Genetic signatures indicate widespread antibiotic resistance and phage infection in microbial communities of the McMurdo Dry Valleys, East Antarctica

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Abstract The McMurdo Dry Valleys of Antarctica support extensive yet cryptic microbial communities but little evidence for 'top-down' herbivory control. A question therefore arises as to how standing microbial biomass is regulated. Here, we present results from a survey of soil and rock microbial community metagenomes using the GeoChip microarray that demonstrate antibiotic resistance and phage infection are widespread. We interrogated a range of dry valley locations from maritime to extreme inland sites. Antibiotic resistance genes were identified in three categories: beta-lactamases, tetracycline and vanomycin plus a range of transporter genes. Frequency of recovery generally reflected microbial diversity, with greatest abundance among Halobacteria, Proteobacteria and the photosynthetic bacteria (Chlorobi, Chloroflexi and Cyanobacteria). However, no clear differences between locations and soil/rock communities were apparent. Phage signals were also recovered from all locations in soil and rock communities. The *Leviviridae*, *Myoviridae*, *Podoviridae* and *Siphoviridae* were ubiquitous . The *Corticoviridae* occurred only in moisture-sufficient hyporheic soils, the *Microviridae* occurred only in maritime and hyporheic sites and an unidentified group within the order *Caudovirales* occurred only at dry inland sites. We postulate that widespread antibiotic resistance indicates potential inter-specific interaction and that phage signals indicate possible 'bottom-up' trophic regulation in the dry valleys.

Keywords Antibiotic resistance · Antarctic Dry Valleys · Phage · Trophic controls · Virus

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Introduction

The McMurdo Dry Valleys (DV) in East Antarctica comprise the coldest and driest deserts on earth (Wynn-Williams 1990; Cowan and Tow 2004). While lichens and mosses are visible as patchily distributed surface biota, the biology of this region is dominated by cryptic microbial communities within soils and rocks (Fraser et al. 2014; Pointing et al. 2014). These sub-surface communities account for the majority of standing biomass in desert systems (Pointing and Belnap 2012) including polar deserts (Cary et al. 2010; Wierzchos et al. 2012). These lithic communities are dominated by cyanobacteria and lichens, but also support a diverse range of archaea, bacteria, chlorophytes and fungi (Friedmann 1982; De La Torre et al. 2003; Broady 2005; De Los Rı´os et al. 2007; Wood et al. 2008; Pointing et al. 2009; Cowan et al. 2010; Rao et al. 2011; Cowan et al. 2012; D e L o s R ´ı os et al. 2014; Richter et al. 2014; Yung et al. 2014). Metazoan life is comparatively sparse, with limited direct evidence for grazing on microbial communities (Wall and Virginia 1999). This system therefore presents the question as to how persistent cryptic microbial communities that are

protected from physical disturbance and are not apparently grazed appreciably are regulated in terms of biomass turnover.

An important feature of microbial community dynamics under resource limitation is inter-specific interaction (Hibbing et al. 2010), which maintains balance and promotes community transitions through competitive forces. This is mediated by antimicrobial (antibiotic) compounds which are secreted by many phyla, and these inhibit the growth and activity of competing taxa (Hibbing et al. 2010). Studies of bacteria isolated from DV soils have shown they are susceptible to antibiotics in vitro (Siebert et al. 1996). However, there is no information on how widespread the ability to defend against antibiotics is among DV microorganisms. Another factor that may be important in extreme environments where microbial communities experience little top-down trophic regulation is phage lysis (Pointing and Belnap 2012). This mechanism, where viral infection causes cell death and thus exerts a control on biomass, would represent a bottom-up control on microbial communities. Detectable phage populations have been demonstrated for extreme environments including hot springs (Breitbart et al. 2004), hot deserts (Adriaenssens et al. 2014), Antarctic lakes (Lo´pez-Bueno et al. 2009) and Antarctic hypoliths and surrounding soil (Zablocki et al. 2014). Investigations of phage–host interaction, including cell lysis, have yet to be addressed in Antarctica. Here, we describe results from a GeoChip-based metagenomic interrogation that supports the contention that inter-specific competition and phage infection may be important factors in DV microbial community regulation.

Materials and methods

Four locations in three separate valleys were selected for this study. McKelvey Valley (77°26′S, 161°33′E, elevation approx. 800 m) is a high altitude inland valley that has no lake system, Victoria Valley (77°23′S, 162°7′E, elevation approx. 400 m) was used as a representative lake-bearing inland valley and Miers Valley (78°45′S, 163°15′E, elevation approx. 200 m) as a maritime lake-bearing valley. An additional hyporheic site (Kite Stream) in Victoria Valley (77°23′S, 162°E, elevation approx. 400 m) was also assessed, and this site experienced moisture sufficiency from glacial melt water for a minimum one-month period during the 2012–2013 austral summer prior to sampling (confirmed visually by the presence of the field team at the site during December 2012–January 2013). Moisture content for all substrates was <2 % w/w with the exception of hyporheic samples where moisture content was >100 % w/w for all samples as measured gravimetrically in the field. At each site, triplicate samples of open soil, hypoliths (communities

beneath quartz stones), chasmoendoliths (communities in rock fissures, granite in Miers Valley and sandstone in McKelvey Valley) and cryptoendoliths (communities within structural cavities of sandstone rock) as defined by Golubic et al. (1981) were recovered aseptically as previously described (Pointing et al. 2009; Rao et al. 2011; Yung et al. 2014). Samples were stored at −80 °C until processed. Recovery of environmental DNA utilized a protocol optimized for edaphic desert microorganisms (Pointing et al. 2007). Microarray interrogation using GeoChip 4.0 was conducted for all samples ($n = 36$) plus negative controls as previously described (Chan et al. 2013). The GeoChip contained probes for 11 antibiotic resistance coding loci (Table S1) and 39 loci for phage spanning eight families (Table S2). The GeoChip dataset reported in this paper is publicly available at http://ieg.ou.edu/4download/.

Results and discussion

Antibiotic resistance genes were identified in three categories: beta-lactamases, tetracycline and vanomycin, together with a range of transporter genes associated with antibiotic resistance (Table S1). Most samples returned signals from the microarray for all categories of antibiotic resistance gene (Fig. 1, Fig. S1). Signals for antibiotic resistance closely tracked observed bacterial and archaeal diversity, such that the strongest antibiotic resistance signals were associated with the Halobacteria, Proteobacteria and the photosynthetic bacteria (Chlorobi, Chloroflexi and cyanobacteria) in all samples. Little location or substrate-specific variation was discernible, although vanomycin genes were absent from hyporheic soil samples. In contrast, a striking difference in the types of organisms from which these resistance genes were derived was apparent between dry and wet samples, mirroring differences in prokaryotic diversity in these communities (Fig. 2). The hyporheic (wetter) samples supported greater relative abundance of antibiotic resistance loci from Halobacteria, photosynthetic bacteria and Spirochaetes. This supports the notion that composition of microbial communities may be influenced by moisture status of the substrate (Yergeau and Kowalchuk 2008; Rao et al. 2011), and our data suggest this may also result in changes in microbial interactions as indicated by antibiotic resistance.

Metagenomic analysis of soils worldwide including DV soils have demonstrated that antibiotic resistance genes are widespread on a global scale, but are relatively less abundant in desert soils (Fierer et al. 2012). The majority of antibiotic resistance signals identified in the GeoChip analysis were of bacterial origin, although antibiotics are produced by both prokaryotic and eukaryotic microorganisms during inter-specific competition. We note that
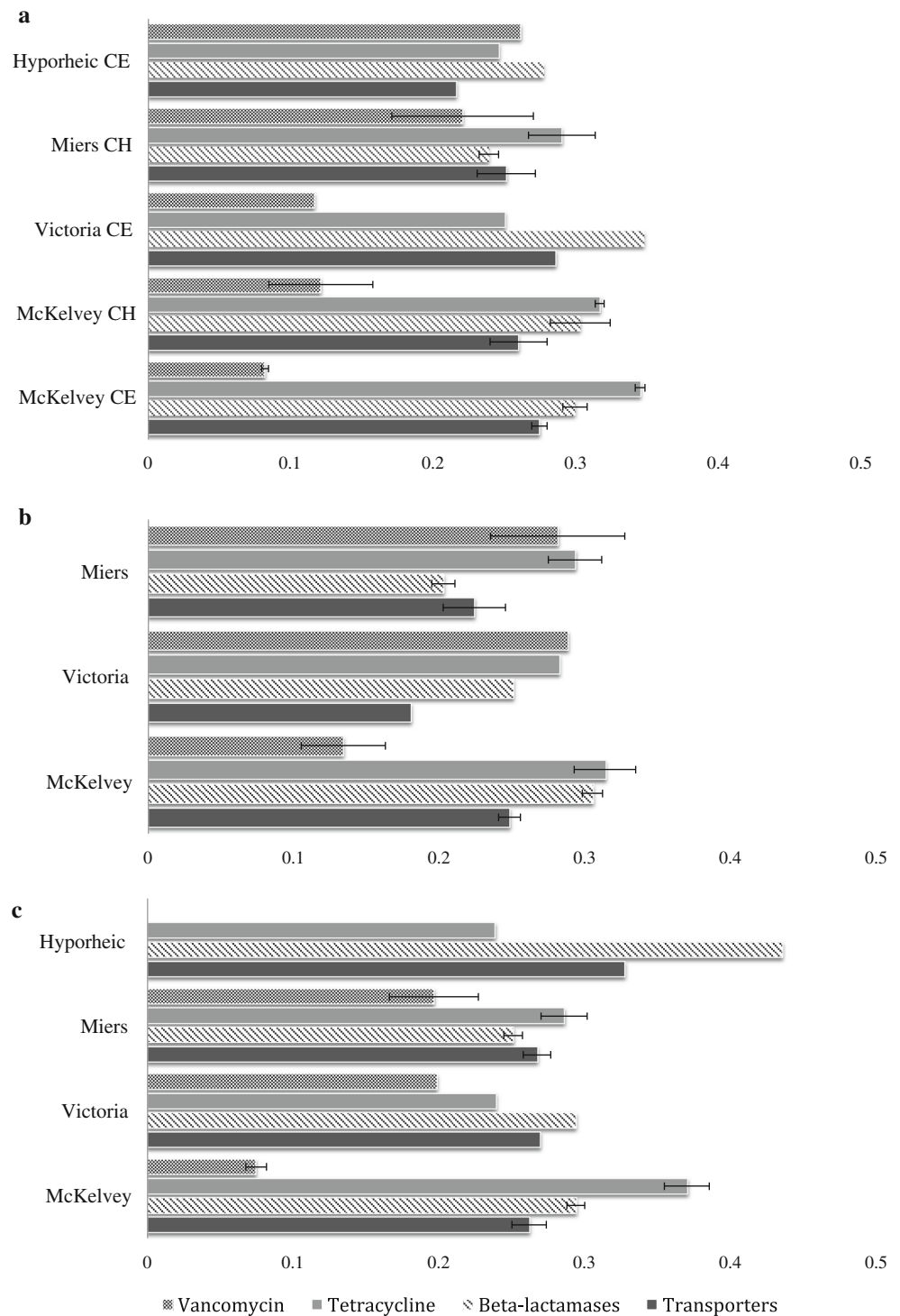
Fig. 1 Antibiotic resistance genes recovered from specific substrates at different dry valleys locations. **a** Endolithic communities (*CH* chasmoendoliths, *CE* cryptoendoliths); **b** hypoliths, **c** soils. *Error bars* represent standard deviation of the mean for three independent GeoChip replicates, where these are not visible standard deviation is less than the minimum scale increment

▨ Vancomycin ▦ Tetracycline ⬡ Beta-lactamases ▪ Transporters

previous studies (Arenz and Blanchette 2011; Rao et al. 2011) reported substantial discrepancies in the diversity and abundance of fungal phylotypes, and so, it is interesting to speculate whether inter-domain level competition may be a factor limiting fungi in the DV edaphic niches.

A range of target loci was used to identify phages, including single-stranded (ss) and double-stranded (ds) DNA phage and ssRNA phage (Fig. S2, Fig. 3). It is interesting to note that ssDNA viruses were not present in all locations; however, these analyses revealed phage spanning eight families in diverse substrates and locations (Fig. 3). The *Leviviridae, Myoviridae, Podoviridae and Siphoviridiae* families were ubiquitous in all samples and all locations (Fig. 3). This is consistent with a recent study
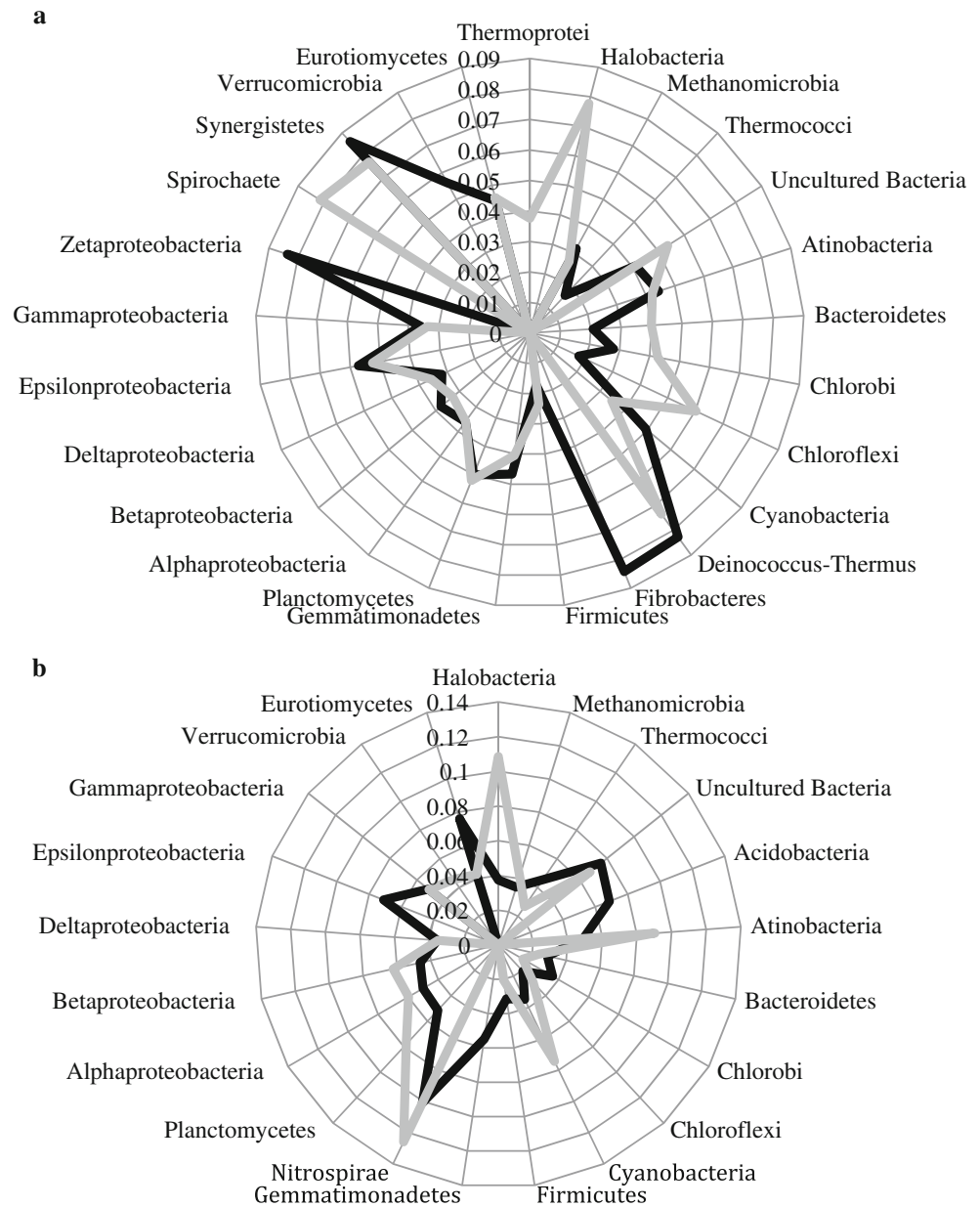
a

Thermoprotei
Halobacteria
Methanomicrobia
Thermococci
Uncultured Bacteria
Atinobacteria
Bacteroidetes
Chlorobi
Chloroflexi
Cyanobacteria
Deinococcus-Thermus
Fibrobacteres
Firmicutes
Gemmatimonadetes
Planctomycetes
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Epsilonproteobacteria
Gammaproteobacteria
Zetaproteobacteria
Spirochaete
Synergistetes
Verrucomicrobia
Eurotiomycetes

0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0

b

Halobacteria
Methanomicrobia
Thermococci
Uncultured Bacteria
Acidobacteria
Atinobacteria
Bacteroidetes
Chlorobi
Chloroflexi
Cyanobacteria
Firmicutes
Gemmatimonadetes
Nitrospirae
Planctomycetes
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Epsilonproteobacteria
Gammaproteobacteria
Verrucomicrobia
Eurotiomycetes

0.14
0.12
0.1
0.08
0.06
0.04
0.02
0

Fig. 2 Relative occurrence of antibiotic resistance genes by phylum for bacteria, archaea and fungi. **a** Cryptoendoliths (*black*, dry; *grey*, wet); **b** soils (*black*, dry; *grey*, wet)

that identified greatest abundance of *Myoviridae* (21 %) and *Siphoviridae* (53 %) in Antarctic hypoliths (Zablocki et al. 2014). The *Levriviridae* are found worldwide, and isolated strains infect gram-negative bacteria (Hulo et al. 2011). The *Podoviridae* have a diverse host range that includes several cyanobacterial genera (Hulo et al. 2011). The study also revealed some interesting patterns with re-gard to phage distribution: The *Corticoviridae* (dsDNA) occurred only in hyporheic soils (Fig. 3), suggesting they infect microbial hosts that are active only during moisture sufficiency. A possible explanation being that most corti-covirus signatures have a marine origin (Hulo et al. 2011). The *Microviridae* occurred only in maritime and hyporheic

soils but not the extreme inland location (Fig. 3). This is suggestive of a host range that spans taxa that occur in wet or ephemerally wet habitats. An unidentified group within the *Caudovirales* was detected only at the dry inland site. Given the paucity of information available on viral diversity in Antarctic environments, this study represents an important incremental step in revealing the extent of phage diversity in Antarctic edaphic and lithic systems. The near-ubiquity of vial signature recovery in this study is consistent with theory regarding phage distribution in natural environments (Breitbart and Rohwer 2005). A recent study of the Antarctic hypolithic metavirome indicated that a range of microbial physiological pathways may also be encoded
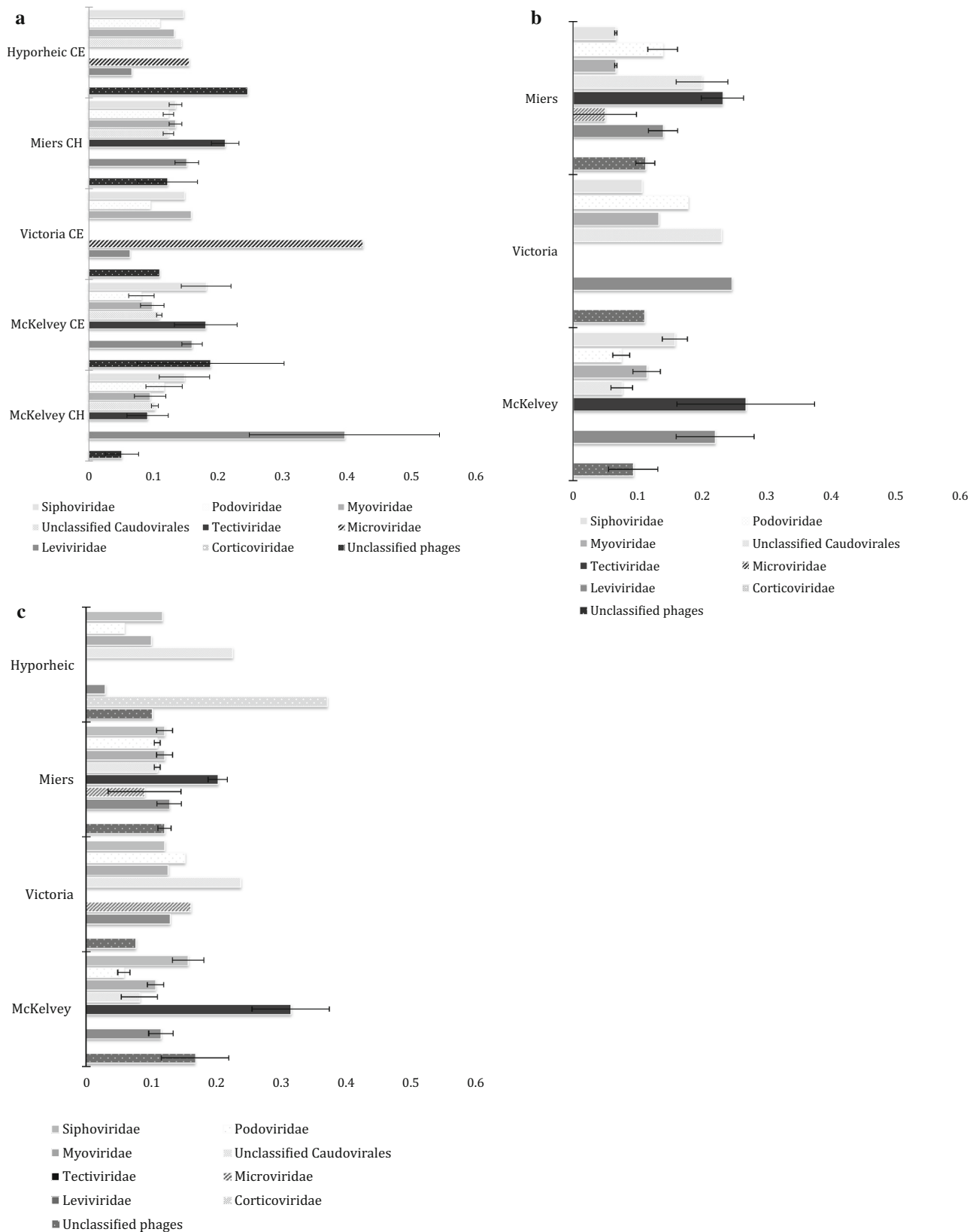
a

Hyporheic CE

Miers CH

Victoria CE

McKelvey CE

McKelvey CH

0   0.1   0.2   0.3   0.4   0.5   0.6

▪ Siphoviridae   ▫ Podoviridae   ▪ Myoviridae
▫ Unclassified Caudovirales   ◼ Tectiviridae   ▨ Microviridae
▪ Leviviridae   ▫ Corticoviridae   ◼ Unclassified phages

b

Miers

Victoria

McKelvey

0   0.1   0.2   0.3   0.4   0.5   0.6

▪ Siphoviridae   ▫ Podoviridae
▪ Myoviridae   ▫ Unclassified Caudovirales
◼ Tectiviridae   ▨ Microviridae
▪ Leviviridae   ▫ Corticoviridae
◼ Unclassified phages

c

Hyporheic

Miers

Victoria

McKelvey

0   0.1   0.2   0.3   0.4   0.5   0.6

▪ Siphoviridae   ▫ Podoviridae
▪ Myoviridae   ▫ Unclassified Caudovirales
◼ Tectiviridae   ▨ Microviridae
▪ Leviviridae   ▫ Corticoviridae
▪ Unclassified phages

Fig. 3 Phage families recovered from specific substrates at different dry valleys locations. a Endolithic communities (CH chasmoen-doliths; CE cryptoendoliths); b hypoliths, c soils. Error bars represent standard deviation of the mean for three independent GeoChip replicates, where these are not visible standard deviation is less than the minimum scale increment

within phage genomes, in addition to virulence, disease and defence functions (Zablocki et al. 2014). Thus, high viral loads in microbial communities in extreme environments may also reflect, in part, acquisition of favourable auxiliary metabolism genes (Roossinck 2011) and may even increase bacterial diversity by selecting for multiple resistance traits (Koskella and Brockhurst 2014).

We suggest that widespread antibiotic resistance loci may indicate high levels of inter-specific competition, and diverse phage signals could indicate a 'bottom-up' trophic regulation in DV edaphic communities. These are likely to be important factors regulating communities in the absence of significant metazoan grazing. Given that high levels of phylogenetic endemism (Bahl et al. 2011; Caruso et al. 2011) and metagenomic novelty (Fierer et al. 2012) are known for Antarctic DV soil habitats, it is also likely that future studies may reveal new mechanisms involved in community regulation. We note that the GeoChip analysis did not target the opposite response that of cooperative interactions such as quorum sensing (Hibbing et al. 2010). In relatively low-diversity systems with low trophic complexity, this might be expected and so offers an interesting avenue for future study.

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