Received: 3 December 2021 | Revised: 24 August 2022 | Accepted: 13 October 2022

DOI: 10.1111/mec.16756

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Metatranscriptomics reveals contrasting effects of elevation on the activity of bacteria and bacterial viruses in soil

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Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: NE 1863/2-1; Funding information
Hessen State Ministry of Higher Education, Research and the Arts (HMWK)

Handling Editor: Henrik Krehenwinkel

Abstract

Soil microbial diversity affects ecosystem functioning and global biogeochemical cycles. Soil bacterial communities catalyse a diversity of biogeochemical reactions and have thus sparked considerable scientific interest. One driver of bacterial community dynamics in natural ecosystems has so far been largely neglected: the predator–prey interactions between bacterial viruses (bacteriophages) and bacteria. To generate ground level knowledge on environmental drivers of these particular predator–prey dynamics, we propose an activity-based ecological framework to simultaneous capture community dynamics of bacteria and bacteriophages in soils. An ecological framework and specifically the analyses of community dynamics across latitudinal and elevational gradients have been widely used in ecology to understand community-wide responses of innumerable taxa to environmental change, in particular to climate. Here, we tested the hypothesis that the activity of bacteria and bacteriophages co-declines across an elevational gradient. We used metatranscriptomics to investigate bacterial and bacteriophage activity patterns at five sites across 400 elevational metres in the Swiss Alps in 2015 and 2017. We found that metabolic activity (transcription levels) of bacteria declined significantly with increasing elevation, but activity of bacteriophages did not. We showed that bacteriophages are consistently active in soil along the entire gradient, making bacteriophage activity patterns divergent from that of their putative bacterial prey. Future efforts will be necessary to link the environment-activity relationship to predator–prey dynamics, and to understand the magnitude of viral contributions to carbon, nitrogen and phosphorus cycling when infection causes bacterial cell death, a process that may represent an overlooked component of soil biogeochemical cycles.

KEYWORDS
altitudinal gradient, bacteriophage, *Caudovirales*, ecosystem functioning, environmental change, microbial interactions, predator–prey dynamics

1 | INTRODUCTION

Soil microbiomes are key for ecosystem functioning and play pivotal roles in global biogeochemical cycles (i.e., C and N cycling) (Braga et al., 2020; Pratama & van Elsas, 2018). The soil microbiome harbours groups of highly diverse organisms, such as bacteria, archaea, fungi and protozoa, including viruses that infect them (Kimura et al., 2008; Pratama & van Elsas, 2018). Bacteria and archaea catalyse a diversity of biogeochemical reactions, many of which are climate relevant (Hallin & Bodelier, 2020; Monteux et al., 2020). Therefore, bacterial and archaeal functional assessment has sparked considerable interest (Hallin & Bodelier, 2020; Kimura et al., 2008; Pratama & van Elsas, 2018). Soil bacteria are increasingly considered in studies, but their respective viruses are readily neglected, resulting in a lack of understanding of how the interaction of bacteria with their viruses influences soil functioning (Ashelford et al., 2003; Kimura et al., 2008; Marsh & Wellington, 1994; Pratama & van Elsas, 2018). Viruses may impact soil communities by (1) controlling microorganismal population dynamics as predators (Breitbart et al., 2018; Morella et al., 2018), and (2) providing genes and functions, which may alter ecosystem properties, for example, carbon and nitrogen cycling (Emerson et al., 2018; Pratama & van Elsas, 2018; Trubl et al., 2018).

Bacteriophages, or short "phages", are viruses that prey on bacteria (Adams, 1959). From the point of view of trophic interactions, the relationship between bacteria and their viruses can be regarded as that of a prey and its predator (Chao et al., 1977; Weinbauer, 2004; Weitz & Dushoff, 2008). Bacterial viruses may affect biogeochemical cycles in soil, when phage mediated lysis of bacterial cells (1) leads to mobilization of bacterial cell carbon that may stimulate heterotrophic consumption leading to carbon loss to the atmosphere as CO_2 and (2) targets bacteria contributing to nitrogen and phosphorus cycling (Emerson et al., 2018; Roux & Emerson, 2022). The full extent of bacteria-phage interactions across different environments, including soil, is poorly understood (Braga et al., 2020; Emerson et al., 2018; Roux et al., 2021; Starr et al., 2019).

The main factor limiting phage occurrence is the presence of bacterial hosts (Olszak et al., 2017; Weitz & Dushoff, 2008). All ecosystems with metabolically active bacterial populations are expected to have abundant and diverse phage populations (Marsh & Wellington, 1994). Recent studies suggest that soil viral communities may not disperse well between bacterial populations, given that viruses are rarely shared between samples (Durham et al., 2022; Santos-Medellín et al., 2022). However, it has also been hypothesized that viruses within soils can cross the distances between bacterial populations (1) via hydrologic conduits and (2) by "leapfrogging" (i.e., a spread of viruses by chain reactions of host population infections over space) (Roux & Emerson, 2022; Santos-Medellín et al., 2022). Accordingly, previous studies using metatranscriptomics found active and diverse phage communities when bacteria were active in soils (Emerson et al., 2018; Starr et al., 2019).

The integration of an ecological framework, such as activity analyses across gradients, into bacteria-virus interaction studies can address questions which have been mostly neglected in virology, but are key to advance our understanding on ecosystem consequences of predator–prey dynamics (Sommers et al., 2021). Numerous studies report changes in bacterial metabolic activity across latitudinal and elevational gradients (Chase et al., 2021; Margesin et al., 2009; Ren et al., 2021; Rivkina et al., 2000; Schinner, 1982). Across these gradients, the metabolic activity of bacteria has been linked to climatic factors, such as mean annual temperature, precipitation, as well as soil properties (bulk density, ammonium nitrogen, and total phosphorus) (Margesin et al., 2009; Ren et al., 2021; Rivkina et al., 2000; Schinner, 1982). Given these changes in bacterial metabolic activity, one would expect changes in phage activity, with a potential impact on bacterial populations (Marsh & Wellington, 1994). Thereby a replicated elevational gradient setup is suitable to generate ground level knowledge on these particular predator–prey interactions and to assess their community-wide responses to environmental change, in particular to climate. However, no study to date has investigated the activity of bacteria and their phage predators across an elevational gradient.

The aim of this study was to establish a baseline approach to simultaneously assess the activity of bacteria and bacteriophages in soil, and investigate potential drivers. To understand community-wide responses to environmental change, in particular to climate, we utilized gradient analyses from an ecological framework (Merges et al., 2021; Sommers et al., 2021) to assess the population-wide activities of bacteria and phage communities across an elevational gradient in the European Alps. Elevational gradients allow the study of broad environmental conditions on a condensed geographic scale (Bergner et al., 2020; Neuschulz et al., 2018). We hypothesized that environmental conditions (such as temperature, soil moisture and soil pH) at high elevations lead to a reduced metabolic activity of bacteria (as in Margesin et al., 2009; Ren et al., 2021; Schinner, 1982) and since phages require metabolically active hosts to support multiplication (Marsh & Wellington, 1994), we hypothesized similar levels of activity in bacteria and phages in a given environment.

2 | MATERIALS AND METHODS

2.1 | Study site and sampling

The study sites were located in the Central Alps in the eastern part of Switzerland in the Sertig valley (46°44′0.76′′N, 9°51′3.5′′E) near Davos (Merges et al., 2018; Neuschulz et al., 2018). The sampling is part of a larger experiment spread along an elevation gradient at nine equidistant elevational levels between 1850 and 2250 m (Merges, Albrecht, et al., 2020; Merges, Bálint, et al., 2020). We measured and recorded soil surface temperatures at each of the nine sites with a total of 54 data loggers (Maxim iButton) every 4 h from May 2015 until September 2017. We calculated the mean temperature of the warmest 3 months (June–August) and the mean of the coldest 3 months (December–February) for each logger at each elevation. We measured soil pH of each soil sample in a 1 M solution of potassium chloride (KCl) with a pH/conductivity metre CPC-401

(Elmetron). Soil moisture measurements were taken each year in September under dry weather conditions by averaging five tensiometre (Theta-Kit version 3) measurements, sampled within 5 cm of each temperature logger per plot.

For assessment of soil bacterial and phage communities, we selected samples from five elevational levels at 1850, 1900, 2000, 2100 and 2250 m a.s.l (Table S1). We conducted two sampling rounds, May 2015, and May 2017, resulting in a total of 10 soil samples (Table S1). Soil samples were taken with a 1 cm soil core sampler (Ehlert & Partner). For each soil sample, we took five 5-cm deep soil cores from a $15 \times 15\,cm^2$ area that we pooled and homogenized in a Ziploc bag (Merges et al., 2018). Then, 10 g of homogenized soil was immediately transferred into a 50 ml Falcon filled with RNA preservative (LifeGuard Soil Preservation Solution, Qiagen). The preserved soil was frozen at −80°C when brought to the laboratory.

2.2 | Laboratory and bioinformatics

RNA was extracted with RNeasy PowerSoil Total RNA Kit (Qiagen) and sequenced to 8 GB depth per sample at Novogene Co., Ltd. Novogene performed the library preparation using NEB Next Ultra RNA Library Prep Kit for Illumina and sequencing on an Illumina NovaSeq6000 with 150 bp paired-end. We received consistently high yields, with a total of 30–40 million reads per sample (NCBI BioProject ID PRJNA849939). Sequences were quality filtered and trimmed of adapters using TRIMMOMATIC (Bolger et al., 2014). Trimming parameters were set to (1) cut adapters and other Illumina-specific sequences from the read (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10), (2) remove low quality bases off the start of a read (LEADING:3), (3) remove low quality bases off the end of a read (TRAILING:3), (4) perform a sliding window trimming, cutting once the average quality within the window falls below a threshold (i.e., 15 phred quality scores), by scanning the read with a 4-base wide sliding window (SLIDINGWINDOW:4:15), (5) drop reads below 36 bases long (MINLEN:36). We assessed the quality of reads with fastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

2.2.1 | Assembly and annotation of bacterial contigs

Trimmed reads were assembled with TRINITY (Grabherr et al., 2013). Assembled contigs were taxonomically binned using the lowest common ancestor (LCA) algorithm of DIAMOND with the NCBI nr protein database and bacterial contigs were selected (Buchfink et al., 2014). Activity was assessed by mapping back raw reads to individual taxa bins using SALMON/DESEQ2 (see below, Patro et al., 2017). PROKKA was used for functional annotation (Seemann, 2014). Bacterial contigs were functionally annotated with PROKKA (Seemann, 2014) and with the DRAM pipeline (Shaffer et al., 2020) which implements annotations from Kyoto Encyclopedia of Genes and Genomes (KEGG), UniRef90 and PFAM databases.

2.2.2 | Assembly and annotation of viral contigs

Trimmed reads were assembled with rnaviralSPAdes (Lapidus & Korobeynikov, 2021; Nurk et al., 2017). Assembled contigs were screened for viral origin using VIRSORTER2 version 2.1 (Guo et al., 2021) and VIBRANT version 1.2 (Kieft et al., 2020). Putative viral contigs were further quality controlled using CHECKV version 0.7.0 (Nayfach et al., 2020). Passing contigs were taxonomically binned using the lowest common ancestor (LCA) algorithm of DIAMOND version 0.9.21.122 with the NCBI nr protein database (Buchfink et al., 2014), KAIJU version 1.7.3 (Menzel et al., 2016) and VCONTACT2 version 0.11.3 (Jang et al., 2019). We used VCONTACT2 to build gene-sharing networks to generate viral clusters at genus level and assign those taxonomically against "ProkaryoticViralRefSeq201-Merged" reference database via DIAMOND version 0.9.21.122 and ClusterONE (vcontact2 --rel-mode Diamond --pcs-mode MCL --vcs- mode ClusterONE) (Liu et al., 2022). Viral clusters identified by VCONTACT2 did not result in clustering between the retrieved viral contigs and the viral sequences from the viral reference database. Thus, to gain an overview of viral families present in the soil metatranscriptomes, we used the DIAMOND and KAIJU assignments of viral taxonomy. The produced data set of viral contigs was subset to taxa exclusively associated with bacteria (i.e., bacteriophages). Viral contigs were functionally annotated in VIBRANT version 1.2 and DRAM-V version 1.3 (Kieft et al., 2020; Shaffer et al., 2020). We followed the default contig length cutoff as implemented in VIBRANT (i.e., 1 kb), since it has been shown to increase viral identification across data sets, without affecting false discovery rate given the minimum open reading frame requirement of 4 (Kieft et al., 2020). Further, VIBRANT version 1.2 was used for identification of lysogenic phages. VIBRANT determines whether a viral sequence was excised from a host sequence or if it encodes an integrase, and classifies these as lysogenic phages (Kieft et al., 2020).

2.2.3 | Activity/transcript expression analyses

For transcript abundance quantification (i.e., estimated counts per transcript) we used SALMON version 0.14.1 with the --gcBias flag (Patro et al., 2017). The --gcBias flag integrates the estimation of a correction factor for systematic biases frequently present in RNA-seq data (Patro et al., 2017). SALMON was run in mapping-based mode with the following mapping parameters: (1) validate mappings to ensure that these may produce reasonable alignments before further use for quantification (--validateMappings) and (2) --libType=A to allow Salmon to automatically infer the library type and keeping other options in default mode (Patro et al., 2017). The tximport package (Soneson et al., 2016) was used to import the quantified data from SALMON into R version 3.6.1 (R Core Team, 2019). For differential expression analysis, a DESeqDataSet was constructed from the tximport object with the DESeqDataSetFromTximport function from the DESEQ2 package (Love et al., 2014). We tested differences between the two sampling years using the likelihood ratio test (LRT) with Benjamini–Hochberg false discovery rate

control as implemented in DESEQ2 (Love et al., 2014). Transcripts were normalized using the relative log expression (RLE) normalization (Love et al., 2014). We found no significantly differentially expressed transcripts between the 2 years (Benjamini-Hochberg adjusted p-values > .05). The plotPCA function was used to visualize similarities between temporal replicates (Figure S1). We tested the effect of elevation on the normalized read counts using generalized linear models (GLM) with negative binomial distribution (Venables & Ripley, 2002). We retrieved all available genome size information on bacterial and phage genomes from NCBI refseq database (n = 5507) using the R/Bioconductor package BIOMART (Durinck et al., 2009) to test whether differences in genome size obscured patterns in transcriptional activity (e.g., if larger genomes attract more reads). We repeated the analysis with a subset of taxa, for which genome size information was available, by additionally normalizing read counts for genome size (i.e., dividing normalized read counts by the mean genome size of the respective taxa; Tables S3 and S4).

2.2.4 | Data analyses of abiotic factors

We modelled each abiotic soil factor as a function of elevation using linear models in R version 3.6.1 (R Core Team, 2019). In all models, we fitted a quadratic and linear term of elevation and selected the best model based on Akaike's information criterion for small sample size (AICc, Hurvich & Tsai, 1989). Based on the best model fit, we retained both the quadratic and the linear term of elevation in the soil moisture model and the linear term of elevation in the models of mean summer temperature, mean winter temperature and soil pH.

2.2.5 | Statistical analyses of bacterial and viral diversity

We assessed the diversity of the active bacteria and viruses across elevation with Hill's series of diversity. Hill's series of diversity (Hill, 1973) leverages a measure accounting for the abundance of taxa along three scale parameters. The series includes three numbers: N0 is the number of species in a sample (i.e., richness); N1 is the antilogarithm of the Shannon diversity (representing the abundant species in a sample), N2 is the inverse Simpson diversity (representing the very abundant species in a sample). N1 and N2 emphasize evenness by putting more weight on abundant taxa. Communities can be considered more diverse if their diversity ranks higher at all three scale parameters. Hill's numbers were calculated on family level with the VEGAN package version 2.6-2 (Oksanen, 2013) and the effect of elevation on diversity indices was tested with linear models in R version 3.6.1 (R Core Team, 2019). Similarity in diversity patterns of viruses and their putative hosts were assessed with correlation tests using Pearson's r in R version 3.6.1 (R Core Team, 2019).

3 | RESULTS

3.1 | Abiotic soil factors across the elevational gradient

Patterns of abiotic soil factors measured at the plots significantly differed across the elevational gradient (Figure 1, Table S2). Mean summer temperature significantly increased with increasing elevation (Figure 1a, Table S2). The increase of temperature towards high elevations reflected the high solar radiation, that was measured by the temperature loggers at the soil surface. Trees at low elevation sites provide shade, resulting in lower temperatures at the soil surface at lower elevations. Mean winter temperature showed a marginally significant decrease with increasing elevation (Figure 1b, Table S2). Percentage of soil moisture showed a u-shaped distribution across the elevational gradient, with the lowest values found at mid-elevations (Figure 1c, Table S2). Soil pH significantly increased with increasing elevation (Figure 1d, Table S2).

3.2 | Taxonomic diversity

3.2.1 | Diversity of bacterial taxa

The retrieved 170.265 bacterial contigs spanned a diversity of 37 phyla encompassing 428 families (Figure 2a). The most expressed transcripts belonged to the phyla Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, and Bacteroidetes (Figure 2a).

3.2.2 | Diversity of phage taxa

We identified 225 bacteriophage contigs belonging to three orders and nine families (Figure 2b). The double stranded DNA (dsDNA) bacterial virus families of *Autographiviridae*, *Demerecviridae*, *Herelleviridae*, *Myoviridae*, *Podoviridae*, *Siphoviridae* of the order *Caudovirales* showed the highest transcriptional activity (Figure 2b). One further virus family with DNA genomes (single stranded DNA) was detected, belonging to the family of *Microviridae* (order *Petitvirales*), as well as one family of single stranded RNA viruses (ssRNA viruses: *Fiersviridae*, formally *Leviviridae*, order *Norzivirales*). None of the bacteriophage contigs were classified as belonging to lysogenic viruses by VIBRANT.

3.2.3 | Bacterial and viral diversity across elevation

The diversity of bacterial and viral communities, as assessed by Hill's N0 (richness), N1 (antilogarithm of the Shannon diversity) and N2 (inverse Simpson diversity) were not significantly affected by elevation (Figure 3, Table 1). The richness of bacterial and viral communities was marginally significantly correlated (Pearson's r = .56, p = .09). Bacterial and viral N1 (antilogarithm of the Shannon diversity)

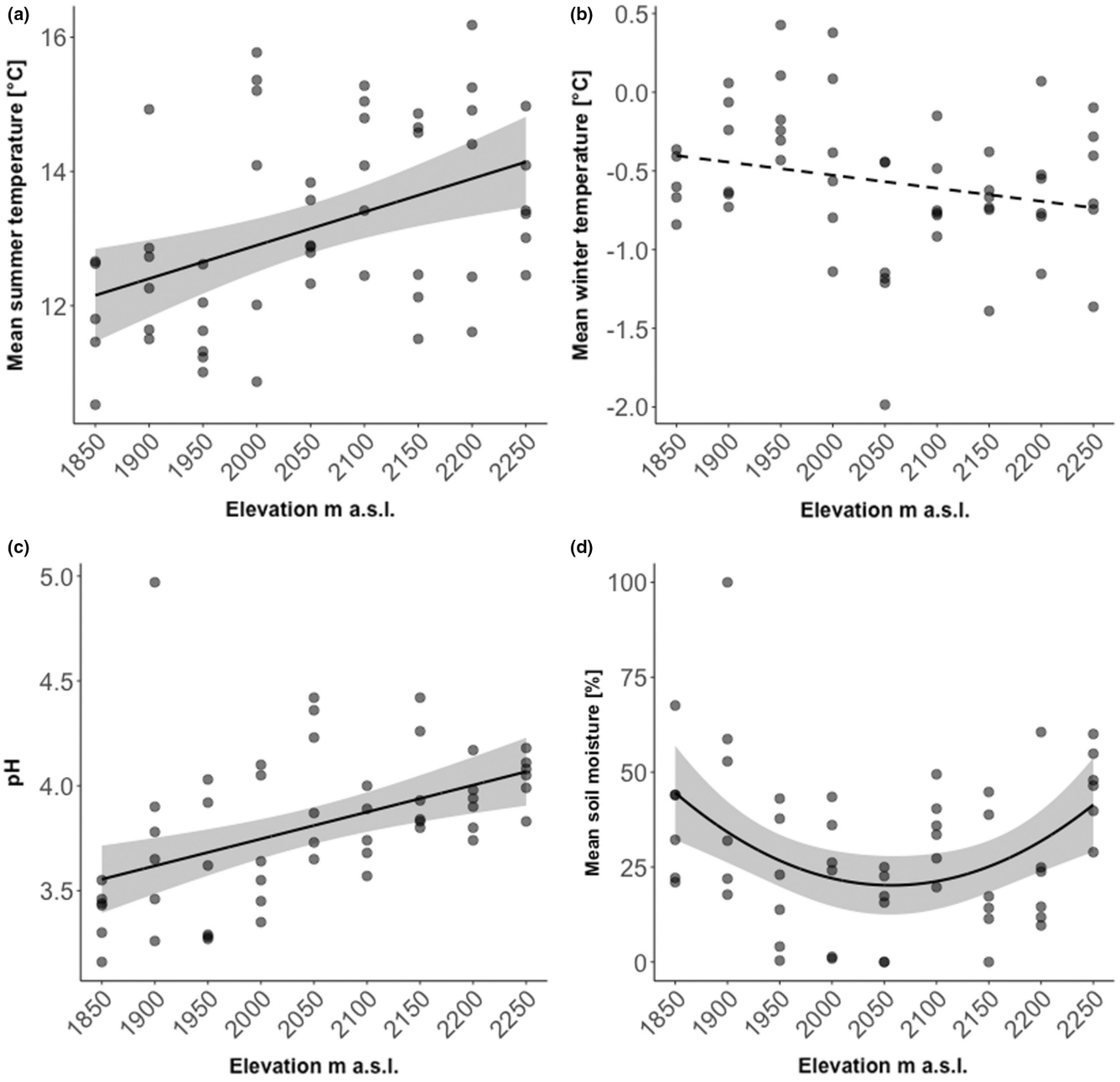


FIGURE 1 Soil abiotic factors (a–d) recorded across the elevational gradient as functions of elevation: (a) mean soil surface temperature of the three warmest months, (b) mean soil surface temperature of the three coldest months, (c) soil pH, (d) proportion of soil moisture. Linear models were fitted using a linear (a–c) and a linear and quadratic term of elevation (d). Circles indicate raw data. Black lines show the model fit (solid: $p < .05$, dashed: $p > .05$). Model statistics are presented in Table S2.

and N2 (inverse Simpson diversity) did not significantly correlate (N1 = Pearson's $r = -.09$, $p = .80$, N2 = Pearson's $r = -.06$, $p = .86$).

3.2.4 | Bacterial and viral activity across years and elevation

Overall activity was not significantly different between the years 2015 and 2017 ($p > .05$; Figure S1). Bacterial activity significantly declined with increasing elevation ($p < .01$, Figure 4a, Table 1), whereas bacteriophage activity was not significantly affected by elevation ($p > .05$, Figure 4b, Table 1). The patterns were robust when normalizing the transcriptional activity by mean genome size.

3.2.5 | Metabolic pathways

Annotation of expressed genes revealed a broad diversity of metabolic pathways across the bacterial taxa (Figure 5a and Figure S2). The majority of annotated genes were directly related to metabolic pathways, tRNA biosynthesis and the biosynthesis of secondary metabolites (Figure 5a and Figure S2). A high diversity of further

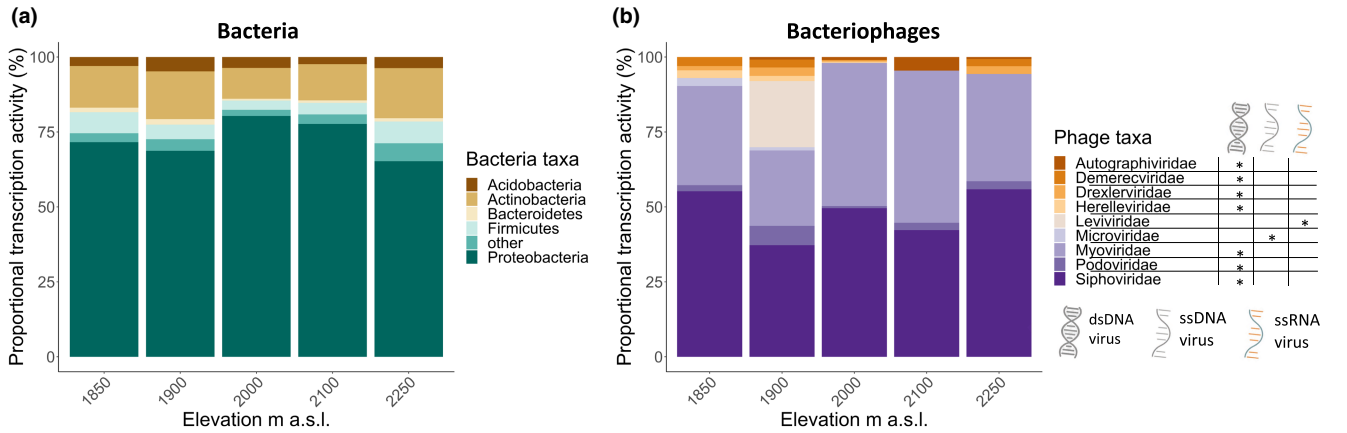
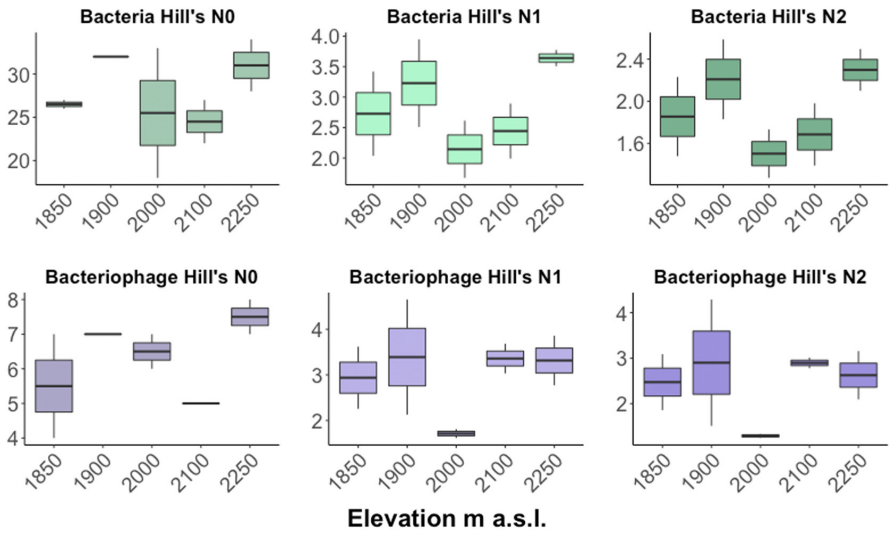


FIGURE 2 Proportional transcription activity of soil bacteria across the elevational gradient. Members of the Proteobacteria showed the highest proportional transcription activity across the elevational gradient, whereas Actinobacteria were the second most transcribed taxa, followed by members of the Acidobacteria and Firmicutes. Proportional transcription activity of soil bacteriophages across the elevational gradient was dominated by members of the *Caudovirales* (dsDNA viruses), that is, *Autographiviridae*, *Demerecviridae*, *Drexlerviridae*, *Herelleviridae*, *Myoviridae*, *Podoviridae* and *Siphoviridae*. ssRNA viruses (*Leviviridea*) and ssDNA viruses (*Microviridae*) showed proportionally higher transcription at lower elevations (1850–1900 m a.s.l).

FIGURE 3 Bacterial and viral diversities, as assessed by Hill's N0 (richness), N1 (antilogarithm of the Shannon diversity) and N2 (inverse Simpson diversity) were not significantly affected by elevation (Table 1).



pathways was detected, such as fatty acid degradation, lipopolysaccharide biosynthesis and transcription of genes involved in nitrogen and sulphur metabolism. The proportional activity of metabolic pathways remained constant across the elevational gradient (Figure 5a). For viral taxa, DRAM annotations revealed the presence of RNA dependent RNA polymerases (RdRP 1 PF00680 and RdRP 2 PF00978), an enzyme that catalyses the replication of RNA from an RNA template (Figure 5b). VIBRANT annotations revealed transcription of a phage specific gene, that is, Capsid proteins alpha (CAPSD_PAV, Q9J7Z0, Figure 5b), which proportionally increased with increasing elevation.

4 | DISCUSSION

Considering bacterial and viral co-occurrence patterns across elevational gradients could provide a template for answering questions regarding the diversity, distribution, infection dynamics, and interactions of viruses with their hosts and their abiotic environment (Merges et al., 2021; Sommers et al., 2021). The integration of an ecological framework in viral metagenomics and -transcriptomics could broadly expand our knowledge on ecosystem-level effects of viruses (Roux et al., 2021; Sommers et al., 2021). So far, the effect of phages on soil bacterial communities has been mostly neglected (Braga et al., 2020) and has only recently been receiving interest enabled by the advance of high throughput sequencing approaches (Braga et al., 2020; Emerson et al., 2018; Starr et al., 2019; Trubl et al., 2018; Wu et al., 2021). In the present study, we applied metatranscriptomics to identify highly diverse bacterial and phage communities in soils across an elevational gradient. In accordance with our hypothesis, bacterial metabolic activities strongly declined with increasing elevation. In contrast, we found that bacteriophages are consistently active in soil along the entire gradient.

Model	Source of variation	Estimate	SE	*p*-value	Adjusted R^2
Bacteria					
(a) Transcription	~ Elevation	−4340.83	1390.82	**.014***	**.493**
(b) Hill's N0	~ Elevation	0.002	0.003	.471	−.05
(c) Hill's N1	~ Elevation	0.001	0.002	.714	−.105
(d) Hill's N2	~ Elevation	0	0.002	.866	−.121
Phages					
(e) Transcription	~ Elevation	1.216	189.992	.995	−.125
(f) Hill's N0	~ Elevation	0.002	0.003	.471	−.05
(g) Hill's N1	~ Elevation	0.001	0.002	.714	−.105
(h) Hill's N2	~ Elevation	0	0.002	.866	−.121

Note: Significant relationships are highlighted in bold.

*$p < .05$.

TABLE 1 Transcriptional activities and Hill diversities of bacteria (a–d) and bacteriophages (e–h) across the elevational gradient as functions of elevation

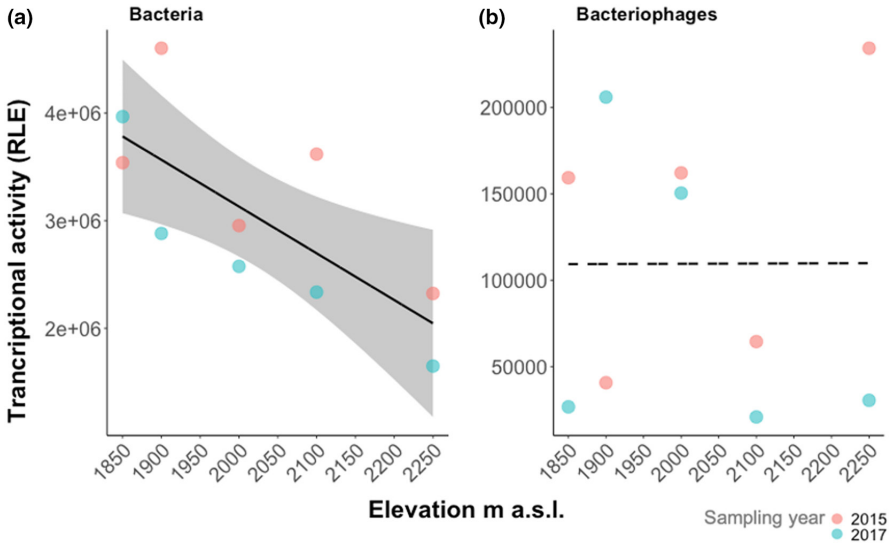


FIGURE 4 Soil bacterial (a) and phage (b) transcriptional activity across the elevational gradient. Bacterial activity (a) significantly declined with increasing elevation, whereas phage activity (b) showed increase in activity in response to increasing elevation. Transcriptional activity was normalized using relative log expression normalization (RLE).

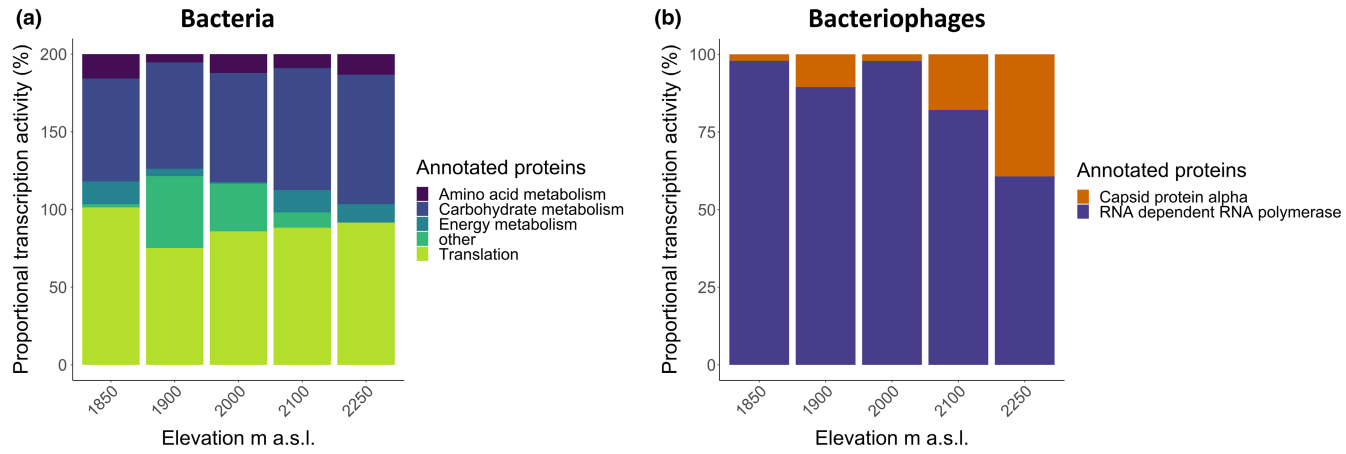


FIGURE 5 Proportional transcription of bacterial and viral pathways across the elevational gradient. (a) The majority of bacterial pathways were attributed to metabolic activity (i.e., amino acid, carbohydrate and energy metabolism) and translation. A high diversity of further pathways was detected, such as the biosynthesis of secondary metabolites, fatty acid degradation, lipopolysaccharide biosynthesis or nitrogen and sulphur metabolism (others). (b) DRAM-v and vibrant annotations revealed RNA dependent RNA polymerase (RDRP) and capsid protein alpha transcription across the elevational gradient.

We found the phyla Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, and Bacteroidetes to be the most metabolically active members of the soil bacterial communities (Figure 2). Their activity strongly declined with increasing elevation (Figure 4a). Similarly, Margesin et al. (2009) could show a decline in the relative amount of these taxa as well as a decrease in activity in a gradient in Austrian Central Alps, based on measurements of soil dehydrogenase activity, with increasing elevation in alpine soils. Ren et al. (2021) reported comparable bacterial community composition and a decreasing abundance with increasing elevation, based on 16S rRNA amplicon sequencing of soils from the Qinling Mountains in central China. Both studies linked the decline in activity and abundance to lower temperatures at high elevational sites (Margesin et al., 2009; Ren et al., 2021). However, elevation is often collinear with many abiotic and biotic factors (Figure 1): in our case both summer soil temperatures and pH were higher at high elevations, while winter temperatures were marginally lower at high elevations (Figure 1). Soil moisture showed a U-shaped relationship with elevation, with higher soil moistures measured at the low and high ends of the elevation gradient (Figure 1). The conditions present at high elevations correlate with decreased bacterial activity (as in Margesin et al., 2009; Ren et al., 2021), but the present study suggests that it is not necessarily the lower temperatures that drive lower activity.

We found dsDNA bacteriophages of the order *Caudovirales* to be the most dominant members of the active soil viral communities. In accordance with our study, dsDNA bacteriophages of the order *Caudovirales* are consistently reported as the most dominant viruses present in soil (Adriaenssens et al., 2017; Emerson et al., 2018; Williamson et al., 2005). Williamson et al. (2005) showed that the majority of soil viruses in Delaware soil were bacteriophages belonging to the *Caudovirales* by combining direct counting of virus-like particles (VLPs) with morphological data gathered using TEM. Additional supporting evidence came from recent metagenomic approaches, which found members of the *Caudovirales* (specifically the families: *Myoviridae*, *Podoviridae* and *Siphoviridae*) made up more than 80% of the relative abundance at all sites in Antarctic soil (Adriaenssens et al., 2017). These are the families which also showed high activity in our samples (Figure 3). A similar dominance of members of the *Caudovirales* order and its families (95% of assigned sequences) was found in soils of a permafrost thaw gradient in northern Sweden (Emerson et al., 2018). Our RNA-based approach now adds evidence that the *Caudovirales* are not only the most abundant, but also the most active members of the soil virome, contributing 97% of the detected viral transcription activity in our data set. However, the putative dominance of *Caudovirales* transcripts in our data set should be taken with caution, since the taxonomic assignment relies on databases of known viruses (with *Caudovirales* being well researched, especially in marine systems, for example, Li et al., 2021; Wommack et al., 1999). The absence of taxonomical clustering at genus level in the gene-sharing networks of our viral contigs also suggests a high number of unknown soil viruses.

Interestingly, the activity of soil bacteria and their putative phages did not co-decline across the elevational gradient. While there is little knowledge on coactivity patterns, previous studies reported correlation between bacterial and phage abundance in marine and soil environments (Weinbauer, 2004; Williamson et al., 2017; Wommack & Colwell, 2000). For example, a meta-analysis of soil viral data sets, revealed viral abundance to be significantly positively correlated with bacterial abundance (Williamson et al., 2017). Such an abundance correlation might be explained by the dependency of phage replication on host availability, where high bacterial host availability is expected to increase phage abundances (Williamson et al., 2017). A positive correlation is expected when viruses are reproducing using the lysogenic cycle, in which phage DNA is inserted into the bacterial genome and cotranscribed when the bacterial genome is copied (Adams, 1959). In our study, we did not detect the transcription of lysogenic phages and a prevalence of lytic reproduction, where the viral DNA is transcribed independently in bacterial cells (Adams, 1959). Here, complex predator–prey dynamics, such as the balance between competitive and defensive abilities of bacteria against strain-specific lytic phage predators in relation to the environment (e.g., an increase in resistant bacteria with elevation) or an increased virulence within declining population of the hosts might be possible explanations of the divergent pattern between bacteria and lytic phage activity, in comparison to abundance-based studies (Breitbart et al., 2018; Trubl et al., 2018). Disentangling infection mechanisms could be possible with increased sample sizes, sequencing depth and fine-scale temporal replication, as well as by pooling and homogenizing more soil from a larger area to account for microsite conditions.

The functional annotation of bacterial and viral contigs revealed low resolution of bacterial metabolic pathways and low diversity of viral functional genes. For bacterial contigs, the majority of the metabolic pathways was attributed to amino acid, carbohydrate and energy metabolism as well as translation (Figure 5a) throughout the elevational gradient. For viral contigs, the functional annotations revealed a dominance of the transcription of RNA-dependent RNA polymerase RNA of viral origin (RdRP_1) throughout the whole gradient (Figure 5b). RdRP_1s are essential enzymes encoded in the genomes of the majority of RNA viruses (Wu et al., 2021) and have been recently utilized to assess RNA viruses in soils (Callanan et al., 2020; Chen et al., 2022; Hillary et al., 2022; Starr et al., 2019; Wu et al., 2021). Overall, the relatively low resolution of bacterial metabolic pathways, as well as the low diversity of viral functional genes, may be due to the underrepresentation of environmental bacterial and viral genomes in the reference databases (Williamson et al., 2017).

Although soil microorganisms have been studied for centuries, the advances in DNA-based surveys suggest that cultivation-based surveys have underestimated the total richness, diversity and activity of soil microbial communities (Fierer et al., 2007; Lu & Salzberg, 2020; Taberlet & Coissac, 2012; Tedersoo et al., 2022). While DNA-centred metabarcoding of taxonomic markers and functional genes (also known as targeted metagenomics or amplicon-based metagenomics) and shotgun whole metagenome sequencing provide a culture-independent insight of microbial communities, RNA-centred approaches (metatranscriptomics) are emerging techniques to profile active structure and function of free-living microbial communities (Fierer et al., 2007; Roux & Emerson, 2022; Tedersoo et al., 2022). Metabarcoding of microbial communities utilizes sequencing of PCR-amplified, ubiquitously conserved genetic

elements, such as 16S and 18S rRNA genes, which are used to characterize the phylogenetic diversity of archaeal, fungal, and bacterial communities in soils (Fierer et al., 2007; Tedersoo et al., 2022). While metabarcoding revolutionized microbial ecology, it is restricted to taxa with ubiquitously conserved genetic elements and confounded by PCR biases (Bálint et al., 2016; Fierer et al., 2007; Tedersoo et al., 2022; Wu et al., 2021).

Shotgun metagenome sequencing and metatranscriptomic sequencing are promising applications overcoming restrains inherent in metabarcoding and enable to simultaneously obtain information on both structure and function of soil communities (Fierer et al., 2007; Hasby et al., 2021; Tláskal et al., 2021; Urich et al., 2008; Žifčáková et al., 2016). Shotgun whole metagenomic sequencing generates information on the total genomic DNA of all microbial organisms in a given sample, avoiding PCR-amplification of conserved genetic elements (Lapidus & Korobeynikov, 2021; Urich et al., 2008). Thereby it does not only provide information on phylogenetic diversity of communities, but also on the functional profile (Lapidus & Korobeynikov, 2021; Urich et al., 2008). However, while revealing the structure and functional profile of soil communities, metagenomics cannot answer whether the organisms detected are actively contributing to soil functions at the time of sampling and hence it is portraying only the genomic potential of communities to provide these functions (Schostag et al., 2019; Wu et al., 2021). This confinement is mainly due to low decay rates of microbial DNA, and the fact that persevered extracellular DNA can obscure short-term changes in microbial communities (Carini et al., 2016, 2020). In contrast, a metatranscriptome approach allows assessment of structure and function of active communities based on the assumption that RNA has a faster turnover than DNA, and is assumed to be transcribed only by metabolically active microorganisms (Romero-Olivares et al., 2019; Schostag et al., 2019; Tláskal et al., 2021; Urich et al., 2008). Thereby, metatranscriptomics of microbial community expression has the potential to reveal which community members are active and whether genes are expressed under certain environmental conditions (Urich et al., 2008; Wu et al., 2021). Further, metatranscriptomics can be applied across a wide range of important topics, for example to gain novel insights into functional consequences of viral dynamics in microbial communities or to monitor RNA viruses. Depending on the research question it might be fruitful to integrate a "multiomics" approach by combining DNA and RNA sequencing to better understand how soil microorganisms and related functions respond to environmental change.

5 | CONCLUSION

By applying a metatranscriptomic approach, we demonstrate the strength of this method to track viral infection dynamics within soil bacterial communities across an elevational gradient. Assessing the transcriptional activity of bacterial and viral communities is a promising way to detect infection dynamics (1) of DNA phages because their RNA is only transcribed while infecting the host and (2) of RNA phages because they lack a DNA phase and thereby are completely missed by metagenomic approaches. A major limitation of the current application of metatranscriptomics is its reliance on reference genome databases, with the majority of environmental bacteria and viruses missing reference genomes. The overrepresentation of certain taxa in references databases (e.g., gut microbiome) can lead to spurious annotations due to sequence similarity. Future genome sequencing efforts will greatly facilitate the applicability of metagenomics and metatranscriptomics and may finally provide insights into quantifiable viral contributions to ecosystem processes. The unexpected lack of co-decline of bacterial and phage activity with increasing elevation indicates gaps in our knowledge of important microbial predator–prey relationships.

AUTHOR CONTRIBUTIONS

Dominik Merges and Miklós Bálint conceived the ideas; Dominik Merges and Eike Lena Neuschulz collected the data, Dominik Merges performed laboratory work; Dominik Merges and Alexandra Schmidt analysed data, Francesco Dal Grande provided analytical guidance; Dominik Merges and Miklós Bálint wrote the manuscript. All authors contributed to the various drafts and gave their final approval for publication.

ACKNOWLEDGEMENTS

We are grateful for permission from landowners for working on their property. The fieldwork was greatly supported by Jan Markus Peters and the Shima Davos. We thank Carola Greve and Damian Baranski for help with laboratory procedures, and Christoph Sinai and Tilman Schell (Frankfurt am Main) for support with bioinformatics. We thank Barbara Feldmeyer and Markus Pfenninger (Frankfurt am Main) for advice on analyses. We thank Kornelia Hardes (Fraunhofer-Institut für Molekularbiologie und Angewandte Oekologie IME, Gießen) for valuable comments on the current version of the manuscript. We greatly appreciate the reviewers' helpful feedback on previous versions of the manuscript. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

FUNDING INFORMATION

This research was funded by the German Research Foundation (DFG; NE 1863/2-1) and Hesse's "State initiative for the development of scientific and economic excellence" (LOEWE).

OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. Raw sequence reads were deposited in the Sequence Read Archive under the BioProject PRJNA849939.

DATA AVAILABILITY STATEMENT

Raw sequence reads were deposited in the Sequence Read Archive under the BioProject PRJNA849939.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Merges, D., Schmidt, A., Schmitt, I., Neuschulz, E. L., Dal Grande, F., & Bálint, M. (2023). Metatranscriptomics reveals contrasting effects of elevation on the activity of bacteria and bacterial viruses in soil. *Molecular Ecology*, *32*, 6552–6563. https://doi.org/10.1111/mec.16756