals from not affected hives compared to those from CCD-affected hives.  
119 Cloning libraries of 16S rRNA by Martison et al. [21] revealed that the most abundant taxon in *Apis*

120 *mellifera* samples was represented by Firm-5 phylotype. *A. mellifera* showed a distinctive bacterial  
121 pattern, made up of the eight typical phylotypes, some of which are also present in closely related  
122 corbiculate bees of the genera *Apis* and *Bombus*. Lately, pyrotag analysis, quantitative PCR (qPCR)  
123 and fluorescent *in situ* hybridization (FISH) confirmed Beta, Firm-5 and Gamma-1 phylotypes  
124 (BFG phylotypes) as main members of *A. mellifera* microbiota, with a characteristic distribution  
125 along the gastrointestinal tract [22]. The crop resulted poor in microbial species, due to continuous  
126 filling and emptying for nectar supply, and also the midgut showed a low BFG load, due to the  
127 presence of the digestive enzymes and the peritrophic membrane that prevents microbial  
128 attachment. On the other hand, the ileum and the rectum were rich in microbes. The ileum showed a  
129 defined microbial distribution with Gamma-1 phylotype gathered in a thick mat, between Beta  
130 phylotypes and the ileum wall, and with Firm-5 phylotype located in small pockets along the ileum  
131 wall. The rectum showed the majority of BFG phylotypes together with the majority of bacterial  
132 diversity [22].

133 A deep sampling of gut microbiota from 40 individuals has been performed by Moran et al. [23].  
134 Four phylotypes were present in all samples, even if with different frequencies, i.e. one  $\gamma$ -  
135 Proteobacterium, classified as *Gilliamella apicola* [24], one  $\beta$ -Proteobacterium corresponding to  
136 *Snodgrassella alvi* [24] and two Firmicutes classified in *Lactobacillus* genus.

137 Yeasts, wide spread microorganisms in the honeybee environment, such as flowers, fruits and plant  
138 leaves [25-26], are also important components of the bee microbiota. Recently by the use of  
139 molecular tools, sequences related to the genera *Saccharomyces/Zygosaccharomyces* and to the  
140 family Saccharomycetaceae have been identified [20], confirming previous results obtained by  
141 cultivation-dependent methods that showed the association of yeasts with honeybee [27].

142

143 **Emerging stressors for honeybee health**

144 Currently, a renewed attention has been directed to the relationship between honeybee health and  
145 the use of pesticides, the occurrence of parasitic mites and the outbreak of viral disease,  
146 emphasizing their interconnection in determining the insect health status [14-28].

147 Pesticides, especially neonicotinoids, which are widely used for their excellent systemic properties,  
148 are indicated by scientists to play a role in CCD phenomenon and, in general, in weakening the  
149 processes of the colony, interacting with other stressors, such as parasites [28]. Honeybees are  
150 exposed to neonicotinoids at sub-lethal doses, and this results in insect behavioral disturbances,  
151 orientation difficulties, and impairment in social activities [28-29]. Experiments to prove these  
152 difficulties have been performed not only in laboratory conditions - by ingestion tests and indirect  
153 contact tests [29] - but also in field trials, where honeybees were exposed to a direct contamination  
154 with the pesticides during the foraging activity or to an indirect contamination with the pesticide-  
155 contaminated materials stored in the hive or exchanged with the sister bees [28]. Sub-lethal doses of  
156 pesticides resulted to be dangerous also for bumble bees, inducing a weight loss of the insect, a low  
157 number of pupae, and a reduced number of queens, thus impacting lastly the bumble bee  
158 populations [30].

159 The worldwide-spread, obligate-ectoparasitic mite *Varroa destructor* represents a severe threat for  
160 apiculture. It can lead to a colony collapse within a 2-3 year period. Periodic treatments with  
161 chemicals increase on one hand the costs for beekeeping, and on the other hand the risk of the  
162 presence of chemical residues in the environment and in the honey [31]. Moreover, *Varroa* mites  
163 act as disseminators of viruses between and within bee colonies [32]. Recent publications  
164 highlighted the multifactorial origin of the honeybee collapse. For instance, *Varroa* can de-stabilize  
165 Deformed Wing Virus (DWV) dynamics making the virus a rapidly replicating killer [14]. When  
166 DWV dynamics are destabilized, a host immunosuppressive status with the down regulation of the  
167 transcriptional factor NF- $\kappa$ B is recorded. The authors suggest that the DWV-mediated  
168 immunosuppressive effect shows a DWV-threshold dependency; below a certain threshold, DWV  
169 infection is maintained under control. If a stress factor, like *Varroa*, subtracts the transcriptional

170 factor NF- $\kappa$ B, the concentration of the latter becomes too low to keep under control DWV that can  
171 finally outbreak, bringing to the collapse the bee population [14].

172 Pesticides, mites and viruses have a serious impact on the health of honeybees, but in all these  
173 studies there is a missing actor, represented by the gut microbial community. We will show in the  
174 next paragraphs how deeply correlated is the insect health with the gut microbiota and the immune  
175 system. Microorganisms could be a key element in managing and preserving honeybee health status  
176 towards different biotic and abiotic stressors.

177

### 178 **Roles of the microbial partners**

179 Recent research has shown that the gut microbiota is strictly linked to host homeostasis and  
180 metabolic diseases, e.g. diabetes and obesity [33]. The gut microbial community is involved in  
181 several aspects of the host life, ranging from the nutritional contribution to the energy salvage  
182 through fermentation, from influencing mating preferences (e.g. this is the case of the gut bacteria  
183 in *Drosophila* [34]) to immunity [5]. The animal immune system works synergistically to contain  
184 the pathogens and to preserve the symbiotic relationships between host and microbiota. A fine  
185 regulation of signaling networks, which control the presence of antimicrobial compounds in the gut,  
186 allows the host to tolerate commensals and to block the proliferation of food-borne pathogens [35].  
187 As presented above, the honeybee microbiota shows a consistency which leads to hypothesize the  
188 possibility of a neutral or beneficial involvement of it, or at least with some members of the  
189 microbiota, in the honeybee's life. Several of the taxa identified in honeybees are known to produce  
190 short chain fatty acids, such as lactic or acetic acid (Lactobacilli, Bifidobacteria, Acetobacteraceae  
191 and *Simonsiella*). These products may act as supplements to honeybee diet. Moreover, gut bacteria  
192 could allow to degrade pollen, which is covered by exine layers recalcitrant to most of digestive  
193 enzymes, using then the intine as a nutrient source [36, 37].

194 While nutritional symbioses between insects and bacteria are well documented [5], the correlation  
195 that exists between the proper function of insect innate immune system and its microbiota is less



196 explored. Symbionts are recently receiving increasing attention because of their recognition as  
197 strong and effective immunomodulators of insects [38-39-40].

198 In their work Ryu and collaborators [38] found that there is a fine equilibrium between the acetic  
199 acid bacterial commensals and the *Drosophila* innate immune system. The normal flora suppresses  
200 the growth of pathogenic bacteria, unless the system is perturbed. If a perturbation of the gut  
201 bacterial community occurs, an increased number of pathogenic bacteria could lead to gut  
202 apoptosis. In a normal condition the fly's immune system allows the dominance of an  
203 Acetobacteraceae strain, which in turn keeps down, by competitive exclusion, the proliferation of  
204 the gut apoptosis inducer.

205 Another case study is represented by the tsetse fly and its obligate symbiont *Wigglesworthia*. The  
206 latter complements the deficient diet of the fly with the products of its metabolism. However, the  
207 symbiosis at the base of tsetse-*Wigglesworthia* interactions goes beyond the nutritional role: larvae  
208 deprived of *Wigglesworthia* are immunocompromised when they reach the adult stage. Weiss and  
209 co-workers [39] show that in aposymbiotic tsetse flies the cellular innate immune system is  
210 seriously compromised and consequently the insects are highly susceptible to infections. When  
211 hemocytes from wild type individuals are transplanted in aposymbiotic adults or *Wigglesworthia*  
212 cell extracts are administered to the aposymbiotic mothers, the innate immune system functionality  
213 is restored.

214 Another study that highlights the multidimensionality of symbionts-host interactions has been  
215 performed on the Hawaiian squid *Euprymna scolopes* and the luminous bacterium *Vibrio fischeri*  
216 [40]. *V. fischeri* is the exclusive partner of the squid light organ and the symbiosis follows a dynamic  
217 balance of symbionts expulsion and re-growth. The well-known mediators involved in animal-  
218 microbe interactions, called "microbe associated molecular patterns" (MAMPs), specifically lipid A  
219 component of lipopolysaccharide (LPS) and peptidoglycan component, interplay synergistically  
220 with the luminescence of symbionts in order to sustain the host development. Researchers found  
221 that MAMPs and luminescence are both critical for the maintenance of the symbiosis.

222 All these findings contribute to state that a finely regulated dialog exists among the symbiotic  
223 partners in order to reach a symbiostasis. This is done through the regulation of pathways  
224 implicated in the substrate availability and pathways that govern host/symbionts population  
225 dynamics. Recently, artificial microcosms have been employed to prove that the high functionality  
226 of a specific system could be maintained, even during stress events, if microorganisms are  
227 distributed in a suitable climax community [41]. In the case of the microbiota associated to the  
228 digestive system, the maintenance and improvement of the host health against pathogens infection  
229 depends on the functionality of the system, which lastly relies on the presence of a suitable climax  
230 community [16]. Cox-Foster and collaborators [19] showed that CCD non-affected honeybees are  
231 mainly colonized by Firmicutes and  $\alpha$ -Proteobacteria, while in CCD affected bees a high abundance  
232 of  $\gamma$ -Proteobacteria is measured. This could be related to a case of dysbiosis, i.e. an unbalance of the  
233 gut microbiota, with the consequent loss of the proper functionality, which in turns negatively  
234 impacts the health status. Further studies are needed to unveil the strict and dynamic interplay  
235 existing between host and symbionts.

236

### 237 **Microbial involvement in the general insect health status**

238 Recent publications highlighted that in different *Drosophila* strains two taxonomically different  
239 bacteria, i.e. *Acetobacter pomorum* and *Lactobacillus plantarum*, modulate the insulin signaling and  
240 TOR pathway, respectively, through different bacterial products [4-42-43]. In *A. pomorum*, the  
241 acetic acid produced by the activity of the pyrroloquinoline quinone-dependent alcohol  
242 dehydrogenase (PQQ-ADH) modulates the insulin signaling which in turn controls several host  
243 homeostatic programs, as the developmental rate, the body size, the energy metabolism and the  
244 intestinal stem cell activity [42]. On the other hand, *L. plantarum* promotes protein assimilation  
245 from the diet, regulating diet-derived branched-chain amino acid (BCAA) levels in the hemolymph.  
246 BCCA activates TOR signaling i) in the fat bodies, which results downstream into the promotion of  
247 growth rate, and ii) in the prothoracic glands, which has an impact downstream on the length of

248 growth phase [43]. In fat bodies TOR pathway normally acts stimulating the systemic production of  
249 insulin-like peptides and thus promoting the growth. It has been hypothesized that 1) the stimulation  
250 of the insulin signaling in presence of commensals could be the result of the evolution conflict  
251 between the host and its microbiota; 2) bacterial metabolites are cues for the host to be informed on  
252 the environmental nutritional availability for the host development [4]. Thus according to this  
253 second hypothesis the host would exploit its microbiota to sense the environment. Bacteria are  
254 known to communicate through quorum sensing which allows the regulation of their activity and  
255 physiological processes. Quorum sensing outcomes in important advantages for bacteria i.e. host  
256 colonization, formation of biofilms, defense against competitors, and adaptation to changing  
257 environments. The kind of interaction here hypothesized implies a higher level of interaction  
258 between symbionts and hosts.

259 The molecular mechanisms that regulate the host microbe cross-talk are still poorly understood.  
260 However, all these studies highlight the key role of microbial partners in influencing the systemic  
261 growth of the host, and preserving its health. As in *Drosophila*, it is possible to hypothesize that  
262 commensals in honeybee could have a higher level of interaction with the host, acting on the growth  
263 regulation of the insect. Components of *Drosophila* microbiota, as Lactobacillales and  
264 Acetobacteraceae members, are widespread in *A. mellifera*. Lactic acid bacteria (LAB) have been  
265 shown to exert a probiotic effect on honeybee larvae, eliciting the innate immune system to  
266 overcome pathogen attacks [15], and have been indicated as major modulators of honeybee health  
267 [44]. Like LAB, well-known for their ability to produce antimicrobial factors, other symbionts such  
268 as sporeforming bacteria are indicated as producers of peptide antibiotics and antibiotic-like  
269 compounds, which in some case possess antagonistic activity [45-46]. Finally, acetic acid bacteria  
270 (AAB), widespread in nature [47], can compete with the pathogen along the host epithelia,  
271 physically occupying the available niches and nutritionally competing with the pathogens.  
272 Moreover acid and exopolysaccharide production may contribute to AAB successful colonization of  
273 the insect gut [48-49].

274

275 **Perspectives**

276 There is increasing evidence that there is a strict interconnection between the intestinal microbiota  
277 balance and the health status of the host [4]. Commensal microbiota drives immune and health  
278 homeostasis by mechanisms that are yet poorly understood and a great effort has to be done in this  
279 direction. Insect symbionts are indeed emerging as a potential tool in biocontrol programs to protect  
280 beneficial insects, ameliorating the innate immune homeostasis and contributing to the general  
281 insect wellbeing [4]. The employment and exploitation of microorganisms in a defined environment  
282 or niche to solve practical problems has been termed as Microbial Resource Management (MRM)  
283 and MRM concepts are applicable to the maintenance and promotion of insect health [3]. A novel  
284 MRM application, the Symbiont Resource Management (SRM), can be defined as the application of  
285 microbial symbionts to manage insect-related problems [3; Fig. 1]. Symbiotic microorganisms can  
286 exert their beneficial contribute towards the host to sustain its health in different ways, i.e. by  
287 competitive exclusion, production of antibiotic compounds, activation/stimulation of the innate  
288 immune system, and communication to the host of the environmental conditions. However, in order  
289 to become able to manage these complex microbial communities within the body of the insects it is  
290 imperative to understand how they interact with the host. Therefore, further research has to be  
291 conducted to clarify the molecular mechanisms at the base of the symbiosis.

292

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452 **Tab. 1. Actual knowledge on the bacterial species associated to the honeybee *Apis mellifera***  
453 **according to cultivation-independent and -dependent methods.** Data from cultivation-  
454 independent studies and some data from cultivation-dependent studies are from Sabree et al. (2012).  
455 Other cultivation-dependent data are from studies that identified the isolates by partial or complete  
456 16S rRNA gene sequencing.

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457 **Tab. 1. Actual knowledge on the bacterial species associated to the honeybee *Apis mellifera* according to cultivation-independent and -dependent methods.**

Case study	Origin	Sample	Method	Total n sequences	% known bee species groups <sup>a</sup>	Alpha-1 <sup>a</sup>	Alpha-2 <sup>a</sup>	Beta <sup>a</sup>	Gamma-1 <sup>a</sup>	Gamma-2 <sup>a</sup>	Firm-4 <sup>a</sup>	Firm-5 <sup>a</sup>	Bifido <sup>a</sup>	Other bacteria
<b>Cultivation-independent techniques</b>														
Jeyaprakash et al. 2003 [50]	South Asia	Dissected guts	Sanger	8	n/a	+ (3)	+ (1)	+ (2)	+ (2)	-	-	+ (1)	+ (1)	<sup>b</sup>
Mohr and Tebbe, 2006 [51]	Germany	Dissected guts	Sanger	13	n/a	-	+ (1)	+ (1)	+ (2)	-	-	-	-	<sup>b</sup>
Babiendrier et al., 2006 [52]	Switzerland	Midgut and hindgut	Sanger	27	n/a	+ (3)	+ (2)	+ (6)	+ (8)	+ (1)	+ (2)	+ (4)	-	<sup>b</sup>
Disayathanoowat et al., 2012 [53]	Thailand	Midgut	Sanger	17	n/a	-	-	+	+ (1)	-	-	+ (2)	+ (1)	<sup>b</sup>
Cox Foster et al., 2007 [19]	Australia, USA, Hawaii	Pooled whole bees	Pyrotags 454	428	97.4	1.9	3.2	16.9	60.9	9.6	0.6	2.8	1.7	2.6
Martinson et al., 2011 [21]	Arizona	Single whole bees	Sanger	271	98.5	0.0	1.1	11.1	11.8	0.0	10.0	63.8	0.7	1.5
Martinson et al., 2011 [21]	Arizona,	Bacterial cells isolated from pooled guts	Sanger	267	98.5	0.7	0.0	3.7	9.7	0.0	10.5	60.7	13.1	1.5
Martinson et al., 2012 [22]	Arizona,	Dissected gut sections	Pyrotags 454	96,505	99.9	0.0	0.3	20.3	10.1	24.2	0.2	44.0	0.8	0.1
Mattila et al., 2012 (re-analysis) [54]	Massachusetts	Dissected guts	Pyrotags 454	106,344	94.8	0.0	0.0	6.74	49.10	1.12	11.05	21.36	5.41	5.2
Moran et al., 2012 [23]	Arizona, Maryland	Dissected guts	Pyrotags 454	329,550	99.1	1.0	1.0	9.1	11.9	2.0	45.4	23.2	5.4	0.9
Engel et al., 2012 [37]	Arizona	Hindguts of worker bees	Illumina sequences	76.6 Mb <sup>d</sup>	82.4	13.8	3.4	4.9	23.9		9.7		3.4	17.6
<b>Cultivation-dependent techniques<sup>c</sup></b>														
Evans and Armstrong, 2006 [55]	USA	Individual larvae	Sanger	11	n/a	-	-	-	-	-	-	+ (1)	-	<sup>b</sup>
Olafsson and Vásquez 2008 [56]	Sweden	Guts	Sanger	17	n/a	-	-	-	+ (3)	+ (1)	+ (1)	+ (4)	+ (5)	<sup>b</sup>
Vásquez and Olafsson 2009 [57]	Arizona	Guts	Sanger	11	n/a	-	-	-	+ (1)	-	+ (1)	+ (2)	+ (4)	-
Sabaté et al., 2009 [58]	Argentina	Pooled intestines	Sanger	1	n/a	-	-	-	-	-	-	-	-	<sup>b</sup>
Loncaric et al., 2011 [59]	Austria	Honey sac	Sanger	11 <sup>e</sup>	n/a	-	-	-	-	-	-	-	-	<sup>b</sup>
Carina Audisio et al., 2011 [60]	Argentina	Intestines	Sanger	5	n/a	-	-	-	-	-	-	-	-	<sup>b</sup>
Vásquez et al., 2012 [44]	Sweden and Kenya	Dissected honey crops	Sanger	137 <sup>e</sup>	n/a	-	-	-	-	-	+ (4)	+ (7)	+ (29)	<sup>b</sup>

<sup>a</sup> For studies with deep sequencing methods, percent values of phylotype abundance are indicated. In those studies where methods do not allow percent representation, “+” indicates the presence of a phylotype. Figures in parentheses indicate the numbers of sequences associated to a bacterial group. “-” indicates no presence of a phylotype.

<sup>b</sup> Sequences of other bacteria, besides the phylotypes presented in the table, have been retrieved but the frequencies cannot be calculated due to the methods employed in these case studies.

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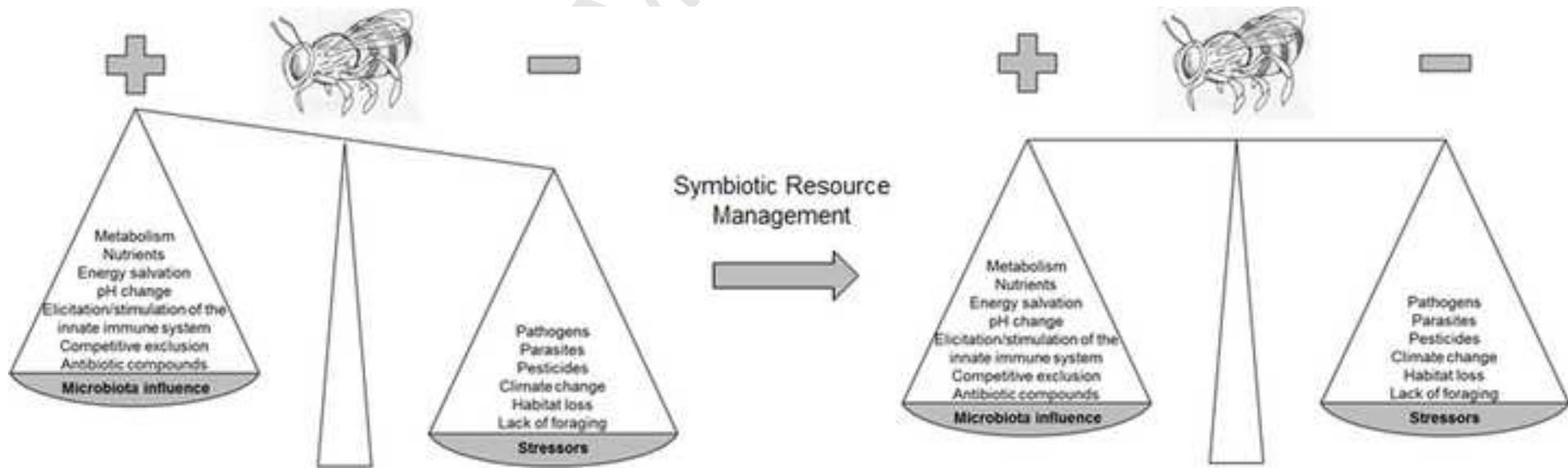
461 ° Cultivation-dependent methods do not allow to represent all bacteria in the gut.  
462 ° These numbers are from a dataset of metagenomic data.  
463 ° These numbers are from a dataset including also, but not only, sequences from *A. mellifera*.  
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465 **Figure 1.** A graphical representation of the ecological concept of Symbiont Resource Management  
466 (SMR) which foresees the management of the insect gut microbiome in order to improve host  
467 health.

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When stressors prevail, dysbiosis occurs and honeybee experiences health problems

Re-balance of dysbiosis contributes to limit stressor effects, restoring healthy conditions