

1 **Drinking Water Microbiome Project: Is it Time?**

2 Natalie M. Hull¹, Fangqiong Ling², Ameet J. Pinto³, Mads Albertsen⁴, H. Grace Jang⁵, Pei-Ying
3 Hong⁶, Konstantinos T. Konstantinidis⁷, Mark LeChevallier⁸, Rita R. Colwell⁹, and Wen-Tso
4 Liu^{10*}

5
6 ¹Civil, Environmental, and Geodetic Engineering, The Ohio State University, Columbus, OH
7 43210, USA.

8 ²Department of Energy, Environmental, and Chemical Engineering, Washington University in St.
9 Louis, St. Louis, MO 63130, USA.

10 ³Department of Civil and Environmental Engineering, Northeastern University, Boston, MA
11 02115, USA.

12 ⁴Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark.

13 ⁵The Water Research Foundation, Denver, CO 80235, USA.

14 ⁶Biological and Environmental Science and Engineering Division, King Abdullah University of
15 Science and Technology, Thuwal 23955, Saudi Arabia.

16 ⁷School of Civil and Environmental Engineering and School of Biological Sciences, Georgia
17 Institute of Technology, Atlanta, GA 30332, USA.

18 ⁸Dr. Water Consulting, LLC, Morrison, CO 80465, USA.

19 ⁹Center for Bioinformatics and Computational Biology, University of Maryland Institute for
20 Applied Computer Studies, College Park, Maryland, and CosmosID, Rockville, MD 20742, USA

21 ¹⁰Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, Urbana, IL
22 61801, USA.

23 *Correspondence: wliu@illinois.edu (W.-T. Liu)

24

25 **Keywords**

26 Microbiome, Meta-omics, Drinking water, Microbial ecology

27 **Glossary**

- 28 • **Meta-Omics, Omics:** Incorporates more than one of the following -omics approaches:
29 metagenomics (study of genomic contents in a sample), metatranscriptomics (study of
30 transcribed genes in a sample), metabolomics (study of metabolites in a sample),
31 metaproteomics (study of proteins in a sample), and interactomics (study of interactions
32 between multiple molecule types in a sample).
- 33 • **Drinking Water:** water that has been treated to be potable, or derives from a protected source.
- 34 • **Community Water Systems:** public systems supplying water for human consumption.
- 35 • **Microbial Ecology:** the relationships of microorganisms (including bacteria, archaea, fungi,
36 protists, and viruses) with each other and with their environment [1].
- 37 • **Microbiome:** the collection of microorganisms, their activities, and all other biotic and abiotic
38 factors in their environment [1].
- 39 • **Biogeography:** distribution of biological communities over space and time.
- 40 • **Biogeochemistry:** chemical, physical, geological, and biological processes and reactions
41 affecting biological systems.
- 42 • **Premise plumbing:** portion of potable drinking water distribution systems in buildings.
- 43 • **Citizen Science:** public participation in research conducted by professional scientists.

44 **Abstract**

45 Now is an opportune time to fund and foster collaborations across sectors and geographical
46 boundaries to enable development of best practices for drinking water (DW) microbiome research,
47 focusing on accuracy and reproducibility of meta-omic techniques (while learning from past
48 microbiome projects). A large-scale coordinated effort that builds on this foundation will enable
49 the urgently needed comprehensive spatiotemporal understanding and control of DW microbiomes
50 by engineering interventions to protect public health. This opinion paper highlights the need to
51 initiate and conduct a large-scale coordinated DW microbiome project by addressing key
52 knowledge gaps, and recommends a roadmap for this effort.

53

54 **Justification for a Large-scale Coordinated Drinking Water Microbiome Project (DWMP)**

55 Drinking water distribution systems (DWDSs) throughout the world host diverse
56 microorganisms in biofilms and bulk water suspensions that make up the drinking water (DW)
57 microbiome, resulting in tap water teeming with microbial life, ranging from 10^6 – 10^9 microbial
58 cells per liter [2,3]. In the United States alone, 39 billion gallons of water are withdrawn every
59 day from the environment by community water systems [4] and pass through DWDSs comprised
60 of conventional or advanced treatment processes, more than 2 million miles of distribution
61 networks, and premise plumbing in buildings before delivery of DW to taps (Figure 1).

62 Although water may be microbiologically safe according to regulations and monitoring
63 designed to limit fecal contamination, other microbial risks may be present in treated water or may
64 increase in distribution systems and premise plumbing, where stagnation and decay of disinfectant
65 residual favor growth of microorganisms, especially in biofilms. For example, biofilms and
66 amoebae in DWDSs can harbor opportunistic pathogens (OPs) (such as *Mycobacterium avium*,
67 *Legionella pneumophila*, and *Pseudomonas aeruginosa*) which can cause infections that are
68 increasing in frequency (e.g., by 3.5-fold between 2000-2011 for *Legionella* [5]) with estimated
69 hospitalization costs of \$2 billion per year [6]. This is further exemplified by the legionnaire's
70 disease outbreak during the Flint water crisis, where changes in water quality in distribution
71 systems associated with interrupted corrosion control were linked with high levels of *Legionella*
72 DNA markers in tap waters [7]. While OPs are frequently present in DW microbiomes and various
73 engineering treatments can cause unintended changes in OP relative abundance, factors that affect
74 presence, proliferation, and control of OPs in DW microbiomes are not yet fully understood [8].
75 Additionally, antibiotic resistant bacteria (ARB) and genes (ARGs) are prevalent in DWDS and
76 tap water [9], and ARGs can be transferred between organisms throughout the DW microbiome
77 continuum, which can escalate illness costs (as has been shown for some OPs [6]) and potential
78 risk of morbidity and mortality among vulnerable and infected individuals.

79 Despite the huge importance that exposure to DW microbiomes plays in public health,
80 current understanding of DW microbiomes and their link to water quality parameters, engineering
81 interventions, and disease is incomplete. Using the recent advances in nucleic acid sequencing
82 technologies and big-data science, broad multi-agency programs have been developed to study and
83 elucidate microbiomes of the human body [10] and built environments [11] (see Box 1).
84 Microbiome studies in natural aquatic systems and in human-associated systems have also enabled

85 development of novel diagnostic biomarkers and treatments for disease [10]. In comparison, DW
86 microbiome study significantly lags behind in both sequencing effort invested and knowledge
87 generated (Figure 2). Recent small-scale DNA sequencing surveys have revealed high
88 spatiotemporal heterogeneity and microbial diversity of DW microbiomes [12–15]. These
89 findings and initial models/meta-analyses [14,16,17] suggest it is challenging to generalize DW
90 microbiomes, for example across >55,000 community water systems in the US. Therefore, it
91 remains difficult without a coordinated effort to devise the next advances in DW microbiome
92 diagnostics and treatments to decrease risks to public health.

93 Understanding of DW microbiomes is also urgently needed to optimize future water
94 infrastructure development and combat increasing global challenges. A 2015 US EPA report
95 estimated a \$472.6 billion need for new US DW infrastructure and for rehabilitation, expansion,
96 or replacement of existing DW infrastructure over the following 20 years [18]. When accounting
97 for population growth from 2010-2035, the American Water Works Association estimated a \$1
98 trillion need for US DW infrastructure [19]. To maximize return on this investment, there is a
99 pressing need to understand the impact of microbiomes on the sustainability of these
100 infrastructures (e.g., impacts of nitrification, corrosion, etc.), and the impact of infrastructure
101 design on DW microbiomes relevant to both operational performance and human health.

102 The US is just one example of impending infrastructure development needed to provide
103 safer drinking water around the world. In other regions, water infrastructure age and designs vary,
104 as do source water quality/quantity and socioeconomic capacity for new and existing
105 infrastructure. Because climate change will pose increasing regional threats on water quality and
106 quantity, the extreme rainfall and flooding in some areas but increasingly arid conditions in other
107 areas will likely lead to DW production relying increasingly on wastewater reuse, desalination,
108 unconventional source waters, and advanced treatment technologies. Existing infrastructure and
109 monitoring programs may not be designed to cope with massive inflow (in the earlier scenario),
110 or to ensure water quality when tapping alternative water sources (in the latter scenario). Rising
111 temperatures may also increase the possibility of waterborne diseases outbreaks [16,20–22]. Thus,
112 developing tools to predict DW microbiome responses to changing treatments, source waters, or
113 water quality is becoming important. Likewise, population changes (e.g., an aging population with
114 increased health vulnerabilities, increased global population, increased urbanization, etc.) will also
115 increase the need to engineer DW microbiomes that are safer for all human exposures.

116 **Knowledge Gaps the DWMP Could Address**

117 The ability to define and successfully engineer safe or healthy DW microbiomes will
118 require the DWMP as a discovery process enabling advanced monitoring, predictive modeling,
119 and optimization of engineering interventions to ensure continued public health protection. By
120 conducting a large-scale coordinated DWMP, we can achieve the required depth and breadth of
121 expertise necessary to address important knowledge gaps and inform DW infrastructure
122 investments while anticipating changes in climate, treatment practices, and exposed populations.

123 The most important knowledge gap is to understand the DW microbiome under different
124 conditions, prior to an attempt to define and engineer a healthy or safe DW microbiome. To
125 achieve this, high quality supporting data must be collected to overcome inherent stochasticity in
126 biological systems, and to integrate characteristics of the larger water and ecological systems
127 affecting DW microbiomes. Because of the influence of past conditions on current DW
128 microbiomes (especially in stagnation-prone distribution systems and buildings), efforts should
129 focus on well-defined, composite and grab sampling methodologies (for both biofilms and bulk
130 waters), and on thorough characterization of key biogeochemistry and physical parameters.
131 Representative parameters include pipe materials and diameters, flow rates and regimes,
132 temperature, sampling time, disinfectant type and residual, and measures of current or potential
133 microbial activity (e.g., ATP or assimilable organic carbon), disinfection by-products (DBPs),
134 turbidity, inorganics such as metals, and nutrients (particularly C and N). Water usage and storage
135 data should also be collected. Such data are useful to identify outliers in microbiome data, although
136 there may be disconnects in time scales for relevant underlying processes. Comprehensively
137 defined samples and well-documented, high-quality sampling procedures and analyses will be
138 essential to tease out confounding interrelationships between these parameters. In-depth studies
139 are particularly needed to understand the underlying mechanisms behind the formation of DW
140 microbiome constituents in biofilms. Most DW microbiome biomass is known to reside in biofilms
141 (including minority microbial populations), and upstream biofilm communities can contribute to
142 downstream DW microbiome populations through sloughing and seeding [20,23,24]. Biofilm
143 communities can have negative biochemical impacts on water quality, facilitate antibiotic
144 resistance transfer, decrease water biostability, and harbor organisms with increased disinfectant
145 resistance [25]. Through the DWMP discovery process, we can better derive knowledge of
146 complex DW microbiomes, and use the knowledge gained to predict them through measurements

147 of associated biotic and abiotic parameters. With this concerted effort, the DW microbiome can
148 be better managed.

149 The Outstanding Questions box lists additional knowledge gaps. One of them is to agree
150 on standard protocol(s) to support DW microbiome studies. Numerous studies have shown that
151 variation in microbiomes of different samples can be caused by bias associated with procedures in
152 sampling, DNA extraction, sequencing, and data analysis [26]. Possible protocols can first be
153 identified from previous and ongoing large-scale microbiome characterization projects (Box 1).
154 Next, participants in the DWMP can participate in round-robin validation tests of various
155 approaches. The use of omics tools alone cannot generate all the required knowledge needed by
156 water utilities to better manage water quality, shape the DW microbiome, and ultimately protect
157 public health. It is also important to evaluate coupling various omics tools (e.g.,
158 metatranscriptomics and metagenomics to confirm viable and nonviable cells), with other
159 molecular methods (e.g., qPCR and metagenomics to derive quantitative measurements), or with
160 routinely collected metadata, and to make the underlying methodologies more accessible. These
161 efforts can support the selection of optimal protocols and ensure that samples and data can properly
162 be collected and processed to minimize biases and allow reproducible comparisons among future
163 DW microbiome studies.

164 Another important gap is the lack of a comprehensive microbial inventory and key
165 biomarkers for rapid monitoring. Currently, the rRNA gene is the most widely used marker for
166 determining microbial diversity, but it offers limited resolution at the species level and cannot
167 differentiate between closely related pathogens and their innocuous commensal counterparts. In
168 addition, viruses and microbial eukaryotes must also be accounted since they are an important but
169 overlooked component of the DW microbiome. Compared to the reference genome databases of
170 bacteria that can provide within-species resolution for some pathogens, reference databases for
171 environmental bacteria, archaea, viruses, and eukaryotes (e.g., fungi and protozoa) are less
172 complete. Omics tools can be applied to establish comprehensive microbial inventories as
173 sequencing costs continue to drastically decrease [27], especially for long-read technologies.
174 Whole genome sequencing, reference database development, and curation of existing databases
175 using ontologies relevant for environmental niches and physiochemical aspects of DW
176 microbiomes will increase efficiency of meta-omics efforts by reducing unclassified/unknown
177 sequences.

178 **Framework and Roadmap for DWMP**

179 Figure 3 depicts the roadmap for a large-scale coordinated DWMP with the goal to involve
180 multi-sector efforts to generate data necessary to link DW microbial ecology with abiotic factors
181 to better understand, monitor, predict, and control DW microbiomes. Phase 1 of the DWMP will
182 start with recruitment of diverse stakeholders across sectors to conduct a comprehensive critical
183 review of existing DW microbiome literature. This could be funded by a workshop/training grant
184 for university labs and other stakeholders including utilities/regulators to work together. The effort
185 would focus on the feasibility and applicability of potential methods, experimental comparisons,
186 pros/cons of various approaches, determining what constitutes value-added microbiome data, and
187 meta-analyses of existing longitudinal full-scale/ pilot-system data. The outcome of this review
188 would be used to direct investment required for the large-scale coordinated effort, and to guide
189 essential round-robin testing to standardize DWMP methodology.

190 Other Phase 1 groundwork would be to devise an appropriate framework for organizing
191 collection, analysis, and storage of samples and data necessary for comprehensive characterization
192 in DWDS under normal operations. This requires leadership by a central lab or network of labs,
193 which might be recruited or self-selected from academia (e.g., the authors of this opinion),
194 industry, or government. The leading labs need to be supported by a network including university,
195 government, and commercial laboratories and entities such as utilities and citizen scientists. The
196 central labs would implement guidance for best practices including sample and data collection and
197 processing, and requirements for the minimum QA/QC procedures and information to accompany
198 samples that were determined in the initial critical review. Guidance from the central leading labs
199 in a top-down approach will enable even those with fewer laboratory resources (e.g., citizen
200 scientists and small utilities) to participate and provide meaningful engagement in a bottom-up
201 support network. This concurrent top-down bottom-up approach will deconstruct the monolithic
202 DWMP into feasible and cohesive projects to advance understanding of DW microbiomes.
203 Finally, the consortium will facilitate sharing data, protocols, and samples.

204 Phase 2 of the DWMP will focus on comprehensive baseline characterizations in diverse
205 full-scale systems (e.g., urban vs. rural, various treatment practices, etc.) to build a strong
206 foundation. Bench-scale experiments should also be used to test hypotheses that fall out of the
207 initial review and ongoing full-scale sampling. This will enable the consortium to build an
208 understanding of ecological processes and contributing factors affecting the DW microbiome

209 through space and time. Although different baselines have been observed for various treatment
210 and distribution processes at different spatiotemporal scales, commonalities and trends that can be
211 modeled and engineered may continue to emerge because finished water quality parameters do not
212 generally fluctuate over a wide range. To illustrate, the water industry is typically required to
213 monitor microbial and physiochemical water quality, and operational data (hydrodynamics,
214 treatment processes, etc.). These valuable long-term continuous data can be leveraged along with
215 epidemiological data to draw connections between nascent microbiome data, functionality of the
216 water treatment and distribution systems, and potentially even with human health outcomes.
217 Additionally, utilities could archive samples obtained during their routine surveillance for
218 microbiome analyses by scientific members of the DWMP. This ongoing effort might be funded
219 through research mechanisms for large centers, such as the NSF ERC (Engineering Research
220 Centers) or GRC (Growing Convergence Research), or the NIEHS (National Institute of
221 Environmental Health Sciences) EHSCC (Environmental Health Sciences Core Centers) in the
222 USA to bring together the biological and chemical aspects of the system. The Phase 1 workshop
223 series will build the coalition and provide the background knowledge and justification necessary
224 for such an endeavor.

225 Phase 3 of the DWMP will establish effective communication developed by scientists,
226 regulators, and utilities to raise awareness of the importance and value of DW microbiomes and
227 share research findings with the public. The DWMP engagement of citizen scientists will enable
228 natural channels for public communication. Questions relative to omics analyses and how to
229 design studies and interpret data with respect to shortcomings of molecular techniques should be
230 disseminated by the leadership lab(s) among scientific communities. Clearly identifying and
231 communicating the importance of determining DW microbiomes as a discovery process and of
232 managing DW microbiomes to protect public health will incentivize this research. This
233 communication and education component will be integral to DWMP success.

234 **Concluding Remarks and Future Perspectives**

235 The time is opportune for a large-scale coordinated drinking water microbiome project that
236 includes cross-directorate funding, involvement of laboratories around the world, and cooperation
237 with utilities and the public. Although we can learn from other microbiome projects, DW
238 microbiomes require a dedicated research focus because of their unique ecosystems and their
239 diverse environmental and engineered parameters. It is critical to determine specific effects of

240 engineering treatments on various source waters and distribution systems, and parameters affecting
241 changes in DW microbiomes, highlighted in the Outstanding Questions Box. Particularly needed
242 is information on distribution systems and buildings to reduce pathogens, and to explore how DW
243 microbiomes respond to new and/or alternative water sources in responses to climate and
244 population changes. Characterization of planktonic and biofilm communities and their functions
245 will help differentiate baseline and impacted DW microbiomes. Reference database development
246 will be required using ontologies relevant to the DW microbiome. The ultimate goal is to provide
247 safe and healthy drinking water for all.

248

249 **Visual Elements**

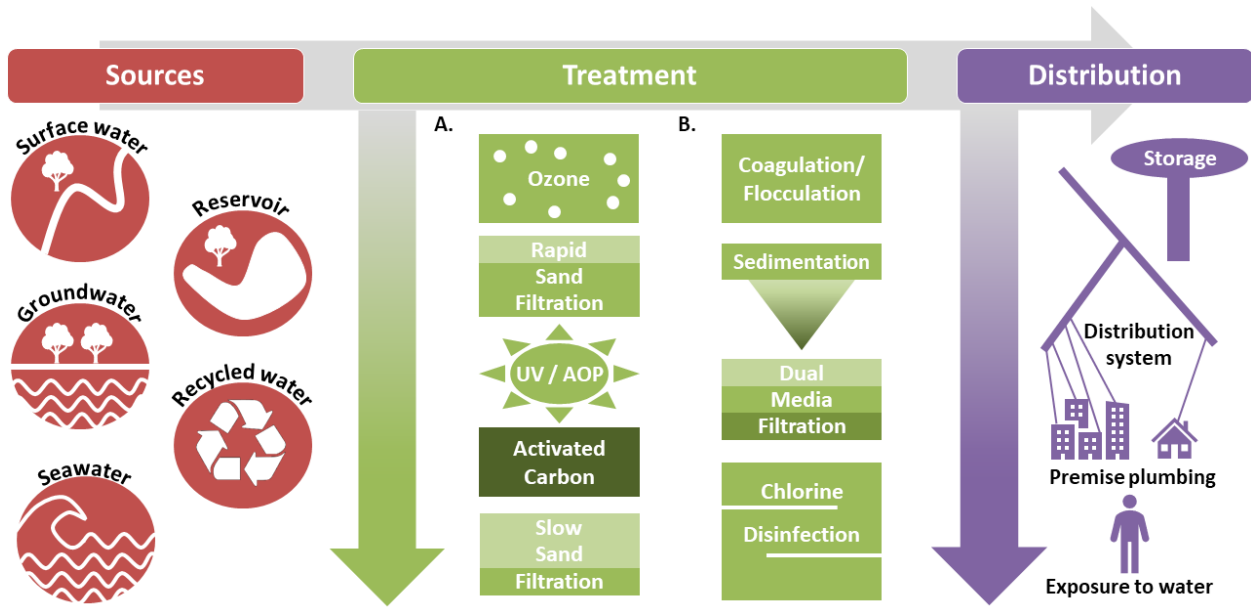
250 **Box 1: It's never too late to learn ... from other microbiome projects.**

251 Broad coordinated efforts to characterize microbiome dynamics have been conducted
252 across a range of different ecosystems, from host-associated [28–30] to environmental [31]
253 ecosystems. These have taken the form of top-down efforts coordinated across multiple research
254 groups (e.g., Human Microbiome Project, Meta-HIT), to citizen science projects (e.g., American
255 Gut), and/or a hybrid involving dissemination of a common set of standardized protocols adopted
256 by numerous research groups (e.g., Earth Microbiome Project). Despite evidence that differences
257 in sample handling, DNA extraction, sequencing, and data processing workflows adopted by
258 different groups can result in sufficient variation to mask underlying biological signal [26,32],
259 there has been a lack of consensus on standardization efforts primarily due to demonstrable
260 limitations of early standardization efforts. More recently, the focus has shifted from standardizing
261 across studies towards development and adoption of best practices [33] in design and
262 implementation of microbiome studies that may be adopted depending on study design and
263 hypothesis [34]. Nonetheless, the key consensus has emerged from these large-scale microbiome
264 efforts around **data recording/reporting standards** and **minimum quality assurance and**
265 **control (QA/QC) requirements**. For instance, the Genome Standards Consortium encouraged the
266 adoption of the MIMARKS and MIxS reporting standards to allow for better comparison of
267 amplicon sequencing data from multiple studies [35]. Similarly, building on the Minimum
268 Information about Genome Sequence (MIGS) specifications proposed by Field et al. in 2008 [36],
269 Bowers et al. recently proposed the MIMAG and MISAG reporting specifications for metagenome
270 assembled and single amplified genomes, respectively [37]. These reporting standards are crucial
271 towards ensuring data quality and re-usability over the long-term. The second key consensus has
272 been around **inclusion of negative controls and mock communities** primarily as a means of
273 identifying both contamination artifacts [38] and batch effects [39,40] that may confound both
274 within- and cross-study data analyses.

275

276

277 **Figure 1: Sources of microbes and factors affecting biogeography of drinking water**
278 **microbiomes to which the public is exposed.** There are many sources of microorganisms and
279 various physiochemical factors shaping microbial biogeography throughout the process of DW
280 production and delivery to taps. The complex relationships in each niche throughout the process
281 all play a role in determining the DW microbiome to which the public may be exposed. Some of
282 the niches for microbiomes throughout this continuum include bulk water and biofilms in source
283 waters (such as surface water from streams or reservoirs, ground water, desalinated seawater,
284 recycled wastewater, or blends of these sources), various treatment processes (such as
285 biostabilization without disinfectant residual treatment approaches shown in A and conventional
286 treatment with disinfectant residual approaches shown in B) to distribution (including storage,
287 transmission, and travel through or stagnation in water main pipes and premise plumbing). Some
288 of the many parameters potentially contributing to the complexity of DW microbiomes from
289 source to tap include differences in source water type and quality (brought on by climate and
290 population changes), differences in engineering treatment technologies, distribution system design
291 and materials (including changes due to infrastructure rehabilitation and redesign), indoor building
292 design and materials (including green building design which increases water stagnation in pipes),
293 and consumer behaviors (such as water conservation). After delivery to taps, DW microbiomes
294 inoculate and may even alter human microbiomes through ingestion (e.g., gastric infection),
295 inhalation (e.g., respiratory infection), and adsorption (e.g., skin infection). UV/AOP: Ultraviolet
296 light and advanced oxidation processes.
297

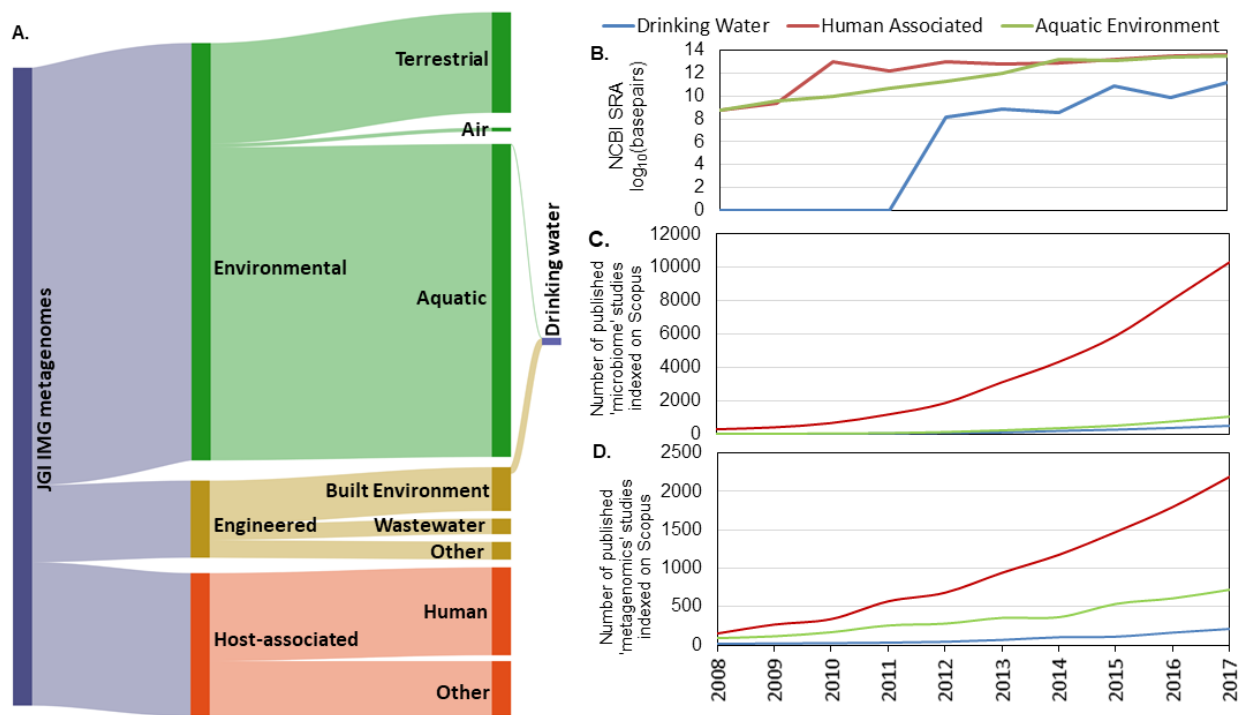


298

299

300

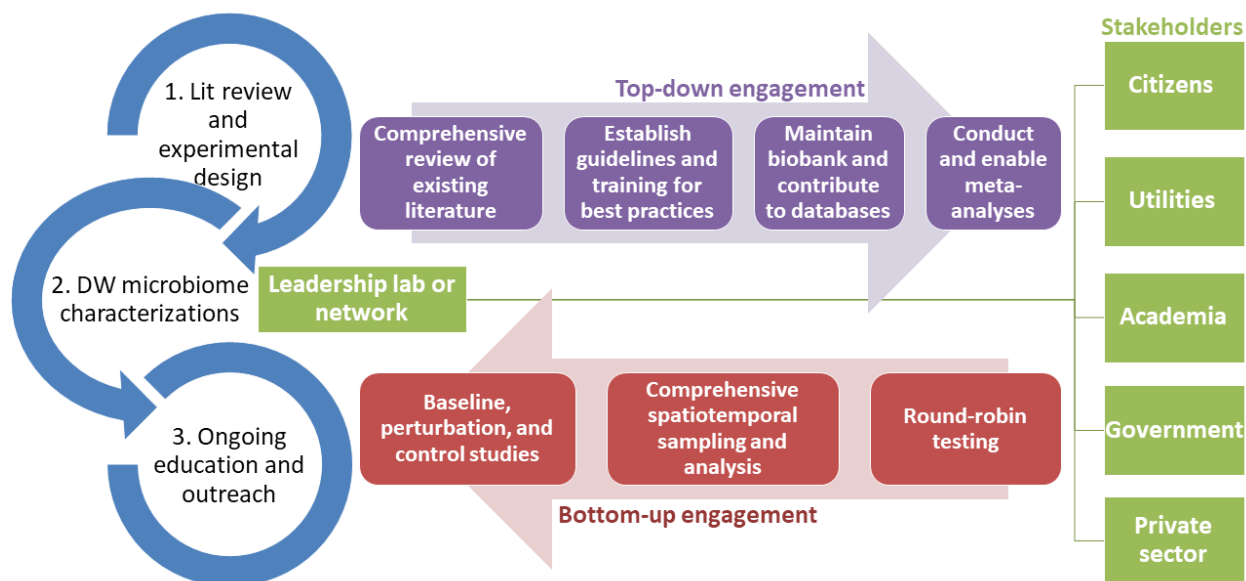
301 **Figure 2 (Key Figure): Knowledge gap in drinking water microbiomes compared to other**
 302 **microbiomes.** A. The total number of metagenome projects in the JGI IMG database (downloaded
 303 Oct 8, 2018) shown in the flow diagram (built on sankeymatic.com) are 2,184; 11,813; and 4,058
 304 for engineered systems; environmental systems; and host-associated systems, respectively. Of the
 305 total 18,055 metagenome projects in the database, only 205 study or sample names mention
 306 drinking water or water treatment. B. The total basepairs in the NCBI SRA database (downloaded
 307 July 2018) depicts similar disparity in 2017 for metagenomic studies of water treatment systems
 308 versus host-associated and other environmental and engineered systems (not shown).
 309 Longitudinally, the total basepairs in the NSBI SRA database show data for DW microbiomes was
 310 unavailable before 2012, and continues to lag behind studies of aquatic environment and human-
 311 associated microbiomes. C. and D. Published manuscripts indexed in Scopus (downloaded
 312 October 2018) comparing study of drinking water to study of other metagenomes (in C) and
 313 microbiomes in general (in D) also demonstrate a large and expanding knowledge gap.



314

315 **Figure 3: Proposed Drinking Water Microbiome Project (DWMP) framework and roadmap.**

316 The roadmap for operation of the DWMP involves three stages including 1) experimental design,
 317 2) the main effort of the DWMP project dedicated to better understanding DW microbiomes, and
 318 3) ongoing outreach. The experimental design phase will consist of a critical review of existing
 319 literature to inform recommendations for best practices for protocols and strategies (which will be
 320 validated in round-robin testing by various stakeholders), and establishment and recruitment of
 321 participants in the stakeholder framework. The framework will enable the primary effort of the
 322 DWMP, which will be to coordinate the collection, analysis, and storage of the vast quantity of
 323 samples and data necessary for comprehensive characterization of DW microbiomes in a
 324 concurrent top-down and bottom-up approach. From the top-down, the central lab/network will
 325 provide guidance and coordination while maintaining databases and a biobank, while the involved
 326 stakeholders across sectors will provide sampling, analysis, and funding effort from the bottom-
 327 up. This will allow meta-analyses by the central lab or any participants that will enable elucidation
 328 of the factors affecting DW microbiomes over space and time in various systems. Throughout the
 329 data analysis effort and engagement with these various stakeholders, high quality and frequent
 330 communication strategies will be essential for effective outreach and education. Stakeholders
 331 should work together to derive guideline or best management practices for their engineering and
 332 distribution operations so as to meet the required drinking water quality that can best protect public
 333 health.



334

335 **Acknowledgements**

336 NH, FL, AP, and WL conceived and outlined the opinion. NH drafted the manuscript with
337 contribution from FL, AP, PH, and WL. GJ, AP, PH, KK, MA, RRC, and ML provided critical
338 review and comments on the manuscript. Authors acknowledge support from NSF (NSF) Division
339 of Chemical, Bioengineering, Environmental, and Transport Systems (CBET) under award
340 number 1701641. Any opinions, findings and conclusions or recommendations expressed in this
341 material are those of the authors and do not necessarily reflect those of NSF. Because many
342 discussions at an NSF Workshop held March 12-13, 2018 in Denver, CO (Advancing
343 understanding of Microbiomes in Drinking Water Distribution Systems and Premise Plumbing
344 Using Meta-omics Techniques) informed this opinion, authors would like to acknowledge input
345 of all workshop participants.

346 **References**

- 347 1 Tipton, L. *et al.* (2019) A Developing Symbiosis: Enabling Cross-Talk Between
348 Ecologists and Microbiome Scientists. *Front. Microbiol.* 10, 292
- 349 2 Hammes, F. *et al.* (2008) Flow-cytometric total bacterial cell counts as a descriptive
350 microbiological parameter for drinking water treatment processes. *Water Res.* 42, 269–
351 277
- 352 3 Lautenschlager, K. *et al.* (2010) Overnight stagnation of drinking water in household taps
353 induces microbial growth and changes in community composition. *Water Res.* 44, 4868–
354 4877
- 355 4 Maupin, M.A. *et al.* (2014) *Estimated Use of Water in the United States in 2010*,
- 356 5 Falkinham, J.O. *et al.* (2015) Epidemiology and ecology of opportunistic premise
357 plumbing pathogens: *Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas*
358 *aeruginosa*. *Environ. Health Perspect.* 123, 749–758
- 359 6 Naumova, E.N. *et al.* (2016) Hospitalizations due to selected infections caused by
360 opportunistic premise plumbing pathogens (OPPP) and reported drug resistance in the
361 United States older adult population in 1991–2006. *J. Public Health Policy* 37, 500–513
- 362 7 Schwake, D.O. *et al.* (2016) *Legionella* DNA Markers in Tap Water Coincident with a
363 Spike in Legionnaires’ Disease in Flint, MI. *Environ. Sci. Technol. Lett.* 3, 311–315
- 364 8 Falkinham, J. *et al.* (2015) Opportunistic Premise Plumbing Pathogens: Increasingly
365 Important Pathogens in Drinking Water. *Pathogens* 4, 373–386
- 366 9 Xi, C. *et al.* (2009) Prevalence of antibiotic resistance in drinking water treatment and
367 distribution systems. *Appl. Environ. Microbiol.* 75, 5714–8
- 368 10 Gilbert, J.A. *et al.* (2018) Current understanding of the human microbiome. *Nat. Med.* 24,
369 392–400
- 370 11 Gilbert, J.A. and Stephens, B. (2018) Microbiology of the built environment. *Nat. Rev.*
371 *Microbiol.* 16, 661–670
- 372 12 Stanish, L.F. *et al.* (2016) Factors Influencing Bacterial Diversity and Community
373 Composition in Municipal Drinking Waters in the Ohio River Basin, USA. *PLoS One* 11,
374 e0157966
- 375 13 Holinger, E.P. *et al.* (2014) Molecular analysis of point-of-use municipal drinking water
376 microbiology. *Water Res.* 49, 225–35

- 377 14 Ling, F. *et al.* (2016) Core-satellite populations and seasonality of water meter biofilms in
378 a metropolitan drinking water distribution system. *ISME J.* 10, 582–595
- 379 15 Bautista-de los Santos, Q.M. *et al.* (2016) Emerging investigators series: Microbial
380 communities in full-scale drinking water distribution systems – A meta-analysis. *Environ.*
381 *Sci. Water Res. Technol.* 2, 631–644
- 382 16 Pinto, A.J. *et al.* (2014) Spatial-temporal survey and occupancy-abundance modeling to
383 predict bacterial community dynamics in the drinking water microbiome. *MBio* 5, e01135-
384 14
- 385 17 Zhang, Y. and Liu, W.-T. (2019) The application of molecular tools to study the drinking
386 water microbiome – Current understanding and future needs. *Crit. Rev. Environ. Sci.*
387 *Technol.* DOI: 10.1080/10643389.2019.1571351
- 388 18 US EPA Drinking Water Infrastructure Needs Survey and Assessment: Sixth Report to
389 Congress. . (2015) , 1–76
- 390 19 AWWA Buried No Longer: Confronting America’s Water Infrastructure Challenge. .
391 (2012) , 1–37
- 392 20 Ling, F. *et al.* (2016) Core-satellite populations and seasonality of water meter biofilms in
393 a metropolitan drinking water distribution system. *ISME J.* 10, 582–595
- 394 21 Levy, K. *et al.* (2016) Untangling the Impacts of Climate Change on Waterborne
395 Diseases: a Systematic Review of Relationships between Diarrheal Diseases and
396 Temperature, Rainfall, Flooding, and Drought. *Environ. Sci. Technol.* 50, 4905–4922
- 397 22 Constantin de Magny, G. and Colwell, R.R. (2009) Cholera and climate: a demonstrated
398 relationship. *Trans. Am. Clin. Climatol. Assoc.* 120, 119–28
- 399 23 Pinto, A.J. *et al.* (2012) Bacterial community structure in the drinking water microbiome
400 is governed by filtration processes. *Environ. Sci. Technol.* 46, 8851–9
- 401 24 Oh, S. *et al.* (2017) Metagenomic characterization of biofilter microbial communities in a
402 full-scale drinking water treatment plant. *Water Res.* DOI:
403 10.1016/J.WATRES.2017.10.054
- 404 25 Fish, K.E. and Boxall, J.B. (2018) Biofilm Microbiome (Re)Growth Dynamics in
405 Drinking Water Distribution Systems Are Impacted by Chlorine Concentration. *Front.*
406 *Microbiol.* 9, 2519
- 407 26 Pollock, J. *et al.* (2018) The Madness of Microbiome: Attempting To Find Consensus

408 “Best Practice” for 16S Microbiome Studies. *Appl. Environ. Microbiol.* DOI:
409 10.1128/AEM.02627-17

410 27 Muir, P. *et al.* (2016) The real cost of sequencing: scaling computation to keep pace with
411 data generation. *Genome Biol.* DOI: 10.1186/s13059-016-0917-0

412 28 Lloyd-Price, J. *et al.* (2017) Strains, functions and dynamics in the expanded Human
413 Microbiome Project. *Nature* 550, 61–66

414 29 McDonald, D. *et al.* (2018) American Gut: an Open Platform for Citizen Science
415 Microbiome Research. *mSystems* DOI: 10.1128/mSystems.00031-18

416 30 Turnbaugh, P.J. *et al.* (2007) The human microbiome project. *Nature* 449, 804–10

417 31 Thompson, L.R. *et al.* (2017) A communal catalogue reveals Earth’s multiscale microbial
418 diversity. *Nature* 551, 457–463

419 32 Sinha, R. *et al.* (2017) Assessment of variation in microbial community amplicon
420 sequencing by the Microbiome Quality Control (MBQC) project consortium. *Nat.*
421 *Biotechnol.* DOI: 10.1038/nbt.3981

422 33 Knight, R. *et al.* (2018) Best practices for analysing microbiomes. *Nat. Rev. Microbiol.*
423 16, 410–422

424 34 Tripathi, A. *et al.* (2018) Are microbiome studies ready for hypothesis-driven research?
425 *Curr. Opin. Microbiol.* 44, 61–69

426 35 Yilmaz, P. *et al.* (2011) Minimum information about a marker gene sequence
427 (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications.
428 *Nat. Biotechnol.* 29, 415–420

429 36 Field, D. *et al.* (2008) The minimum information about a genome sequence (MIGS)
430 specification. *Nat. Biotechnol.* 26, 541–547

431 37 Bowers, R.M. *et al.* (2017) Minimum information about a single amplified genome
432 (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. *Nat.*
433 *Biotechnol.* 35, 725–731

434 38 Salter, S.J. *et al.* (2014) Reagent and laboratory contamination can critically impact
435 sequence-based microbiome analyses. *BMC Biol.* DOI: 10.1186/s12915-014-0087-z

436 39 Costea, P.I. *et al.* (2017) Towards standards for human fecal sample processing in
437 metagenomic studies. *Nat. Biotechnol.* DOI: 10.1038/nbt.3960

438 40 Yeh, Y.-C. *et al.* (2018) Taxon Disappearance from Microbiome Analysis Reinforces the

439 Value of Mock Communities as a Standard in Every Sequencing Run. *mSystems* DOI:
440 10.1128/mSystems.00023-18
441
442