Meeting report

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Meeting Report: International Soil Virus Conference 2024

María Touceda-Suárez[1], Matthew A. Perry[2], Riccardo Frizzo[3], John Henry Lotz-McMillen[4], Ruby Ann Gilmore[4], Shauna Marie Bennet[4], Jonelle T.R. Basso[5], William Donovan[6], Jane D. Fudyma[2], Sara E. Geonczy[2], Marissa Gittrich[7], Grant Gogul[2], Christina Hazard[8], Ellie Jameson[9], Lucie Jiraska[2], Sarah Stewart Johnson[4], James C. Kosmopoulos[10,11], Ikaia Leleiwi[6], Bin Ma[12], Catherine M. Mageeney[13], Andrew Millard[14], Uri Neri[5], Josué Rodríguez-Ramos[15], Simon Roux[5], Di Tong[12], Yiling Wang[12], Kurt Williamson[16], Ruonan Wu[15], Paula Dalcin Martins[17,18], Rumakanta Sapkota[19], Joanne B. Emerson[2], Gareth Trubl[11,*]

[1] Department of Environmental Science, University of Arizona, Tucson, AZ, USA

[2] Department of Plant Pathology, University of California, Davis, Davis, CA, USA

[3] Department of Biology, University of Padova, Italy

[4] Department of Biology, Georgetown University, Washington DC, USA

[5] DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

[6] Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA

[7] Environmental and Resource Sciences, Zhejiang University, Hangzhou, China.

Department of Microbiology, The Ohio State University, Columbus, OH, USA

[8] Université Claude Bernard Lyon 1, CNRS, INRAE, VetAgro Sup, Laboratoire d'Ecologie Microbienne LEM, CNRS UMR5557, INRAE UMR1418, Villeurbanne, France

[9] School of Environmental and Natural Sciences, Bangor University, Gwynedd, LL57 2TH, United Kingdom

[10] Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA

[11] Microbiology Doctoral Training Program, University of Wisconsin-Madison, Madison, WI, USA

[12] Institute of Soil and Water Resources and Environmental Science, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China

[13] Biotechnology & Bioengineering, Sandia National Laboratories, Livermore, CA, USA

[14] Leicester Centre for Phage Research, Dept Genetics, Genomics, and Cancer Sciences, University of Leicester, University Road, Leicester, LE1 7RH, United Kingdom

[15] Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA, USA

[16] Institute of Soil and Water Resources and Environmental Science, College of Biology Department, College of William & Mary, Williamsburg, VA, USA

[17] Department of Microbiology, Radboud Institute for Biological and Environmental Sciences, Radboud University, Nijmegen 6525 AJ, The Netherlands

[18] Department of Ecosystem and Landscape Dynamics, Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam 1098 XH, The Netherlands

[19] Department of Environmental Science, Aarhus University, Roskilde, Denmark

*Corresponding author: Gareth Trubl (Trubl1@llnl.gov)

Abstract

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

1. Introduction

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

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

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

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

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

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

2. Talks

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2.4. The soil RNA virome

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

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

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

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

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

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

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

3. Discussions

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

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

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

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

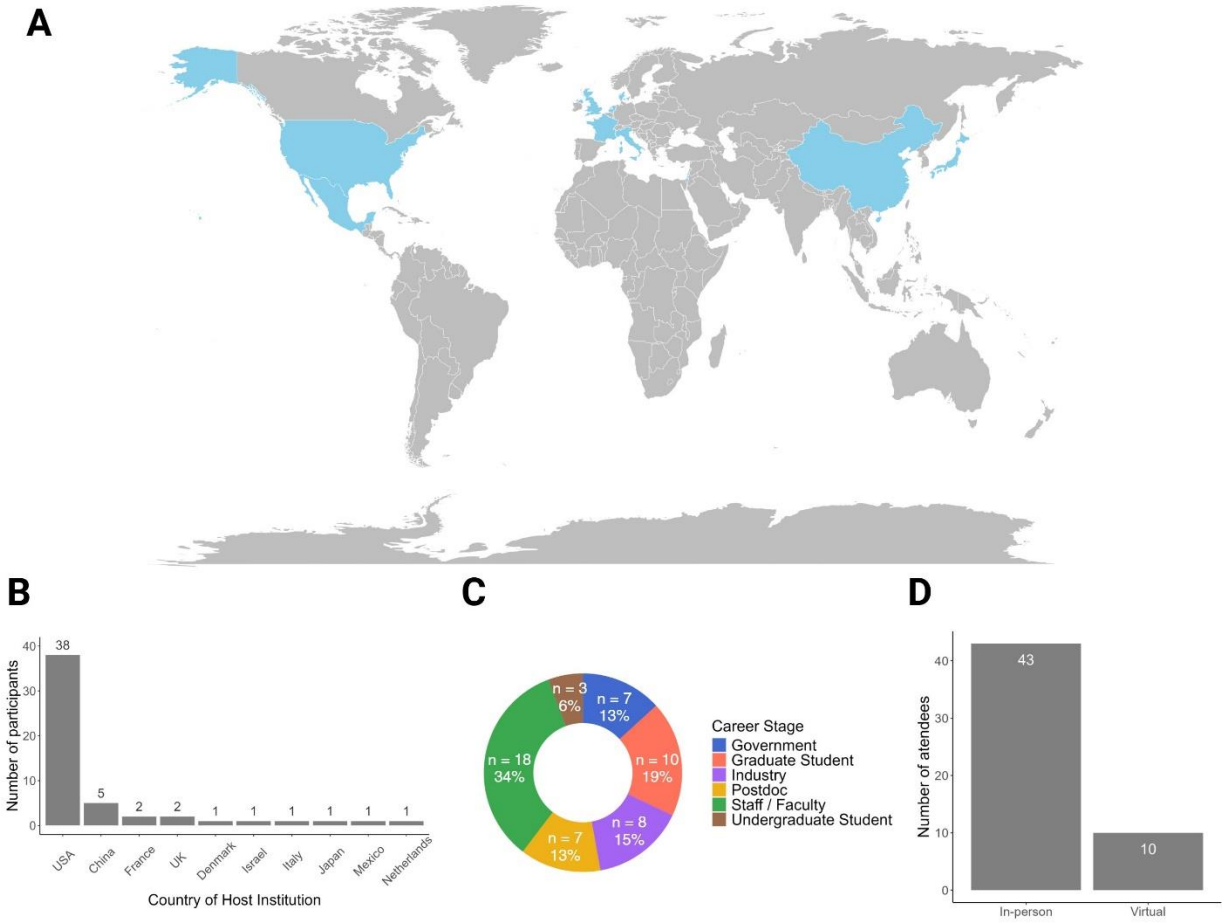
to prioritize, iii) relevant new directions for research, and constitute some of the topics that attendees envisioned as some of the most important issues to address in the near future. First, the study of auxiliary metabolic genes (AMGs) – metabolic genes of bacterial origin encoded in viral genomes–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

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

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

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

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

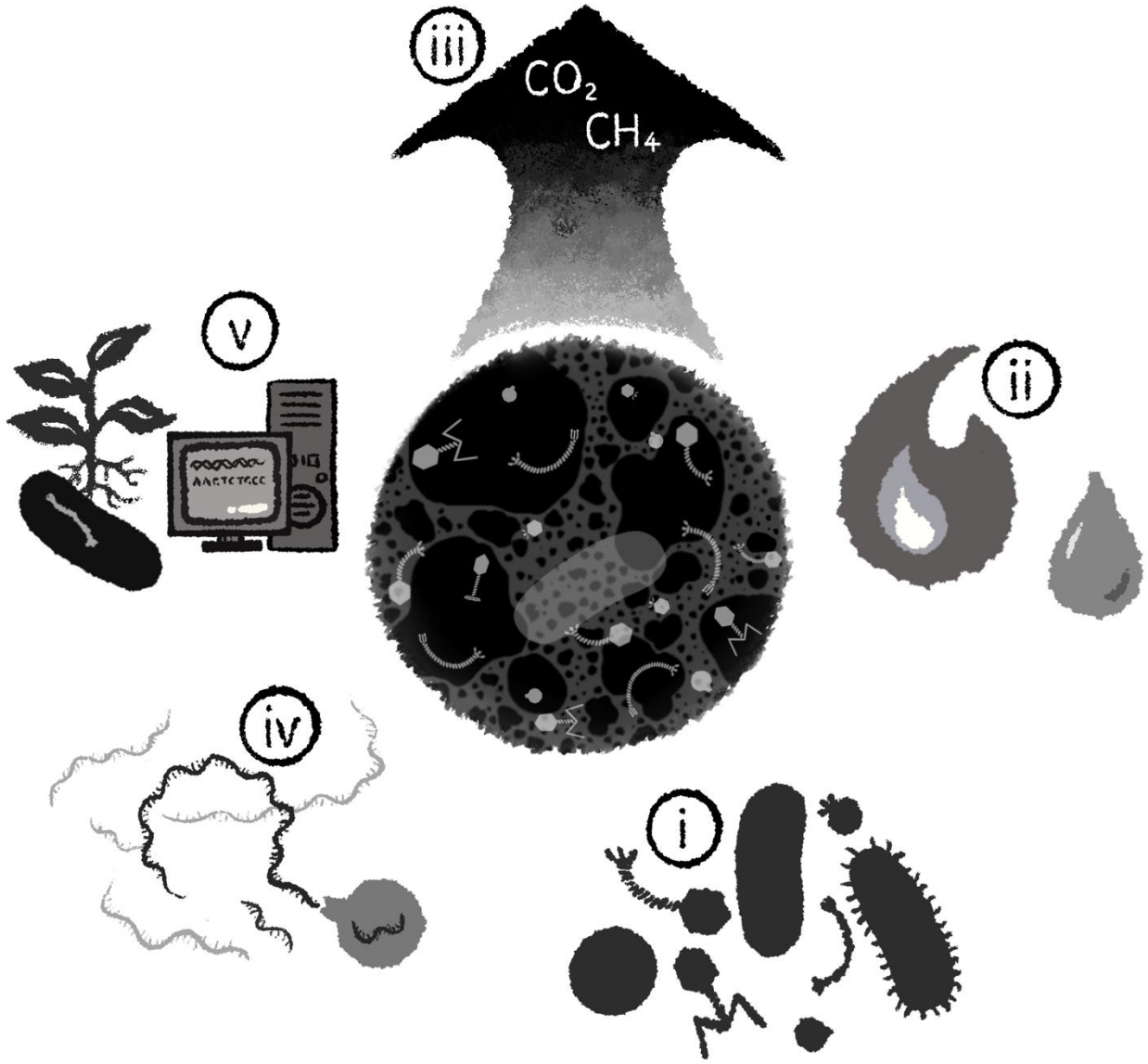


355 **Figure 1. Demographic summary of attendees of the International Soil Virus Conference 2024.**

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).



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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.

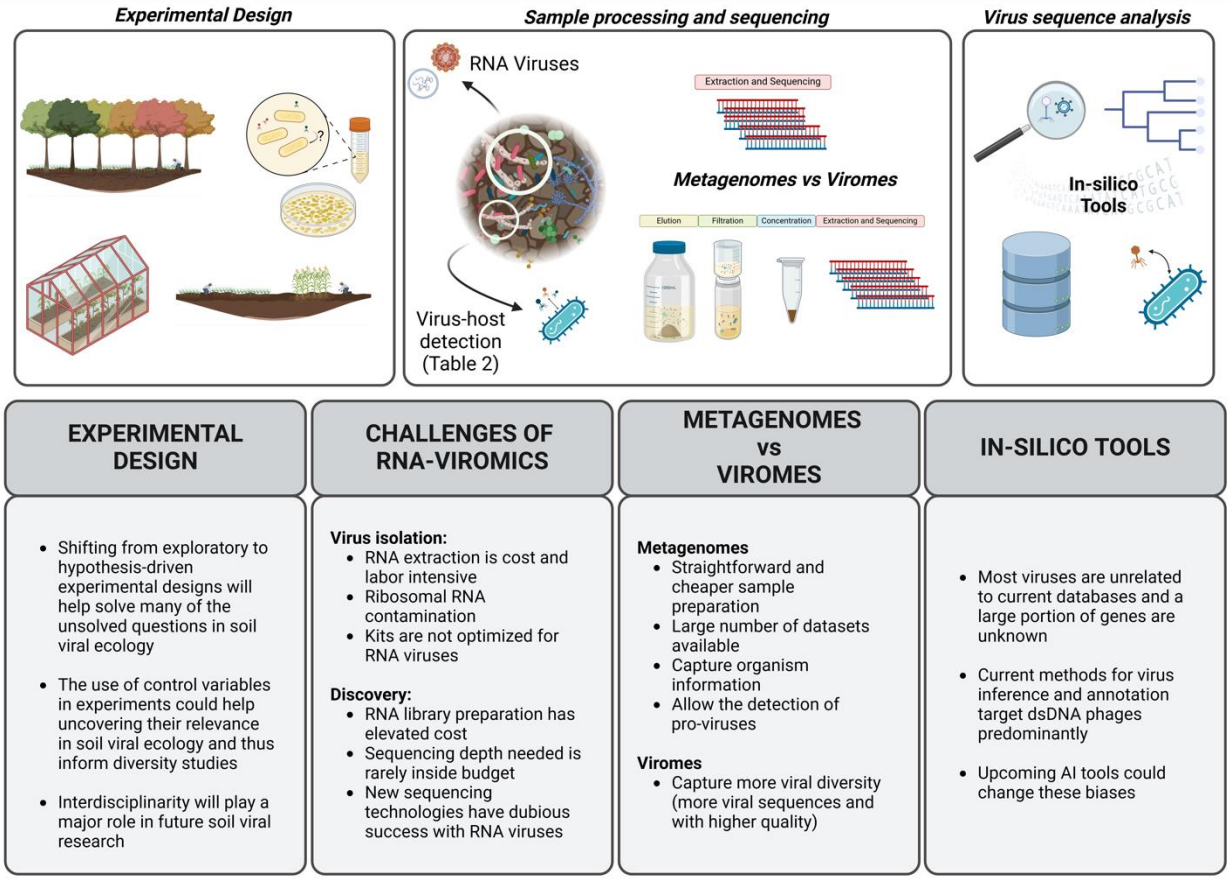

Figure 3. Soil viral research framework with discussion key points. Visual summary of the phases that form a soil viral study. Some of these phases were discussion topics, other topics are illustrated in the figure within the phase they belong to. Boxes summarize discussion points.

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Authors' Contributions

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 listed first. Conceptualization by G.T. and J.B.E. Review and editing by all authors. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interests

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

Data availability

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

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Supplementary Table 1. Presentations key points

Session	Key points
1. Viral diversity and community structure in the soil 2. Response of soil viral communities to ecosystem disturbance	- Viromes and active viral populations are more sensitive than metagenomes to changes in the environment - Viral communities suffer from small to large scale spatial heterogeneity, both horizontally (across space) and vertically (across depths) - The effect of spatial heterogeneity can dampen the effect of changes in the environment
3. Impacts of soil viruses on the soil microbiome and associated ecosystem processes	- Studies on the viral role on soil processes can benefit from combined *in vivo* and *in silico* approaches - Additionally, they can benefit from targeting a single function or functional microbial group - Soil viral activity might be associated with increases in available organic matter
4. RNA soil virome	- RNA viruses are understudied, but abundant in soil and have key roles in ecosystem processes - Methods need to be developed that specialize in the inference and analysis of RNA viruses
5. *In vivo* and *in silico* approaches to the study of soil viruses	- The development of new *in vivo* and *in silico* 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

Supplementary Table 2. Priorities for RNA virus discovery/annotation pipeline
• Improved user experience

- 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

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

Method	Infection step detected	Type	Limitations	Reference
iPHoP	• Host recognition • Present of past interaction with host	Suite of computational tools for host prediction (*In silico*)	• Slow • Relies on databases • Indirect	Roux et al. 2023
VirMatcher	• Host recognition • Present of past or predicted interaction of host	*In silico*	Indirect • Relies on databases	Gregory et al. 2020; Bolduc et al. 2021
HiC	• Host recognition • Viral DNA replication/expression	Chemically link viral genome to host	• Expensive • Identifies co-occurrence and/or presence	Marbouty et al. 2021; Wu et al. 2023

		chromosome (*In vivo/ in silico*)	of viral genome in microbial cell, not infection • Downstream analyses are not standardized • High likelihood of false positives	
Plaque assay + efficiency plating	• Host recognition • Viral DNA replication/expression • Viral production in host • Host Lysis	Semi-solid agar with culturable host (*In vivo*)	• Requires culturable host • Not all phages produce plaques on agar plates • Low throughput	
Viral Ribo-Seq	• Host recognition • Viral DNA replication/expression	Isolate and sequence ribosomes to see what is translated (*In vivo*)	• Few studies • Lots of optimization • Under development	Gerovac et al. 2024

vOTU + MAGs	• Host recognition • Viral DNA present • Lysogeny	*In silico*	• Can be misleading as viral contigs are sometimes binned with non-host MAGs • Small subset of viruses are matched to host	
Induction assay	• Viral DNA replication • Viral production in host • Host Lysis • Lifestyle switch	Induce with inducing agent (e.g. mitomycin C, UV) and test by plaque assay (*In vivo*)	• Relies on culturable host • Need host for prophage • Lysogeny switch not always known	
qPCR	• Host recognition • Viral DNA replication/expression	Test if phage genome present and/or	• Phage sequence required • Can not make universal primers	

		expressed (*In vivo*)		
Droplet PCR	• Present of past interaction with host	High throughput PCR based method to study single viral-bacteria interaction (*In vivo*)	• Relies on predicted phage-host pair for probe design • Primer bias • No quantification	Sakowski et al. 2021
Single cell sequencing	• Viral DNA replication/expression • Present of past interaction with host	Sequence one bacteria cell *In vivo / in silico*	• Relatively low-throughput, sensitive to small amount of cross-talk between samples or libraries • Methods limited • Low coverage	Labonté et al. 2015

			impacts results	
microscopy/nano-SIMs	• Host recognition • Viral production in host • Host Lysis • Lifestyle switch	*In vivo*	• Relies on culturable host, otherwise is a needle-in-a-haystack • Specialized equipment	
Viral tag and grow	• Host recognition	DNA-tag phage and watch growth (*In vivo*)	• Host cultivation • Diverse expertise needed • Unknown if lytic infection • Requires a flow cytometer	

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

Institution	**Number of attendees**
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, Richland, USA	2
Aarhus University	1
Bangor University	1
Cal State East Bay	1
College of William & Mary, Williamsburg, USA	1
East China Normal University, Shanghai, China	1
Kaneka Corporation, China	1
Lawrence Berkeley National Laboratory, Berkeley, USA	1
Michigan State University, East Lansing, USA	1
Ohio State University, Columbus, USA	1
San Francisco State University, San Francisco, USA	1
Southern New Hampshire University, 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

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