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3 **Soil pH influences the structure of virus communities at local and global scales**

4

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27 **Abstract**

28 The influence of biotic and abiotic factors on viral communities across environmental  
29 gradients in soil is relatively unknown. While soil pH strongly influences microbial  
30 community structure, it is unclear whether there is a similar influence on soil viruses.  
31 In this study, prokaryotic and viral communities were characterized in soils from a long-  
32 term pH-manipulated soil gradient (pH 4.5 and 7.5), and viral populations also  
33 compared to those of other soils ranging in pH (4.0-7.5). Viral communities were  
34 significantly influenced by pH at the local scale with 99% of viral operational taxonomic  
35 units restricted to pH 4.5 or 7.5 soil only. Analysis of viromes from six other European  
36 and North American soil systems demonstrated that a selection of viral clusters from  
37 acidic and neutral pH soils were more associated with those from the local gradient  
38 pH 4.5 or 7.5 soils, respectively. While direct pH effects on virion integrity and indirect  
39 selection via host composition were not distinguished, the results reveal that soil pH  
40 is a factor in structuring viral communities at local and global scales.

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42 key words: viruses; prokaryotes; community structure; pH

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52 **Main**

53 Viruses play a major role in controlling the abundance, structure and evolution of  
54 microbial communities through cell lysis and the release of nutrients and modulation  
55 of host cell metabolism (Pratama et al., 2018). In soils, viruses are diverse (Emerson  
56 et al., 2018), abundant (Williamson et al., 2017), and have potential to impact nutrient  
57 cycling and other ecosystem functions (Emerson et al., 2018; Kuzyakov and Mason-  
58 Jones, 2018; Trubl et al., 2018). However, fundamental knowledge gaps in soil viral  
59 ecology include a basic understanding of the biotic and abiotic drivers of viral  
60 communities.

61 Soil bacterial and viral community dynamics co-vary, with the susceptibility of  
62 hosts to infection from individual viruses varying over time (Vos et al., 2009) or viral  
63 community shifts occurring as an indirect result of nutrient input altering host  
64 community structure (Srinivasiah et al., 2015). While host communities are likely the  
65 strongest factor for defining viral community structures, the host range of viruses may  
66 also play a role in defining whether their community dynamics vary to the same extent  
67 over physicochemical gradients (de Jonge et al., 2019). Closely related host  
68 populations may be adapted to growth under different conditions, resulting in niche  
69 differentiation and contrasting distribution across an ecological gradient. However, it  
70 is unclear whether narrow or broad host ranges of their associated viruses reduce the  
71 relative variation in virus community structure compared to prokaryotes. In addition,  
72 changes in soil physicochemical characteristics may also directly impact the physical  
73 integrity and dispersal of viruses (Trubl et al., 2016). As soil pH is a major determinant  
74 of prokaryotic community composition at local and global scales (e.g. Bahram et al.,  
75 2018; Bartram et al., 2014; Griffiths et al., 2011; Lammel et al., 2018; Lauber et al.,

76 2009), it is possible that virus communities may also exhibit pH-influenced community  
77 structures.

78 Soil samples were taken from the extreme ends of an extensively characterized  
79 contiguous soil pH gradient (pH 4.5 to 7.5) where total carbon, nitrogen and organic  
80 matter do not change significantly across the gradient (Nicol et al., 2008; Bartram et  
81 al., 2014). At this site, prokaryotic communities have been shown to vary in response  
82 to pH (Nicol et al., 2008; Bartram et al., 2014), and distinct virus populations infecting  
83 methylophilic communities associated with contrasting soil pH have recently been  
84 observed (Lee et al., 2021). Both non-targeted total community metagenome and  
85 virus-targeted virome libraries were prepared as previously described (Lee et al.,  
86 2021), and the assembled predicted viral contigs obtained were clustered into 1,910  
87 viral operational taxonomic units (vOTUs) (Table S1), and prokaryotic 16S rRNA gene  
88 fragments were extracted and classified into 2,312 OTUs (Supplementary Methods).  
89 Sequencing depth was sufficient to capture vOTU richness, although further  
90 sequencing and sampling may have increased 16S rRNA OTU recovery (Fig. S1).

91 Of the metagenomic reads, 22.7% were taxonomically defined, with  
92 Actinobacteria and Proteobacteria dominating in both pH soils (Fig. 1a). Similar to  
93 other soil viral studies, only a small proportion of the prokaryotic viral community (7.0%  
94 of vOTUs) was taxonomically defined. A total of 29.1% of vOTUs were linked to  
95 predicted hosts, with the majority for both pH soils belonging to Actinobacteria and  
96 Proteobacteria (Fig. 1a, Table S2).

97 Analysis of individual 16S rRNA OTUs and vOTUs demonstrated distinct  
98 structures between pH 4.5 and 7.5 soils for both prokaryote and viral communities  
99 (Fig. 1b). Specifically, 38.6% of OTUs (pH 4.5 OTUs, 263; pH 7.5 OTUs, 630) and  
100 99.0% of the vOTUs (pH 4.5 vOTUs, 524; pH 7.5 vOTUs, 1,361) were found in only

101 one soil pH. Prokaryote and viral (virome) alpha-diversity were significantly greater in  
102 pH 7.5 soil (Table S3). Although absorption of viruses to soil organic particles can  
103 decrease with increasing pH (Sobsey et al., 1980), the effect of soil pH biasing  
104 measurements of alpha-diversity may be small as organic matter content does not  
105 change significantly across the gradient and a neutral pH buffer was used for  
106 extracting viral particles from both soils, with no significant difference in the yields of  
107 virome DNA.

108         While viromes produced 73x more assembled viral contigs than metagenomes,  
109 read mapping of individual reads to all vOTUs demonstrated that both approaches  
110 produced distinct viral community profiles between soils (Fig. 1b). Decreasing the  
111 breadth (length of contig covered by mapped reads) threshold (<75%) for defining  
112 vOTU detection disproportionately increased vOTU detection in metagenomes  
113 compared to viromes (Fig. S2). This suggests that the appropriate breadth thresholds  
114 for detection may be different in viromes compared to total metagenomes, with a cut-  
115 off  $\geq 75\%$  potentially too conservative for the total metagenomes. However, care  
116 should be taken before reducing breadth threshold in other datasets that do not have  
117 paired viromes to corroborate viral detection.

118         Soil viromes from the gradient soils were compared with those from six other  
119 ecosystems varying in pH, soil type, land use and location and where viral contigs  
120 were predicted using the same tools and standards (Table S4) (Emerson et al., 2018;  
121 ter Horst et al., 2021; Santos-Medellin et al., 2021). Using gene-sharing network  
122 analysis (Jang et al., 2019), the number of clusters containing vOTUs from these  
123 additional soils and the local gradient (pH 4.5 only, pH 7.5 only or both) were  
124 determined (Fig. 2, Table S5). On average, 31% of clusters were shared (range 1-  
125 62%) in pairwise comparisons between all soils (Fig. S3). A clear trend was also

126 observed in comparison with the gradient soils (Fig. 2b) with shared viral clusters in  
127 acidic and neutral pH soils more associated with those from the local gradient pH 4.5  
128 or 7.5 soils, respectively. While this demonstrates that certain viral genes were  
129 restricted to acidic vs neutral pH soils, no candidate genes were identified as  
130 potentially conferring host adaptation to a particular pH range, with the majority  
131 encoding uncharacterized hypothetical proteins. In addition, the relative proportion of  
132 virus-encoded putative auxiliary metabolic genes for different metabolic functions was  
133 similar between soils with no trends associated with pH (Fig. S4).

134         If individual viruses can infect multiple host populations at different soil pH,  
135 there would be a potential for virus community structures to be less distinct over an  
136 ecological gradient. However, analysis of samples from a continuous pH gradient  
137 demonstrated that contrasting soil pH results in the selection for virus community  
138 structures that are at least as distinct as prokaryote host community structures. While  
139 a relatively limited number of sites for analysis of viromes in different soil systems and  
140 that these analyses do not separate direct effects of pH on virion integrity from indirect  
141 effects of host composition, they demonstrate that, as with prokaryote communities,  
142 soil pH correlates with distinct patterns of virus community structures.

143

#### 144 **Data availability**

145 Metagenome sequence reads are deposited in NCBI's GenBank under BioProject  
146 accession nos. PRJNA621436–PRJNA621447. Metagenome draft assemblies are  
147 accessible through the JGI Genome Portal (DOI: 10.25585/ 1487501). Assembled  
148 metagenome-derived 16S rRNA gene sequences are available at [ftp://ftp-adn.ec-](ftp://ftp-adn.ec-lyon.fr/)  
149 [lyon.fr/](ftp://ftp-adn.ec-lyon.fr/). Metagenome sequence reads from 'Agriculture 2' site are available through  
150 NCBI BioProject PRJNA767554.

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166 processing the 'Agriculture 2' site samples.

167

168 **Competing Interests**

169 The authors declare no competing interests.

170

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273 **Figure Legends**

274 **Fig. 1:** Taxonomic composition and community structure of prokaryotic and virus  
275 communities in pH 4.5 and 7.5 replicate soil samples taken from the ends of a  
276 contiguous pH gradient. a) Relative abundance of taxonomically-defined prokaryotes,  
277 taxonomically-defined viruses and the predicted hosts of viruses. For prokaryotes,  
278 reads from metagenomes were annotated at the phylum level using the NCBI nr  
279 database. Numbers in parenthesis denote the number of mapped reads:total reads  
280 analyzed. Viral contigs  $\geq 10$  kb were taxonomically defined at the family level based on  
281 gene-sharing network analysis (Jang et al., 2019). Host prediction of viruses was  
282 determined by using the gene-sharing network, and gene homology analysis (Al-  
283 Shayeb et al., 2020). Numbers in parenthesis denote the number of annotated:total  
284 reads or contigs for each sample, and plots display the relative proportion of annotated  
285 reads only (i.e. annotated reads of prokaryotes or reads mapped to annotated viral  
286 contigs). b) Normalized relative abundance of individual 16S rRNA OTUs and vOTUs  
287 in soil samples determined by read-mapping. Only vOTUs where reads were mapped  
288 with  $\geq 1x$  coverage over 75% contig breadth were included. Ordinations show the  
289 principal coordinate analysis of Bray-Curtis dissimilarities derived from relative  
290 abundance tables. For virus communities, reads from both viromes (V) and  
291 metagenomes (M) were analyzed for each sample. Details of all methods used are  
292 provided in Supplementary Information.

293

294 **Fig. 2:** Network analysis describing linkages of Gradient 4.5 and 7.5 vOTUs with six  
295 sets of soils samples ranging in pH from 4.0 to 7.5 from Europe and North America  
296 (Table S4). a) Gene sharing network of vOTUs showing viral clusters containing  $\geq 25$   
297 vOTUs. b) Relative abundance of clusters from each soil that contain vOTUs shared

298 with those from gradient pH 4.5 (blue) and gradient pH 7.5 soil (red) or both (grey).  
299 Numbers in parenthesis denote total number and percentage of shared viral clusters  
300 with Gradient soils. Details of all methods used are provided in Supplementary  
301 Information.

Figure 1

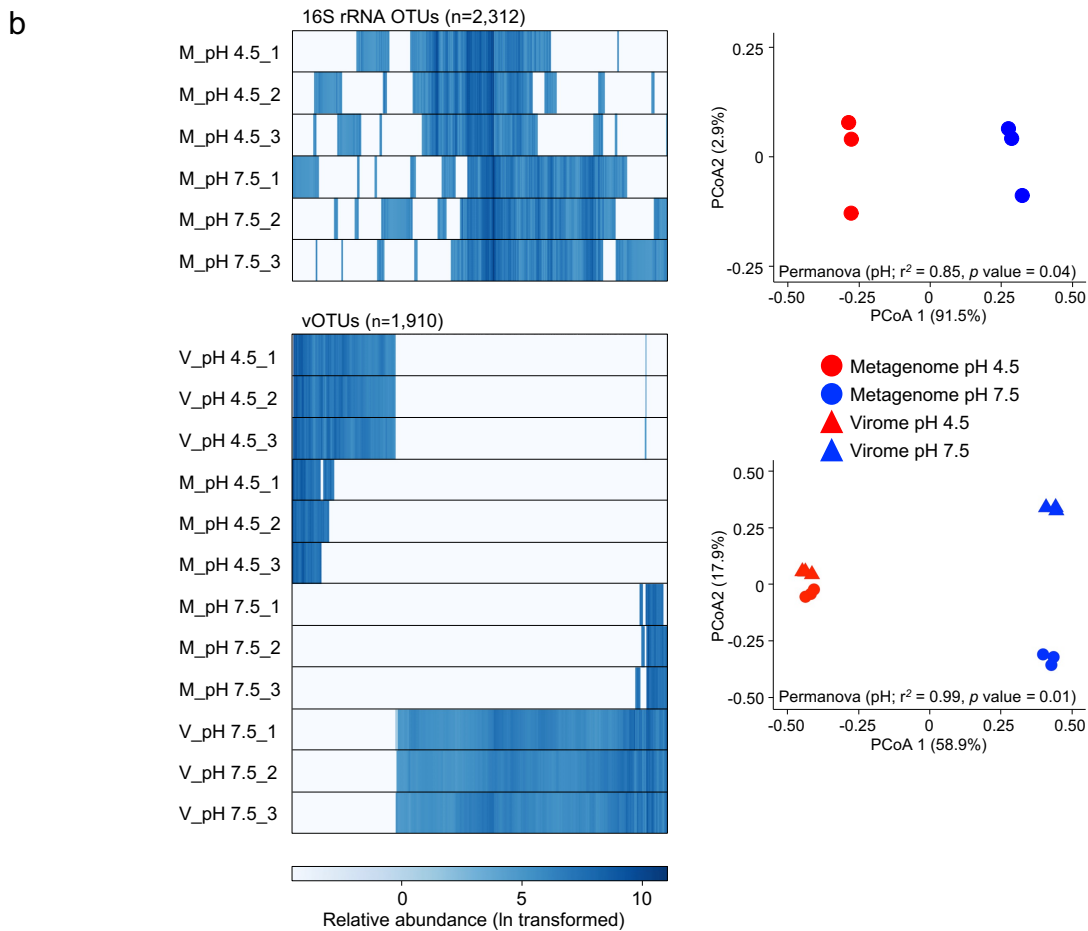
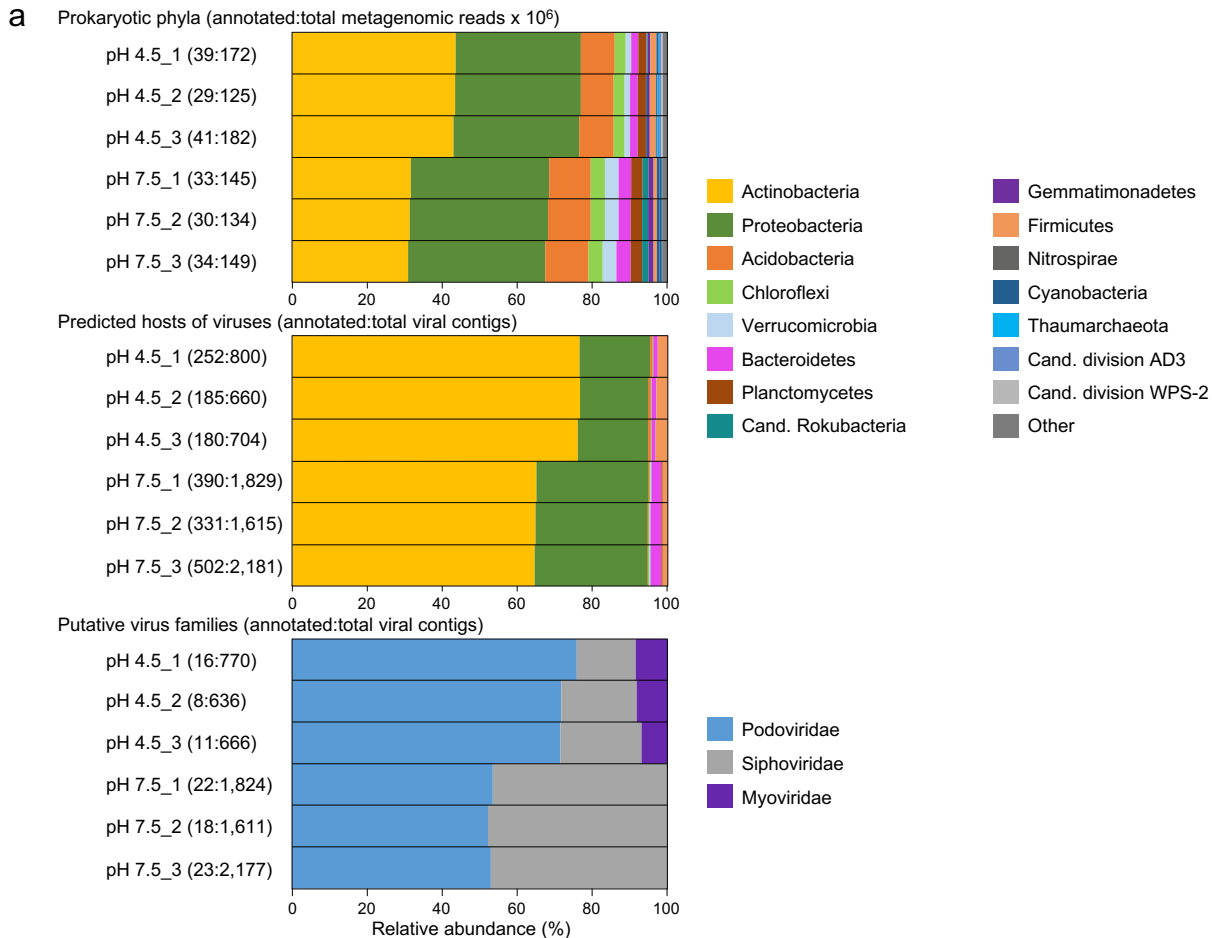


Figure 2

