

## Meeting report

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1 **Meeting Report: International Soil Virus Conference 2024**

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41

## 42 **Abstract**

43 The research field of soil viral ecology continues to advance rapidly as the roles of viruses in  
44 the functioning of soil ecosystems are increasingly recognized. To address recent developments  
45 in the field, the second International Soil Virus Conference was held in Livermore, California,  
46 USA, from June 25 to 27<sup>th</sup>, 2024, providing soil viral ecologists the opportunity to share new  
47 findings and suggest guidelines for future research, while encouraging international scientific  
48 discussion and collaboration. The meeting was held in person with sessions simultaneously  
49 streamed online. Fifty researchers attended from ten different countries and spanned a wide  
50 range of subfields and career stages. A total of 21 oral presentations were presented, followed  
51 by discussions covering key themes in soil viral research. This report summarizes the main  
52 takeaways and recommendations from the talks and discussions.

53

## 54 **1. Introduction**

55 Viruses are abundant biological entities in soil and key components of trophic networks that  
56 play a major part in soil ecosystem function (Carreira et al. 2024). Briefly, viruses can directly  
57 and indirectly impact biogeochemical cycles, reshape microbial diversity, and act as genetic  
58 reservoirs (Chevallereau et al. 2022; Piel et al. 2022). Soil viruses have sparked the interest of  
59 researchers across disciplines, including: microbial ecology due to their roles in structuring  
60 microbial communities and metabolism (Luo et al. 2022), theoretical ecology because of the  
61 variety of ecological interactions with their hosts and environment (Breitbart et al. 2018), and  
62 soil science to better understand soil health and ecosystem services (Tong et al. 2023).

63 However, the study of soil viruses still faces significant challenges, both technical—due to  
64 limitations in our ability to isolate, sequence, and adequately analyze viral genomes—and  
65 conceptual—due to high levels of community complexity and small-scale diversity  
66 (Williamson et al., 2017; Dion et al. 2020). These challenges highlight the need for the  
67 international soil virus research community to come together, exchange ideas, discuss optimal  
68 approaches, and solidify methodological protocols.

69 The International Soil Virus Conferences aim to create a biennial platform for  
70 interactions among researchers in the field. The first meeting took place in 2022 in Denmark  
71 (Buivydaite et al. 2023). While some of the knowledge gaps identified in this previous meeting  
72 have been tackled, many remain. Similarly, both computational and experimental tools  
73 continue to advance, prompting new discoveries but also uncovering new gaps in our  
74 knowledge. Therefore, continued regular meetings create a forum for researchers interested in  
75 soil viruses to come together, fostering collaboration and establishing a solid foundation for  
76 advancing this emerging field. These gatherings provide a platform for sharing new ideas,  
77 addressing ongoing challenges, and identifying directions for future research in soil viral  
78 ecology. In this report, we summarize what was presented and discussed at the second  
79 International Soil Virus Conference held in June of 2024 at the University of California  
80 Livermore Collaboration Center in Livermore, California, USA. Our hope is that this  
81 communication will serve as a reference to researchers who could not be present and as a  
82 record of the recent advances and driving questions in soil viral ecology.

83           Since the first conference in 2022, participation increased twofold from 23 to 53  
84 participants. This year's participants came from ten different countries (Figure 1. A,B).  
85 Unfortunately, we observed a bias towards the Northern Hemisphere, specifically towards  
86 participants from institutions in the USA (72% of the total participants, and 85% of in-person  
87 participants), potentially due to the conference being hosted in the USA. This year's  
88 conference brought a higher participation rate from early career researchers: out of the 53  
89 participants, 20 (38%) were early career (three undergraduate students, ten graduate students,  
90 and seven postdocs, Figure 1. C). While the strong showing of early-career researchers suggests  
91 a robust and vibrant future for the study of soil viruses (Bankston et al. 2020; Smoliński et al.  
92 2022), the demographic observations suggest that the field should make a stronger effort to  
93 connect with researchers in the Global South and to support more international participation  
94 of early-career researchers, including by holding conferences in different countries and by  
95 finding ways to provide funding opportunities such as travel grants to assist with attendance.

96           The workshop followed a structured format that fostered discussion on key themes in  
97 soil viral research. Each day began with a keynote speaker, followed by two or three  
98 presentations, with discussion following the talks. The workshop was purposefully organized  
99 without a daily thematic structure to encourage the presentation of a wide range of research  
100 topics and to avoid limiting the type of work that could be shared. Although the talks had no  
101 prior grouping, five core topics naturally emerged (Figure 2; Supplementary Table 1): (i) viral  
102 diversity and community structure in the soil, (ii) responses of soil viral communities to  
103 ecosystem disturbances, (iii) impacts of soil viruses on the soil microbiome and ecosystem

104 processes, (iv) the soil RNA virome, and (v) *in vivo* and *in silico* approaches to studying soil  
105 viruses. On the last day, a group discussion and breakout discussion sessions covered the  
106 following themes: i) host detection methods, ii) RNA viruses, iii) limitations of bioinformatics  
107 methods for viral annotation, and iv) overall state of soil viral research.

## 108 **2. Talks**

### 109 2. 1. Viral diversity and community structure in the soil

110 The presentations in this theme focused on exploring the key drivers of viral  
111 community composition and structure in soil microbiomes, with attention to edaphic  
112 parameters and seasonality. For instance, Grant Gogul (PhD student, University of California-  
113 Davis, USA; UC-National Laboratory In-Residence Graduate Fellow, Lawrence Livermore  
114 National Laboratory, USA) found dramatic changes in the community composition of viromes  
115 and transcriptionally active viral populations caused by wet-up and phosphate amendment of  
116 a Mediterranean grassland soil in northern California. Interestingly, modifications in soil  
117 phosphorus content only affected the transcriptional activity of viral populations (unpublished  
118 data). Next, Ikaia Leleiwi (Postdoctoral scholar at Lawrence Livermore National Laboratory,  
119 USA) presented evidence for soil redox conditions influencing viral communities and driving  
120 virus-host dynamics and plant biomass degradation in tropical soils (Trubl et al., Biorxiv). Both  
121 presentations provided evidence of how sensitive viruses are to changes in the soil  
122 environment.

123 Several researchers reported the strong spatial structuring of soil viral communities.  
124 Ellie Jameson (Lecturer, Bangor University, UK) showed that the viral community was

125 surprisingly conserved across all depths in seasonally waterlogged soils, however there were  
126 changes in soil viral community composition and virus-host interactions (predicted lysogeny  
127 and defense systems) down the soil depth profile (Muscatt et al. 2023). Work presented by  
128 John Henry Lotz-McMillen, Ruby Gilmore (undergraduate students, Georgetown University,  
129 USA), and Shauna Bennett (Assistant Teaching Professor, Georgetown University, USA)  
130 further reported depth as a driver of viral community composition in their metagenomic  
131 analysis of viral populations along a soil depth series from Taylor Valley in the Antarctic Dry  
132 Valleys. Their ecological analyses suggested that viruses in this extreme ecosystem were mostly  
133 novel, with 21 unclassified double-stranded DNA bacteriophages identified, overall viral  
134 diversity was relatively low across samples, and species richness significantly increased with  
135 depth (unpublished data). Yiling Wang (PhD student, Zhejiang University, China) observed  
136 strong distance-decay patterns in soil viral community composition from a global genome  
137 database of 1,824 samples from five continents. Wang also reported finding higher viral  
138 diversity in agricultural soils compared to natural shrubland and forest soils and a strong  
139 correlation between viral diversity and soil moisture content (Ma et al. 2024). Conversely, in  
140 a different dataset, Josué Rodríguez-Ramos (Postdoctoral scholar at Pacific Northwest National  
141 Laboratory, USA) found that while season did not have a significant impact on DNA or RNA  
142 virus communities, both soil moisture and sample location were explanatory of viral  
143 community composition (unpublished data). Given the growing evidence for substantial  
144 spatial differences in soil viral diversity, Jane Fudyma (PhD student, University of California-  
145 Davis, USA) evaluated the scales at which spatial heterogeneity affects viral community



146 structure. Fudyma presented a study that addressed changes in soil viral community  
147 composition over scales ranging from 10 cm to 100 m in one high- and one low-precipitation  
148 grassland, reporting large differences in viromes and metagenomes across all scales  
149 (unpublished data).

150 In summary, the studies in this section suggested that spatial factors (soil depth and  
151 geographic distance) are important drivers of soil viral diversity, as are edaphic properties such  
152 as phosphorus and carbon content, redox chemistry, and moisture. Multiple presenters also  
153 reported a lack of consistency between viral and microbial diversity patterns and highlighted  
154 the major challenge of trying to disentangle the impact of geography, soil properties, and host  
155 community composition when comparing soil viral communities.

## 156 2. 2. Responses of soil viral communities to ecosystem disturbance

157 Three oral and two poster presentations extended the question of how viral community  
158 structure shifts to cases of ecosystem disturbances. Ecosystem disturbances featured in these  
159 presentations were related to either fire and/or heat, drying and rewetting, or both. Luke  
160 Hillary (Postdoctoral scholar at the University of California-Davis, USA), for example,  
161 conducted a pyrocosm experiment to study the severity and depth-dependence of the effects  
162 of fire on soil DNA viral communities, using viromes, identifying putative fire-responsive  
163 bacteriophage populations of the endospore-forming *Bacillota* (unpublished data). Studying a  
164 natural fire, Sara Geonczy (PhD student, University of California-Davis) found strong patterns  
165 of viral community compositional differences according to habitat and in response to wildfire  
166 (Geonczy, et al., 2024). Sam Barnett (Postdoctoral scholar at Michigan State University, USA)

167 studied heat disturbance in a slowly spreading coal seam fire in Centralia (PA, USA), observing  
168 that DNA viral community composition did not change consistently with time post-  
169 disturbance as bacteria did in the same system (Barnett & Shade 2024). In fact, Barnett  
170 observed that these soil viral communities exhibited significant spatial heterogeneity.

171       Turning to responses of soil viral communities to drying and rewetting, María (Mery)  
172 Touceda-Suárez (PhD student, University of Arizona, USA) studied how soil viral community  
173 structure changed in a 60-day drought and posterior recovery in an artificial rainforest housed  
174 at the Biosphere 2 (Oracle, AZ, USA). Touceda-Suárez observed that, while viral community  
175 structure was affected by the changes in soil moisture, spatial heterogeneity had a greater  
176 effect, and community composition did not return to pre-drought state (unpublished data).  
177 Along those lines, Lucie Jiraska (Postdoctoral scholar at University of California-Davis, USA)  
178 presented data from monthly soil sampling of a California grassland site, showing a clear  
179 successional pattern of viral community composition in time, corresponding to changes in soil  
180 moisture (unpublished data). Further, Jiraska also reported findings from soil microcosm  
181 experiments on the historical effects of drying conditions that can influence viromic DNA  
182 yields (a proxy for viral particle abundances) and viral community composition; compared to  
183 moderate drying at 20°C, exposure to higher temperatures (35°C) resulted in a seven-fold  
184 reduction in DNA yields after rewetting and led to significant differences in viral community  
185 composition observed 24 hours post-rewetting.

186       Together these results suggest that viral communities may not always return to their  
187 “pre-disturbance” composition, and their recovery and resilience may be dependent on the

188 drying/drought history of the soil, severity and duration of the disturbance event, and spatial  
189 heterogeneity in the soil environment.

### 190 2.3. Impacts of viruses on the soil microbiome and ecosystem processes

191 A primary reason for studying the response of viral communities to both expected and  
192 unexpected changes in environmental conditions is their influence on the soil microbiome and  
193 soil processes. However, the ability of viruses to affect the diversity and functions of their hosts  
194 as well as their impacts on soil composition, biogeochemistry and plant-microbe interactions  
195 are not fully understood. Some of the presenting researchers shared their attempts at tackling  
196 these questions. Ruonan Wu (Staff scientist, Pacific Northwest National Laboratory, USA)  
197 synthesized the state of our knowledge on the mechanisms by which soil viruses can impact  
198 soil microbial community composition and function (host targeting, lifestyle switching, and  
199 expression of auxiliary metabolic genes). Wu presented an approach that combined field,  
200 incubation, and molecular studies that was successfully used to overcome some of the  
201 challenges posed specifically by the soil environment (high spatial heterogeneity, high  
202 microbial diversity, and complex physicochemical properties) (Wu et al. 2021, 2022, 2023;  
203 Graham et al. 2024). Additionally, keynote speaker Christina Hazard (Research Scientist,  
204 Université Claude Bernard Lyon 1, France) presented a high-resolution approach for studying  
205 viral impacts on soil microbial communities targeting specific functional microbial groups  
206 (nitrifiers and methanotrophs) in stable isotope probing incubations linked to viromics and  
207 metagenomics (Lee et al. 2023). Finally, Bin Ma (Associate Professor, Zhejiang University,  
208 China) presented a combined in silico and in vitro approach using time-series data to reveal

209 how temperate phage infections enhance arsenic oxidation in the rhizosphere by enriching  
210 arsenic oxidase genes and facilitating horizontal gene transfer.

211 We also heard from researchers who studied the mechanics underlying viral impacts  
212 on soil processes. Keynote speaker Paula Dalcin Martins (Assistant Professor, University of  
213 Amsterdam, Netherlands) proposed that the viral shunt in agricultural peatland soils could  
214 lead to higher methane and carbon dioxide emissions due to virus-induced increases in labile  
215 soil organic matter pools (unpublished data). James Kosmopoulos (PhD student, University of  
216 Wisconsin-Madison, USA) further showed that restoration of degraded peatlands modifies the  
217 soil DNA viral community composition, and that the predominant host phyla of viruses vary  
218 across peatland ecosystem health statuses (unpublished data). Di Tong (PhD student, Zhejiang  
219 University, China) showed evidence that the “viral shuttle” exists in the soil environment  
220 (Tong et al. 2023), and quantified the contributions of both free extracellular viruses and  
221 prophages to soil organic matter under anaerobic conditions (unpublished data).

222 This group of presentations highlight the increasing efforts in the field to understand  
223 the effects of soil viruses on ecosystem processes, through both devising new methodological  
224 approaches and creating experimental designs that center around these questions.

#### 225 2.4. The soil RNA virome

226 A fourth theme addressed the untapped diversity of soil RNA viruses in different  
227 habitats, and how to study it via sequencing data. RNA viruses are still largely uncharacterized  
228 in soils, despite their potential role in soil carbon cycling (Starr et al. 2019; Hillary et al. 2022),  
229 likely due to challenges with sampling and processing methodology (e.g., low yields, folding

230 of single-stranded RNA, removal of ribosomal RNA). Keynote speaker Uri Neri (Data Scientist,  
231 The Joint Genome Institute, USA) discussed the challenges and potential solutions to infer  
232 RNA viral diversity from sequencing data. Neri presented an *in silico* method to robustly  
233 extract RNA viruses from sequencing data using RNA-dependent RNA polymerases as a  
234 hallmark gene (Neri et al. 2022). Rumakanta Sapkota (Associate Professor, University of  
235 Aarhus, Denmark) illustrated the high diversity of RNA viruses across many soil ecosystems,  
236 including beech forest, pine forest, grassland and especially in agricultural soils (unpublished  
237 data). Both presentations underscored the imbalance between the potential importance of  
238 RNA viruses and our current understanding of their diversity, host range, and activity.

#### 239 2.5. *In vivo* and *in silico* approaches to the study of soil viruses

240 Due to the inherent complexities of soil microenvironments, and particularly the  
241 highly diverse pool of soil viruses, the last major theme of the conference addressed the need  
242 for innovations in methodology. This topic focused on new *in silico*, experimental, and  
243 combined methods for the discovery of viruses, plasmids, and virus-host interactions, as well  
244 as microbe-plant associations and bacteriophage engineering. This theme included two  
245 keynote presentations, one focused on whole community approaches from Kurt Williamson  
246 (Associate Professor, College of William and Mary, USA), and one focused on *in silico*  
247 approaches from Andrew Millard (Professor, University of Leicester, UK). Williamson posed  
248 the question of how viruses infect their hosts and maintain their abundance in the soil, given  
249 its inherent spatial and structural limitations. He described experimental assays to answer this  
250 question from two perspectives: 1) testing different agents of prophage induction to examine

251 whether lysogeny in soil exhibits a seasonal or temporal component (Jacoby et al. 2024) and 2)  
252 developing infectivity assays with individual phage isolates to estimate the limits of viral  
253 persistence and decay in soils, and its relationship with soil moisture (DiPietro et al. 2023).  
254 Meanwhile, Millard's group identified a unique set of double-stranded DNA viral populations  
255 that were not recovered in viromic or metagenomic data but were identified in  
256 metatranscriptomics data, highlighting the importance of integrating multiple sampling and  
257 sequencing methods. Work from his group expanded the number of *Leviviricetes* genomes  
258 (RNA bacteriophages) by more than five-fold (Muscatt et al. 2022). Also using *in silico*  
259 methods, Jonelle Basso (Research Scientist, The Joint Genome Institute, USA) introduced a  
260 tool for studying the role of rhizosphere viruses in microbe-plant associations using targeted  
261 metabolomics. Resident viruses were bioinformatically detected (using VirSorter2; Guo et al.  
262 2021 and geNomad; Camargo, Roux et al. 2023) in the model plant growth-promoting  
263 rhizobacterium, *Pseudomonas simiae* WCS417, which was experimentally validated using  
264 classical induction experiments with mitomycin C. Her team used homologous recombination  
265 and transformation methods to generate a prophage deletion mutant, used for experimental  
266 comparisons with wild-type in root colonization assays. Metabolomics results sought to  
267 highlight that resident phages may hold functional potential to modulate their bacterial host's  
268 ability to colonize plant roots, as well as influence root exudate composition in ways that may  
269 benefit plant health (unpublished data). Simon Roux (Research Scientist, The Joint Genome  
270 Institute, USA) introduced IMG/VR and IMG/PR, publicly available databases of viruses and  
271 plasmid sequences, respectively, identified from genomes and metagenomes (Camargo,

272 Nayfach, et al. 2023), and geNomad (Camargo, Roux, et al. 2023), a new bioinformatics tool  
273 for detecting viruses and plasmids from metagenomes.

274 Finally, two researchers presented methods for bacteriophage-host interactions  
275 through the rapid isolation of phages and identification of bacterial genes required for phage  
276 infection. Catherine Mageeney (Research Scientist, Sandia National Laboratories, USA)  
277 presented a combined *in silico* and experimental approach for the identification, validation,  
278 and engineering of bacteriophages (Mageeney et al. 2020) and the application of these methods  
279 to *Pseudomonas putida*. Marissa Gittrich (Ph.D. student, The Ohio State University, USA)  
280 presented an experiment for identifying bacterial genes required for 24 phages infecting  
281 *Klebsiella* sp. M5al and examined the patterns of bacterial gene requirements based on phage  
282 taxonomy and found.

### 283 3. Discussions

284 On the last day of the conference, attendees participated in breakout discussions  
285 focused on topics commonly agreed upon as the most relevant or pressing. These topics  
286 included i) host detection or prediction, ii) RNA viruses, iii) current methodologies, and iv) an  
287 open-ended category aiming to capture the most outstanding questions in the field. A summary  
288 of the discussions is illustrated in Figure 3, which also includes a schematic of the typical  
289 methodological steps in soil virus studies. Briefly, participants agreed on the importance of  
290 generating hypothesis-driven studies to help address the outstanding unknowns. Controlled  
291 experiments, in which variables can be manipulated, could shed light on the factors that  
292 impact viral communities the most. Along those lines, the advantages and disadvantages of

293 studying soil viral communities using DNA viromes versus metagenomes were compared.  
294 While metagenomic sequencing allows us to study viruses inside cells (López-Pérez et al.  
295 2017), DNA viromes have been shown to capture more diversity, and more accurately  
296 represent the active DNA viral community (Santos-Medellin et al. 2021; Kosmopoulos et al.  
297 2023). Therefore, the choice of experimental design and methodology should be carefully  
298 selected to best suit the resource availability, environmental context, and ecological question  
299 being addressed.

300 Discussions surrounding the knowledge gaps related to RNA viruses continued  
301 throughout the conference. Participants identified several limitations and challenges  
302 associated with RNA viral isolation and discovery that will be important to address to further  
303 advance the study of RNA viruses (Figure. 1 and Supplementary Table 2). Specifically, RNA  
304 virus isolation and discovery are hindered by non-optimized kits, suboptimal sequencing  
305 technologies, and high costs and labor requirements at multiple steps, primarily due to the  
306 difficulty of separating RNA viruses from other RNA sequences. As a result, research tends to  
307 focus on double-stranded DNA viruses, particularly bacteriophages. However, conference  
308 participants agreed on the necessity of expanding our studies to include micro-eukaryotic  
309 viruses, fungal viruses (mycoviruses), and archaeal viruses to fully capture viral diversity and  
310 understand their roles in soil ecosystems.

311 Finally, attendees collectively discussed the next steps and remaining outstanding  
312 questions for the field. The remarks made during these discussions could be broadly grouped  
313 as follows: i) subfields in need of standardization and definitions, ii) methodological advances



314 to prioritize, iii) relevant new directions for research, and constitute some of the topics that  
315 attendees envisioned as some of the most important issues to address in the near future. First,  
316 the study of auxiliary metabolic genes (AMGs) – metabolic genes of bacterial origin encoded  
317 in viral genomes– has caused widespread interest given their potential to alter ecosystem  
318 function (Zheng et al. 2022). However, the full span of functions encoded in AMGs and the  
319 level of influence that AMGs have in overall soil processes are still underexplored. On the  
320 other hand, the identification of potential AMG has been streamlined through the mining of  
321 sequencing data, creating a surge in the reporting of putative AMG sequences. Thus, attendees  
322 agreed that standardized notation and minimum reporting standards for AMGs are necessary  
323 to ensure the reliability of the data.

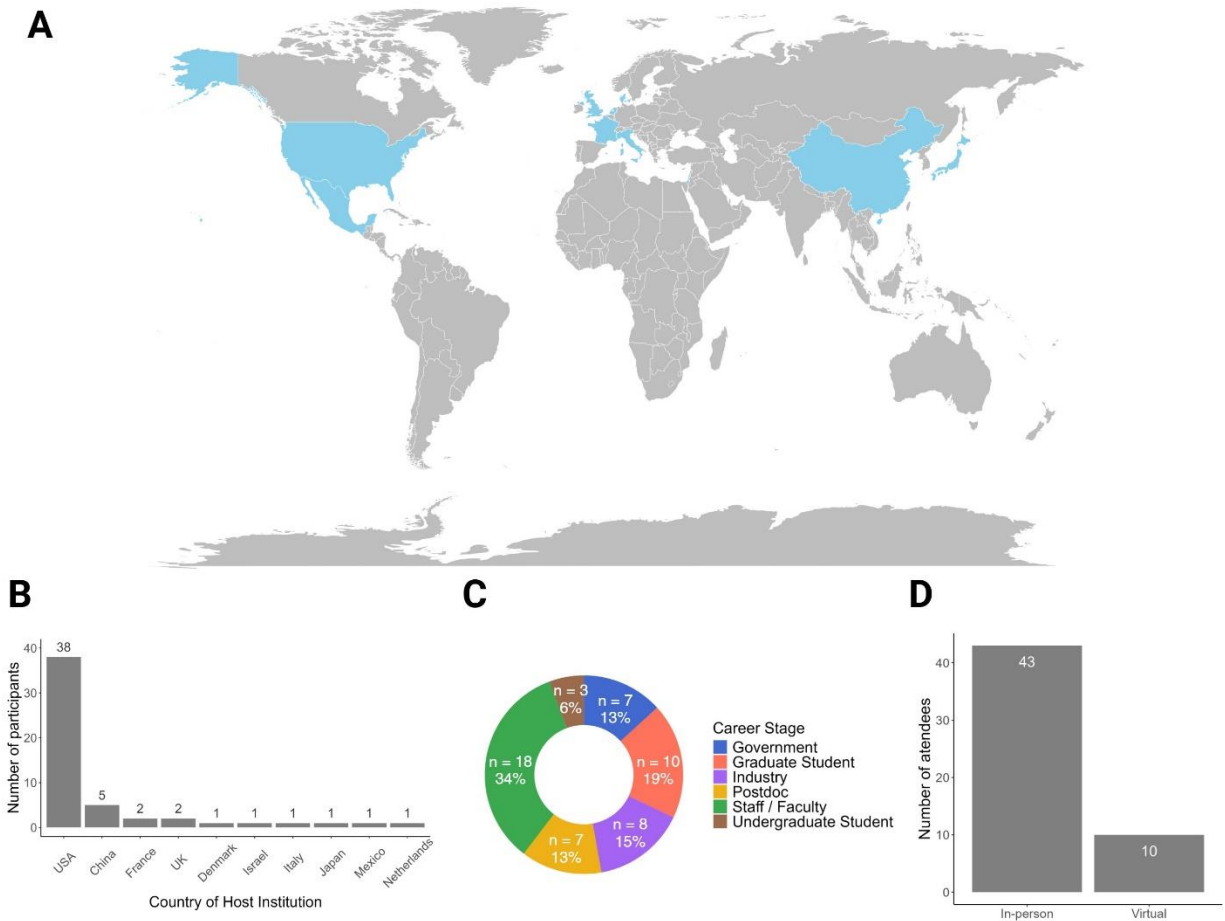
324 Bioinformatic and experimental techniques for linking viruses to their hosts are  
325 advancing rapidly, offering powerful tools to deepen our understanding of virus-host  
326 interactions. With these new methodologies, we highlighted the need to establish clear  
327 guidelines for defining virus-host interactions, tailored to the capabilities and limitations of  
328 each approach. For example, some bioinformatic methods predict virus-host associations by  
329 analyzing metagenomic sequences, while experimental techniques link viruses to hosts  
330 through DNA proximity ligation, viral transcription screening, or direct evidence of successful  
331 infection. These methods are critical for addressing the challenges of studying viral impacts in  
332 soil communities. However, it is essential to standardize interpretations of each technique's  
333 outputs and carefully evaluate their potential for training new virus-host linkage tools.  
334 Similarly, several bioinformatic tools predicting virus-host interactions and laboratory

335 techniques linking viruses to hosts are improving upon viral host detection and have led to the  
336 need for definitions and guidelines to define a viral host. These definitions should be  
337 accompanied by new methodologies that assess viral-host interactions, overcoming some of the  
338 challenges of current methods (Supplementary Table 3).

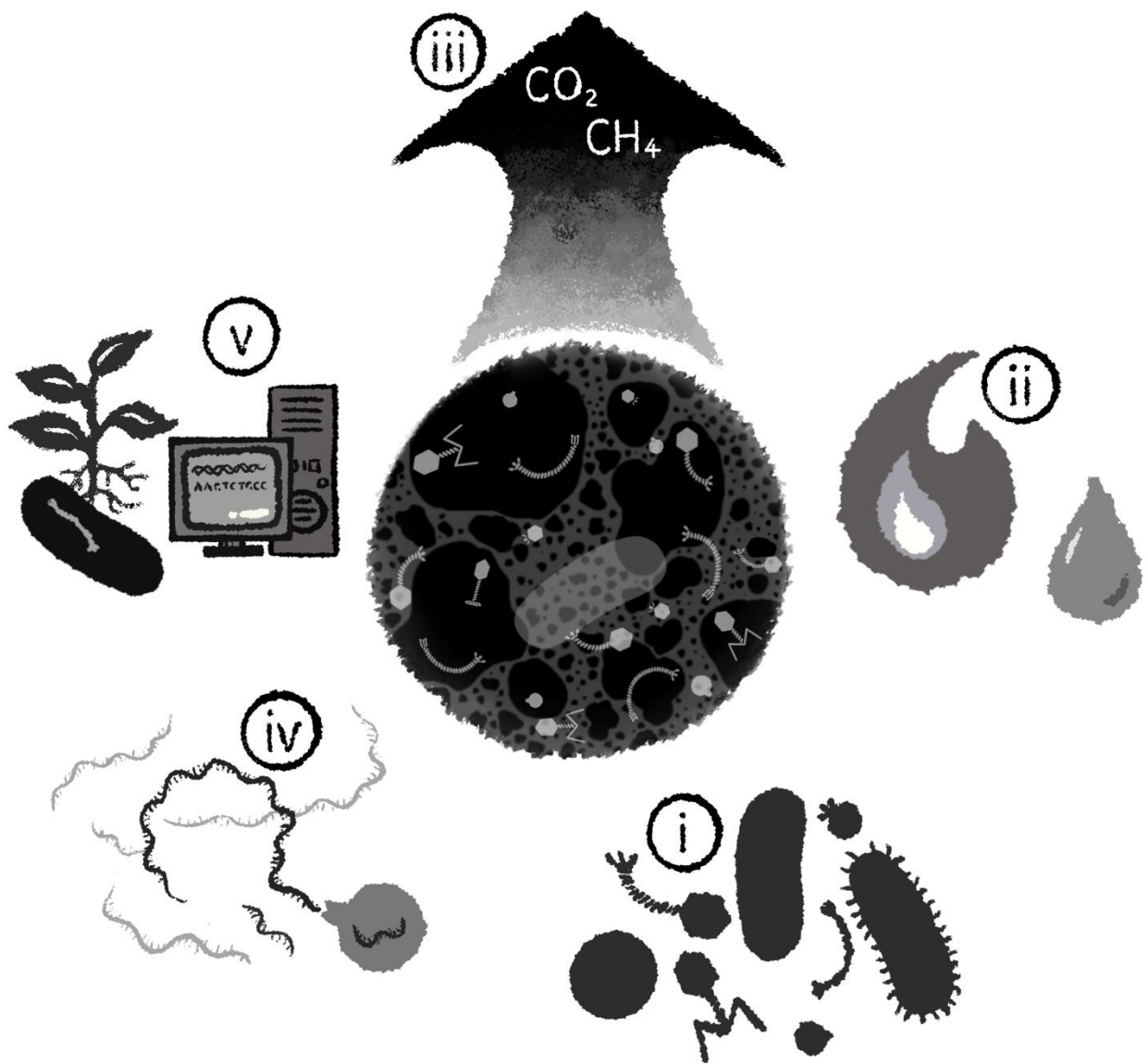
339 Another methodological advance that scholars agreed upon was soil sterilization  
340 techniques with minimum soil disturbance. Current soil sterilization methods (e.g. gamma  
341 irradiation) aim to generate sterile soils for comparing biotic versus abiotic processes or  
342 allowing the reconstruction of microbial communities. However, these methods can alter  
343 physical and chemical structures that should be taken into consideration. Discussions  
344 concluded with two topics that attendees believed should guide future research steps in the  
345 field, the study of viruses that are not double-stranded DNA and/or bacteriophages, given that  
346 these two types of viruses have been at the center of research until now, and the uncovering  
347 of interactions viruses have with organisms other than bacteria, which could lead to important  
348 discoveries on the impacts of soil viruses on the ecosystem.

349 In conclusion, the 2024 International Soil Virus Conference offered the opportunity for  
350 researchers in this growing field to gather, create professional relationships, and participate in  
351 exciting conversations around the current state of the field, its future, and how, as a  
352 community, we can advance the field. With this report we aim to record the outcomes of these  
353 provocative discussions.

354



355 **Figure 1. Demographic summary of attendees of the International Soil Virus Conference 2024.**  
 356 Countries of origin of the 53 attendees (A, and B), see the institution for those countries with  
 357 more than two attendees in Supplementary Table 4. Career stage of attendees (C), and  
 358 attendance mode (D).



359

360 **Figure 2. Soil viral talk topic areas.** Topic areas covered by the talks during the International

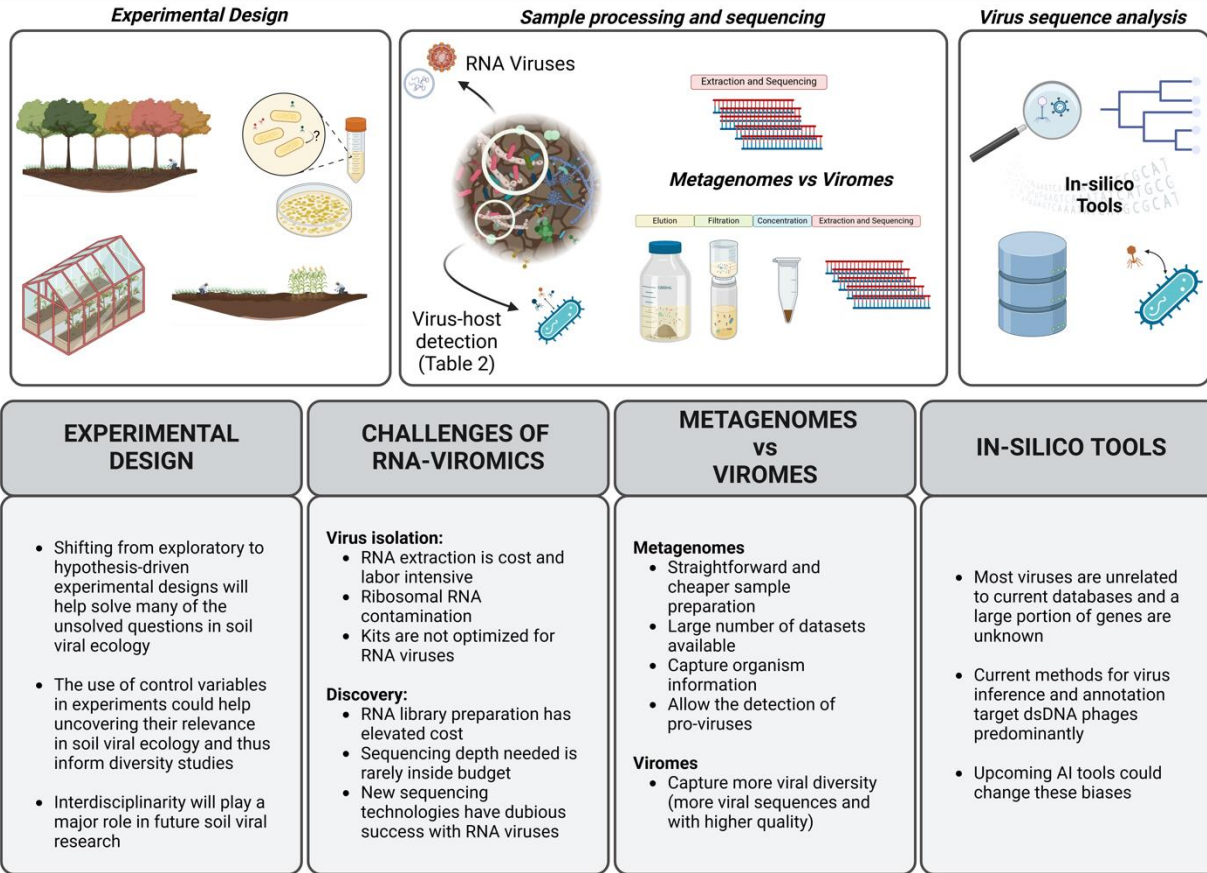
361 Soil Virus Conference 2024: (i) Viral diversity and community structure in the soil; (ii)

362 Response of soil viral communities to ecosystem disturbance; (iii) Impacts of viruses on the soil

363 microbiome and ecosystem processes; (iv) Soil RNA virome; (v) *In vivo* and *in silico* approaches

364 to the study of soil viruses. All talks contributed to building up a more comprehensive picture

365 of the soil virome and its wider impacts.



366

367 **Figure 3. Soil viral research framework with discussion key points.** Visual summary of the

368 phases that form a soil viral study. Some of these phases were discussion topics, other topics

369 are illustrated in the figure within the phase they belong to. Boxes summarize discussion

370 points.

371

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378

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380 Report writing was led by M.T-S., M.A.P., R.F., JH. L-M., R.A.G., S.M.B, who are therefore  
381 listed first. Conceptualization by G.T. and J.B.E. Review and editing by all authors. All authors  
382 have read and agreed to the published version of the manuscript.

383

### 384 **Declaration of Competing Interests**

385 The authors declare that they have no known competing financial interests or personal  
386 relationships that could have appeared to influence the work reported in this paper.

387

### 388 **Data availability**

389 No data was used for the research described in the article.

390

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400

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519

520 **Supplementary Table 1. Presentations key points**

| Session  | Key points   |
|--|--|
| 1. Viral diversity and community structure in the soil<br>2. Response of soil viral communities to ecosystem disturbance | <ul style="list-style-type: none"> <li>- Viromes and active viral populations are more sensitive than metagenomes to changes in the environment</li> <li>- Viral communities suffer from small to large scale spatial heterogeneity, both horizontally (across space) and vertically (across depths)</li> <li>- The effect of spatial heterogeneity can dampen the effect of changes in the environment</li> </ul> |
| 3. Impacts of soil viruses on the soil microbiome and associated ecosystem processes                                     | <ul style="list-style-type: none"> <li>- Studies on the viral role on soil processes can benefit from combined <i>in vivo</i> and <i>in silico</i> approaches</li> <li>- Additionally, they can benefit from targeting a single function or functional microbial group</li> <li>- Soil viral activity might be associated with increases in available organic matter</li> </ul>                                    |
| 4. RNA soil virome   | <ul style="list-style-type: none"> <li>- RNA viruses are understudied, but abundant in soil and have key roles in ecosystem processes</li> <li>- Methods need to be developed that specialize in the inference and analysis of RNA viruses</li> </ul>  |
| 5. <i>In vivo</i> and <i>in silico</i> approaches to the study of soil viruses   | <ul style="list-style-type: none"> <li>- The development of new <i>in vivo</i> and <i>in silico</i> methodologies and approaches is crucial to the advancement of soil viral research field, such as viral sequence inference, lifestyle prediction, taxonomic annotation, and host prediction</li> </ul>  |

521

| <b>Supplementary Table 2. Priorities for RNA virus discovery/annotation pipeline</b> |
|--|
| <ul style="list-style-type: none"> <li>• Improved user experience</li> </ul>         |

- Customization for RNA virus peculiarities (multi-segmented genomes, overlapping genes, divided RNA-directed RNA polymerase (RdRp) genes, etc)
- Explicit thresholds for each tool/ stage with scores explained
- Modularity to allow people to bring in outputs from other tools
- Assembly to RdRp identification to taxonomy to host prediction
- Improved contig binning, identifying multiple segments from the same virus (currently challenging in soil environment due to high diversity)
- Tools made to be easily installable/ runnable/ explainable

522

523 **Supplementary Table 3. Current methods in soil viral research for viral host detection.** List  
524 of current *in silico* and *in vivo* methods for viral host detection.

| Method     | Infection step detected  | Type  | Limitations   | Reference                               |
|------------|--|---|---|---|
| iPHoP      | <ul style="list-style-type: none"> <li>• Host recognition</li> <li>• Present of past interaction with host</li> </ul>            | Suite of computational tools for host prediction ( <i>In silico</i> ) | <ul style="list-style-type: none"> <li>• Slow</li> <li>• Relies on databases</li> <li>• Indirect</li> </ul>       | Roux et al. 2023                        |
| VirMatcher | <ul style="list-style-type: none"> <li>• Host recognition</li> <li>• Present of past or predicted interaction of host</li> </ul> | <i>In silico</i>  | Indirect <ul style="list-style-type: none"> <li>• Relies on databases</li> </ul>                                  | Gregory et al. 2020; Bolduc et al. 2021 |
| HiC        | <ul style="list-style-type: none"> <li>• Host recognition</li> <li>• Viral DNA replication/expression</li> </ul>                 | Chemically link viral genome to host                                  | <ul style="list-style-type: none"> <li>• Expensive</li> <li>• Identifies co-occurrence and/or presence</li> </ul> | Marbouty et al. 2021; Wu et al. 2023    |

|  |  |   |  |                            |
|--|--|---|--|----------------------------|
|  |  | <p>chromosome</p> <p>(<i>In vivo/ in silico</i>)</p>                                    | <p>of viral genome in microbial cell, not infection</p> <ul style="list-style-type: none"> <li>• Downstream analyses are not standardized</li> <li>• High likelihood of false positives</li> </ul> |                            |
| <p>Plaque assay + efficiency plating</p> | <ul style="list-style-type: none"> <li>• Host recognition</li> <li>• Viral DNA replication/expression</li> <li>• Viral production in host</li> <li>• Host Lysis</li> </ul> | <p>Semi-solid agar with culturable host</p> <p>(<i>In vivo</i>)</p>                     | <ul style="list-style-type: none"> <li>• Requires culturable host</li> <li>• Not all phages produce plaques on agar plates</li> <li>• Low throughput</li> </ul>                                    |                            |
| <p>Viral Ribo-Seq</p>                    | <ul style="list-style-type: none"> <li>• Host recognition</li> <li>• Viral DNA replication/expression</li> </ul>   | <p>Isolate and sequence ribosomes to see what is translated</p> <p>(<i>In vivo</i>)</p> | <ul style="list-style-type: none"> <li>• Few studies</li> <li>• Lots of optimization</li> <li>• Under development</li> </ul>   | <p>Gerovac et al. 2024</p> |

|                 |   |   |   |  |
|-----------------|---|---|---|--|
| vOTU + MAGs     | <ul style="list-style-type: none"> <li>• Host recognition</li> <li>• Viral DNA present</li> <li>• Lysogeny</li> </ul>   | <i>In silico</i>  | <ul style="list-style-type: none"> <li>• Can be misleading as viral contigs are sometimes binned with non-host MAGs</li> <li>• Small subset of viruses are matched to host</li> </ul> |  |
| Induction assay | <ul style="list-style-type: none"> <li>• Viral DNA replication</li> <li>• Viral production in host</li> <li>• Host Lysis</li> <li>• Lifestyle switch</li> </ul> | <p>Induce with inducing agent (e.g. mitomycin C, UV) and test by plaque assay</p> <p><i>(In vivo)</i></p> | <ul style="list-style-type: none"> <li>• Relies on culturable host</li> <li>• Need host for prophage</li> <li>• Lysogeny switch not always known</li> </ul>                           |  |
| qPCR            | <ul style="list-style-type: none"> <li>• Host recognition</li> <li>• Viral DNA replication/expression</li> </ul>  | Test if phage genome present and/or   | <ul style="list-style-type: none"> <li>• Phage sequence required</li> <li>• Can not make universal primers</li> </ul>   |  |

|                        |   |   |  |                      |
|------------------------|---|---|--|----------------------|
|                        |   | expressed<br><i>(In vivo)</i>   |  |                      |
| Droplet PCR            | <ul style="list-style-type: none"> <li>• Present of past interaction with host</li> </ul>   | High throughput PCR based method to study single viral-bacteria interaction<br><br><i>(In vivo)</i> | <ul style="list-style-type: none"> <li>• Relies on predicted phage-host pair for probe design</li> <li>• Primer bias</li> <li>• No quantification</li> </ul>   | Sakowski et al. 2021 |
| Single cell sequencing | <ul style="list-style-type: none"> <li>• Viral DNA replication/expression</li> <li>• Present of past interaction with host</li> </ul> | Sequence one bacteria cell<br><br><i>In vivo / in silico</i>  | <ul style="list-style-type: none"> <li>• Relatively low-throughput, sensitive to small amount of cross-talk between samples or libraries</li> <li>• Methods limited</li> <li>• Low coverage</li> </ul> | Labonté et al. 2015  |

|                                      |  |  |   |  |
|--------------------------------------|--|--|---|--|
|                                      |  |  | impacts results   |  |
| microscopy<br>/nan<br>o-<br>SI<br>Ms | <ul style="list-style-type: none"> <li>• Host recognition</li> <li>• Viral production in host</li> <li>• Host Lysis</li> <li>• Lifestyle switch</li> </ul> | <i>In vivo</i>   | <ul style="list-style-type: none"> <li>• Relies on culturable host, otherwise is a needle-in-a-haystack</li> <li>• Specialized equipment</li> </ul>                                 |  |
| Viral tag<br>and<br>grow<br>w        | <ul style="list-style-type: none"> <li>• Host recognition</li> </ul>   | DNA-tag<br>phage<br>and<br>watch<br>growth<br><br><i>(In vivo)</i> | <ul style="list-style-type: none"> <li>• Host cultivation</li> <li>• Diverse expertise needed</li> <li>• Unknown if lytic infection</li> <li>• Requires a flow cytometer</li> </ul> |  |

525

526 **Supplementary Table 4. Institutions of origin of attendees and number of attendees**  
527 **belonging to each institution.**

| <b>Institution</b>                                     | <b>Number of attendees</b> |
|--|----------------------------|
| University of California, Davis, USA                   | 7                          |
| Lawrence Livermore National Laboratory, Livermore, USA | 7                          |
| Oxford Nanopore Technologies, USA                      | 6                          |
| Georgetown University, Washington DC, USA              | 4                          |
| The Joint Genome Institute, Berkeley, USA              | 4                          |



|   |   |
|---|---|
| Zhejiang University, Hangzhou, China                    | 3 |
| Sandia National Laboratory, USA                         | 2 |
| Pacific Northwest National Laboratory,<br>Richland, USA | 2 |
| Aarhus University                                       | 1 |
| Bangor University                                       | 1 |
| Cal State East Bay                                      | 1 |
| College of William & Mary, Williamsburg,<br>USA         | 1 |
| East China Normal University, Shanghai,<br>China        | 1 |
| Kaneka Corporation, China                               | 1 |
| Lawrence Berkeley National Laboratory,<br>Berkeley, USA | 1 |
| Michigan State University, East Lansing,<br>USA         | 1 |
| Ohio State University, Columbus, USA                    | 1 |
| San Francisco State University, San<br>Francisco, USA   | 1 |
| Southern New Hampshire University,<br>Manchester, USA   | 1 |
| University of Padova                                    | 1 |
| Tel Aviv University                                     | 1 |
| Université Claude Bernard Lyon                          | 1 |
| University of Leicester                                 | 1 |
| University of Amsterdam                                 | 1 |
| University of Arizona, Tucson, USA                      | 1 |
| University of Wisconsin, Madison, USA                   | 1 |

528

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