EFFECTS OF CONVENTIONAL AND ORGANIC MANAGEMENT ON VIRAL COMMUNITIES IN A NON-EXPERIMENTAL AGROECOSYSTEM IN HONG KONG, CHINA

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Abstract. Soil viral communities are affected by factors such as agricultural management, crop type and soil compartment. In this study, metagenomics analysis was used to investigate the characteristics of viral communities in farmland under conventional and organic management in Hong Kong, China, in order to assess the effects of land management, crop type, and rhizosphere on soil viral communities and identify specific viruses they affect. We hypothesized that agricultural management had the greatest impact on viral communities. Genome annotation revealed double-stranded DNA viruses were dominant viruses, with *Siphoviridae* being the most prevalent viral family (account for 42.4%). Hierarchical clustering, principal coordinate analysis and redundancy analysis demonstrated significant distinctions in viral communities between organic and conventional farming systems. Organic management was associated with reduced viral diversity as indicated by lower Shannon, Simpson, and Chao diversity indices, alongside a notable increase in viral abundance. Specifically, *Siphoviridae* exhibited an 84.5% increase, while *Marseilleviridae* and *Baculoviridae* surged by approximately 99% and 93.8%, respectively. The structure of viral communities was primarily influenced by management mode, followed by crop type. Viral structure was mainly affected by soil pH, available phosphorus and available potassium. Organic agricultural management could significantly increase soil pH, organic matter content and available nitrogen, which may help to maintain the health of plants and soil. Our findings suggest that organic management practices have a large effect on increasing viral diversity, promoting healthier soils via viral taxa and functions.

Keywords: *soil viral communities, non-experimental agroecosystem, agricultural management, viral diversity*

Introduction

Viruses exhibit a remarkable diversity and stand as the most abundant community on Earth, estimated at a staggering total number of 10^{31} (Breitbart and Rohwer, 2005), with 97% of the global viruses existing in soil and sediments (Cobián Güemes et al., 2016). Interactions between phage and hosts in soil may be uniquely shaped by soil heterogeneity and physical constraints on the mobility of bacteria and viruses (Roy et al., 2020). Studies have shown that soil viruses are affected by a variety of factors in soil. For instance, temperature is the most critical factor in the survival and growth of phage through affecting the host bacteria (Ye et al., 2019). Soil pH also exerts a significant influence on viral communities, with soil viral communities from different regions showing clustering patterns based on similar pH values (Lee et al., 2022). High soil water content notably increases viral abundances, with wet soils displaying highly diverse viral compositions and heightened viral activity (Wu et al., 2021; Huang and Zhang, 2022). Additionally, metal ions such as Ag^+ and Cu^{2+} have been observed to inactivate Phi6 bacteriophage (Molan et al., 2022). Factors such as host biomass, host growth rate, and soil nitrogen addition elicit robust viral production responses (Whitaker et al., 2015). Soil viruses play pivotal roles in the soil ecosystem; for instance, rhizosphere phage can influence plant health by regulating rhizosphere microbial communities (Pratama et al., 2020). Moreover, soil viruses serve ecological functions such as participating in the geochemical cycle of elements (Jover et al., 2014), controlling bacterial diversity, and maintaining the youth of bacterial populations (Kuzyakov and Mason-Jones, 2018). There were differences in RNA viral communities in grassland ecosystems with different geographic distribution and even in grasslands with different degrees of management clustered together, which was different from that in unmanaged grassland viruses. Viruses are diverse and come from different hosts, so they may affect soil ecosystems at multiple trophic levels (Hillary et al., 2022). Viral communities and their hosts exist on the basis of root litter, and viral communities may participate in soil carbon cycling by mobilizing cellular carbon (Starr et al., 2019). The viral community in soil is affected by land management and has great dynamic properties, which can affect the function of bacterial community (Roy et al., 2020). The diversity of viral community regulates the balance of soil bacterial community, and the co-evolution of virus and bacteria has many effects on soil ecosystem (Wang et al., 2024).

The application of organic fertilizers has been noted to alter the structure of viral communities and enhance interactions between viruses and bacteria in soil (Chen et al., 2021). Organic fertilizers contribute to increased soil organic matter and soil pH, thereby enhancing soil conditions and subsequently elevating viral abundance (Chen et al., 2014). Agricultural management practices in farmland have profound implications for crops, which represent a fundamental food source for humanity and are intimately linked to human welfare. While chemical fertilizers can boost crop yields, improper fertilization practices can lead to soil degradation, including nutrient imbalances, soil acidification and abnormal accumulations of elements, among other soil health issues (Cai, 2019).

Furthermore, pesticide pollution poses severe environmental hazards and threatens non-target organisms, while long-term exposure to pesticides poses significant health risks to humans (Tudi et al., 2021; Tudi et al., 2022). Hence, there is a pressing need to adopt environmentally friendly organic agricultural management measures. Organic practices, such as the application of organic fertilizers, crop rotation, and minimizing tillage and synthetic chemical usage (Mazzon et al., 2021), can enhance soil quality, bolster soil microbiome activity, and thus fortify agro-ecosystem health and improve farmland productivity. Moreover, organic management fosters the development of sustainable soil systems and promotes soil health (Lori et al., 2022). Our previous study demonstrated significant differences in bacterial and fungal communities between conventional and organic systems. Organic management led to increases in soil pH, organic matter content, available phosphorus, available nitrogen, and alkali-hydrolysed nitrogen content, significantly impacting the diversity of soil bacterial and fungal communities and fostering a more complex and interconnected bacterial community structure. Organic management facilitates soil nutrient cycling, promoting healthier plants and higher soil quality (Zhou and Fong, 2021). However, it remains unclear whether differences exist in viral communities between conventional and organic management systems.

Currently, metagenomics technology has been used to analyse the characteristics of soil viral communities under various land use patterns (Bi et al., 2021). However, it is still unclear that how different agricultural management practices influence on the structure of soil viral communities. In this study, topsoil samples under conventional and organic management modes in Hong Kong, China were selected as the focal point. Metagenomics technology was employed to investigate the impacts of agricultural management on the diversity, structural composition, and function of soil viral communities. This endeavour aims to provide valuable insights into soil virus characteristics and facilitate comparisons of the strengths and weaknesses of different agricultural management modes.

Materials and methods

Soil sample collection and soil properties

The sampling area is located in an agroecosystem site (22.42792N 114.09297E) in Tsang Uk village, Shek Kong, Yuen Long District, New Territories, Hong Kong, China. This region experiences a subtropical monsoon climate characterized by an average annual temperature of 23.1℃ and an annual rainfall of 2043.4 mm, with soil texture classified as sandy loam. Sampling was carried out between November 2019 and January 2020, during a period of relatively stable weather conditions. The map in this study site section to clearly indicate the location of the sampling site was shown in our previous study (Zhou and Fong, 2021). Two parcels of land have been designated for conventional and organic agricultural management, with each treatment subdivided into three plots measuring 20 m×1 m. The plots were laid out in a randomized block design to minimize positional effects, ensuring uniform environmental conditions across the site. Plots were separated by 1-meter buffer zones to avoid potential nutrient or chemical interference between treatments.

Organic management practices entail the application of 50-150 kg/ha of organic fertilizer, comprising spent mushroom substrate, bean curd dregs, and homemade compost. Seeds are sown to a depth of 25 cm using hoes, with crops protected through manual weeding and artificial mosquito nets. Conversely, conventional management involves the application of various synthetic fertilizers totaling 30-50 kg/ha, supplemented with organic manure composed of cow manure, bone meal, and peanut press pulp. Seeds are sown to a depth of 25 cm using a ploughing machine, with herbicides, fungicides, and insecticides employed for pest control.

Cabbages (*Brassica oleracea* var. *Capitata* L.) and India lettuces (*Lactuca indica*) are concurrently cultivated in both management modes over a period of four months. The exact dates of crop transplantation into the soil were November 10th and 12th, 2019, followed by initial fertilization according to the respective management practices. A second round of fertilization was conducted in December 2019, during which organically managed cabbages were piled at the base of their plants following fertilization.

Before crop harvest in January 2020, rhizosphere soil and bulk soil were collected separately. The method involved excavating pits measuring 5 to 20 cm in width and depth around randomly selected plants. Soil sampling was conducted on January 15th, 2020, after a period of relatively consistent environmental conditions. The plant and soil were then removed, and the plant was gently shaken to remove irrelevant soil, after which the soil attached to the root cap of the plants was collected (Semenov et al., 2020). Bulk soil was collected from a depth of 15 to 20 cm near the plant using a sampling tube. Samples were categorized into eight groups based on three factors: management mode, crop type, and soil compartment. Each group comprised three letters, with the first letter denoting organic management (O) or conventional management (C), the second letter representing cabbage (C) or lettuce (L), and the third letter indicating bulk soil (B) or rhizosphere soil (R). Each treatment was repeated three times, resulting in a total of 24 soil samples collected.

After randomly sampling, the soil samples were air-dried at room temperature and then sieved through a 1-mm screen. Soil properties were measured by the Analysis and Testing Center in Chinese Academy of Agricultural Sciences (IEDA, CAAS, Beijing, China), including soil pH, organic matter (OM), available nitrogen (AN), available phosphorus (AP) and available potassium (AK). The five characteristics were measured following (Zhou et al., 2017): soil pH was measured by glass electrode; OM was determined using the potassium dichromate external heating method; AN was quantified by extraction with 2M KCl, steam distillation and titration; AP was determined using the molybdenum blue method and AK was determined by flame photometry.

DNA extraction and metagenomics analyzing

Total DNA was extracted from 24 soil samples and subsequently sequenced using the Illumina sequencing platform Novaseq 2000. Initial processing involved removing adapter sequences from both the 3' and 5' ends of the sequences. Reads shorter than 50 base pairs, those with an average quality value below 20, and those containing N bases were filtered out using the fastp software. High-quality reads, including paired-end reads and single-end reads, were retained for further analysis. Subsequently, reads were aligned with host DNA using the BWA software, and reads showing high similarity to host DNA were removed to mitigate contamination. The Megahit software was utilized for the assembly and splicing of quality-controlled sequences, resulting in contigs with a minimum length of 300 base pairs. These contigs were then used for predicting open reading frames (ORFs) of genes with nucleic acid lengths greater than or equal to 100 base pairs, facilitated by the MetaGene software (Noguchi et al., 2006).

Statistical analysis

The non-redundant gene set was constructed, and species annotation results were obtained by comparing the obtained non-redundant gene set with the NR database using DIAMOND. Various diversity indices including Shannon, Simpson, Evenness, Chao, and Sobs were calculated. The data are represented as means ± standard deviations. Viral beta diversity analysis was examined based on Cluster tree analysis and principal coordinate analysis (PCoA). Hierarchical clustering analysis using the Bray-Curtis distance algorithm was conducted at the species level to elucidate similarities and differences between samples. Regression analysis based on PCoA on bray curtis distance matrix was conducted at the generic level to visualize differences in viral communities between different groups (conventional/organic, cabbage/lettuce, bulk/rhizosphere). The Wilcoxon rank-sum two-tail test was utilized to assess significant differences in viral families among the samples of three treatments separately. Additionally, RDA analysis was used to examine the relationship between viral community and environmental factors (pH, OM, AN, AK and AP). Physicochemical properties and Alpha diversity indices were presented analyzed by performing one way analysis of variance (ANOVA). Tukey test was used to compare the data for the sample and contrasted among the treatments. All the statistical analyses were conducted using SPSS 21.0 software. Differences were considered significant base on a P value of < 0.05.

Results

Physical and chemical properties of soil

As shown in *Table 1*, the pH value of organic soil was significantly higher than that of conventional soil, approaching neutrality. Particularly, the pH of cabbage soil managed organically tended towards neutrality and significantly exceeded that of lettuce soil. However, no significant disparity was observed between the two crop types under conventional management. The rhizosphere exhibited no significant influence on soil pH.

Table 1. *Physicochemical properties of different treated soils*

	pH	OM (mg/kg)	AP (mg/kg)	AK (mg/kg)	AN (mg/kg)
CBC	5.77±0.29^{ab}	22.03±3.48^{a}	164.00±5.29^{ab}	72.33±23.63^{ab}	114.33±6.51^{a}
CBO	7.23±0.25^{c}	41.23±3.85^{a}	135.57±2.21^{a}	47.23±1.66^{a}	168.90±11.46^{ab}
CRC	5.60±0.30^{ab}	29.53±9.70^{a}	151.00±2.65^{a}	43.67±11.24^{a}	140.67±34.96^{a}
CRO	7.20±0.10^{c}	81.07±32.45^{b}	184.00±51.07^{abc}	86.67±12.50^{ab}	288.33±86.69^{b}
LBC	5.40±0.10^{a}	20.30±0.92^{a}	239.33±22.05^{c}	199.00±47.57^{b}	113.97±17.75^{a}
LBO	6.20±0.20^{b}	43.57±10.59^{a}	135.33±3.51^{a}	193.00±90.14^{ab}	194.33±28.75^{ab}
LRC	5.23±0.15^{a}	21.47±2.80^{a}	222.33±37.82^{bc}	215.33±96.36^{b}	147.33±66.76^{a}
LRO	6.23±0.40^{b}	46.10±3.20^{ab}	141.90±3.73^{a}	139.77±48.37^{ab}	216.23±8.08^{ab}

OM: organic matter, AP: available phosphorus, AK: available potassium, AN: available nitrogen. CBC: Cabbage Bulk Conventional; CBO: Cabbage Bulk Organic; CRC: Cabbage Rhizosphere Conventional; CRO: Cabbage Rhizosphere Organic; LBC: Lettuce Bulk Conventional; LBO: Lettuce Bulk Organic; LRC: Lettuce Rhizosphere Conventional; LRO: Lettuce Rhizosphere Organic. One-way ANOVA. n=3, the data are represented as means ± SD, different letters varied significantly at $P < 0.05$ levels, a<b

Organic management increased the content of OM in soil, especially in cabbage rhizosphere soil. Conversely, Lettuce cultivation under conventional management significantly increased soil AP content. Moreover, the content of AN in rhizosphere soil of cabbage under organic management was significantly higher than that under conventional management. Nonetheless, no significant differences were observed in AK or AN among three treatments.

Alpha diversity of viral communities

A total of 1,818,294,008 raw reads were obtained from the 24 soil samples, and 1,790,079,354 clean reads were obtained after quality control. 12,749,141 contigs was obtained in total, and 16,045,283 ORFs were predicted. Subsequent species annotation identified 273,460 viral sequences, comprising 3 orders (*Caudovirales*, *Herpesvirales*, and *Picornavirales*), 18 families, and several unclassified viruses.

The alpha diversity indices Shannon, Simpson, Chao and Sobs are shown in *Table 2*. Results indicated that the diversity indices Shannon, Simpson and richness index Sobs in organic rhizosphere and bulk soils of lettuce and cabbage were significantly lower than those of conventional soil samples. Notably, no significant difference was observed in viral alpha diversity (Shannon, Simpson, Sobs indices) between rhizosphere and bulk soils under the same management. Furthermore, viral diversity (Shannon index) in cabbage soil was higher than that in lettuce under both management modes, although the difference was not statistically significant. However, in conventional soils, viral richness in cabbage soil was significantly lower than that in lettuce soil, as indicated by the Sobs and Chao indices.

Table 2. *Alpha diversity index of viral communities*

	CBC	**CBO**	**CRC**	**CRO**	**LBC**	**LBO**	**LRC**	**LRO**
Shannon	4.63±0.09^{b}	4±0.15^{a}	4.67±0.18^{b}	4.02±0.08^{a}	4.55±0.21^{b}	3.75±0.21^{a}	4.47±0.14^{b}	3.86±0.05^{a}
Simpson	0.032±0.003^{a}	0.059±0.023^{ab}	0.024±0.003^{a}	0.055±0.008^{ab}	0.042±0.020^{a}	0.106±0.018^{c}	0.048±0.016^{ab}	0.086±0.005b^{c}
evenness	0.797±0.013^{c}	0.754±0.018^{abc}	0.795±0.012^{c}	0.772±0.014^{bc}	0.733±0.049^{abc}	0.690±0.028^{a}	0.704±0.025^{ab}	0.702±0.007^{a}
Chao	335±14^{bc}	203.33±29.09^{a}	358.67±58.35^{c}	182.67±18.15^{a}	501±86.63^{d}	230±26.89^{ab}	576±38.20^{d}	245.67±21.55^{abc}
Sobs	335±14^{bc}	203.33±29.09^{a}	358.67±58.35^{c}	182.67±18.15^{a}	501±86.63^{d}	230±26.89^{ab}	576±38.20^{d}	245.67±21.55^{abc}
Ace	335±14^{bc}	203.33±29.09^{a}	358.67±58.35^{c}	182.67±18.15^{a}	501±86.63^{d}	230±26.89^{ab}	576±38.20^{d}	245.67±21.55^{abc}

CBC: Cabbage Bulk Conventional; CBO: Cabbage Bulk Organic; CRC: Cabbage Rhizosphere Conventional; CRO: Cabbage Rhizosphere Organic; LBC: Lettuce Bulk Conventional; LBO: Lettuce Bulk Organic; LRC: Lettuce Rhizosphere Conventional; LRO: Lettuce Rhizosphere Organic. One-way ANOVA. n=3, the data are represented as means ± SD, different letters varied significantly at $P < 0.05$ levels, a<b

Beta diversity of viral communities

Hierarchical clustering analysis in *Figure 1* illustrates the grouping of viral communities across the 24 soil samples. Upon closer examination of branch lengths, it becomes evident that these communities segregate into two distinct clusters based on different management practices. Similarly, viral communities from different crop types roughly align with their respective management methods. Interestingly, there was no significant contrast between rhizosphere and bulk soil samples.

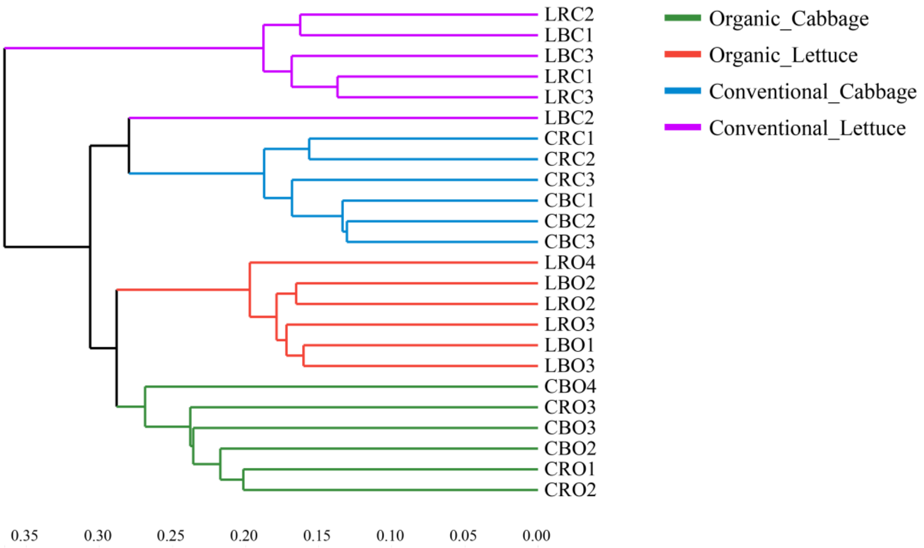

Figure 1. *Cluster tree analysis of multiple samples at the species level, viral communities segregate into two distinct clusters based on different management practices and crop types. Organic cabbage treatment is highlighted green, organic lettuce is highlighted red, conventional cabbage is highlighted blue and conventional lettuce is highlighted purple*

In *Figure 2*, PCoA analysis at the generic level illustrates that the first and second principal coordinates (PC1 and PC2) account for 66.54% and 15.05% of the variation in soil viral community structure, respectively, within a 95% confidence interval. Notably, samples under the same management exhibit a distinct tendency to cluster together, indicating a cohesive community distribution within each agricultural management treatment (*Figure 2a*). This observation corroborates the findings from the cluster tree analysis, suggesting that different agricultural management practices exert a significant influence on soil viruses.

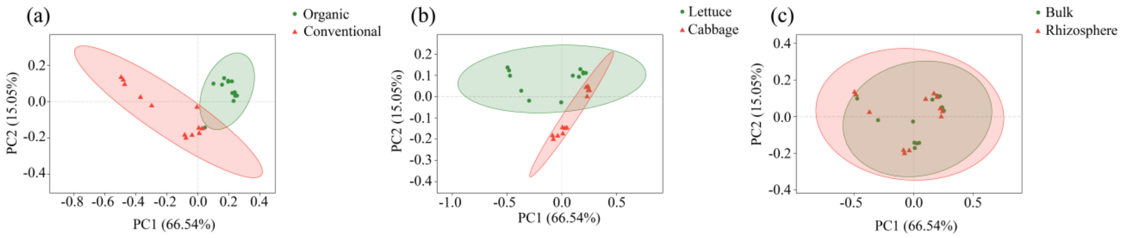

Figure 2. *PCoA analysis of each treatment at the generic level (a) Organic and conventional; (b) Cabbage and lettuce; (c) Rhizosphere and bulk*

Differences in composition of viral community

In analyzing the composition of viral communities at the family level, a notable difference emerges between various agricultural management practices. Under conventional management, the prevalence of three specific viral families—*Siphoviridae*, *Marseilleviridae*, and *Baculoviridae*—was significantly higher compared to organic management, accounting for a larger proportion of the total viral population in conventional soils as illustrated in *Figure 3*. Conversely, viruses from the *Phycodnaviridae*, *Retroviridae*, *Herpesviridae*, and *Microviridae* families were found in higher proportions under organic management than under conventional practices.

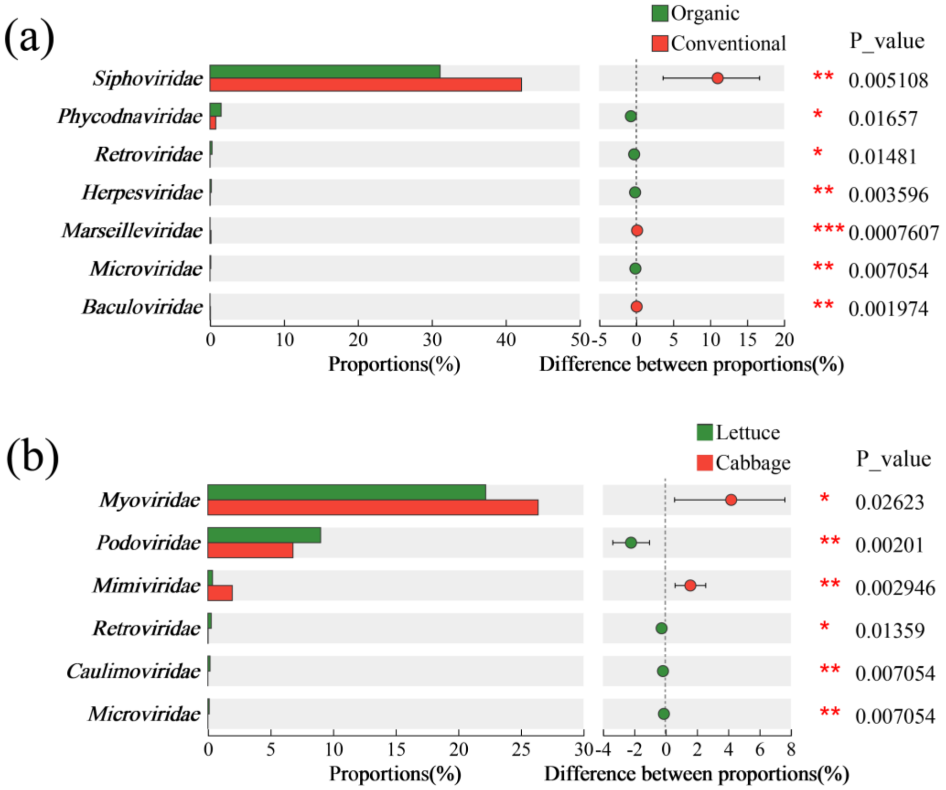

Figure 3. *Comparative analysis results of two groups in each treatment at the family level, the bar section shows the proportion of viral family, and the line section shows the differences between proportion (a) Viral communities with significant differences between organic and conventional management; (b) Viral communities with significant differences between two types of crops*

Furthermore, the study highlights differences in viral abundance based on crop type. In soils cultivated with lettuce, viruses from the *Podoviridae*, *Retroviridae*, *Caulimoviridae*, and *Microviridae* families were more abundant compared to those in cabbage soils. Conversely, the *Myoviridae* and *Mimiviridae* viral families were more prevalent in soils associated with cabbage.

Interestingly, *Caulimoviridae* viruses were exclusively detected in rhizosphere soil, although the presence of the rhizosphere itself did not significantly impact the overall viral community composition.

Overall, agricultural management practices exert the most significant influence on the structure of viral communities, followed by the type of crops grown. The impact of the rhizosphere, compared to bulk soil, on viral community structure appears to be minimal.

RDA analysis

The scatter plot from the RDA analysis vividly illustrates the sample distribution, with arrows indicating both the direction and magnitude of environmental influence on sample species/function. This visual representation succinctly depicts the relative impacts of environmental factors such as pH, organic matter (OM), ammonium nitrogen (AN), available potassium (AK), and available phosphorus (AP) on the samples.

As depicted in *Figure 4*, soil pH exhibited a pronounced effect on cabbage, with the viral community showing a positive correlation with pH, OM, and AN, while displaying a negative correlation with AP and AK. In aggregate, these five soil properties collectively explained 80.09% of the variance in the viral community, with soil AP, pH, and AK significantly influencing community structure ($P < 0.05$).

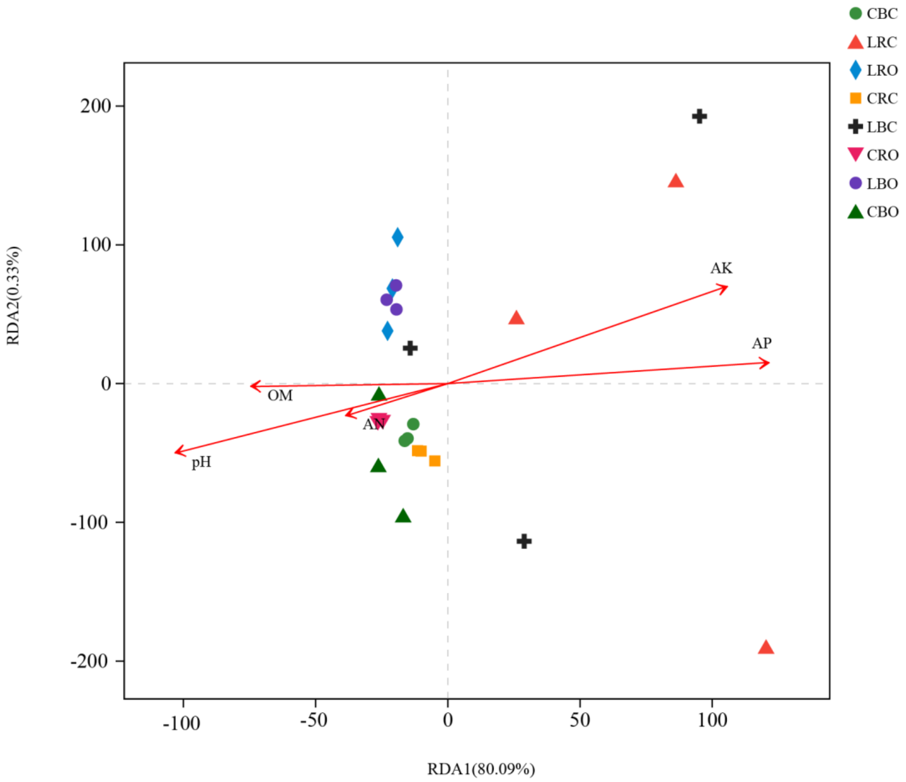

Figure 4. *RDA analysis of each treatment at the generic level. The arrows indicate the physical and chemical properties, and the different shapes represent the combination of the three treatments, for a total of eight. The length of the arrow represents the degree of influence of physical and chemical properties on the distribution of viral community, the projected distance from the sample point to the vector of quantitative environmental factors represents the degree of influence of the sample by environmental factors, the Angle between environmental factors represents the positive and negative correlation between environmental factors, the acute Angle represents the positive correlation, the right Angle has no correlation, and the obtuse Angle represents the negative correlation*

Discussion

In this study, organic and conventional management were conducted on lettuce and cabbage fields in Hong Kong to explore the effects of agricultural management, crop types and crop rhizosphere on soil viral communities, Metagenomics analysis showed that organic management significantly reduced the alpha diversity of viral communities, specifically Shannon, Simpson and Sobs index. The distribution of viral community was relatively concentrated under the same management, and different management changed the composition of viral community. Organic management decreased the abundance of *Siphoviridae*, *Marseilleviridae* and *Baculoviridae*, and soil pH, AP and AK increased due to organic management, which also affected the viral community. Crop type also affected soil viral community, and it was found that the intensity of the influence was not as strong as that of management, and there is no significant effect of plant rhizosphere on soil viral community.

Effects of management mode on viral alpha diversity in soil

In this study, organic management reduced the abundance and variety of viruses, which are important for soil health and crop condition due to their adsorption to the soil and the effects on their host. And the predominant soil viruses detected were phages, implying that changes in bacterial communities profoundly influenced viral community dynamics. Phages, as bacterial viruses, play a pivotal role in shaping the composition and functionality of rhizosphere bacterial communities (Yang et al., 2023). Our previous study showed that the bacterial alpha diversity in organic soil was significantly higher than that in conventional soil (Zhou and Fong, 2021). Specifically, organic management was associated with decreased alpha diversity of soil viral communities. This observation aligns with prior studies demonstrating a negative correlation between bacterial diversity and viral abundance in soil, suggesting that lower bacterial diversity is linked to higher viral abundance (Liang et al., 2021), who found that bacterial diversity was significantly negatively correlated with viral abundance in soil in Tennessee, USA that lower bacterial diversity leads to higher viral abundance. Studies have found that cultivated soil with low bacterial diversity has high viral diversity (Muñoz-Arenas et al., 2020). In general, the alpha diversity of bacteria was higher than that of viruses. However, some studies have shown that the application of organic fertilizer significantly increased the richness and diversity of soil viruses (Nwokolo and Enebe, 2021). The different effects of organic fertilizer on viruses may be due to different fertilizer types and application time. Viruses may respond to the addition of different fertilizers by adjusting their survival strategies, such as cracking nitrogen-fixing bacteria to prevent excessive nitrogen accumulation (Li et al., 2019). The application of various chemical fertilizers in conventional soil may lead to massive viral replication to crack related bacteria, resulting in fewer bacteria and more viruses. It has been reported that bacteria isolated from animal feces can attack the capsid of hepatitis A virus and inactivate it (Deng and Cliver, 1995). In this study, organic fertilizer applied in organic management were made up of spent mushroom substrate, bean curd dregs and homemade compost, while in conventional management, synthetic fertilizers supplemented with cow manure were applied. Since the amount of animal feces used in organic management is larger than that in conventional management, it’s possible that the decrease in viral abundance may be caused by bacteria present in feces defending against phages (Georjon and Bernheim, 2023). Systems with low biodiversity of other groups may lead to the increase of viral diversity (Han et al., 2017), while biodiversity is negatively correlated with the intensity of pesticide application (Geiger et al., 2010).

The presence of pesticides in conventional management could contribute to lower bacterial and fungal diversity in the soil, thereby possibly resulting in higher viral diversity (Walder et al., 2022). It’s noteworthy that pesticide-contaminated soil often exhibits reduced bacterial diversity but higher viral diversity, highlighting the substantial influence of viral communities on bacterial ecology within soil microbial communities (Zheng et al., 2022). Our study further revealed that organic soil harbored higher microbial diversity, including Acidobacteria and Rokubacteria, likely facilitated by looser soil conditions conducive to the survival of aerobic bacteria. This increased presence of aerobic microorganisms may enhance the rate of virus inactivation (Hurst, 1988).

Effects of management mode on soil viral community composition

The viral community in this study mainly consisted of three orders and 18 families, most of which were double-stranded DNA viruses. There were significant differences in the relative proportion of viral families when comparing conventional and organic management; the families *Siphoviridae*, *Baculoviridae* and *Marseilleviridae* were found in higher proportions in conventional management than under organic management. We provide a discussion on all three of these families. Firstly, *Siphoviridae,* highly abundant in conventionally managed soils, are widely distributed in nature and have been identified as the most prevalent viral family in red soil during the corn heading period (Bi et al., 2020). *Siphoviridae* are known to parasitize rhizobia, possibly reducing their population in the soil. Therefore, the reduction of these bacteriophages under organic management may be advantageous for rhizobia populations, thereby benefiting plant growth (Msimbira et al., 2016). The family *Siphoviridae*, and one of its hosts Bacteria of the genus *Streptomyces* play significant roles in soil ecosystems (Nikulina et al., 2023). Previous research has identified *Siphoviridae* as a major viral family in marine and soil ecosystems, with variations in community structure and functional potential based on lifestyle and altitude (Zhu et al., 2023). The relative abundance of *Siphoviridae* has been linked to enhanced mutualistic symbiosis between soil phages and bacteria (Huang et al., 2021). The presence of *Siphoviridae* in unique soil habitats, such as the Kogelberg Biosphere Reserve fynbos soil, underscores their role in influencing plant diversity and productivity in terrestrial ecosystems (Segobola et al., 2018). Secondly, *Baculoviridae*, a family of DNA viruses, exclusively infect the larvae of certain insects like lepidoptera, hymenoptera, and diptera (Harrison et al., 2018). Baculoviruses are DNA viruses that play a crucial role in controlling insects and are utilized in the development of biological insecticides (Williams, 2023). These viruses have arthropod hosts (Szewczyk et al., 2006), and the higher detection frequency of *Baculoviridae* in conventional soil may indicate a greater abundance of these insects and, consequently, a higher viral load carried by them. Future research could explore insect diversity under different agricultural management practices to elucidate these patterns further. Thirdly, *Marseilleviridae,* belonging to the nuclear cytoplasmic large DNA viruses (NCLDVs), are giant viruses that infect amoeba (Sahmi-Bounsiar et al., 2021). They have been found in freshwater environments, diptera, and even humans (Aherfi et al., 2014). The higher detection frequency of *Marseilleviridae* in conventional soil suggests a potentially greater presence of dipteran insects, which aligns with the *Baculoviridae* findings. Members of this family infect exclusively larvae of insect orders Lepidoptera, Hymenoptera and Diptera, indicating a possible higher abundance of dipteran insects under conventional management. In summary, the differential abundance of these viral families between conventional and organic management systems suggests varying ecological dynamics influenced by agricultural practices. Further research into insect diversity and viral transmission pathways under different management regimes could provide valuable insights into these ecological interactions.

The following four viral families were significantly higher in organic soils than in conventional soils. Firstly, *Microviridae* are single-stranded DNA viruses, and their hosts are Proteobacteria, Chlamydia, Spiroplasma and so on (Brentlinger et al., 2002). Most of the hosts of *Microviridae* come from feces, such as Bacteroidetes, Proteobacteria and Tenericutes (Zhong et al., 2015), so it is frequently obtained from animal feces. Long-term application of cattle manure enriched gut microbiota and heterotrophic bacteria in farmland (Kim et al., 2021), thus there were more viruses of *Microviridae* in organic management soil. Proteobacteria is the dominant group of soil bacteria and plays an important role in maintaining the stability of soil ecology. Secondly, viruses of *Herpesviridae* family are double-stranded DNA viruses belonging to the phylum capsid virus and parasitize vertebrates (Gatherer et al., 2021), which might be present in animal feces. Thirdly, *Retroviridae* are RNA viruses that also have vertebrates as their hosts and are associated with a variety of animal diseases (Coffin et al., 2021). Fourthly, the *Phycodnaviridae* are double-stranded DNA viruses that parasitize algae and also belong to nuclear cytoplasmic large DNA viruses (NCLDVs) (Mönttinen et al., 2021). *Phycodnaviridae* only infects algae in seawater or fresh water and may have been introduced by water sources. Animal viruses in organic soils may be introduced by animal manure in fertilizers. Further studies may focus on the effects of fertilization on the distribution of soil viral communities and the specific causes of the emergence of some viral families.

In addition to the significant differences in viral family, there were also significant differences in beta diversity of viral communities. Clustering and PCoA analysis showed that different management modes had significant impact on the viral communities. The results of one-way analysis of variance, ANOSIM analysis (one-way ANOVA) and PERMANOVA analysis confirmed the significance of the results. RDA analysis showed that pH was the most important environmental factor affecting structure of viral community, and organic management changed soil pH. This study found that organic management brought soil pH closer to neutral and increased soil organic matter content. Although neutral pH is symbol of healthy soil (Wang et al., 2017), low pH means more positive charges in the soil, which can enhance the adsorption of viruses, and high content of organic matter in soil may hinder the adsorption of viruses (Yoshimoto et al., 2012). Fewer soil viruses mean less damage to crops, healthier crops and higher yields. Organic management can keep crops healthier, thus affecting rhizosphere microorganisms such as bacteria and fungi, which further affect viruses (Windisch et al., 2021). Phosphorus is necessary for plant growth and development, and soil available phosphorus is the main phosphorus source for plant growth (Liang et al., 2022). Potassium is also an essential macronutrient for plant growth. The content of available potassium is low in the soil and easy to be absorbed by plants (Geisseler and Miyao, 2016). Luo et al. (2016) found that the bacterial community dynamics in paddy soils were significantly correlated with total carbon, moisture, available potassium, and pH. A healthy and stable pH is important for both plant and soil health, which is an important part of sustainable agriculture, and soil microbial communities maintain soil health by improving soil fertility and productivity (Monther et al., 2020). As the host of bacteriophages, bacteria play an important role in the community structure of soil viruses, so they are closely linked and play essential roles in soil fertility and plant growth. Fertilizers are important in shaping microbial communities and soil properties. Understanding the interactions between these factors is crucial for sustainable land management practices and ecosystem health.

Effects of crop types and rhizosphere on soil viral communities

Crops also exerted discernible effects on viral communities, albeit not as pronounced as those of soil management practices. Organic cabbage soil exhibited a higher pH compared to lettuce soil and approached neutrality. Research has shown that the stress resistance of Chinese cabbage, belonging to the cruciferous family like cabbage, significantly improves after the application of organic fertilizer (Shi et al., 2021). Under organic management, there was no significant difference in the alpha diversity of soil viruses between the two crop species. However, in conventional soil, the viral richness of lettuce soil was notably higher than that of cabbage soil. The presence of *Caulimoviridae*, a family of viruses that infect plants (Teycheney et al., 2020), was exclusively detected in the rhizosphere soil of lettuce Larger land cover measurements related to plant presence were positively correlated with viral abundance, suggesting that the presence of crops and crop residues may aid in viral survival by mitigating soil stress, such as high year-round soil temperatures (Cornell et al., 2021). The use of biochar in combination with *Podoviridae* could reduce the number of pathogenic bacteria in the soil where lettuce is grown and is conducive to the recovery of microbial communities (Ye et al., 2018). *Myoviridae*, *Siphoviridae* and *Podoviridae* could parasitise vegetable soft rot pathogen, which was isolated from the soil of Korean cabbage growing area (Jee et al., 2012). The microbial communities in the rhizosphere and bulk soils of Chinese cabbage plantations have been studied in a Karst region, demonstrating the importance of understanding the interactions between plants and soil microbes in agricultural settings (Wei et al., 2023). Additionally, the succession of soil bacterial communities in response to plant viruses such as tobacco mosaic virus highlights the complex interactions between plants, microbes, and viruses in agricultural ecosystems (Zhao et al., 2024). Further research is needed to elucidate the role of viral communities in lettuce and cabbage soil and their impact on agricultural productivity. Cluster tree analysis of multiple samples at the species level revealed that the viral community in each sample could be categorized into two groups based on crop type, although this effect was less pronounced than that of management mode. The influence of the rhizosphere on viral communities was less evident, possibly because some viruses are more affected by rhizosphere litter than by growing roots (Starr et al., 2019). Additionally, the selection of rhizosphere bacteria by root exudates may render them less susceptible to viral infection (Pratama and van Elsas, 2018). Further research is needed to confirm these findings, as it has been observed that although the number of bacteria in wheat rhizosphere soil is substantial, the number of rhizosphere bacteriophages remains unchanged, suggesting that the number of rhizosphere viruses does not necessarily reflect the rhizosphere effect (Swanson et al., 2009).

Conclusions

In this study, we conducted a comprehensive analysis of three factors potentially influencing the viral community in soil in Hong Kong, China: agricultural management, crop type, and the rhizosphere effect. Our findings revealed that agricultural management had the most substantial impact on viral communities, followed by crop type, while the influence of the rhizosphere was relatively minor. Organic management emerged as a significant factor in altering the structure of the viral community in soil, leading to reduced diversity and a lower relative abundance of pathogenic viruses, thereby promoting plant health. Moreover, organic management resulted in increased soil pH and organic matter content, contributing to overall soil health improvement. Significant disparities in viral communities were observed under different agricultural management practices, and crop type also exerted a notable effect on the structure of soil viral communities. Soil pH emerged as the primary environmental factor driving differences in viral communities. Agricultural management can directly or indirectly affect viral communities, which can affect soil, plants, microbes and further affect soil health. These findings provide valuable insights that can aid in predicting the rates of biogeochemical processes in farmland across various scales, from the field level to landscape and ecosystem scales. Because this study aimed to investigate effects in a non-experimental agroecosystem, we had access to a limited sample and collected area, which may have affected the generalizability of this work. And the limited technology may underestimate the diversity of soil viruses. Further research is needed to elucidate the specific mechanisms by which viruses influence soil-plant-microbe and to fill existing knowledge gaps in soil viral ecology.

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Data availability statement. The data presented in this study are available in the article. The sequences obtained have been submitted to the NCBI SRA database under the accession number PRJNA852672.

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