


# Microbial and macroinvertebrate communities, but not leaf decomposition, change along a mining-induced salinity gradient

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## Abstract

1. Natural levels of salinity in aquatic ecosystems drive biodiversity patterns across broad spatial scales; however, less is known about changes in biotic communities and the ecosystem functions they support along anthropogenic salinisation gradients. Resource extraction often causes salinisation of freshwater ecosystems which may extirpate salinity-sensitive macroinvertebrates and microbes and thus reduce rates of organic matter decomposition.
2. We quantified bacterial (16S), fungal (ITS) and macroinvertebrate taxonomic richness, composition, and  $\beta$ -diversity across the resulting gradient of specific conductance (annual mean: 25–1,383  $\mu\text{S}/\text{cm}$ ) in 24 headwater streams in the eastern U.S.A. variously influenced by surface coal mining but selected to minimise habitat and water-quality differences other than salinity. Furthermore, we measured rates of organic matter decomposition in submersed leaf packs (*Quercus alba*) across these same sites.
3. Bacterial and reach-wide macroinvertebrate richness was reduced along the mining-induced salinity gradient whereas fungal or leaf-pack macroinvertebrate richness remained similar. Community composition of microbes and macroinvertebrates changed along the salinity gradient, with mining-influenced sites becoming increasingly dissimilar to reference sites as salinity increased. Beta-diversity was driven by taxonomic replacement rather than nestedness for microbial and macroinvertebrate communities.
4. Organic matter decomposition rates in mining-influenced streams were not reduced even at mean specific conductance levels 10 $\times$  higher than reference sites. We attribute maintenance of decomposition rates to salinity tolerance of both fungi and macroinvertebrate shredders found in leaf packs.
5. Our study informs theory linking anthropogenic alteration of biotic communities to rates of ecosystem functions by providing evidence that taxonomic replacement and stressor-tolerance of specific functional groups along a mining-induced salinity gradient can maintain certain ecosystem functions, at least within the studied salinity range. We therefore advise against generalising biodiversity–ecosystem function relationships in other salinised systems, but caution that organic matter decomposition may be altered at salinity levels above those observed in

our study and that other ecosystem functions may be affected by loss of salinity-sensitive taxa.

**KEYWORDS**

$\beta$ -diversity, coal mining, community composition, freshwater ecosystem, stressor

## 1 | INTRODUCTION

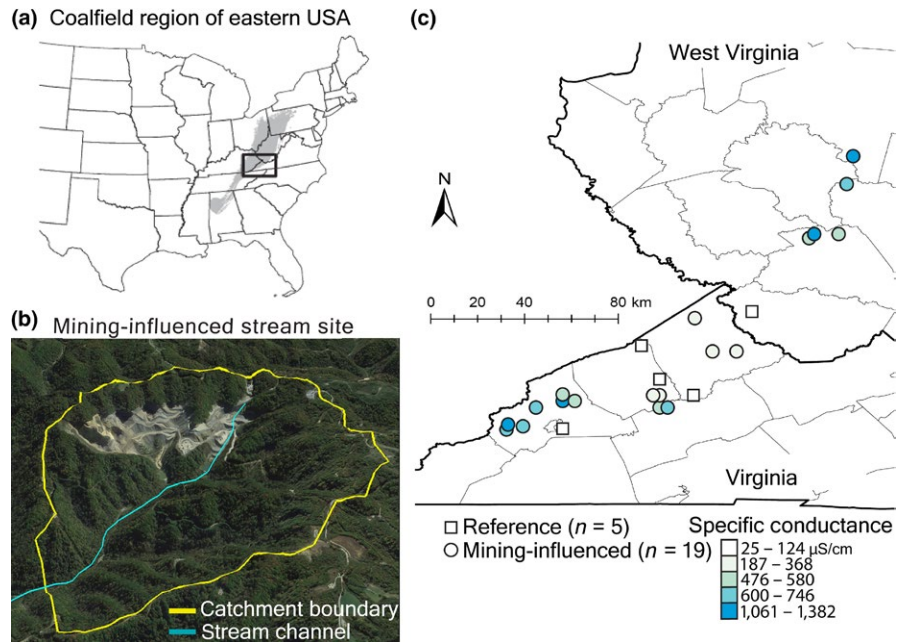
Salinity is a primary determinant of biotic communities over broad spatial gradients, differentiating aquatic ecosystems at global scales (Kefford et al., 2016; Lozupone & Knight, 2007; Mohamed & Martiny, 2011). Although natural levels of salinity are critical for physiological processes of aquatic organisms, anthropogenic salinisation—defined as human-caused increase in dissolved major-ion concentrations—is an emerging stressor with potential to alter biotic community richness and composition (Cañedo-Argüelles et al., 2013; Canhoto, Simões, Gonçalves, Guilhermino, & Bärlocher, 2017; Kefford et al., 2016). Globally, freshwater ecosystems are subject to anthropogenic salinisation from land-use activities such as agriculture, resource extraction, land clearing, road de-icing, and other activities (Cañedo-Argüelles et al., 2016; Kaushal et al., 2018). In the contiguous U.S.A. alone, salinisation affects an estimated 37% of catchment areas with increasing trends in many regions, such as the eastern U.S.A. (Kaushal et al., 2018). Anthropogenic salinisation raises concerns for loss of freshwater biodiversity and ecosystem functions; however, responses of common freshwater groups such as microbes and macroinvertebrates and important ecosystem functions, including organic matter decomposition, remain difficult to predict.

Salinisation has potential to alter richness and composition of microbial and macroinvertebrate communities, which are major functional components of freshwater ecosystems (Barlocher, 2016; Cañedo-Argüelles et al., 2013; Hieber & Gessner, 2002; Sauer et al., 2016). Freshwater microbes and macroinvertebrates use osmoregulation to balance their bodily water and salt concentrations but salinisation alters this process, potentially increasing energy costs, reducing fitness, and causing mortality (Oren, 2002; Soucek & Kennedy, 2005). Therefore, salinisation could reduce richness and alter microbial and macroinvertebrate composition, unless the species loss can be offset via replacement by salinity-tolerant species (Kefford et al., 2016). For example, anthropogenically-salinised rivers could be colonised by salinity-tolerant macroinvertebrates including Diptera, Coleoptera, Odonata, and Hemiptera but replacement of salinity-sensitive species will be highly dependent on regional species pools and individual dispersal abilities (Kefford et al., 2016). For microbial communities, growth rates of some taxa may even increase under moderate levels of salinisation (Canhoto et al., 2017). Understanding how microbial and macroinvertebrate communities change in salinised aquatic ecosystems is especially important considering their important roles in key ecosystem functions (Hieber & Gessner, 2002).

Aquatic biodiversity and ecosystem functions are often linked, with taxonomic richness serving as a common biodiversity measure (Cardinale et al., 2006; Jonsson & Malmqvist, 2000). If taxonomic replacement does not occur as a result of salinisation, reductions in richness could negatively affect rates of ecosystem functions (Cardinale et al., 2006; Jonsson & Malmqvist, 2000). For example, organic matter decomposition in freshwater ecosystems is often positively affected by species richness of detritivorous microbes and macroinvertebrates (Jonsson & Malmqvist, 2000; Tiunov & Scheu, 2005). However, species identity and abundance also influence decomposition (Dangles & Malmqvist, 2004), challenging the idea of a universal relationship between species richness and ecosystem function. Field-based studies along gradients of environmental change, such as salinisation, are needed to better understand biodiversity-ecosystem function relationships (De Laender et al., 2016).

Headwater streams contribute greatly to regional freshwater species richness, ecosystem functions and downstream water quality (Finn, Bonada, Múrria, & Hughes, 2011; Gomi, Sidle, & Richardson, 2002; Meyer et al., 2007); however, their intricate link to surrounding landscapes makes them susceptible to anthropogenic change. In central Appalachia—a land area covering approximately 60,000 km<sup>2</sup> in the eastern U.S.A. (Bernhardt et al., 2012)—resource extraction in the form of mountaintop-removal coal mining is an important source of headwater stream salinisation (Palmer et al., 2010). Specific conductance (SC), a surrogate measure of dissolved major ions composing salinity (Hem, 1985), ranges from 20 to 130  $\mu$ S/cm in relatively undisturbed central-Appalachian streams to >2,500  $\mu$ S/cm in mining-influenced streams (Bernhardt et al., 2012; Timpano, Zipper, Soucek, & Schoenholtz, 2018). In mining-influenced Appalachian streams, salinisation has been identified as the primary stressor, or limiting factor (Cormier, Suter, Zheng, & Pond, 2013; Pond, Passmore, Borsuk, Reynolds, & Rose, 2008; Timpano, Schoenholtz, Soucek, & Zipper, 2015), for macroinvertebrates, despite its co-occurrence with other potential stressors, such as elevated levels of suspended solids, alkalinity, selenium, manganese, and nitrogen, and with altered physical habitat (Bernhardt et al., 2012; Lindberg et al., 2011; Pond et al., 2008). Although macroinvertebrate richness in mining-influenced streams is typically reduced (Fritz et al., 2010; Pond et al., 2008; Voss & Bernhardt, 2017), less is known about potential changes in microbial communities and organic matter decomposition along gradients of mining-induced salinisation (Lecerf & Chauvet, 2008; Pond et al., 2008).

In this study, we measured microbial and macroinvertebrate richness, composition, and  $\beta$ -diversity across a mining-induced salinity gradient in Appalachian headwater streams, where sites



**FIGURE 1** (a) Map of the Appalachian coalfield region (shaded) in eastern U.S.A. (box shows study region), (b) aerial image example of a mining-influenced stream study site (grey land area indicates barren mine lands), and (c) study region map showing stream sites ( $n = 24$ ) located in southwestern Virginia and southern West Virginia

were selected for similarity in all characteristics other than salinity. We predicted that: (1) taxonomic richness of microbes and macroinvertebrates would decrease as SC increases; (2) community composition in mining-influenced streams would become increasingly dissimilar to that in reference streams as SC increases; and (3) variability in composition across streams, or  $\beta$ -diversity, would be likely to occur by losses of salinity-sensitive taxa creating more nestedness than taxonomic replacement of both microbial and macroinvertebrate communities. We quantified leaf litter decomposition, as a measure of organic matter decomposition, and predicted that (4) decomposition rates would be reduced as SC increases and species richness decreases, unless microbial and macroinvertebrate detritivores were among the remaining salinity-tolerant taxa.

## 2 | METHODS

### 2.1 | Study sites

We studied 24 first-order headwater streams in the central Appalachian coalfield region of Virginia and West Virginia, U.S.A. (Figure 1). Five streams were classified as *reference*, as they are not salinised by mining, are relatively unimpacted by anthropogenic activities (Timpano et al., 2015), and have SC comparable to that of estimated natural background levels in the region (Griffith, 2014). Nineteen streams were classified as *mining-influenced*, as they receive waters discharged by or draining from active or completed surface coal mines (Figure 1). We endeavoured to select mining-influenced stream sites with non-salinity attributes comparable to those of reference streams so as to minimise potential confounding of the salinity variable. All streams had intact riparian forest, and 21 had  $\geq 50\%$  forest cover in their catchments (Timpano, Zipper, et al., 2018). Other characteristics were similar across study sites (Table 1,

see Appendices S1 and S2) including instream habitat, which was comparable among reference and mining-influenced streams as determined using Rapid Bioassessment Protocols (Barbour, Gerritsen, Snyder, & Stribling, 1999, Table 1).

### 2.2 | Salinity and water chemistry

We measured SC at 15–30-min intervals using automated data loggers (HOBO Freshwater Conductivity Data Logger, model U24-001, Onset Computer Corp., Bourne, Massachusetts; Timpano, Zipper, et al., 2018). We measured physicochemical parameters and collected grab samples of stream water in October 2015 and April 2016. We analysed water for major cations and select trace elements by ICP-MS, for sulfate and chloride by ion chromatography, and for bicarbonate by calculation from alkalinity titration. See Timpano, Schoenholtz, Soucek, and Zipper (2018) for details of analytical chemistry methods.

### 2.3 | Leaf packs

We used standardised leaf packs to assess influence of salinity on organic matter decomposition and microbial and macroinvertebrate richness and composition. We collected freshly abscised leaves of *Quercus alba* (white-oak), a common species throughout the region. Leaves were uniformly mixed, air-dried indoors, and weighed until constant mass was achieved. Dried leaves ( $6.5 \pm 0.2$  g) were then placed in c. 28 cm  $\times$  30 cm nylon coarse-mesh (6 mm) and fine-mesh (1 mm) packs. We anchored coarse- and fine-mesh packs to the streambed at three separate locations in each stream in November 2015. We placed leaf packs in runs (transitional zones between riffles and/or pools) to maximise the likelihood of them remaining submerged and to minimise loss of packs from high streamflow during the study. We collected leaf packs approximately every 30 days until

TABLE 1 Characteristics of study sites grouped into reference and mining-influenced streams

Site type	Catchment area (ha)	Specific conductance prior 365 days ( $\mu\text{S}/\text{cm}$ )	Specific conductance prior 150 days ( $\mu\text{S}/\text{cm}$ )	Specific conductance prior 90 days ( $\mu\text{S}/\text{cm}$ )	Water temperature November 2015–April 2016 ( $^{\circ}\text{C}$ )	Habitat score		pH	
						October 2015	April 2016	October 2015	April 2016
Reference (n = 5)									
Mean $\pm$ SE	256 $\pm$ 90	66 $\pm$ 16	53 $\pm$ 16	51 $\pm$ 15	8.3 $\pm$ 1.3	165 $\pm$ 3.3	170 $\pm$ 2.1	7.9 $\pm$ 0.1	7.6 $\pm$ 0.1
Range	82–596	25–124	20–112	19–108	4.0–11.8	159–177	164–177	7.5–8.2	7.4–7.9
Mining-influenced (n = 19)									
Mean $\pm$ SE	368 $\pm$ 54	644 $\pm$ 72*	608 $\pm$ 68*	596 $\pm$ 66*	9.7 $\pm$ 0.3	169 $\pm$ 1.4	168 $\pm$ 1.5	8.1 $\pm$ 0.04	8.1 $\pm$ 0.1*
Range	67–977	187–1383	180–1302	151–1147	6.5–11.6	154–178	151–178	7.6–8.3	7.7–8.5

Asterisk indicate mining-influenced site mean is different from reference site mean (Welch's t-test;  $p < 0.05$ ).

April 2016. In the field, we stored collected leaf packs on ice and immediately returned them to the laboratory for further processing.

## 2.4 | Microbial community characterisation

We characterised bacterial and fungal communities by amplifying the V4 region of the bacterial 16S rRNA gene and the fungal ITS1 region using polymerase chain reaction (PCR), following previously published protocols (Caporaso et al., 2012; Sun, Li, Avera, Strahm, & Badgley, 2017). Briefly, total DNA was extracted from approximately 0.25 g of leaf material collected from coarse-mesh packs submersed for 150 days using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, U.S.A.). The PCR used targeted primer pairs Eub338 (5'-ACT CCT ACG GGA GGC AGC AG-3') and Eub518 (5'-ATT ACC GCG GCT GCT GG-3') for bacteria (Caporaso et al., 2012), and ITS1f (5'-TCC GTA GGT GAA CCT GCG G-3) and 5.8s (5'-CGC TCGCTTCTTCAT CG-3') for fungi (Bellemain et al., 2010). We amplified samples in triplicate on thermal cyclers (Bio-Rad, Hercules, CA, U.S.A.), then pooled and visualised them on agarose gels. Negative (no template) amplification controls were conducted for each sample using the correct barcoded primer pair. Each amplification control was visualised using gel electrophoresis to verify that no contamination was present prior to sequencing. We used an UltraClean PCR cleanup kit (Mo Bio Laboratories) to purify PCR products following amplification. Amplicons were sequenced on the Illumina MiSeq platform (250 bp paired end) at the Virginia Tech Biocomplexity Institute. Raw sequencing reads were deposited in the NCBI BioProject database (accession number: PRJNA484970).

## 2.5 | Macroinvertebrate community characterisation

We collected benthic macroinvertebrates using two methods to characterise richness and composition reach-wide and in leaf packs. For reach-wide characterisation, we composited macroinvertebrate samples from six 1  $\times$  0.3-m habitats using a 0.3-m D-frame kicknet (500- $\mu\text{m}$  mesh) along a 100-m reach at each study site in October 2015 (Barbour et al., 1999). Before preserving samples in 95% ethanol, we removed crustaceans and molluscs to avoid collecting at-risk species found in this region. We randomly sub-sampled reach-wide macroinvertebrates in the laboratory to obtain 200 ( $\pm$ 20) organisms (Barbour et al., 1999).

For leaf-pack characterisation, we removed all macroinvertebrates from leaf packs following 90 days of submersion. In the laboratory, we removed leaves from coarse-mesh packs and rinsed them over a 250- $\mu\text{m}$  sieve. Leaf fragments  $>1 \text{ cm}^2$  were removed from the sieve and the remaining sample (fine sediments, macroinvertebrates) was placed into a small white pan. Under a stereo microscope, we enumerated and identified macroinvertebrates to genus-level using a standard key (Merritt, Cummins, & Berg, 2008), except organisms in the families Chironomidae, Capniidae, Leuctridae, and sub-class Oligochaeta. Damaged or immature organisms were identified to the lowest practicable level. We assigned functional feeding groups

to identified taxa using a functional trait niche database (Poff et al., 2006).

## 2.6 | Organic matter decomposition

We measured leaf litter decomposition as a surrogate of organic matter decomposition following a standard protocol that involved removing leaf packs from streams at regular intervals (Benfield, 2006). In the laboratory, we removed leaves from leaf packs and rinsed samples over 250- $\mu$ m sieves to separate leaf material from mineral deposits and macroinvertebrates. We dried leaves until reaching constant dry mass (DM) in an oven (60°C) before aggregating, milling, and ashing leaves at 550°C. We calculated the % organic matter and multiplied by DM to obtain ash-free dry mass, from which % ash-free dry mass remaining was determined.

## 2.7 | Data analysis

We cleaned raw microbial sequencing reads using standard protocols (details in Supporting Information Appendix S3). Any operational taxonomic units (OTUs) identified as non-bacterial (chloroplast, mitochondrial, or archaeal) were removed. For downstream analyses, 10,000 bacterial 16S and 1,062 fungal ITS sequences were randomly selected for each sample to correct for differences in sequencing depth. We removed OTUs that (1) did not account for >0.1% of relative abundance in at least one sample and (2) were only found in one sample to prevent potential bias from misidentified or rare taxa from influencing microbial analyses. Rarefaction and removing rare species was done using *vegan* (Oksanen et al., 2013) and *OTUtable* (Linz et al., 2017) packages in R (version 3.2.2; R Core Team, 2017), respectively.

We tested effects of SC on microbial and macroinvertebrate taxa richness using non-parametric Spearman rank correlation. We averaged taxonomic richness at each site from all within-site replicate samples collected on the same day for microbes and leaf-pack macroinvertebrates. We used separate SC values in these tests based on the antecedent 150-, 365-, and 90-day periods preceding sample collection for microbial and for reach-wide, and leaf-pack macroinvertebrate samples to most accurately represent salinity experienced by these groups during the study period. We calculated rho using the R base package and JMP Pro (version 13; SAS Institute, Cary, NC, U.S.A.) software and a test  $\alpha = 0.05$ .

We tested effects of SC on microbial and macroinvertebrate community composition by correlating SC with non-metric multidimensional scaling (NMDS) axis scores. We averaged taxonomic relative abundance at each site from within-site replicate samples as described above. We then calculated the Bray-Curtis dissimilarity index from the abundance data and ordinated samples in two-dimensional space. We rotated NMDS solutions to maximise correlation of axis 1 with SC before computing rho of axis 1 scores and SC (Legendre & Legendre, 2012). Macroinvertebrate taxa axis 1 scores from NMDS were extracted to allow visualisation of taxa associations with sites along the salinity gradient. We used the

*vegan* package in R for dissimilarity index calculation, ordination, and NMDS rotation (Oksanen et al., 2013).

We performed Spearman rank correlations to determine responses to SC for the 127 and 69 most abundant (by mean relative abundance) bacterial and fungal OTUs, respectively. These OTUs constituted >70% and c. 90% of total OTU relative abundance, respectively.

We partitioned dissimilarity in community composition into two components, taxonomic replacement and nestedness, of  $\beta$ -diversity using a Sorenson-index with presence-absence data (Baselga, 2010). The replacement component accounts for taxa that are replaced by others across sites, whereas nestedness occurs when taxa are lost without replacement; that is, taxa at one site are a subset of taxa found at another site (Baselga, 2010). We used the *betapart* package in R for  $\beta$ -partitioning (Baselga, 2010).

Leaf decomposition rates were determined based on a first-order decay model (Benfield, 2006), with adjustments for mass loss incurred from handling during transport and installation of leaf packs. Rates were calculated separately for 90- and 150-day submersion periods for coarse-mesh packs and 90 days for fine-mesh packs.

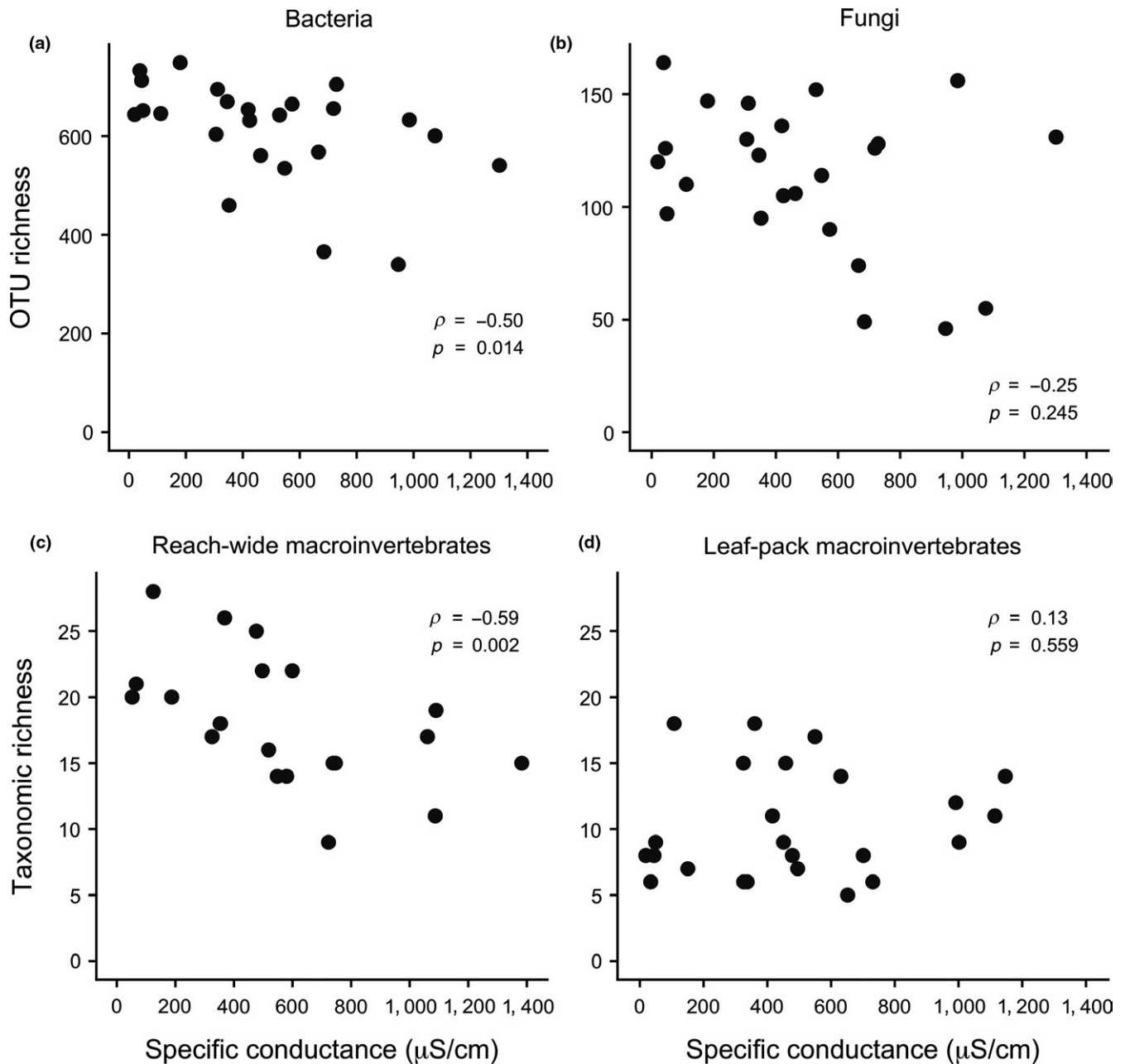
## 3 | RESULTS

### 3.1 | Salinity and water chemistry

Mean SC in the 24 streams ranged from 20 to 1,302  $\mu$ S/cm during the 150-day study period (November 2015–April 2016; Table 1) and similar to the antecedent 4 years (2011–2015) when mean SC over that period ranged from 24 to 1,431  $\mu$ S/cm (Timpano, Zipper, et al., 2018). Ionic composition of water was generally consistent among streams of the same type but did vary between reference and mining-influenced streams (Supporting Information Appendix S1). Reference stream waters were dominated by calcium ( $\text{Ca}^{2+}$ ), sodium ( $\text{Na}^+$ ), and bicarbonate ( $\text{HCO}_3^-$ ), whereas mining-influenced streams were dominated by  $\text{Ca}^{2+}$ , magnesium ( $\text{Mg}^{2+}$ ), sulfate ( $\text{SO}_4^{2-}$ ), and  $\text{HCO}_3^-$  (Supporting Information Appendix S1). Relative proportions of  $\text{Na}^+$ , potassium ( $\text{K}^+$ ), and chloride ( $\text{Cl}^-$ ) were generally low in both mining-influenced and reference streams (Supporting Information Appendix S1).

### 3.2 | Biological samples

A total of 2,308,907 bacterial (16S) and 300,443 fungal (ITS) sequences were processed from raw sequencing reads, averaging 39,134 and 4,622 reads per sample for bacterial ( $n = 59$ ) and fungal ( $n = 65$ ) samples, respectively. From these sequences, we identified 10,345 and 1,244 bacterial and fungal OTUs, respectively. For macroinvertebrates, we identified a total of 4,703 individuals from 120 taxa among all reach-wide samples ( $n = 24$ ) and 1,798 individuals from 56 taxa among all leaf-pack samples ( $n = 72$ ). *Allocapnia* (Plecoptera; 29%), *Diplectrona* (Trichoptera; 17%), Chironomidae (Diptera; 10%), *Cheumatopsyche* (Trichoptera; 5%), and *Hydropsyche* (Trichoptera; 4%) were the dominant macroinvertebrates in reach-wide samples



**FIGURE 2** Correlations of microbial and macroinvertebrate richness with specific conductance at 24 headwater stream sites. Lettering corresponds to bacterial (a), fungal (b), reach-wide macroinvertebrate (c) and leaf-pack macroinvertebrate (d) richness. Specific conductance values are site means from the 150, 365, and 90 days prior to collection of microbial, reach-wide, and leaf-pack macroinvertebrate communities, respectively

in terms of relative abundance. In leaf packs, relative abundance was highest for Chironomidae (59%), *Amphinemoura* (Plecoptera; 7%), *Diplectrona* (5%), Capniidae/Leuctridae (Plecoptera; 4%), and Oligochaeta (3%).

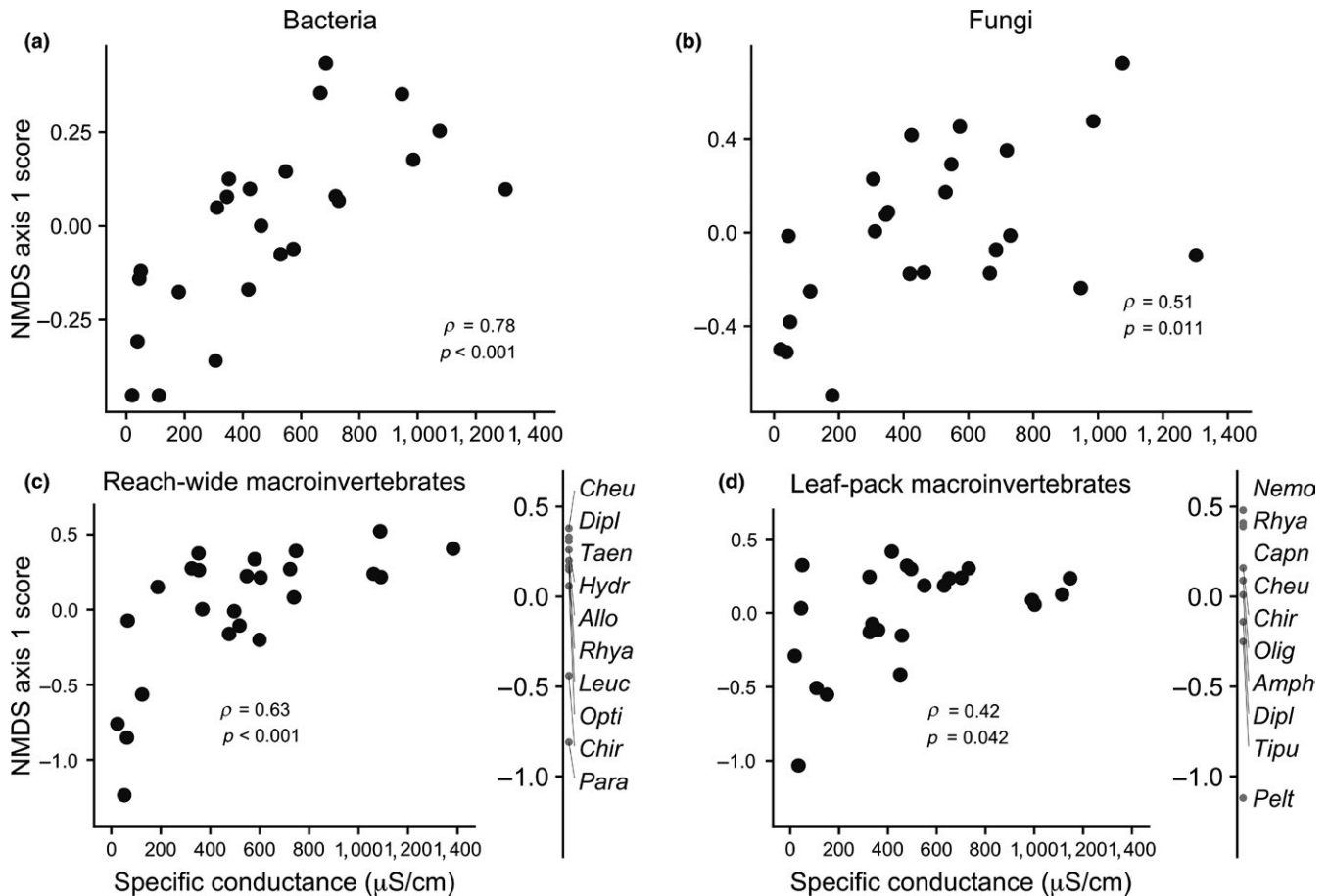
### 3.3 | Microbial and macroinvertebrate richness along salinity gradient

Bacterial richness was negatively correlated with SC ( $\rho = -0.50$ ,  $p = 0.014$ ); however, fungal richness showed no correlation with SC

( $\rho = -0.25$ ,  $p = 0.245$ , Figure 2a,b). Reach-wide macroinvertebrate richness was negatively correlated with SC ( $\rho = -0.59$ ,  $p = 0.002$ ), whereas leaf-pack macroinvertebrate richness was not correlated with SC ( $\rho = 0.13$ ,  $p = 0.559$ , Figure 2c,d).

### 3.4 | Microbial and macroinvertebrate community composition along salinity gradient

Microbial and macroinvertebrate taxonomic composition became increasingly dissimilar along the salinity gradient. Ordination revealed



**FIGURE 3** Correlations of microbial and macroinvertebrate taxonomic composition with specific conductance at 24 headwater streams. Non-metric multidimensional scaling axis 1 scores were generated from Bray–Curtis dissimilarity indices and rotated to best visualise changes in composition in relation to specific conductance. Lettering corresponds to bacterial (a), fungal (b), reach-wide macroinvertebrate (c), and leaf-pack macroinvertebrate (d) composition. Specific conductance values are site means from the 150, 365, and 90 days prior to collection of microbial, reach-wide, and leaf-pack macroinvertebrate communities, respectively. Insets show macroinvertebrate species scores NMDS axis 1 (Allo, *Allocapnia*; Amph, *Amphinemura*; Capn, Capniidae/Leuctridae; Cheu, *Cheumatopsyche*; Chir, Chironomidae; Dipl, *Diplectrona*; Hydr, *Hydropsyche*; Leuc, *Leuctra*; Nemo, *Nemoura*; Oligo, Oligochaeta; Optio, *Optioservus*; Para, *Paracapnia*; Pelt, Peltoperlidae; Rhya, *Rhyacophila*; Taen, *Taeniopteryx*; Tipl, Tipulidae)

that SC was correlated with bacterial ( $\rho = 0.78$ ,  $p < 0.001$ ) and fungal ( $\rho = 0.51$ ,  $p = 0.011$ ) composition (Figure 3a,b). For macroinvertebrates, taxonomic composition was correlated with SC for reach-wide ( $\rho = 0.63$ ,  $p < 0.001$ ) and leaf-pack samples ( $\rho = 0.42$ ,  $p = 0.042$ ; Figure 3c,d).

Bacterial and fungal OTUs varied in the direction and strength of their response to SC (Supporting Information Appendix S4). Relative abundance of 25 of the 127 most abundant bacterial OTUs varied negatively with increasing SC, whereas relative abundance of 13 bacterial OTUs increased with SC (Supporting Information Appendix S4). When bacterial OTUs were analysed at the phylum and sub-phylum levels, relative abundances of Verrucomicrobia increased with SC, whereas relative abundances of Cyanobacteria and Gammaproteobacteria decreased with SC (Supporting Information Appendix S4). Relative abundances of 15 of the 69 most abundant fungal OTUs varied negatively with increasing SC, whereas relative abundances of two fungal OTUs increased with SC (Supporting Information Appendix S4). Fungal relative abundances at the

phylum and sub-phylum level did vary in response to SC (Supporting Information Appendix S4).

*Cheumatopsyche*, *Diplectrona*, Taeniopterygidae, *Hydropsyche*, *Allocapnia*, *Rhyacophila*, *Leuctra*, and *Optioservus* were the dominant taxa associated with sites in the higher range of SC, whereas Chironomidae and *Paracapnia* associated with sites in the lower SC range in reach-wide samples (Figure 3c). For leaf-pack samples, *Nemoura*, *Rhyacophila*, Capniidae/Leuctridae, Chironomidae, *Cheumatopsyche*, and Oligochaeta were the dominant taxa associated with sites in the higher SC range, whereas *Amphinemura*, *Diplectrona*, Tipulidae, and *Peltoperla* were associated with sites in the lower SC range (Figure 3d).

### 3.5 | Beta diversity of microbial and macroinvertebrate communities

Dissimilarity of microbial and macroinvertebrate communities was dominated by taxonomic replacement rather than nestedness (Table 2).

$\beta$ measure	Bacteria	Fungi	Reach-wide macroinvertebrates	Leaf-pack macroinvertebrates
$\beta_{SIM}$	0.623	0.803	0.828	0.835
$\beta_{SNE}$	0.112	0.060	0.050	0.059
$\beta_{SOR}$	0.735	0.863	0.878	0.894

**TABLE 2** Microbial and macroinvertebrate dissimilarity partitioned into replacement ( $\beta_{SIM}$ ) and nestedness ( $\beta_{SNE}$ ) components of total dissimilarity ( $\beta_{SOR}$ )

Replacement ( $\beta_{SIM}$ ) represented 83% and 93% of total dissimilarity ( $\beta_{SOR}$ ) for bacteria and fungi, respectively (Table 2). For macroinvertebrates, replacement represented 88% and 94% of reach-wide and leaf-pack macroinvertebrate total dissimilarity, respectively (Table 2).

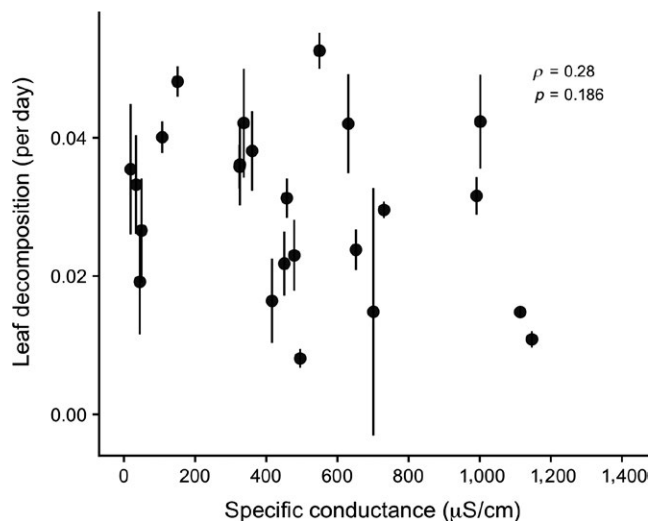
### 3.6 | Organic matter decomposition across mining-induced salinity gradient

Leaf decomposition rates were not correlated with SC in coarse-mesh ( $\rho = 0.28$ ,  $p = 0.186$ , Figure 4) and fine-mesh ( $\rho = 0.05$ ,  $p = 0.809$ , Supporting Information Appendix S5) leaf packs during a 90-day submersion period. Similarly, coarse-mesh decomposition rate based on a 150-day submersion period was not correlated with SC ( $\rho = 0.04$ ,  $p = 0.840$ , not shown).

A total of 12 macroinvertebrate shredder taxa were found in coarse-mesh leaf packs, with *Amphinemura* (Plecoptera:Nemouridae), Capniidae/Leuctridae (Plecoptera), *Tipula* (Diptera:Tipulidae) and *Peltoperla* (Plecoptera:Peltoperlidae) being the most abundant. Leaf-pack shredder abundance was positively correlated with SC ( $\rho = 0.55$ ,  $p = 0.005$ , Figure 5), whereas leaf-pack shredder richness was not correlated with SC ( $\rho = 0.28$ ,  $p = 0.176$ , not shown).

## 4 | DISCUSSION

Natural levels of salinity shape the richness and composition of aquatic communities but less is known about how communities



**FIGURE 4** Leaf decomposition rates (mean  $\pm$  1 SE) in coarse-mesh packs at 24 headwater streams over a 90-day period. Specific conductance values are site means during the same period

respond to anthropogenic salinisation. Across a mining-induced salinity gradient ranging from SC of 25–1,383  $\mu\text{S}/\text{cm}$  (annual mean) in 24 headwater streams, we found bacterial and reach-wide macroinvertebrates richness were reduced, but contrary to our predictions we did not find changes in fungal and leaf-pack macroinvertebrates richness. Bacteria, fungi, and both reach-wide and leaf-pack macroinvertebrate community composition in mining-influenced streams became increasingly dissimilar to those at reference streams as mining-induced salinity increased. Beta-diversity across sites was driven mainly by taxonomic replacement rather than nestedness in all communities.

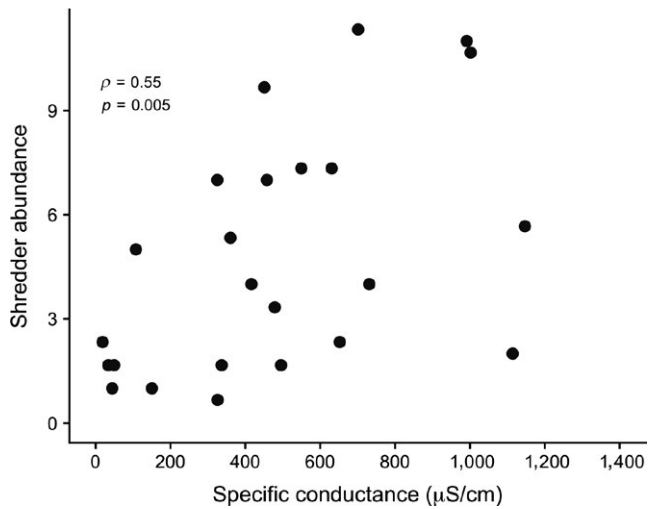
To better understand potential changes in organic matter decomposition, we measured leaf litter decomposition rates but found no statistically significant relationship between decomposition and salinity. Leaf decomposition was probably maintained through taxonomic replacement of sensitive taxa by salinity-tolerant fungi and macroinvertebrate shredders in our study streams. Our results address the relatively unexplored relationships between anthropogenic salinisation and aquatic communities and inform a critical aspect of freshwater management-related salinisation effects on ecosystem functions.

### 4.1 | Salinity as a limiting factor for biological communities in mining-influenced streams

In our study, we selected sites that allowed measuring ecosystem responses along a salinity gradient where most non-salinity factors, such as habitat quality, hydrology, and water-quality differences other than major-ion concentrations (Timpano et al., 2015) in mining-influenced streams were similar to those of reference streams. Mining-influenced and reference streams in this study had similar habitat quality (Table 1) and all study sites were first-order streams with similar mean catchment areas (Table 1), which suggests similarities in mean discharge. Although mining activities can influence infiltration and baseflows (Evans, Zipper, Hester, & Schoenholtz, 2015), a prior study demonstrated that seasonal variation of salinity is similar for our mining-influenced and reference sites and that these similarities are a result of similar seasonal hydrologic variation (Timpano, Zipper, et al., 2018).

We also attempted to minimise potential differences of ion matrix and trace elements across study sites. First, ionic composition of stream water varied among sites (Appendix S1) as a direct consequence of the geochemical processes that produce elevated salinity in mined landscapes (Clark, Daniels, Zipper, & Eriksson, 2018). Therefore, ionic composition did not vary independently from SC (Timpano et al., 2017), making SC a suitable surrogate for





**FIGURE 5** Macroinvertebrate shredder abundance in leaf packs at 24 headwater streams after 90 days. Specific conductance values are site means during the same period

salinity while limiting our ability to assess the differential effects of individual ions as done in previous studies (Clements & Kotalik, 2016; Mount, Gulley, Hockett, Garrison, & Evans, 1997). Second, trace elements Al, Cu, Fe, and Zn were below criteria continuous concentrations (CCC) for aquatic life in quarterly samples collected during our study (Appendix S2) indicating a likely lack of toxicity for macroinvertebrates (USEPA, 2004). Selenium was generally below the CCC of 3.1 µg/L for macroinvertebrates (USEPA, 2016), but we observed Se greater than the CCC at four mining-influenced streams in this study and in a prior study (Timpano, Schoenholtz, et al., 2018) and therefore cannot rule out the potential influence of Se in our results. Selenium concentrations observed in four of our mining-influenced streams were within the range of those with potential to influence fecundity but below those likely to influence survival of an Ephemeroptera taxon, *Centroptilum triangulifer* (Conley, Funk, & Buchwalter, 2009; Whitmore, Schoenholtz, Soucek, Hopkins, & Zipper, 2018). For microbial communities, less is known about tolerance to trace elements; hence, we cannot assume microbial tolerance for all trace elements associated with mining effluent though bacteria are considered tolerant to Se (Janz et al., 2010).

Lastly, alteration of nutrients (e.g. N, P) and C downstream of mined lands are potential confounding factors. Prior studies of Appalachian mining have found N enrichment to co-occur with salinisation, but with P remaining at levels below detection limits of 0.05 mg/L (Pond et al., 2008) and 0.007 mg/L (Krenz, Schoenholtz, & Zipper, 2016). Although nutrients were not assessed in this study, careful selection of our study sites in catchments where non-mined areas are predominantly forests and devoid of other developed land uses (Timpano, Zipper, et al., 2018) ensures minimal influence by nutrients from non-mining sources, and suggests a likelihood of similar C dynamics across our salinity gradient. Therefore, we conclude that salinity was probably the primary limiting factor of our observed responses in bacteria, fungi, macroinvertebrates, and leaf

litter decomposition but we cannot rule out influence by all other potential co-occurring factors associated with mining.

Several studies have concluded that salinisation is the primary causative stressor, likely due to osmoregulatory effects, despite the presence of potential confounding influences (Bernhardt et al., 2012; Cormier et al., 2013; Pond et al., 2008; Timpano et al., 2015). Pond et al. (2008) applied causal-influence logic and concluded that the altered ionic strength of streams receiving mining effluents satisfied six of eight relevant criteria for causation of the macroinvertebrate community alterations observed. Similarly, Cormier et al. (2013) applied a six-step weight-of-evidence approach to assess causation for observed extirpations of multiple macroinvertebrate taxa from mining-influenced streams in Central Appalachia and found salinity to be the primary cause. Appalachian mining effects on microbial communities, however, are not well studied, and we are aware of no similar mining induced causality assessments for alterations of microbial communities.

#### 4.2 | Microbial and macroinvertebrate richness along mining-induced salinity gradient

Our results confirm potential consequences of salinisation on aquatic community richness by showing that negative salinity-richness relationships can occur even over relatively moderate gradients of salinity. Previous studies attributed reductions in microbial richness to mining pollution (Bier, Voss, & Bernhardt, 2015; Lecerf & Chauvet, 2008). Lecerf and Chauvet (2008), using microscope identification, found fungal species richness was reduced by >50% in a mining-influenced stream compared to a control stream (SC mean = 575 µS/cm versus 170 µS/cm). In our study, bacterial richness decreased and fungal richness did not change with an increase in salinity. Despite broad-scale efforts to measure aquatic microbial richness across salinity gradients (Herlemann et al., 2011; Lozupone & Knight, 2007; Mohamed & Martiny, 2011), additional studies of salinity effects on freshwater bacterial and fungal communities are clearly required before generalising salinity-microbial richness relationships.

Macroinvertebrate richness frequently declines with increasing salinity and our study partially corroborates previous research on this topic, at least at the reach scale (Cañedo-Arguelles et al., 2013; Pond et al., 2008). Conceptually, salinisation acts as an environmental filter of the regional macroinvertebrate species pool that limits local species richness to salinity-tolerant species (Kefford et al., 2016; Voss & Bernhardt, 2017). Indeed, several macroinvertebrate taxa inhabiting headwater streams are locally-extirpated at elevated salinity levels, especially members of the orders Ephemeroptera, Plecoptera, and Trichoptera (Pond, 2012; Pond et al., 2008). At the reach scale, our results here and in a previous study of these sites (Timpano, Schoenholtz, et al., 2018; Timpano, Zipper, et al., 2018) bring further empirical support to the environmental filtering concept as it relates to mining-induced salinity. Within leaf packs, however, we found no correlation between richness and salinity, indicating the importance of scale when investigating biotic responses to salinity. A likely explanation for this lack of relationship with

salinity is that we artificially imposed a separate and influential environmental filter by measuring richness from leaf packs, essentially filtering the local species richness to mainly detritivorous invertebrates. Therefore, our results suggest that salinisation can have negative consequences on aquatic richness but careful consideration must be made regarding the spatial scales and ecological roles to which these relationships are inferred. In efforts aiming to explore salinisation effects on specific ecological processes, such as organic matter decomposition, the leaf-pack scale, or the media of choice, is arguably the most appropriate scale at which to measure richness.

### 4.3 | Microbial and macroinvertebrate community composition and $\beta$ -diversity along mining-induced salinity gradient

Although alteration of microbial and macroinvertebrate community composition has been shown in mining-influenced streams, our study helps isolate the importance of salinity from other commonly occurring changes. Our results show bacterial and fungal community composition is strongly influenced by salinity in streams where mining-induced salinity is the dominant stressor, whereas previous work exploring mining impacts on microbial communities suggests that alteration occurs through heavy-metal and pH changes (Bier et al., 2015; Duarte, Pascoal, & Cássio, 2004; Ferreira, Gulis, Pascoal, & Graca, 2014). For example, zinc altered the relative abundances of aquatic hyphomycetes (fungi) on leaves (*Alnus glutinosa*) without major reductions of species richness in laboratory microcosms (Duarte et al., 2004). Although some taxonomic changes observed in our study are difficult to interpret because of limited ecological knowledge of the groups, such as Verrucomicrobia, other changes suggest that anthropogenic salinisation led to important compositional shifts. For example, the decrease in Cyanobacteria with increasing salinity found here indicates potential negative impacts to instream primary productivity, unless other primary producers (e.g. algae) are thus freed from competition with Cyanobacteria (Danger, Oumarou, Benest, & Lacroix, 2007). However, identifying taxonomic groups that responded to salinity was difficult because of current taxonomic resolution of microbial communities. To further understand microbial compositional changes along mining-induced salinity gradients and links to ecosystem functions, more precise taxonomic and functional characterisation of OTUs is required (Bier et al., 2015).

Alteration of macroinvertebrate communities is evident from mining-induced stressors in Appalachian streams (Fritz et al., 2010; Pond et al., 2008; Voss & Bernhardt, 2017). For example, Pond et al. (2008) showed changes in composition across 27 mining-influenced stream sites compared to 10 reference sites in the central Appalachians of West Virginia. Changes in macroinvertebrate community composition are often related to the loss of salinity-sensitive Ephemeroptera (Fritz et al., 2010; Pond et al., 2008; Voss & Bernhardt, 2017). Our results and those from a concurrent study (Timpano, Schoenholtz, et al., 2018), confirm that Ephemeroptera (except Baetidae) are greatly reduced or absent from mining-influenced

Appalachian streams. In contrast, some Trichoptera (e.g. *Hydropsche*, *Cheumatopsyche*, *Diplectrona*, *Rhyacophila*) and Plecoptera (e.g. *Leuctra*, *Allocapnia*, *Amphinemura*, Taeniopterygidae) appeared tolerant to the salinity gradient studied here.

Partitioning dissimilarity in community composition provides insights into the processes structuring communities along our salinity gradient. For bacteria, fungi, and macroinvertebrates, dissimilarity in composition was mainly caused by taxonomic replacement, meaning that as salinity increased, taxa lost were replaced by others. Evidence of replacement in bacterial communities along salinity gradients from high-mountain lakes (Wu, Zwart, Schauer, Kamst-Van Agterveld, & Hahn, 2006) to estuaries (Bouvier & Del Giorgio, 2002) suggests this may be a common process for microbial communities. Indeed, diverse bacterial communities exist in non-saline and hypersaline environments (Lozupone & Knight, 2007) making it likely that salinity-tolerant taxa colonised salinised habitats. Similarly, Kefford et al. (2016) hypothesised that some replacement by salinity-tolerant macroinvertebrates could occur in salinised streams but there are likely to be fewer salinity-tolerant than sensitive taxa able to colonise. Our results do not support this hypothesis but this could be because the salinity gradient studied here was relatively moderate in comparison to other systems (e.g. Kefford et al., 2011), including other mining-influenced streams (Fritz et al., 2010; Ladrera, Cañedo-Argüelles, & Prat, 2017). We expect that macroinvertebrate replacement occurs at low to moderate increases in salinity above natural levels, as shown here, but nestedness is likely to occur at higher salinity levels. For example, Fritz et al. (2010) found SC was up to 57× higher (max SC > 3,000  $\mu\text{S}/\text{cm}$ ) and leaf-pack macroinvertebrate richness was c. 50% lower in mining-influenced streams compared to forested streams. This suggests that, at higher levels of salinisation than studied here, fewer salinity-tolerant taxa will be able to replace those lost and communities in salinised streams will become increasingly nested.

### 4.4 | Maintaining organic matter decomposition in salinised freshwater ecosystems

Mining-induced salinisation had no adverse effect on leaf decomposition rates despite the aforementioned alterations to microbial and macroinvertebrate communities, suggesting the importance of species identity and abundance to ecosystem function. For example, we found fungi and shredder macroinvertebrates, two key decomposers of organic matter, were relatively tolerant to mining-induced salinisation. Fungi are the dominant microbial group involved in decomposition in streams and in some instances constitute up to 95–99% of total microbial biomass on decomposing leaves, whereas macroinvertebrate shredders can contribute greatly to leaf mass loss (e.g. 51–64%; Hieber & Gessner, 2002). Therefore, increases in shredder abundance found here likely helped maintain decomposition rates, especially if total biomass also increased (Dangles & Malmqvist, 2004). Future studies of microbial and macroinvertebrate biomass along salinity gradients could provide additional insights into ecosystem function responses to salinity.

Our results showing no reduction in leaf litter decomposition differ from findings in other studies examining litter decomposition in mining-influenced streams. However, comparing the salinity gradient studied here to other systems suggests that salinity is likely to affect leaf decomposition at much higher levels. For example, Cañedo-Argüelles et al. (2014) found that decomposition (*Populus nigra*) in mesocosms with repeated salt pulses was only reduced in treatments with SC concentrations of 15,000  $\mu\text{S}/\text{cm}$ . At lower salinity levels, such as those studied here, both invertebrates and fungal communities may be able to maintain normal levels of leaf decomposition (Cañedo-Argüelles et al., 2014; Canhoto et al., 2017). Interestingly, fungal communities may shift energy resources from sporulation to biomass accrual at elevated salinity levels as a stress response, such that decomposition can increase at elevated salinity levels despite lower diversity (Canhoto et al., 2017). However, when salinisation is coupled with other stressors, leaf decomposition has been shown to be drastically reduced. For example, Schäfer et al. (2012) found as much as a 4 $\times$  reduction in organic matter decomposition (*Eucalyptus camaldulensis* and cotton strips) associated with the combination of agricultural pesticides and salinity (>1,000  $\mu\text{S}/\text{cm}$ ) compared to less-impacted sites in Australia. In our study region, Fritz et al. (2010) found leaf breakdown rates per degree day were reduced >50% in valley-fill-mining streams, most of which had been reconstructed, compared to those in forested catchments as a likely result of the combination of elevated SC and alterations to stream habitat quality that influences biotic communities. Similarly, Krenz et al. (2016) found reduced rates of leaf litter decomposition in reconstructed streams with elevated SC. However, Krenz et al. (2016) found no relationship of leaf-litter breakdown rates to mean SC (range: c. 200–1,800  $\mu\text{S}/\text{cm}$ ) within the reconstructed streams, suggesting the importance of habitat quality rather than moderately elevated salinity in leaf decomposition rates. Our results build upon these previous studies to show maintenance of leaf decomposition at salinity levels up to 1,383  $\mu\text{S}/\text{cm}$  when other potential stressors are minimised and habitat quality is maintained.

Future work should aim to better understand potential effects of anthropogenic salinisation on other important ecosystem functions. Although leaf decomposition is an important function in headwater streams, alteration of species richness and composition could influence primary and secondary production. For example, Timpano et al. (2015) found that the proportion of macroinvertebrate scrapers, which feed on attached algae and biofilms, decreased by nearly 70% in mining-influenced versus reference streams within our study region. Effects of scraper loss on primary production should therefore be quantified in salinised streams. Local extirpation of sensitive aquatic insects (e.g. Ephemeroptera) caused by salinisation could reduce total insect emergence and alter this subsidy to terrestrial systems in mining-influenced streams, highlighting potential effects of salinity on secondary production (Voss & Bernhardt, 2017).

There is a growing need to understand how anthropogenic stressors such as salinity influence ecosystem functions via changes in the communities driving those functions. Quantifying

ecosystem functions has even become a way to assess the ecological integrity of freshwater ecosystems, moving beyond the use of structural measures of communities (Castela, Ferreira, & Graça, 2008). Our results suggest that conclusions about the impact of salinity based on measures of richness and composition will not always match those based on measures of ecosystem functions. We show that richness is not a robust measure of community structural response to salinity in these systems, given the substantial taxonomic replacement that occurred over the salinity gradient. Community composition was altered along the salinity gradient; however, these changes did not manifest as reductions in organic matter decomposition. Therefore, changes in community structure and ecosystem function as influenced by salinity are a critical consideration when designing research questions and management objectives related to anthropogenic salinisation.

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## CONFLICT OF INTEREST

The authors have no conflicts of interest to acknowledge.

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## REFERENCES

- Barbour, M. T., Gerritsen, J., Snyder, B., & Stribling, J. (1999) Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC.
- Barlocher, F. (2016). Aquatic hyphomycetes in a changing environment. *Fungal Ecology*, 19, 14–27. <https://doi.org/10.1016/j.funeco.2015.05.005>
- Baselga, A. (2010). Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 19, 134–143. <https://doi.org/10.1111/j.1466-8238.2009.00490.x>
- Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., & Kausserud, H. (2010). ITS as an environmental DNA barcode for fungi: An in silico approach reveals potential PCR biases. *BMC Microbiology*, 10, 189. <https://doi.org/10.1186/1471-2180-10-189>
- Benfield, E. (2006). Decomposition of leaf material. In F. R. Hauer, & G. A. Lamberti (Eds.), *Methods in stream ecology* (2nd ed., pp. 711–720). San Diego, CA: Academic Press.

- Bernhardt, E. S., Lutz, B. D., King, R. S., Fay, J. P., Carter, C. E., Helton, A. M., ... Amos, J. (2012). How many mountains can we mine? Assessing the regional degradation of central Appalachian rivers by surface coal mining. *Environmental Science & Technology*, 46, 8115–8122. <https://doi.org/10.1021/es301144q>
- Bier, R. L., Voss, K. A., & Bernhardt, E. S. (2015). Bacterial community responses to a gradient of alkaline mountaintop mine drainage in Central Appalachian streams. *ISME Journal*, 9, 1378–1390. <https://doi.org/10.1038/ismej.2014.222>
- Bouvier, T. C., & Del Giorgio, P. A. (2002). Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. *Limnology and Oceanography*, 47, 453–470. <https://doi.org/10.4319/lo.2002.47.2.0453>
- Cañedo-Argüelles, M., Bundschuh, M., Gutiérrez-Cánovas, C., Kefford, B. J., Prat, N., Trobajo, R., & Schäfer, R. B. (2014). Effects of repeated salt pulses on ecosystem structure and functions in a stream mesocosm. *Science of the Total Environment*, 476–477, 634–642. <https://doi.org/10.1016/j.scitotenv.2013.12.067>
- Cañedo-Argüelles, M., Hawkins, C., Kefford, B., Schäfer, R., Dyack, B., Brucet, S., ... Lazorchak, J. (2016). Saving freshwater from salts. *Science*, 351, 914–916. <https://doi.org/10.1126/science.aad3488>
- Cañedo-Argüelles, M., Kefford, B. J., Piscart, C., Prat, N., Schafer, R. B., & Schulz, C. J. (2013). Salinisation of rivers: An urgent ecological issue. *Environmental Pollution*, 173, 157–167. <https://doi.org/10.1016/j.envpol.2012.10.011>
- Canhoto, C., Simões, S., Gonçalves, A. L., Guilhermino, L., & Bärlocher, F. (2017). Stream salinization and fungal-mediated leaf decomposition: A microcosm study. *Science of the Total Environment*, 599–600, 1638–1645. <https://doi.org/10.1016/j.scitotenv.2017.05.101>
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., ... Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, 6, 1621. <https://doi.org/10.1038/ismej.2012.8>
- Cardinale, B. J., Srivastava, D. S., Emmett Duffy, J., Wright, J. P., Downing, A. L., Sankaran, M., & Jouseau, C. (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*, 443, 989–992. <https://doi.org/10.1038/nature05202>
- Castela, J., Ferreira, V., & Graça, M. A. (2008). Evaluation of stream ecological integrity using litter decomposition and benthic invertebrates. *Environmental Pollution*, 153, 440–449. <https://doi.org/10.1016/j.envpol.2007.08.005>
- Clark, E. V., Daniels, W. L., Zipper, C. E., & Eriksson, K. (2018). Mineralogical influences on water quality from weathering of surface coal mine spoils. *Applied Geochemistry*, 91, 97–106. <https://doi.org/10.1016/j.apgeochem.2018.02.001>
- Clements, W. H., & Kotalki, C. (2016). Effects of major ions on natural benthic communities: An experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity. *Freshwater Science*, 35, 126–138. <https://doi.org/10.1086/685085>
- Conley, J. M., Funk, D. H., & Buchwalter, D. B. (2009). Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. *Environmental Science and Technology*, 43, 7952–7957. <https://doi.org/10.1021/es9016377>
- Cormier, S. M., Suter, G. W., Zheng, L., & Pond, G. J. (2013). Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions. *Environmental Toxicology and Chemistry*, 32, 277–287. <https://doi.org/10.1002/etc.2059>
- Danger, M., Oumarou, C., Benest, D., & Lacroix, G. (2007). Bacteria can control stoichiometry and nutrient limitation of phytoplankton. *Functional Ecology*, 21, 202–210. <https://doi.org/10.1111/j.1365-2435.2006.01222.x>
- Dangles, O., & Malmqvist, B. (2004). Species richness–decomposition relationships depend on species dominance. *Ecology Letters*, 7, 395–402. <https://doi.org/10.1111/j.1461-0248.2004.00591.x>
- De Laender, F., Rohr, J. R., Ashauer, R., Baird, D. J., Berger, U., Eisenhauer, N., ... Van Den Brink, P. J. (2016). Reintroducing environmental change drivers in biodiversity-ecosystem functioning research. *Trends in Ecology & Evolution*, 31, 905–915. <https://doi.org/10.1016/j.tree.2016.09.007>
- Duarte, S., Pascoal, C., & Cássio, F. (2004). Effects of zinc on leaf decomposition by fungi in streams: Studies in microcosms. *Microbial Ecology*, 48, 366–374. <https://doi.org/10.1007/s00248-003-2032-5>
- Evans, D. M., Zipper, C. E., Hester, E. T., & Schoenholtz, S. H. (2015). Hydrologic effects of surface coal mining in Appalachia (U.S.). *JAWRA Journal of the American Water Resources Association*, 51, 1436–1452. <https://doi.org/10.1111/1752-1688.12322>
- Ferreira, V., Gulis, V., Pascoal, C., & Graca, M. A. S. (2014). Stream pollution and fungi. In E. B. Gareth Jones, K. D. Hyde & K. Pang (Eds.), *Freshwater fungi and fungal-like organisms* (pp. 388–412). Berlin, Germany: Walter de Gruyter.
- Finn, D. S., Bonada, N., Múrria, C., & Hughes, J. M. (2011). Small but mighty: Headwaters are vital to stream network biodiversity at two levels of organization. *Journal of the North American Benthological Society*, 30, 963–980. <https://doi.org/10.1899/11-012.1>
- Fritz, K. M., Fulton, S., Johnson, B. R., Barton, C. D., Jack, J. D., Word, D. A., & Burke, R. A. (2010). Structural and functional characteristics of natural and constructed channels draining a reclaimed mountaintop removal and valley fill coal mine. *Journal of the North American Benthological Society*, 29, 673–689. <https://doi.org/10.1899/09-060.1>
- Gomi, T., Sidle, R. C., & Richardson, J. S. (2002). Understanding processes and downstream linkages of headwater systems. *BioScience*, 52, 905–916. [https://doi.org/10.1641/0006-3568\(2002\)052\[0905:upadl0\]2.0.co;2](https://doi.org/10.1641/0006-3568(2002)052[0905:upadl0]2.0.co;2)
- Griffith, M. B. (2014). Natural variation and current reference for specific conductivity and major ions in wadeable streams of the conterminous USA. *Freshwater Science*, 33, 1–17. <https://doi.org/10.1086/674704>
- Hem, J. D. (1985). *Study and interpretation of the chemical characteristics of natural water* (3rd ed., 263 pp.). U.S. Geological Survey Water-supply Paper 2254. Alexandria, VA: U.S. Department of Interior.
- Herlemann, D. P. R., Labrenz, M., Jurgens, K., Bertilsson, S., Waniek, J. J., & Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *The ISME Journal*, 5, 1571–1579. <https://doi.org/10.1038/ismej.2011.41>
- Hieber, M., & Gessner, M. O. (2002). Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, 83, 1026–1038. [https://doi.org/10.1890/0012-9658\(2002\)083\[1026:cosdfa\]2.0.co;2](https://doi.org/10.1890/0012-9658(2002)083[1026:cosdfa]2.0.co;2)
- Janz, D., De Forest, D., Brooks, M., Chapman, P., Golron, G., Hoff, D., ... Wayland, M. (2010). Selenium toxicity to aquatic organisms. Chapter 6, ecological assessment of selenium in the aquatic environment. In P. M. Chapman, W. J. Adams, M. L. Brooks, C. G. Delos, S. N. Luoma, W. A. Maher, H. M. Ohlendorf, T. S. Presser, & D. P. Shaw (Eds.), *Ecological assessment of selenium in the aquatic environment* (pp. 141–231). Boca Raton, FL: CRC Press - Taylor & Francis Group, LLC. <https://doi.org/10.1201/ebk1439826775>
- Jonsson, M., & Malmqvist, B. (2000). Ecosystem process rate increases with animal species richness: Evidence from leaf-eating, aquatic insects. *Oikos*, 89, 519–523. <https://doi.org/10.1034/j.1600-0706.2000.890311.x>
- Kaushal, S. S., Likens, G. E., Pace, M. L., Utz, R. M., Haq, S., Gorman, J., & Grese, M. (2018). Freshwater salinization syndrome on a continental scale. *Proceedings of the National Academy of Sciences of the United States of America*, 115(4), E574–E583. <https://doi.org/10.1073/pnas.1711234115>
- Kefford, B. J., Buchwalter, D., Cañedo-Argüelles, M., Davis, J., Duncan, R. P., Hoffmann, A., & Thompson, R. (2016). Salinized rivers: Degraded

- systems or new habitats for salt-tolerant faunas? *Biology Letters*, 12, 20151072. <https://doi.org/10.1098/rsbl.2015.1072>
- Kefford, B. J., Marchant, R., Schäfer, R. B., Metzeling, L., Dunlop, J. E., Choy, S. C., & Goonan, P. (2011). The definition of species richness used by species sensitivity distributions approximates observed effects of salinity on stream macroinvertebrates. *Environmental Pollution*, 159, 302–310. <https://doi.org/10.1016/j.envpol.2010.08.025>
- Krenz, R. J., Schoenholtz, S. H., & Zipper, C. E. (2016). Riparian subsidies and hierarchical effects of ecosystem structure on leaf breakdown in Appalachian coalfield constructed streams. *Ecological Engineering*, 97, 389–399. <https://doi.org/10.1016/j.ecoleng.2016.10.012>
- Ladrera, R., Cañedo-Argüelles, M., & Prat, N. (2017). Impact of potash mining in streams: The Llobregat basin (northeast Spain) as a case study. *Journal of Limnology*, 76(2), 343–354. <https://doi.org/10.4081/jlimnol.2016.1525>
- Lecerf, A., & Chauvet, E. (2008). Diversity and functions of leaf-decaying fungi in human-altered streams. *Freshwater Biology*, 53, 1658–1672. <https://doi.org/10.1111/j.1365-2427.2008.01986.x>
- Legendre, P., & Legendre, L. (2012). *Numerical ecology* (3rd ed.). Amsterdam, The Netherlands: Elsevier B.V.
- Lindberg, T. T., Bernhardt, E. S., Bier, R., Helton, A. M., Merola, R. B., Vengosh, A., & Di Giulio, R. T. (2011). Cumulative impacts of mountaintop mining on an Appalachian watershed. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 20929–20934. <https://doi.org/10.1073/pnas.1112381108>
- Linz, A. M., Crary, B. C., Shade, A., Owens, S., Gilbert, J. A., Knight, R., & McMahon, K. D. (2017). Bacterial community composition and dynamics spanning five years in freshwater bog lakes. *mSphere*, 2, e00169–17. <https://doi.org/10.1128/msphere.00169-17>
- Lozupone, C. A., & Knight, R. (2007). Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 11436–11440. <https://doi.org/10.1073/pnas.0611525104>
- Merritt, R. W., Cummins, K. W., & Berg, M. (2008). *An introduction to the aquatic insects of North America* (4th ed.). Dubuque, IA: Kendall Hunt.
- Meyer, J. L., Strayer, D. L., Wallace, J. B., Eggert, S. L., Helfman, G. S., & Leonard, N. E. (2007). The contribution of headwater streams to biodiversity in river networks. *Journal of the American Water Resources Association*, 43, 86–103. <https://doi.org/10.1111/j.1752-1688.2007.00008.x>
- Mohamed, D. J., & Martiny, J. B. H. (2011). Patterns of fungal diversity and composition along a salinity gradient. *The ISME Journal*, 5, 379–388. <https://doi.org/10.1038/ismej.2010.137>
- Mount, D. R., Guley, D. D., Hockett, J. R., Garrison, T. D., & Evans, J. M. (1997). Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (fathead minnows). *Environmental Toxicology and Chemistry*, 16, 2009–2019. [https://doi.org/10.1897/1551-5028\(1997\)016<2009:smtptt>2.3.co;2](https://doi.org/10.1897/1551-5028(1997)016<2009:smtptt>2.3.co;2)
- Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., ... Wagner, H. (2013) *Vegan: Community ecology package. R package version 2.0-9*. Retrieved from <http://cran.r-project.org/package=vegan>
- Oren, A. (2002). Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. *Journal of Industrial Microbiology and Biotechnology*, 28, 56–63. <https://doi.org/10.1038/sj/jim/7000176>
- Palmer, M. A., Bernhardt, E. S., Schlesinger, W. H., Eshleman, K. N., Foufoula-Georgiou, E., Hendryx, M. S., ... Wilcock, P. R. (2010). Mountaintop mining consequences. *Science*, 327, 148–149. <https://doi.org/10.1126/science.1180543>
- Poff, N. L., Olden, J. D., Vieira, N. K. M., Finn, D. S., Simmons, M. P., & Kondratieff, B. C. (2006). Functional trait niches of North American lotic insects: Traits-based ecological applications in light of phylogenetic relationships. *Journal of the North American Benthological Society*, 25, 730–755. [https://doi.org/10.1899/0887-3593\(2006\)025\[0730:ftnna\]2.0.co;2](https://doi.org/10.1899/0887-3593(2006)025[0730:ftnna]2.0.co;2)
- Pond, G. J. (2012). Biodiversity loss in Appalachian headwater streams (Kentucky, USA): Plecoptera and Trichoptera communities. *Hydrobiologia*, 679, 97–117. <https://doi.org/10.1007/s10750-011-0858-2>
- Pond, G. J., Passmore, M. E., Borsuk, F. A., Reynolds, L., & Rose, C. J. (2008). Downstream effects of mountaintop coal mining: Comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. *Journal of the North American Benthological Society*, 27, 717–737. <https://doi.org/10.1899/08-015.1>
- R Core Team. (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <https://www.R-project.org/>.
- Sauer, F. G., Bundschuh, M., Zubrod, J. P., Schafer, R. B., Thompson, K., & Kefford, B. J. (2016). Effects of salinity on leaf breakdown: Dryland salinity versus salinity from a coalmine. *Aquatic Toxicology*, 177, 425–432. <https://doi.org/10.1016/j.aquatox.2016.06.014>
- Schäfer, R. B., Bundschuh, M., Rouch, D. A., Szöcs, E., Peter, C., Pettigrove, V., ... Kefford, B. J. (2012). Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services. *Science of the Total Environment*, 415, 69–78. <https://doi.org/10.1016/j.scitotenv.2011.05.063>
- Soucek, D. J., & Kennedy, A. J. (2005). Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. *Environmental Toxicology and Chemistry*, 24, 1204–1210. <https://doi.org/10.1897/04-142.1>
- Sun, S., Li, S., Avera, B. N., Strahm, B. D., & Badgley, B. D. (2017). Soil bacterial and fungal communities show distinct recovery patterns during forest ecosystem restoration. *Applied and Environmental Microbiology*, 83, e00966–00917.
- Timpano, A. J., Schoenholtz, S. H., Soucek, D. J., & Zipper, C. E. (2015). Salinity as a Limiting Factor for Biological Condition in Mining-Influenced Central Appalachian Headwater Streams. *JAWRA Journal of the American Water Resources Association*, 51, 240–250. <https://doi.org/10.1111/jawr.12247>
- Timpano, A. J., Schoenholtz, S. H., Soucek, D. J., & Zipper, C. E. (2018). Benthic macroinvertebrate community response to salinization in headwater streams in Appalachia USA over multiple years. *Ecological Indicators*, 91, 645–656. <https://doi.org/10.1016/j.ecolind.2018.04.031>
- Timpano, A. J., Vander Vorste, R., Soucek, D. J., Whitmore, K. M., Zipper, C. E., & Schoenholtz, S. H. (2017). Stream ecosystem response to mining-induced salinization in central appalachia. Final report to US Office of Surface Mining Reclamation and Enforcement, Cooperative Agreement S15AC20028 (121 pp).
- Timpano, A. J., Zipper, C. E., Soucek, D. J., & Schoenholtz, S. H. (2018). Seasonal pattern of anthropogenic salinization in temperate forested headwater streams. *Water Research*, 133, 8–18. <https://doi.org/10.1016/j.watres.2018.01.012>
- Tiunov, A. V., & Scheu, S. (2005). Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. *Ecology Letters*, 8, 618–625. <https://doi.org/10.1111/j.1461-0248.2005.00757.x>
- USEPA. (2004). National recommended water quality criteria. U.S. Environmental Protection Agency (EPA 4304T), Washington, DC. Retrieved from <https://www.epa.gov/sites/production/files/2015-06/documents/nrwqc-2004.pdf>
- USEPA. (2016) Aquatic life ambient water quality criterion for Selenium - Freshwater (EPA 822-R-16-006). U.S. Environmental Protection Agency, Washington, D.C. Retrieved from <http://www.epa.gov/waterscience/criteria/aqlife.html>
- Voss, K. A., & Bernhardt, E. S. (2017). Effects of mountaintop removal coal mining on the diversity and secondary productivity of Appalachian rivers. *Limnology and Oceanography*, 62, 1754–1770. <https://doi.org/10.1002/lno.10531>

- Whitmore, K. M., Schoenholtz, S. H., Soucek, D. J., Hopkins, W. A., & Zipper, C. E. (2018). Selenium dynamics in headwater streams of the central Appalachian coalfield. *Environmental Toxicology and Chemistry*, 37, 2714–2726. <https://doi.org/10.1002/etc.4245>
- Wu, Q. L., Zwart, G., Schauer, M., Kamst-Van Agterveld, M. P., & Hahn, M. W. (2006). Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. *Applied and Environmental Microbiology*, 72, 5478–5485. <https://doi.org/10.1128/aem.00767-06>

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## SUPPORTING INFORMATION

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