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5 **Synthetic microbial ecosystems: an exciting tool to understand and**

6 **apply microbial communities**

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11 Authors: Karen De Roy¹, Massimo Marzorati¹, Pieter Van den Abbeele¹, Tom Van de Wiele¹

12 and Nico Boon^{1*}

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14

15 ¹Laboratory of Microbial Ecology and Technology (LabMET), Gent, Belgium

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17

18 *Corresponding author: Nico Boon, Coupure links 653, 9000 Gent, Belgium, tel.: +32(0)9/264

19 5976, fax: +32(0)9/264 6248, Nico.Boon@UGent.be

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21 **Abstract**

22 Many microbial ecologists have described the composition of microbial communities in a
23 plenitude of environments, which has greatly improved our basic understanding of
24 microorganisms and ecosystems. However, the factors and processes that influence the
25 behaviour and functionality of an ecosystem largely remain black boxes when using
26 conventional approaches. Therefore, synthetic microbial ecology has gained a lot of interest in
27 the last few years. Because of their reduced complexity and increased controllability, synthetic
28 communities are often preferred over complex communities to examine ecological theories.
29 They limit the factors that influence the microbial community to a minimum, allowing their
30 management and identifying specific community responses. However, besides their use for
31 basic research, synthetic ecosystems also found their way towards different applications, like
32 industrial fermentation and bioremediation. Here we review why and how synthetic microbial
33 communities are applied for research purposes and for which applications they have been and
34 could be successfully used.

35

36 **Introduction**

37 Microorganisms are ubiquitous on earth, with an estimated amount of 10^6 bacterial species

38 (Lopez-Garcia and Moreira, 2008) and 4×10^{30} microbial cells globally (Horner-Devine *et al.*,

39 2004). Their genetic and physiological diversity result in an enormous metabolic potential.

40 They contribute to nearly all biogeochemical cycles as they are the drivers of global and local

41 nitrogen, oxygen, carbon, sulphur and phosphorus cycles (Schmidt, 2006), what makes them

42 essential for maintaining the earth's biosphere and for the survival of plants and animals.

43 Most of these processes are accomplished by joint effort of microorganisms with different

44 functional roles. These microorganisms do not act as individuals, but rather act as a

45 dynamically changing microbial community, where all cells interact and communicate with one

46 another (Little *et al.*, 2008; Klitgord and Segre, 2010). They influence each other's behaviour

47 and possibly alter the biochemical phenotypes of the participating strains (Wintermute and

48 Silver, 2010).

49 Understanding the factors that shape and influence these microbial ecosystems is essential from

50 a microbiological, ecological and biotechnological point of view. According to Prosser *et al.*

51 (2007), this knowledge can be achieved by using a theory driven approach: theories are

52 generated based on existing observational data, after which they are verified using quantitative

53 research. A deliberate choice of the experimental setup, methodology and microbial model

54 systems is indispensable for optimal hypothesis testing. Pure cultures and complex microbial

55 communities are conventionally used, however synthetic ecosystems with intermediate

56 complexity and high controllability are becoming increasingly popular.

57 **From synthetic biology to synthetic microbial ecology**

58 Culture-dependent methods allow the isolation of single microbial community members for in-
59 depth analysis of their genetic and physiological characteristics. The body of literature on
60 research with single microorganisms is tremendous (Jessup *et al.*, 2005). Since the -omics era,
61 a lot of knowledge on these simple model systems is gained. Over 4000 complete microbial
62 genomes have been sequenced, while more than 12000 are in progress
63 (www.genomesonline.org). Transcriptomics, proteomics and metabolomics gave further insight
64 into their functionality, resistance to stress and adaptation. This increased understanding on
65 how microorganisms function, led to the urge to steer and manipulate them. Synthetic biology,
66 which is the application of engineering methodology to biology, was proven to be very useful
67 (Endy, 2005; Leonard *et al.*, 2008). Microorganisms have been engineered to improve their
68 resistance to stress, to have a higher productivity and functional redundancy, to degrade toxic
69 and recalcitrant compounds, to synthesize new chemical compounds or to have other particular
70 –unnatural - characteristics (Benner and Sismour, 2005). The numerous capacities of both
71 genetically engineered and wild-type microorganisms make them interesting for different
72 applications. They are used as probiotics in the medical and food industry (Steidler *et al.*, 2000;
73 Huijbregtse *et al.*, 2012), as cell factories for valuable products in the food, pharmaceutical,
74 chemical and agriculture industry, with products ranging from anticancer drugs to biofuels (Du
75 *et al.*, 2011; Waegeman and Soetaert, 2011).

76 The fact that (i) only a small fraction of the microorganisms present in a microbial community
77 can be cultured and (ii) the behaviour of microorganisms as pure cultures is different from their
78 behaviour in a microbial community has caused a shift from single-organism studies to whole
79 community studies. Molecular fingerprinting and high-throughput sequencing techniques are
80 used to characterize these microbial communities. These techniques use a top-down approach
81 and target microbial communities as a whole. Metagenomics, metatranscriptomics and

82 metaproteomics give information on the taxonomic and functional diversity, the population
83 structure, the presence of genes as well as their levels of expression and translation into
84 proteins (VerBerkmoes *et al.*, 2009; Temperton and Giovannoni, 2012). A drawback is the
85 complex post-processing of the big amount of data obtained by these high-throughput
86 techniques (Raes and Bork, 2008). Even with the most advanced bioinformatics tools and
87 sequencing technology, it is almost impossible to assign the (expressed) genes and proteins,
88 and thus the functionality, to specific species (Temperton and Giovannoni, 2012; Zengler and
89 Palsson, 2012). Furthermore, it is not possible to fully map and understand the microbial
90 interactions, which are often the driving force of a community.

91 Compared to the amount of literature available on single organisms and complex microbial
92 communities, only a small fraction of microbial ecology research makes use of synthetic
93 microbial communities. Synthetic microbial ecology is a collective term for all rationally
94 designed ecosystems that are created by a bottom-up approach where two or more defined
95 microbial populations are assembled in a well-characterised and controlled environment
96 (Figure 1). These synthetic ecosystems have a lower complexity, higher controllability, higher
97 reproducibility and are a simplified representation or simulation of natural ecosystems.

98 Synthetic ecosystems are used (i) to gain insight in fundamental principles such as metabolic
99 processes, interactions, networking, diversity-functionality relation and nutrient cycling and (ii)
100 to create interactions and communities with desired characteristics and functionality.

101 Alternative terms for similar experimental setups are microcosms or artificial ecosystem, while
102 other terms have been mistakenly used for synthetic ecology: (i) synthetic biology, which is the
103 engineering of cells and (ii) systems biology, which considers the use of a top-down approach
104 to understand a system by characterizing the different parts.

105 **Synthetic microbial ecology for theory testing**

106 While a microbial community as such is already complex, numerous environmental factors
107 further increase the level of complexity (Figure 2). Microorganisms live in close contact with
108 each other as they continuously **interact** and **communicate (A)** with one another (Little *et al.*,
109 2008; Klitgord and Segre, 2010). These interactions may be unidirectional or bidirectional
110 (West *et al.*, 2006). Molecules are produced that can be beneficial or detrimental for both the
111 actor and recipient. Different kinds of interactions and cooperation are present in nature:
112 mutualism, syntrophy or cross-feeding (beneficial to the actor/beneficial to the recipient; +/+),
113 selfishness (beneficial to the actor/costly to the recipient; +/-), spite (-/-) and altruism (-/+ or
114 parasitism (+/-) (West *et al.*, 2007; Faust and Raes, 2012). Microorganisms can **communicate**
115 with one another through mechanisms like quorum sensing, which allow them to express
116 certain genes only under favorable circumstances (Manefield and Turner, 2002). Next to the
117 abundant microorganisms that actively contribute to the **functionality** of the ecosystems,
118 numerous species are present in lower abundance. They are regularly categorized as redundant
119 and are responsible for the **resilience (E)** of the community (Bissett *et al.*, 2013). **Abiotic**
120 **factors (C)** like temperature, salinity and pH can alter the environment in such a way that they
121 cannot perform their role in the community anymore (Wu and Conrad, 2001; Sharma *et al.*,
122 2006). Under these circumstances, redundant species can take over and guarantee the
123 ecosystem functionality. The resilience of a community is thus also strongly dependent on the
124 **community diversity (B)** (Loreau *et al.*, 2001). Both the number of microorganisms (richness)
125 and their relative abundance (evenness) influence the resistance to stress, invasion and
126 predation (Wittebolle *et al.*, 2009; Saleem *et al.*, 2012; De Roy *et al.*, 2013). Next to the
127 microbial diversity, also the **spatial organization (F)** as it exists in a biofilm, can be of
128 importance (Tolker-Nielsen and Molin, 2000). It allows only those species that are located in

129 close proximity to interact and communicate with each other; furthermore, it provides
130 microenvironments and niches for specific microbes.

131 All these factors shape, characterize and influence an ecosystem and its functioning. By
132 interfering with one of these parameters, a complete ecosystem might collapse. However, also
133 the opposite might happen as an ecosystem may perform better or new functions can be
134 introduced. By doing research and gaining knowledge on these fundamental principles, it will
135 become possible to steer, manage and create ecosystems to optimize their performance.

136
137 *In situ* or *in vivo* models are complex systems in which nearly all of the above-mentioned
138 influencing factors are present, thus giving a good representation of the real situation. The
139 complexity of the microbiota in these systems is useful for the validation of different products
140 or treatments, but may also be a confounding factor for research purposes, as most of the
141 influencing factors are hard to control. Intrinsic system effects and reciprocal interactions may
142 even lead to opposite conclusions on the role of a specific parameter in closely related
143 ecosystems (Wilsey and Polley, 2002; Emery and Gross, 2007). For this reason, synthetic
144 ecosystems are a powerful tool to investigate fundamental principles in natural and engineered
145 systems. They limit the influencing factors to a minimum, allowing their management and
146 tracking of the effects of the above-mentioned parameters. Furthermore, fully characterized
147 microorganisms with a well-defined genetic background can be used in synthetic ecosystems.
148 In the following paragraphs, we provide several examples of how synthetic microbial
149 ecosystems have been used to study the role of specific influencing factors.

150 The first synthetic ecosystems were used to study microbial **interactions and signalling**, as
151 reviewed by Yu *et al.* (2012). For this type of research, communities mainly consist of only two
152 or three microbial species, which are often also being genetically engineered to create the
153 interaction of interest or to simplify tracking of the parameters of interest. In this way,

154 hypotheses can be tested that would otherwise not be accessible (Wintermute and Silver, 2011).
155 Next to creating an interacting community by genetically engineering the organisms, Klitgord
156 and Segre (2010) showed it is also possible to create interactions by changing the environment:
157 for every two species-consortia, a cooperation-inducing environment could be identified.
158 **Environmental factors**, like the availability of nutrients, temperature, presence of toxic
159 compounds and oxygen-level not only influence microbial interactions, but also influence the
160 **resilience** of a community, which on its turn is influenced by the microbial **diversity**. To get
161 insight in the biodiversity-productivity relationship along different kinds of stress, researchers
162 also opted for synthetic microbial ecosystem experiments. This allows controlling the evenness
163 and richness, the applied stress and the follow up of the functionality, which is not possible in
164 natural environments. Doing so, Wittebolle and coworkers investigated the effect of
165 community evenness on the functionality of a denitrifying bacterial community in the presence
166 and absence of salinity stress. They created over 1000 synthetic ecosystems in 96-well plates
167 with the same 18 denitrifying strains, but with different levels of initial evenness. It was
168 concluded that highly uneven communities (low biodiversity) are less resistant to
169 environmental stress than even communities (high biodiversity). The latter could better retain
170 their functionality under stress conditions (Wittebolle *et al.*, 2009). In another study regarding
171 the effect of richness on resistance to cadmium pollution, 330 synthetic ecosystems
172 characterized by changing numbers of algal species were created. It was shown that the
173 conservation of biodiversity (richness) may reduce the future impacts of increasing
174 environmental stresses (Li *et al.*, 2010). A positive relationship between richness and
175 functionality was also shown by Bell *et al.* (2005) by using synthetic microcosms with up to 72
176 bacterial species. Finally, Gravel *et al.* (2011) showed that the loss of specialists - strains that
177 exploit only few resources - has a stronger effect on ecosystem functioning, compared to loss of
178 generalists, which are able to use a spectrum of substrates.

179 The effect of trophic interactions - such as **predation** - on ecosystem functioning was
180 investigated by altering the predator and prey richness. Predators were simulated by three
181 bacterivorous protists, while five bacterial strains were used as model organisms of the prey. It
182 was shown that the presence of multiple predators resulted in increased bacterial diversity,
183 which had a positive effect on bacterial yields (Saleem *et al.*, 2012; Saleem *et al.*, 2013).

184 As the effect of **invasion** is mainly studied during observational studies in natural ecosystems,
185 many controversies on the outcome of invasion exist (Lambertini *et al.*, 2011; Lockwood *et al.*,
186 2011). By using more than 3000 synthetic ecosystems, it was show by De Roy *et al.* (2013) that
187 the contradicting results can be explained by the environmental condition under which invasion
188 occurs. In the absence of salt stress, invasion by non-native species in an uneven community
189 had adverse effects on the community functionality. In contrast under stress, invasion of the
190 same strain can help the community to perform better. Invasion was also shown to be higher in
191 uneven communities compared to even communities in the absence of salt stress. On the
192 contrary, evenness has no effect on invasion in the presence of stress. The importance of the
193 environment on interactions between different species was also shown by Hu *et al.* (2010). By
194 using two quorum-sensing circuits, they designed a synthetic ecosystem in which different
195 antibiotic and initial cell density levels resulted in different interactions and population
196 dynamics, such as extinction and mutualism.

197 Finally, the **spatial organization and architecture** of microbial communities is also crucial to
198 maintain a stable and functional community. By combining FISH with a digital image analysis
199 software that quantifies the spatial localization patterns of microorganisms in complex samples,
200 it was shown that functionally linked species cluster together in a microbial community (Daims
201 *et al.*, 2006). Kim *et al.* (2008) controlled the spatial organization of a community by using a
202 microfluidic device that controls the distance between three wild type soil bacterial populations
203 with syntrophic interactions. In this community each species is required for the survival of the

204 community. It was shown that spatial organization is necessary to balance competition and
205 beneficial interactions to create a stable community (Kim *et al.*, 2008). Brenner *et al.* (2011)
206 used two genetically engineered *E. coli* populations to study the benefits of the formation of
207 physical structures like biofilms. Species associated in a biofilm were shown to be more
208 productive than non-associated community members.

209 In conclusion, the use of synthetic ecosystems increased our knowledge regarding factors that
210 shape and influence microbial communities. Such advances would have been difficult to obtain
211 in natural ecosystems due to the presence of confounding factors which are hard to control or
212 measure. As a result, the research regarding synthetic ecosystems initiates many opportunities
213 to manage ecosystems. By changing one of the parameters, the community can be steered and a
214 desired effect can be created. This approach is generally known as microbial resource
215 management (MRM) and will be elaborated in the following section.

216 **Synthetic communities for applications**

217 Microbial Resource Management (MRM) has been defined as the optimal management of
218 microbial resources in order to develop novel products and processes to improve the
219 environment or human health in the most sustainable way (Verstraete *et al.*, 2007; Read *et al.*,
220 2011). Management may occur at the level of single cells, i.e. engineering of individual
221 microbial populations to improve their resistance to stress, to have a higher productivity or to
222 degrade toxic compounds (Benner and Sismour, 2005). Furthermore, management may also
223 occur at the level of the complex microbial community, which inhabits natural and
224 anthropogenic environments and whose final functionalities often result from metabolic
225 networking among the different members. As described above, both extremes have distinct
226 advantages and disadvantages. Next to research purposes, synthetic ecology can therefore be of

227 importance for the development of specific applications, representing a good balance in terms
228 of complexity, relevance and manageability.

229 Synthetic communities can be used, for instance, to recycle waste products. The European
230 Space Agency (ESA) designed MELiSSA (Micro-Ecological Life Support System Alternative),
231 a bioregenerative life support system for the complete recycling of gas, liquid and solid wastes
232 during long distance space exploration (Fulget *et al.*, 1999; Hendrickx *et al.*, 2006). In
233 MELiSSA, cyanobacteria and plants are use as food sources. As both cyanobacteria and plants
234 preferentially take up nitrogen as nitrate, the ammonium-enriched liquid waste derived from
235 human activities needs to be nitrified to nitrate to create the most optimal recycling system.
236 Therefore, ammonia is oxidized to nitrite by ammonia-oxidizing bacteria (i.e. genera
237 *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio*) and then nitrite to
238 nitrate by nitrite oxidizers (i.e. genera *Nitrobacter*, *Nitrococcus*, and *Nitrospira*). Considering
239 that MELiSSA has been designed for space exploration, the stability of the system is a key
240 aspect in order to assure long-term functionality. In this respect, the choice of a synthetic
241 community should assure both a functional and compositional stability as the environment is
242 well-defined and the required metabolic conversions are not complex. In fact, according to
243 Pimm (1984), the more the functionality of one species depends on the activity of another
244 species, the fewer species will be necessary to maintain ecosystem stability. Moreover, as the
245 loss of a species would lead to the disruption of the whole ecosystem, the designed synthetic
246 community should be also resilient to perturbation (Pimm, 1984).

247 Synthetic communities also play a key role in the industrial fermentation and production of
248 chemical compounds. In industrial bioethanol production, most ethanol is produced by the
249 fermentation of glucose or sucrose from corn, sugar cane or beets. Because this competes with
250 food production, alternative sources of sugar are investigated, such as lignocellulosic biomass.
251 Glucose and xylose are the two dominant sugars. But current approaches are inefficient, since

252 no native microorganisms can convert all sugars into ethanol at high yield. Therefore co-
253 cultures of strains that have a high yield for different sugars are used (Chen, 2011). Patle and
254 Lal (2007) showed that a very simple community composed of *Zymomonas mobilis* and
255 *Candida tropicalis* was able to transform enzymatically hydrolysed lignocellulosic biomass in
256 ethanol with a yield of 97.7%. Mixed-culture fermentation from lignocellulosic biomass for
257 ethanol production can increase ethanol yield and production rate and reduce process cost.
258 Synthetic microbial communities consisting of *Ketogulonicigenium vulgare* and *Bacillus*
259 *megaterium* have been used in industry to produce 2-keto-gulonic acid (2-KGA), the precursor
260 of vitamin C (Ma *et al.*, 2011). By means of quantitative systems biology analysis, it was
261 shown that the cell lysis of *B. megaterium* provided key elements necessary for *K. vulgare* to
262 grow better and produce more 2-KGA, as compared to the production as a pure strain. Also
263 Masset *et al.* (2012) demonstrated the benefits of working with a synthetic community as
264 compared to pure strains in the field of hydrogen production from starch. Traditionally, pure
265 strains give better H₂-yields as compared to mixed communities. However, the main limitation
266 of this approach is the need to work under sterile conditions. Communities composed by
267 *Clostridium pasteurianum* and *Clostridium felsineum* and by *Clostridium butyricum* and
268 *Clostridium pasteurianum* were shown to offer better performance in terms of H₂ production
269 from different carbon sources than the single strains. Moreover, in contrast with the pure
270 cultures, the co-cultures were able to use starch without any need for pre-hydrolysis.
271 Another field of application for synthetic communities is the bioremediation of contaminated
272 areas. This approach often relies on the addition of microorganisms with the metabolic
273 potential to degrade a specific contaminant, i.e. bioaugmentation. Given the high complexity of
274 some contaminants, bioaugmentation of single strains may not be sufficient to achieve a good
275 'removal efficiency', as demonstrated in the case of the pesticide linuron (Dejonghe *et al.*,
276 2003). *Variovorax* sp. strain WDL1 could degrade linuron using it as C, N and energy source.

277 Conversely, *Delftia acidovorans* WDL34 and *Pseudomonas* sp. strain WDL5 were not able to
278 use linuron but only some intermediate of its degradation. When these strains were mixed in a
279 synthetic community, the rate of linuron degradation improved due to the synergistic
280 interaction of the strain WDL1 with the other bacteria. A similar case is represented by the
281 degradation of 4-chlorosalicylate (4-CS). This compound can only be degraded if *Pseudomonas*
282 *reinekei* (MT1), *Wautersiella falsenii* (MT2), *Achromobacter spanius* (MT3) and *Pseudomonas*
283 *veronii* (MT4) work together (Pawelczyk *et al.*, 2008).

284 A final example is the application of synthetic microbial communities as a safe alternative for
285 human faecal transplants. Because the human gut contains a dense (10^{13} - 10^{14} microbial cells)
286 and diverse microbial community (Eckburg *et al.*, 2005), consisting of several hundred
287 microbial species, severe disturbances of this ecosystem are unlikely to be resolved by the
288 administration of a single probiotic strain. Indeed, recurrent *Clostridium difficile*-associated
289 diarrhoea (Khoruts *et al.*, 2010; Guo *et al.*, 2012), which is thought to result from persistent
290 disruption of the commensal gut microbiota, was cured upon transplantation of a complex
291 faecal microbiota derived from a healthy human donor (Shahinas *et al.*, 2012). This approach is
292 however only applied in severe cases given the high complexity of a human faecal sample,
293 which is inherently associated with a certain risk for transmitting disease. As a result, there is a
294 large potential for synthetic ecology to mix a well-characterized and safe set of gut
295 microorganisms. Petrof *et al.* (2013) synthesized a synthetic microbiota consisting of 33
296 individual microbial species and indeed demonstrated the potential of such synthetic microbiota
297 in the eradication of *Clostridium difficile* infections. Such approaches may result in a
298 replacement of commonly used antibiotics.

299 All the cases described in this section demonstrate the potential that synthetic communities may
300 cover in practical applications. Despite this potential, the road to translate MRM into practice is
301 still long and several aspects require further investigation, as outlined below.

302 **Future perspectives**

303 The majority of synthetic ecosystems consist of only two to four species. Although being very
304 useful to study ecological theories, the resemblance with natural ecosystems and potential for
305 practical applications may be more limited. Therefore, a next step in synthetic ecology is to
306 create synthetic ecosystems with increasing resemblance to natural ecosystem. The better a
307 model can simulate the actual complexity of nature, the higher its scientific value. Firstly, this
308 can be achieved by using sophisticated experimental models that better simulate the
309 environmental factors. An example of a sophisticated model is a high-pressure reactor to
310 simulate the deep-sea environment (Zhang *et al.*, 2011). Secondly, synthetic ecosystems can be
311 optimized by increasing the number of species and optimizing their composition, structure and
312 functionality. Such studies have mostly been restricted to short-term experiments, due to
313 stability issues of synthetic communities. It was theoretically shown by ecological models that
314 some species and specific mixtures of agonistic and mutualistic interactions between species
315 are necessary to obtain a stable ecosystem (Boyd, 2012; Mougi and Kondoh, 2012). The
316 integration of such models in microbial ecology would be of high value. Research with
317 synthetic microbial ecosystems created an enormous amount of complementary data, in
318 addition, the genomes of numerous microorganisms have been sequenced. By combining these
319 data and information, *in silico* models making use of ‘digital microorganisms’ could be created
320 and used for the construction of synthetic ecosystems with desired characteristics (Figure 3)
321 (Yedid *et al.*, 2009). Furthermore, these models could be used to predict an ecosystem’s
322 behaviour like stability, resistance and functionality. The problem with many ecological models
323 is the lack of validation and overparameterization. Therefore, we argue to use real ecosystem,
324 *in vivo* models, sophisticated *in vitro* models or synthetic ecosystems for the validation of *in*
325 *silico* theoretical models and correct for possible overparameterization. But also to use real
326 ecosystems to check the relevance of synthetic ecosystems, since numerous important factors

327 could be missed. Only when this is done, models can really contribute to the understanding,
328 prediction and management of ecosystems.

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505 **Figure legends**

506 **Figure 1. Strategy of how to create synthetic ecosystems.** Synthetic communities are created
507 by a bottom-up approach. This includes that microorganisms are initially isolated from their
508 natural environment, via conventional culture-based techniques. Upon growth in liquid media,
509 they are quantified via flow cytometry and diluted to the desired cell numbers. Synthetic
510 communities are then created by mixing microbial species in specific proportions under desired
511 conditions, after which they are incubated. Finally, all parameters of interest, like functionality
512 and cell count, are analysed.

513

514 **Figure 2. Synthetic ecosystems for research purposes.** Natural ecosystems are complex as
515 many factors influence and shape microbial communities. These factors are include: A)
516 microbial metabolic interactions, signalling and communication B) diversity, C) abiotic or
517 environmental factors, D) biotic factors like invasion and predation, E) resilience and
518 redundancy and F) architecture and spatial organization. Research with pure cultures provides
519 information on genetic, physiological and morphological characteristics of specific microbes
520 (a), as well as on their resistance and sensitivity to stress (b). However, they do not allow
521 researchers to investigate the factors that shape and influence microbial communities. For this
522 purpose, synthetic ecosystems are a powerful tool as they have a reduced complexity and
523 higher controllability compared to natural ecosystems. They also allow to focus on specific
524 parameters of interest while excluding other influencing factors.

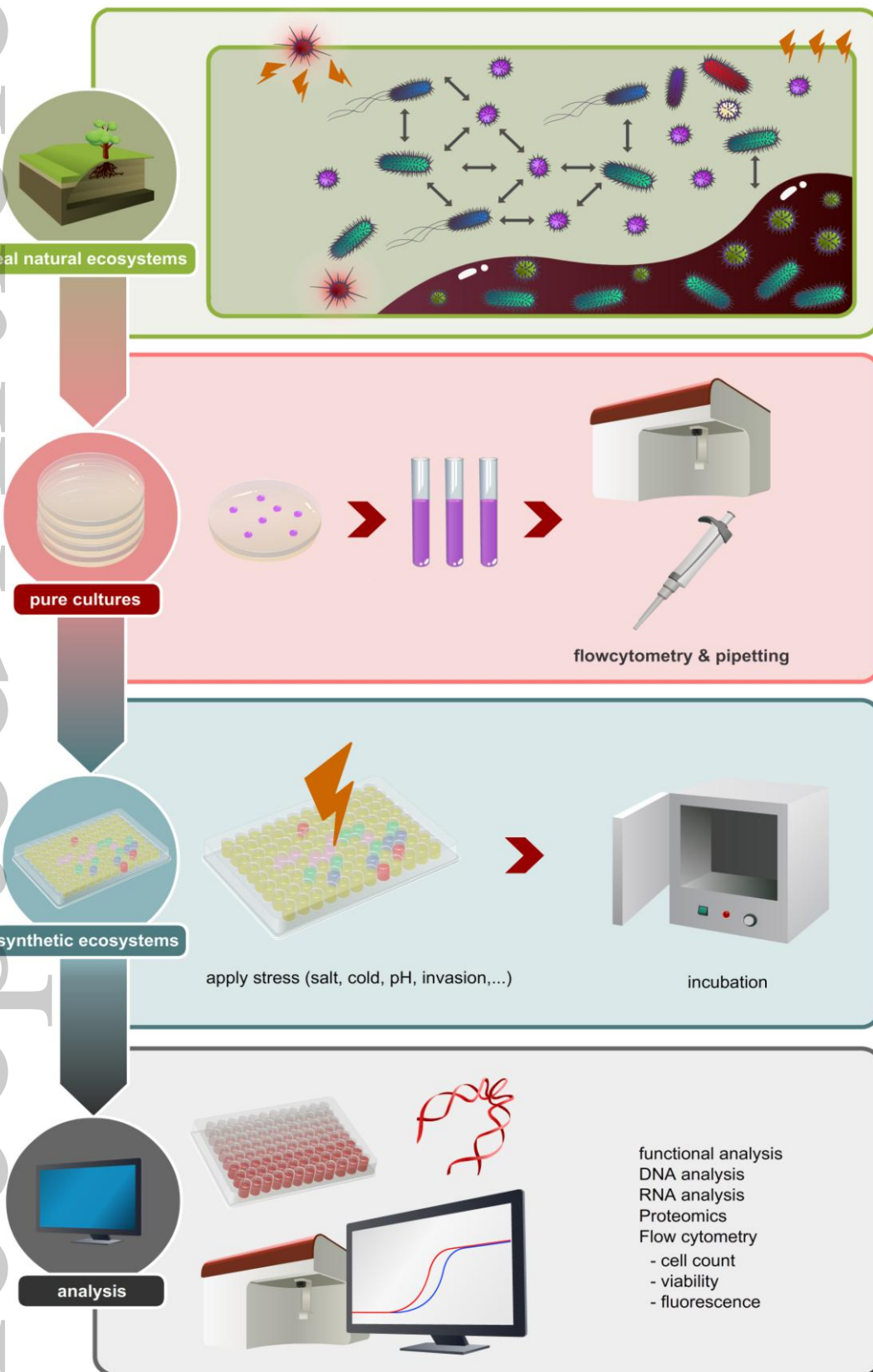
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526 **Figure 3. The future of synthetic ecosystem research.** Research with synthetic ecosystems
527 drastically increased the knowledge on microbial ecosystems. All this information could be

528 used to create *in silico* models that can predict an ecosystem 's behaviour. After validation and
529 correction for possible over- or underparameterization, these models could be used to
530 understand, predict, manage and create ecosystems.

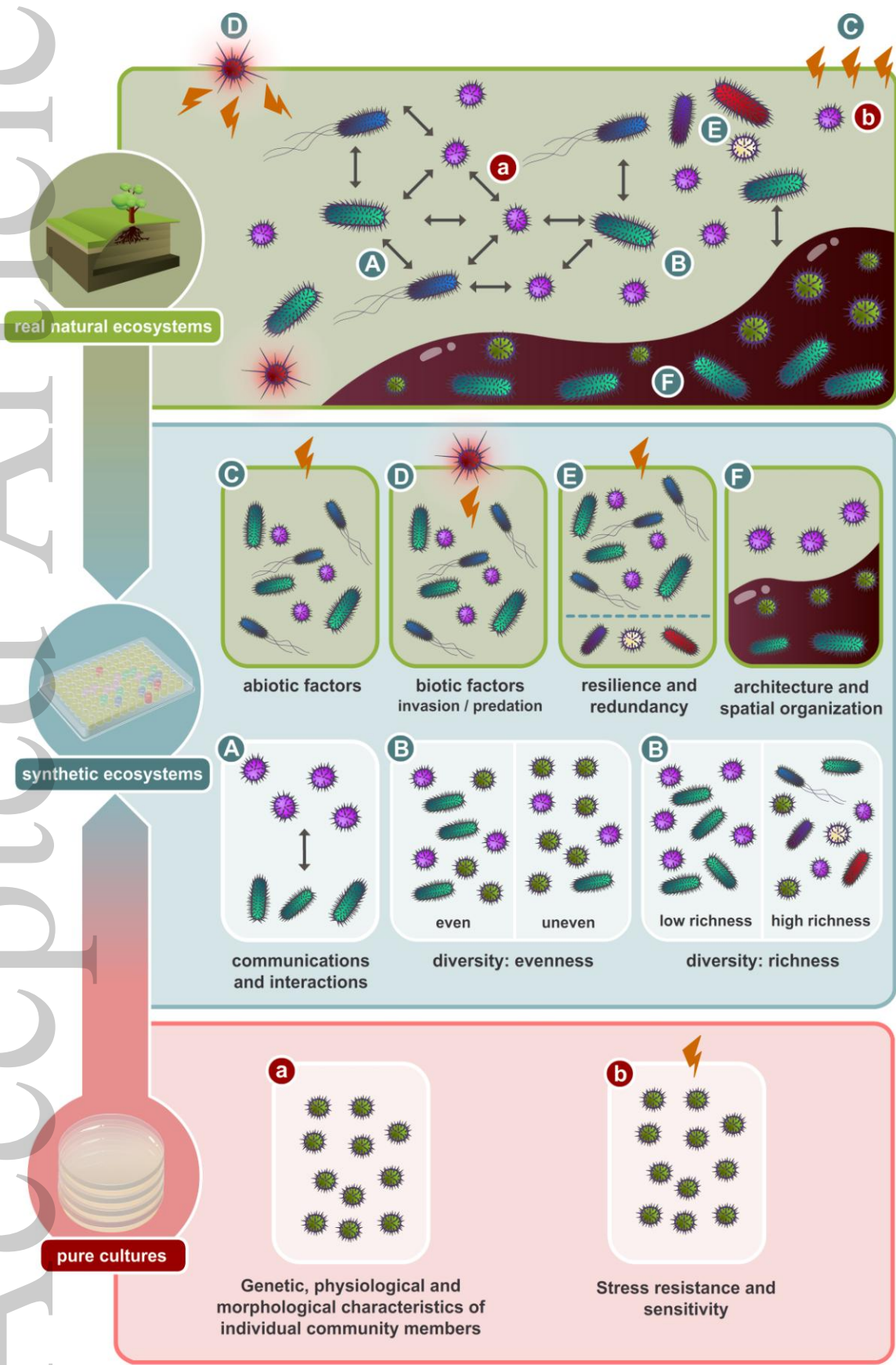
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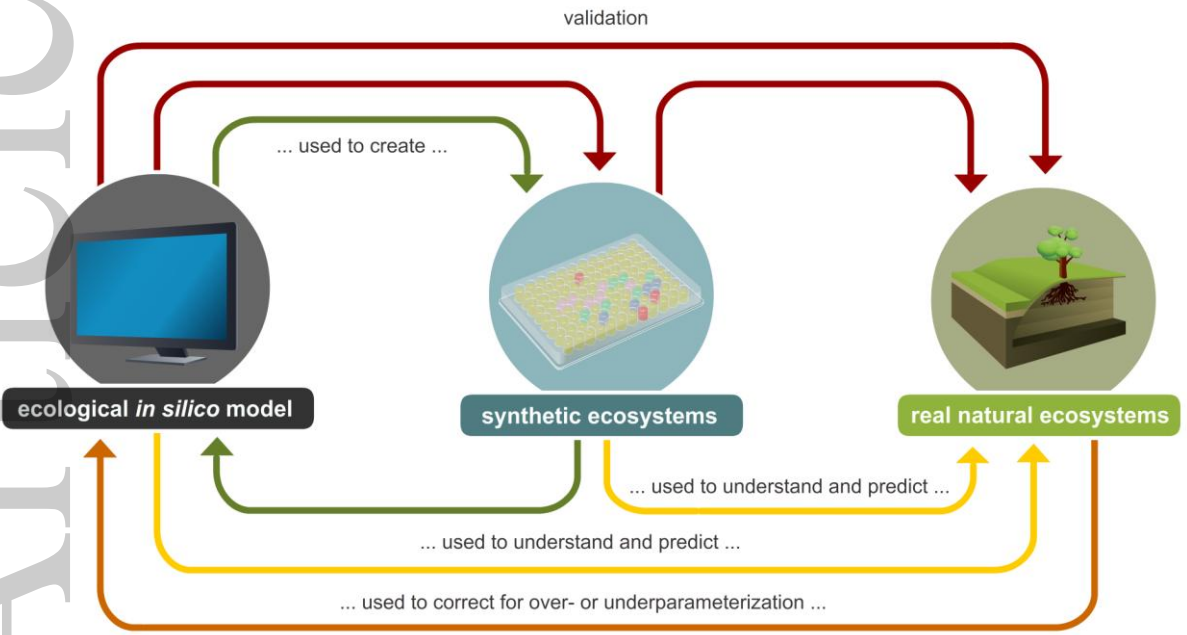
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