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

Sungeun Lee[1], Jackson W. Sorensen[2], Robin L. Walker[3], Joanne B. Emerson[2], Graeme W. Nicol[1], Christina Hazard[1]

1. Environmental Microbial Genomics Group, Laboratoire Ampère, École Centrale de Lyon, CNRS UMR 5005, Université de Lyon, Écully, 69134, France

2. Department of Plant Pathology, University of California, Davis, One Shields Avenue, Davis, 95616, California, USA

3. School of Rural Land Use, Scotland's Rural College, Craibstone Estate, Aberdeen, AB21 9YA, Scotland, United Kingdom

Corresponding author

Email: christina.hazard@ec-lyon.fr

Tel: +33 4 72 18 60 91

Abstract

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

key words: viruses; prokaryotes; community structure; pH

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 methylotrophic 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 ≥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-
149 lyon.fr/. Metagenome sequence reads from 'Agriculture 2' site are available through
150 NCBI BioProject PRJNA767554.

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Competing Interests

The authors declare no competing interests.

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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 ≥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 ≥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 ≥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

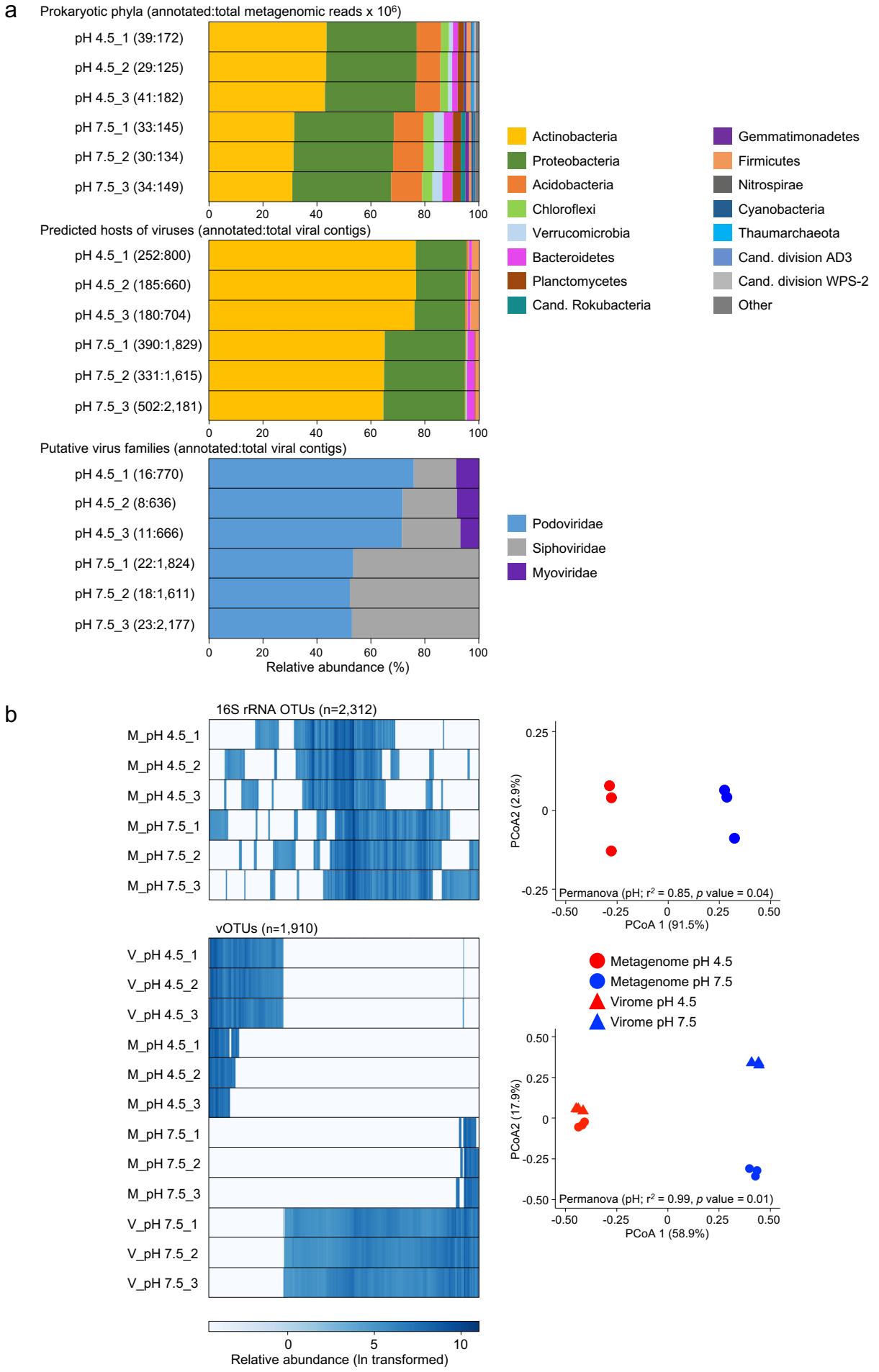
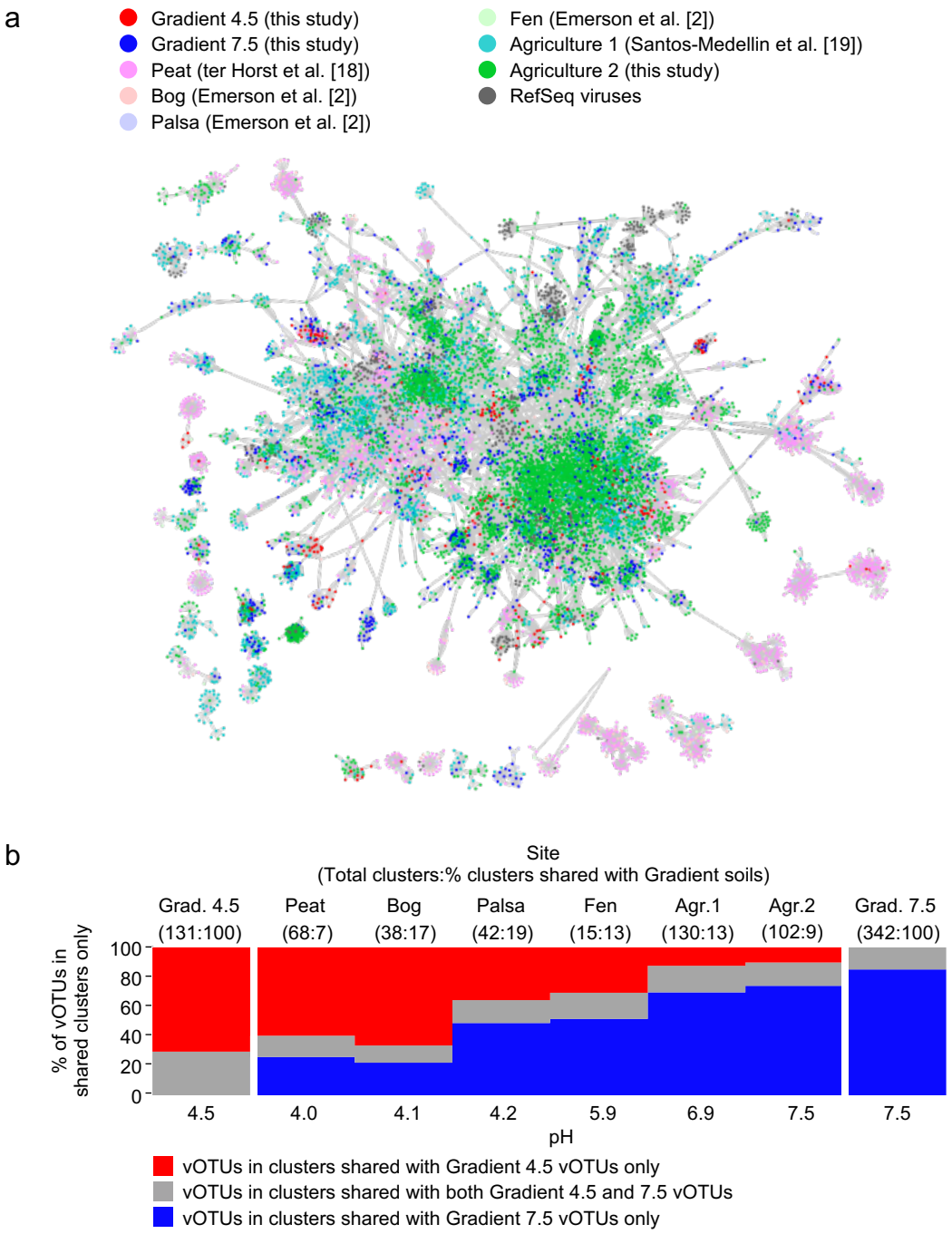

a
Prokaryotic phyla (annotated:total metagenomic reads x 10^6)
pH 4.5_1 (39:172)
pH 4.5_2 (29:125)
pH 4.5_3 (41:182)
pH 7.5_1 (33:145)
pH 7.5_2 (30:134)
pH 7.5_3 (34:149)
0 20 40 60 80 100
Predicted hosts of viruses (annotated:total viral contigs)
pH 4.5_1 (252:800)
pH 4.5_2 (185:660)
pH 4.5_3 (180:704)
pH 7.5_1 (390:1,829)
pH 7.5_2 (331:1,615)
pH 7.5_3 (502:2,181)
0 20 40 60 80 100
Putative virus families (annotated:total viral contigs)
pH 4.5_1 (16:770)
pH 4.5_2 (8:636)
pH 4.5_3 (11:666)
pH 7.5_1 (22:1,824)
pH 7.5_2 (18:1,611)
pH 7.5_3 (23:2,177)
0 20 40 60 80 100
Relative abundance (%)
Actinobacteria
Proteobacteria
Acidobacteria
Chloroflexi
Verrucomicrobia
Bacteroidetes
Planctomycetes
Cand. Rokubacteria
Gemmatimonadetes
Firmicutes
Nitrospirae
Cyanobacteria
Thaumarchaeota
Cand. division AD3
Cand. division WPS-2
Other
Podoviridae
Siphoviridae
Myoviridae
b
16S rRNA OTUs (n=2,312)
M_pH 4.5_1
M_pH 4.5_2
M_pH 4.5_3
M_pH 7.5_1
M_pH 7.5_2
M_pH 7.5_3
vOTUs (n=1,910)
V_pH 4.5_1
V_pH 4.5_2
V_pH 4.5_3
M_pH 4.5_1
M_pH 4.5_2
M_pH 4.5_3
M_pH 7.5_1
M_pH 7.5_2
M_pH 7.5_3
V_pH 7.5_1
V_pH 7.5_2
V_pH 7.5_3
0 5 10
Relative abundance (ln transformed)
PCoA2 (2.9%)
0.25
0
-0.25
Permanova (pH; r² = 0.85, p value = 0.04)
-0.50 -0.25 0 0.25 0.50
PCoA 1 (91.5%)
Metagenome pH 4.5
Metagenome pH 7.5
Virome pH 4.5
Virome pH 7.5
PCoA2 (17.9%)
0.50
0.25
0
-0.25
-0.50
Permanova (pH; r² = 0.99, p value = 0.01)
-0.50 -0.25 0 0.25 0.50
PCoA 1 (58.9%)

Figure 2


a
Gradient 4.5 (this study)
Gradient 7.5 (this study)
Peat (ter Horst et al. [18])
Bog (Emerson et al. [2])
Palsa (Emerson et al. [2])
Fen (Emerson et al. [2])
Agriculture 1 (Santos-Medellin et al. [19])
Agriculture 2 (this study)
RefSeq viruses
b
Site
(Total clusters:% clusters shared with Gradient soils)
Grad. 4.5 (131:100)
Peat (68:7)
Bog (38:17)
Palsa (42:19)
Fen (15:13)
Agr.1 (130:13)
Agr.2 (102:9)
Grad. 7.5 (342:100)
% of vOTUs in shared clusters only
100
80
60
40
20
0
4.5
4.0
4.1
4.2
5.9
6.9
7.5
7.5
pH
vOTUs in clusters shared with Gradient 4.5 vOTUs only
vOTUs in clusters shared with both Gradient 4.5 and 7.5 vOTUs
vOTUs in clusters shared with Gradient 7.5 vOTUs only