





Context-dependent role of abiotic and biotic factors structuring nematode communities along two environmental gradients

Kaitlin Gattoni¹  | Eli Michael Sokol Gendron¹  | Abigail Borgmeier² |
 J. Parr McQueen¹  | Peter G. Mullin² | Kris Powers² | Thomas O. Powers² |
 Dorota L. Porazinska¹ 

¹Department of Entomology and Nematology, University of Florida, Gainesville, Florida, USA

²Department of Plant Pathology, 406 Plant Science, University of Nebraska-Lincoln, Lincoln, Nebraska, USA

Correspondence

Dorota L. Porazinska, Department of Entomology and Nematology, University of Florida, Gainesville, Florida, USA.
 Email: dorotalp@ufl.edu

Funding information

University of Nebraska Collaborative Initiative, Grant/Award Number: Seed Grant Program; University of Florida

Handling Editor: Aurélie Bonin

Abstract

Although abiotic environmental factors have been historically regarded as the dominant deterministic process in microbial community assembly, recent studies indicate that biotic interactions may be equally significant. However, the extent to which both processes are important in assembly of belowground communities is unknown. Along two environmental gradients: alkalinity (ranging from pH ~7 to ~11) and habitat type (lakes, shorelines, and prairies around lakes) present in the Western Nebraska Sandhills, we used 18S rRNA gene marker metabarcoding and statistical analyses, including generalized dissimilarity modelling (GDM), to evaluate the dynamics between abiotic and biotic factors that might play a role in nematode community assembly. Lakes supported the least diverse and prairies the most diverse communities with completely distinct compositions. We also observed a potential role of alkalinity in shaping these communities but only in lakes. Generally, GDMs indicated the influence of both abiotic and biotic factors. However, their relative importance in explaining community variability was dependent on the habitat. Biotic factors influenced the lake communities most, followed by shorelines and prairies, explaining ~47%, 27% and 8% of the variation, respectively. In contrast, the role of abiotic factors was relatively similar in lakes, shorelines and prairies (~15%, 18% and 14% of the variation, respectively). Most variation in the shorelines (62%) and prairies (82%) remained unexplained, suggesting the potential importance of factors associated with specific traits or a stronger role of stochastic processes. Nevertheless, our findings suggest both deterministic processes are important in nematode community assembly, but their specific contributions are context-dependent.

KEYWORDS

biotic interactions, community assembly, deterministic, environmental factors, microbial community, soil, stochasticity

1 | INTRODUCTION

A plethora of ecological theories have been used to explain patterns of community diversity and assembly, including that of microorganisms (Zhou & Ning, 2017). Among the most popular theories are those that focus on stochastic (neutral theory) and deterministic processes (niche theory). Stochastic processes are defined by random events resulting from priority effects and ecological drift (Chase & Meyer, 2011; Chave, 2004; Hubbell, 2005; Nemergut et al., 2013). Deterministic processes are defined by abiotic (e.g., soil chemistry) factors and biotic interactions (e.g., competition) (Dini-Andreote et al., 2015; Fargione et al., 2003; Vellend, 2010; Zhou & Ning, 2017). Despite significant differences between these two theories, recent research suggests that they are not mutually exclusive, with microbial communities driven at varying degrees by both deterministic and stochastic processes (Caruso et al., 2011; Dini-Andreote et al., 2015; Nemergut et al., 2013) particularly along environmental gradients (Stegen et al., 2012; Zhao et al., 2019). For example, the assembly of Antarctic soil bacterial communities has been shown to be driven mainly by stochasticity when soil moisture is low, with the influence of deterministic processes becoming more important when moisture increases (Lee et al., 2018).

Among deterministic processes, abiotic factors have been studied more than biotic interactions. For example, substrate pH is considered a major abiotic driver of belowground community diversity and composition in both aquatic and terrestrial ecosystems (Lauber et al., 2009; Percent et al., 2008; Siles & Margesin, 2016; Xiong et al., 2012). Other abiotic factors important to belowground microbial and nematode community assembly include elevation, water availability and temperature (Fierer et al., 2009; Porazinska et al., 2018, 2021; Wu et al., 2011). Along environmental gradients, the diversity of belowground organisms often decreases as harshness increases, suggesting a negative influence of abiotic extremes (Afzal et al., 2021). In high alpine soils, for example, restricted soil water availability, high depth of snow cover, and low pH curtail the diversity of microbes and nematodes (Porazinska et al., 2018, 2021).

In comparison to abiotic factors, biotic interactions have been overlooked and understudied. This is because focus on community-level biotic interactions requires comprehensive sampling schemes involving characterization of microorganisms across domains, kingdoms and phyla (Lee et al., 2018). In addition, modelling tools to simultaneously quantify effects of abiotic factors and complex biotic interactions were unavailable until recently (Lee et al., 2018). The few studies that have successfully undertaken this task are limited to environments with relatively simple nematode communities. For example, the structure of nematode communities in the Antarctic Dry Valleys soils was originally believed to be driven exclusively by abiotic factors (i.e., soil water and salinity) (Adams et al., 2014; Nielsen et al., 2011; Wlostowski et al., 2018) but has recently been redefined and shown to be strongly influenced by nematode–nematode interactions (Caruso et al., 2019). These biotic interactions were also observed in the composition of other eukaryotes, as well as bacteria and fungi (Lee et al., 2018). Although support for the role of

biotic interactions in belowground community assembly is slowly gaining traction, more studies in complex ecosystems are needed to assess the interplay of both factors across different environmental gradients.

As the most numerous animals on the planet (van den Hoogen et al., 2019), with rapid population turnover and high levels of diversity (Reiss et al., 2010), nematodes provide a model system to assess the differential role of deterministic processes. With representation of all major trophic groups (e.g., bacterivores, fungivores, herbivores, omnivores and predators) (Yeates et al., 1993), nematodes act as connectors between primary producers, decomposers and higher trophic levels, and thereby are capable of modifying ecosystem functions (de Mesel et al., 2004; Heidemann et al., 2014; Majdi & Traunspurger, 2015; Pascal et al., 2008). For example, bacterivorous and fungivorous nematodes contribute to carbon and nutrient dynamics by feeding on microbes, which keeps them in their active growth phase (Bonaglia et al., 2014; Creamer et al., 2016; Gerlach, 1978) and excreting excess of ingested N back to the environment (Schratzberger et al., 2019; Trap et al., 2016). We used nematodes as a model system to examine the relative role of abiotic vs. biotic factors in shaping their communities.

Known for their unique biological diversity and the presence of environmental gradients (Gosselin, 1997; Loope et al., 1995), the Western Nebraska Sandhills provide an ideal place to study community assembly processes. The Sandhills are stabilized sand dunes covered by mixed grasses (e.g., *Andropogon hallii*, *Calamovilfa longifolia* and *Schizachyrium scoparium*) and forbs (e.g., *Helianthus pauciflorus*) (Keeler et al., 1980). Embedded within the dunes are hundreds of naturally eutrophic, shallow lakes (~2.5 m in depth) (Zhang et al., 2013) exhibiting an alkalinity gradient driven by potassium (K) and sodium (Na) ion concentrations (Gosselin, 1997; Loope et al., 1995). The gradient can range from neutral (pH of ~7 and salinity of ~200 mg L⁻¹) to highly alkaline (pH of ~11 and salinity >100,000 mg L⁻¹). Despite the presence of diverse bacterial and microbial eukaryotic communities in the highly alkaline lakes of the Western Nebraska Sandhills (Amaral-Zettler, 2012; Fiore et al., 2019), neither deterministic nor stochastic components of community assembly in this ecosystem have been described. Similarly, belowground communities in the prairies and shorelines surrounding the lakes await discovery. In one of the only two similar ecosystems in the world (i.e., Tibetan Plateau), alkalinity has been shown to play a major role in the assembly of bacterial and microbial eukaryotes not only in lakes but also soils surrounding the lakes (Liu et al., 2020; Shen et al., 2019; Wang et al., 2011; Wu et al., 2016; Xiong et al., 2012).

The goal of this study was to examine nematode communities along habitat (lake, shoreline, prairie) and alkalinity (pH 7–10) gradients across five lake basins in the Western Nebraska Sandhills to determine the degree to which abiotic (biogeochemistry) and biotic (diversity of bacterial, fungal and nonfungal eukaryotic communities) factors are key to their diversity and assembly. We expected nematode communities in lake sediments to display limited diversity and simplistic composition due to potential direct toxicity of the abiotic conditions (i.e., concentrations of Na, K and Cl ions), particularly in

the most alkaline lakes. In contrast, prairie communities would be more diverse and complex primarily due to innate soil heterogeneity, presence of plants and the capacity of soils to buffer nematodes from the direct negative effects of alkalinity. The shoreline communities would fall in between and represent a transitional zone. We also hypothesized that due to these differences, the relative role of abiotic, biotic and stochastic factors would vary among habitats. More specifically, abiotic factors (especially pH) would be the dominant driver of lake communities. In contrast, due to their high diversity and complexity, prairie communities would be shaped predominantly by biotic factors. Finally, shoreline communities would be driven mostly by stochasticity, due to their transitional nature.

2 | METHODOLOGY

2.1 | Study site and sampling

The study was conducted in the Nebraska Sandhills at the Crescent Lake National Wildlife Refuge (41°45'41.004"N, 102°26'12.012"W). The western region of the Sandhills receives ~400 mm of yearly precipitation (Szilagyi et al., 2011). Embedded within the prairies are shallow lakes (~2.5 m in depth) (Zhang et al., 2013) that range in alkalinity. For this study, we selected five lake basins representing an alkalinity gradient (pH ~7–10) with two of the most neutral lakes, Island and Gimlet, situated ~14 km from Bean, and the two most alkaline lakes, Kokjohn and Border. Island lake is the largest (~2,153,000 m²), followed by Bean, Gimlet, Border and the smallest, Kokjohn (~11,000 m²). The alkalinity range across lake basins for lakes, shorelines and prairies was 7.4–10.1, 6.9–9.9 and 6.9–8.4, respectively.

In late October 2019, we collected soil and sediment samples at each lake basin along a habitat gradient, spanning lake sediments (denoted hereafter as lake), shoreline sediments/soils (hereafter shoreline) and prairie soils (hereafter prairie) by taking three replicate composite samples per each habitat (3 replicates × 3 habitats × 5 lake basins = total of 45 samples). To ensure sample independence among habitats and replicates within each habitat, they were separated from each other by 30–100 m. Each composite sample consisted of ~15–20 bulk soil cores (~2 × 10 cm) from an area equivalent to 20 m². In the prairies, samples were taken in proximity to plant stems. Generally, plant diversity was limited (~10 plant species) and similar among lake basins. Soil samples were placed in sterile Whirlpaks and stored on ice while in the field. Upon return to the University of Nebraska, samples were gently homogenized and stored at 4°C until further processing.

2.2 | Sample processing

Gravimetric soil moisture (%) was measured by drying 5 g of soil and sediment subsamples for 24 h at 60°C. Biogeochemical analyses of soil and sediment samples were performed at Ward Laboratories for pH, % organic matter (OM), dissolved salts, nitrogen-nitrate (NO₃⁻),

phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sulphur (S), zinc (Zn), iron (Fe), copper (Cu), boron (B), chloride (Cl) and manganese (Mn) (Table S1).

To ensure maximum nematode recovery from potentially sub-optimal subsample sizes (~50 g) (Wiesel et al., 2015), nematodes were extracted using two concurrent methods. First, subsamples were passed through a series of sieves (25, 60 and 325 μm mesh) with tap water. Nematodes collected on the 325-μm mesh sieve were then processed by sugar floatation/centrifugation (Jenkins, 1964). Second, plant material and organic matter collected by the 25 and 60 mesh sieves was placed in a modified Baermann funnel for further nematode extraction for 24 h (Baermann, 1917). All nematodes extracted by sugar floatation/centrifugation and Baermann funnel per each sample were then combined before enumeration and morphological identification under inverted microscopy using keys (Goodey & Goodey, 1963). All nematodes were identified to at least the family level and further categorized into trophic groups (i.e., bacterivore, fungivore, omnivore, predator, plant parasite, root associate or animal parasite) (Yeates et al., 1993) (Figure S1). They provided baseline information to ensure the accuracy of DNA-based analyses.

Following nematode morphological identification, the volume of water was reduced to 0.5 ml and nematodes were transferred to bead beating tubes from the Qiagen DNeasy PowerSoil Kit and processed for DNA extraction according to the manufacturer's instructions (Qiagen). Bacterial, fungal and other eukaryotic DNA was extracted from ~0.35 g of soil or sediment subsamples. A Qubit assay was used to quantify all DNA concentrations prior to DNA amplification. Extracted DNA concentrations were standardized to 1 g of dry soil to serve as a proxy for microbial biomass (Sommers et al., 2020).

2.3 | DNA amplification and sequencing

Nematode DNA samples were amplified using the 18S rRNA gene delimited by the NF1/18Sr2b primers (Porazinska et al., 2009) using standard EMP (Earth Microbiome Project) 18S PCR protocols. Bacterial, fungal and other eukaryotic DNA samples were amplified using 16S (515f/806r) (Apprill et al., 2015; Parada et al., 2016) and 18S (1391f/EukBr) (Amaral-Zettler et al., 2009; Caporaso et al., 2012) rRNA metabarcoding markers following EMP protocols (Thompson et al., 2017). Amplifications were done in triplicates per each sample and all amplicons were verified by gel electrophoresis. Triplicates were pooled and all samples sent to the Hubbard Center for Genome Studies, University of New Hampshire, for the attachment of primer indexes (using Golay barcodes) (Caporaso et al., 2012), library preparation and sequencing using Illumina sequencing (HiSeq 2500, 2 × 250 bp, Illumina).

2.4 | Amplicon data processing

QIIME2 version 2021.4 was used to remove primer regions of the demultiplexed sequences using the CUTADAPT software (Martin, 2011).

Forward and reverse reads were joined using the vsearch plugin for QIIME2 (Rognes et al., 2016) and joined sequences were filtered for quality (Bokulich et al., 2013). The average length of nematode reads was 369 bp, 16S rRNA bacterial reads 252 bp, and 18S rRNA eukaryotic reads 146 bp. Because clustering algorithms producing exact amplicon variants (e.g., DADA2) excessively remove genuine nematode reads (Schenk et al., 2020), sequences were clustered into operational taxonomic units (OTUs) using the vsearch plugin at 99% similarity and subsequently used for the construction of the nematode community OTU table. For consistency, the 16S and 18S rRNA OTU tables were generated using the same bioinformatic methods but at 97% similarity. Taxonomic assignment of OTUs was performed using BLAST and an in-house curated reference database, ARB-SILVA SSU version 111 (Quast et al., 2013; Yilmaz et al., 2014).

The nematode OTU table was filtered to retain only nematode sequences (~30% of total sequences). Nematode OTUs were further condensed using head–tail patterns (Porazinska et al., 2010) and maximum-likelihood (ML) phylogenetic trees were created in MEGA version 10.1.8 to help delimit “species-equivalent” OTUs (Table S2). The 16S rRNA OTU table (Table S3) was filtered of mitochondrial, chloroplast and archaeal sequences. The 18S rRNA OTU table was filtered of all bacterial, nematode, and plant (Viridiplantae) sequences. In addition, the 18S rRNA table was split into a fungal table (Table SI 4) and a nonfungal nonnematode eukaryotic OTU table (represented mostly by protists and algae) (Table S5). All tables were filtered of unassigned OTUs. The number of reads was standardized to per 100g of dry soil/sediment for nematodes and to 1 g for bacteria, fungi and other eukaryotes, respectively. Lastly, trophic groups were assigned to each nematode OTU following Yeates et al. (1993) as described above.

2.5 | Statistical analyses

All statistical analyses were performed in R version 3.6.1 (R Core Team, 2020). Fifteen total treatments are defined by five lake basins (Island, Gimlet, Bean, Kokjohn and Border) along the alkalinity gradient and three habitats (lake, shoreline and prairie). Alpha diversity metrics (OTU Richness, Shannon, and Faith's PD) were calculated using the “estimate richness” function in the “phyloseq” package version 1.34.0 (McMurdie & Holmes, 2013). To detect whether alkalinity or habitat type explained variation in alpha diversity, generalized linear models (GLMs) followed by a post-hoc Tukey's honest significant difference (HSD) test were used with the “betadisper” function in the “Vegan” package version 2.5-6. Community dissimilarity based on Aitchison distances (Aitchison et al., 2000) was used to measure compositional differences among communities using “cmultRepl” in the “zCompositions” package version 1.3.4 and “vegdist” in the “Vegan” package version 2.5-6. The significance of treatment on compositional differences among communities was tested using permutational analysis of variance (PERMANOVA) using 1000 permutations with the R package “Vegan” version 2.5-6 (Oksanen et al., 2012) using the “adonis” function and visualized with principal coordinate analysis (PCoA) ordination plots using “vegdist.”

GLMs were used to evaluate the direction and strength of relationships between alpha diversity (Shannon's) and abiotic factors (e.g., pH, K, Na, OM) (Table S6–S9). Pearson's correlations between alpha diversity and abiotic factors described above were evaluated with the “cor” function in the “stats” package version 3.6.2 and visualized using the R package “corrplot” version 0.84 (Wei et al., 2017) with the “corrplot” function.

The degree to which abiotic and biotic factors played the most significant roles in the assembly of nematode communities was evaluated with generalized dissimilarity modelling (GDM) (Ferrier et al., 2007) using the R package “GDM” version 1.3.11 (Fitzpatrick et al., 2021) and the “gdm” function. The most potentially predictive variables for the GDM analyses were selected based on prior knowledge of the biogeochemical composition of the lake basins (Table S1) (Gosselin, 1997; Loope et al., 1995) and included pH, OM, K, Na, Cl, Zn, Fe, Ca and soil moisture as abiotic factors, and bacterial, fungal and other eukaryotic alpha diversity (Shannon's) and microbial biomass as biotic factors. Backward elimination (Mokany et al., 2021) was used to account for potentially high correlations between pH and Na, K and Cl. Only factors that explained a proportion (>0%) of nematode compositional dissimilarity models were retained in the final models. The GDM analyses seek to establish relationships based on input data consisting of distance dissimilarity matrices of nematode composition against abiotic/biotic predictors. Using the variables deemed important in the model for each habitat, abiotic and biotic variable sets were created and used for variance partitioning with the “gdm.partition.deviance” function. In addition to the abiotic and biotic sets involving variables utilized in the GDM, geography using latitude and longitude was also considered. Results from this analysis indicate the total amount of deviance explained by each variable set individually as well as in combination. To better explain the differential effect of abiotic and biotic predictors within prairie nematode communities, separate GDMs for individual nematode trophic groups were also performed. In these models, all factors included in the whole prairie model were included regardless of the proportion of dissimilarity explained.

3 | RESULTS

3.1 | Nematode community diversity

Nematode communities in the lakes were less diverse (Shannon's) than those in the shorelines and prairies ($p < .05$; Figure 1a). In addition, the diversity decreased with increasing alkalinity, but only in lakes ($p < .05$; Figure 1a). Other metrics (Richness and Faith's) indicated similar patterns of decreasing diversity as alkalinity increased ($p < .05$; Figures S2a and S3a). Different lake basins and habitats supported different nematode communities (PERMANOVA, $p < .05$; Figure 2a; Table S10). All prairie communities were most alike each other in composition regardless of the lake basin and completely distinct from the shorelines and lakes. In addition to differences between shorelines and lakes, lake communities varied among basins (PERMANOVA, $p < .05$; Figure 2a).

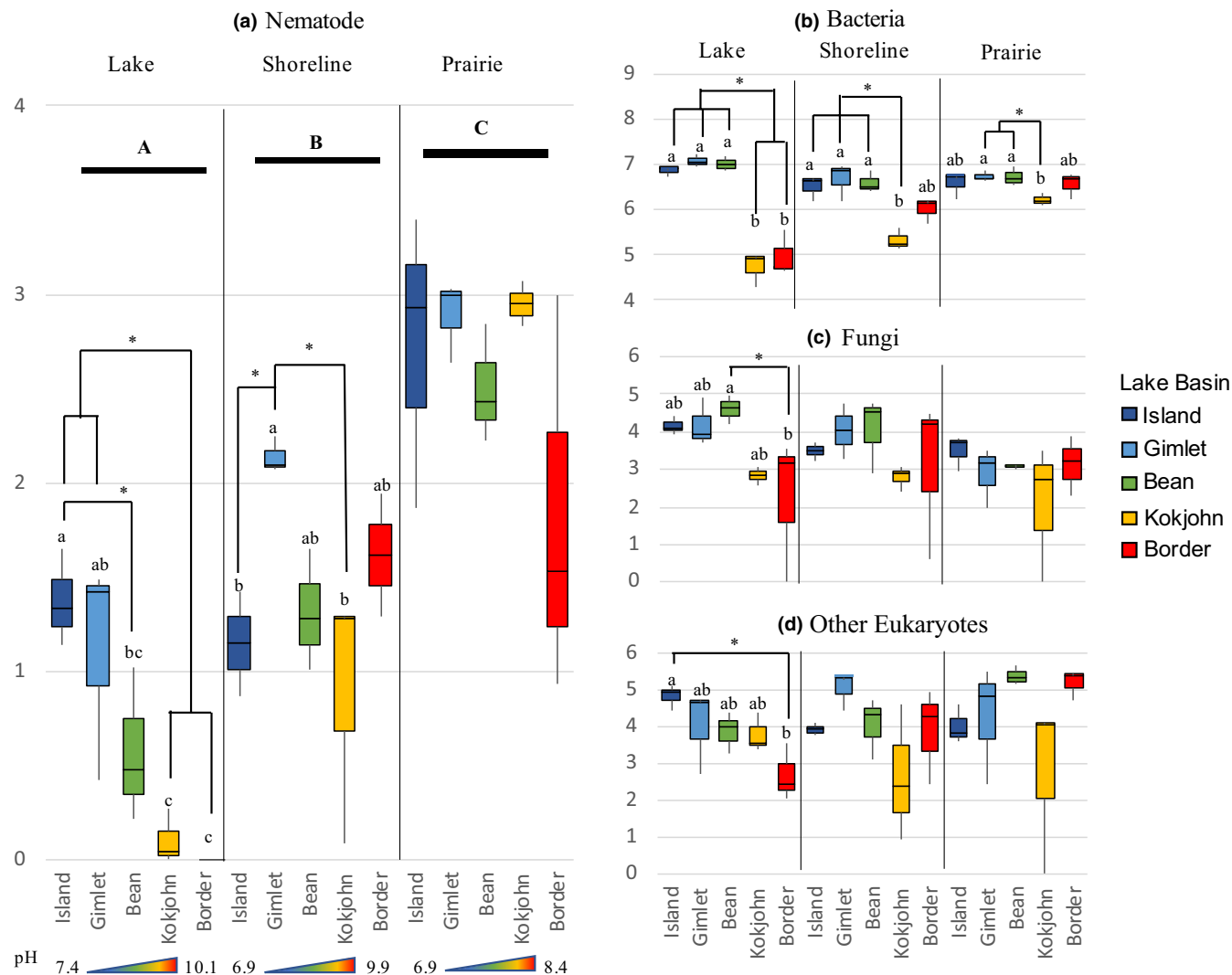


FIGURE 1 Alpha (Shannon's) diversity of: (a) nematode, (b) bacterial, (c) fungal and (d) other eukaryotic communities in lake sediment (lake), shoreline sediment/soils (shoreline) and prairie soils (prairie) in and around five lakes (Island, Gimlet, Bean, Kokjohn and Border) ranging from neutral (pH ~7) to highly alkaline (pH >10). Uppercase letters indicate statistically significant differences among habitats ($N = 3$) and lowercase letters indicate statistically significant differences within the habitats among different lake basins ($N = 5$) as determined by generalized linear models and $p < .05$

Although both lakes and shorelines were dominated by the predatory family Tobrilidae, lake communities displayed the simplest trophic diversity. This was especially evident in the most alkaline lakes (Bean, Kokjohn and Border) where ~90% of detected taxa consisted of predatory Tobrilidae (Figure 3). Only ~42% of nematodes were unique to shorelines and ~58% of taxa were shared with either the prairies or lakes. In general, prairie communities were characterized by the greatest trophic diversity and the greatest taxonomic richness (Figure 3). The most abundant nematodes in the prairies included species of bacterivorous *Acrobeles* (Cephalobidae) and *Plectus* (Plectidae), omnivorous *Aporcelaimellus* (Aporcelaimidae), fungivorous *Aphelenchoides* (Aphelenchidae) and plant-parasitic *Xiphinema* (Longidoridae). Unlike the other two habitats, there were few predatory nematodes in the prairies, all of which were representative of typical terrestrial habitats (e.g., *Paractinolaimus* sp. [Actinolaimidae]) and no Tobrilidae.

3.2 | Microbial community diversity

Bacterial diversity (Shannon's, Richness and Faith's PD) in all three habitats decreased significantly with increasing pH, predominantly due to low diversity in Kokjohn shoreline and prairie (GLM, $p < .05$; Figure 1b; Figures S2b and S3b), but there were no differences among habitats. Likewise, fungal and other eukaryotic Shannon's diversity was similar among habitats (Figure 1c,d). However, richness of fungal communities (GLM, $p < .05$; Figure S2c) and Faith's PD of other eukaryotic communities ($p < .05$; Figure S3d) were lowest in prairies. In general, all alpha diversity measures of both fungal and other eukaryotic decreased in the most alkaline lakes (GLM, $p < .05$; Figure 1c,d; Figures S2c,d and S3c,d).

Overall, compositions of bacterial, fungal and other eukaryotic communities were different among both lake basins and habitats (PERMANOVA, $p < .001$; Figure 2b-d; Table S10). Prairie

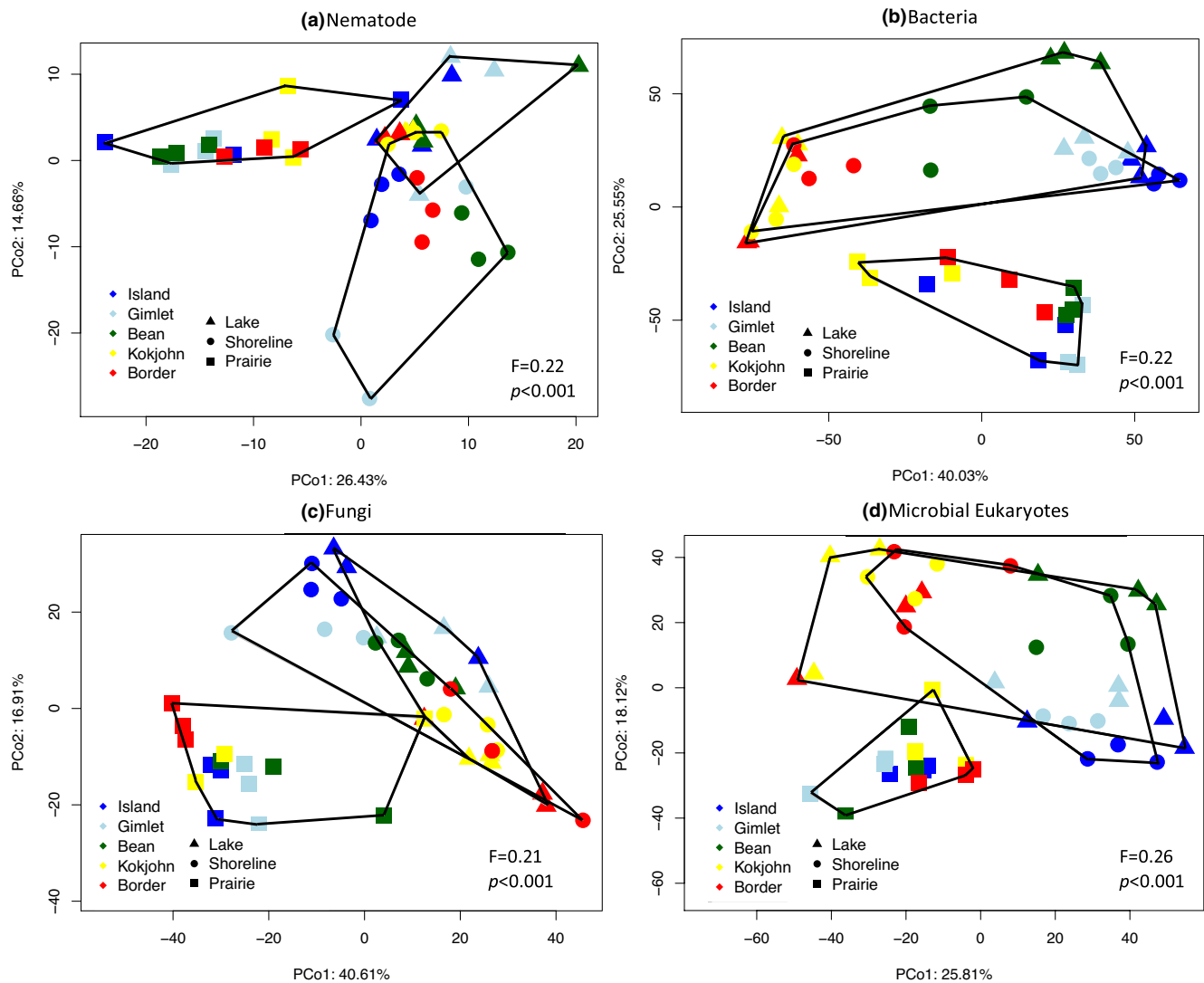


FIGURE 2 Principal coordinate analysis (using Aitchison's distances) of compositional community dissimilarity for: (a) nematodes, (b) bacteria, (c) fungi and (d) other eukaryotes. Dissimilarity of the nematode communities of the lakes Island (blue), Gimlet (turquoise), Bean (green), Kokjohn (yellow) and Border (red) show a general clustering by both lake basins and habitats (lake sediment [triangle], shorelines [circle] and surrounding prairies [square])

communities were most alike regardless of the lake basin and most distinct from shorelines and lakes (Figure 2b-d). In contrast, communities of shorelines and lakes clustered more along the alkalinity gradient (e.g., the most alkaline Kokjohn and Border communities clustered apart from the least alkaline Gimlet and Island communities) (PERMANOVA, $p < .5$; Figure 2b-d; Table S10).

3.3 | Relationships between diversity and biogeochemistry

Nematode diversity had a negative relationship with Na and K in all habitats to varying degrees (GLM, $p < .05$; Figure S4), and only in lakes and shorelines was it positive with OM, microbial biomass, and bacterial, fungal and other eukaryotic diversity (GLM, $p < .05$; Figure S4). Additionally, lake nematode diversity was negatively

related to several other biogeochemical variables (i.e., pH, cation exchange capacity [CEC], B, Cl, Ca and Mg) (GLM, $p < .05$; Figure S4). Relationships of soil microbial groups and biogeochemical features remained relatively consistent among all habitats (Figure S4).

3.4 | Drivers of nematode compositional dissimilarity

GDMs were used to examine the degree to which abiotic and biotic predictors explained nematode compositional dissimilarity. While the heights of GDM splines indicate their relative importance in models, the shapes suggest the degree of compositional change in response to the predictor's change (Ferrier et al., 2007). The displayed coefficients of deviance provide additional credence to the overall predictor's importance in the model. Overall, nematode

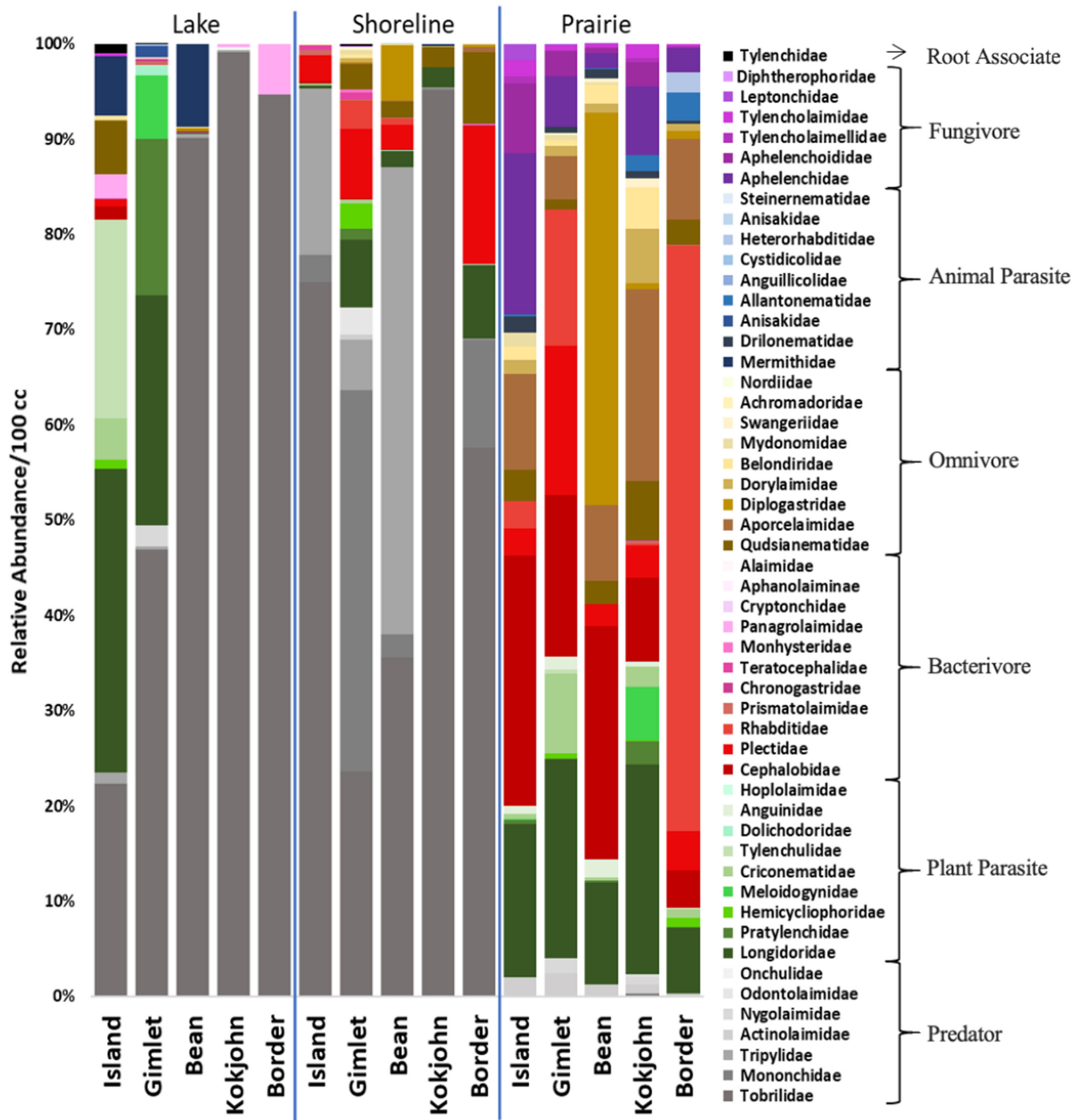


FIGURE 3 Relative abundance of nematode families in lakes, shorelines and prairies. Colour scales correspond to nematode trophic groups (greys = predators, greens = plant parasites, reds = bacterivores, golds = omnivores, blues = animal parasites, purples = fungivores, black = root associates). The prairie nematode communities had the most diverse trophic- and family-level diversity. The shorelines and the lake communities were dominated by predatory Tobrilidae

community composition was predictable by both abiotic and biotic factors, but the proportion of compositional variability explained by GDMs varied among habitats and ranged from 59.2% to 32.4% to 17.9% in lakes, shorelines and prairies, respectively (Table 1; Figure S5). In addition, the identity of the most notable predictor was habitat-specific. The biotic factors, including bacterial (proportion deviance explained = 0.64), fungal (0.48) and other eukaryotic

diversity (0.34), were the most predictive of lake communities when all other variables were accounted for in the model (Figure 4a). However, the abiotic factors such as Zn, Na and Cl were also important, particularly at low levels of change within predictor values and compositional dissimilarities (<0.20) (Figure 4a). Shoreline communities were most explained by concentrations of Cl (0.47) closely followed by bacterial (0.32) and fungal (0.31) diversity (Figure 4b). In

TABLE 1 Results of GDM variance partitioning illustrating the percentage of deviance explained by abiotic, biotic and geographical variables in isolation and in combination. Unexplained refers to deviance that could not be explained by the variables in the model

	Lake	Shoreline	Prairie
Abiotic	15.0	18.7	14.5
Biotic	47.5	27.4	7.8
Geographical	11.0	10.6	0.9
Abiotic+Biotic	59.2	32.4	17.9
Abiotic+Geographical	15.3	26.9	14.6
Biotic+Geographical	52.0	35.0	8.4
All variables	59.2	38.0	18.0
Unexplained	40.8	62.0	82.0

prairies, nematode communities were mostly explained by soil moisture (0.64) across the entire range of soil moisture and compositional variability, although biotic factors such as fungal (0.23) and other eukaryotic (0.21) diversity as well as microbial biomass (0.20) were also important (Figure 4c).

To further examine the role of the abiotic and biotic factors, we used deviance partitioning by setting all variables to biotic, abiotic and geography. Biotic variables alone explained 47.5%, 27.4% and 7.8% of the compositional dissimilarity in lakes, shorelines and prairies, respectively (Table 1). Abiotic factors alone explained 15.0%, 18.7% and 14.5% of the variation in lakes, shorelines and prairies, respectively. The interaction of these factors contributed to the explained total variation in lakes the most (11.8%) and prairies the least (3.4%). Additionally, geography was minimally important (~6%) but only in shorelines.

To better understand the low variation explained in the prairies that could result from divergent functional traits of nematodes, we reran the GDMs for each trophic group separately. Both abiotic and biotic variables explained 49.2% of the variation in prairies for bacterial feeders, 42.6% for omnivores, 20.2% for predators, 20.0% for fungivores and 12.8% for plant parasites (Figure S6). Only soil moisture content was important for all trophic groups, except plant parasites, and other factors were more trophic group-specific.

4 | DISCUSSION

4.1 | Microbial community diversity in the Western Nebraska sandhills

Here we describe the diversity of nematode communities in and around lakes of the Western Nebraska Sandhills along a naturally occurring alkalinity gradient as well as deterministic processes that shape the assembly of their communities. We provided evidence that both abiotic and biotic factors are important in the nematode community assembly, but their relative importance is context-dependent.

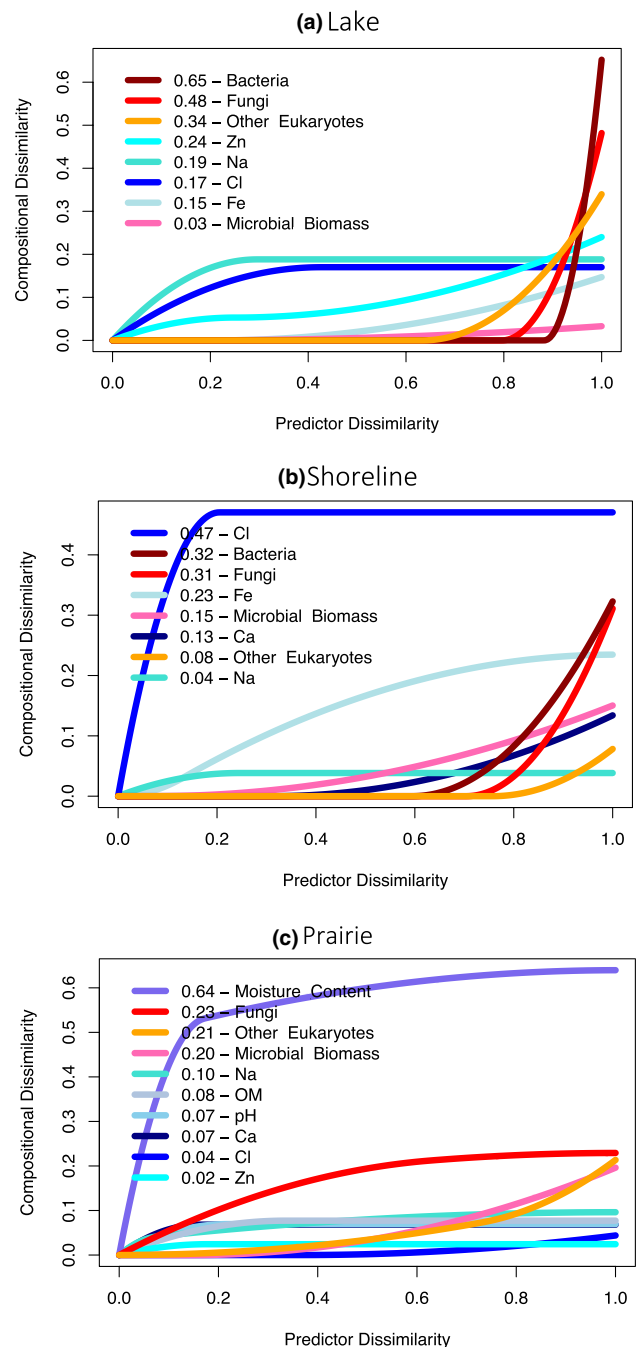


FIGURE 4 Generalized dissimilarity models (GDMs) with abiotic and biotic predictors for (a) lake, (b) shoreline and (c) prairie nematode communities. The Shannon diversity index of Bacteria, Fungi and other Eukaryota (Other_Eukaryotes) and Microbial Biomass were used as biotic predictors. GDM splines are the partial regression fits. The shape of the spline depicts the rate of community compositional changes (y-axis) as the predictor variable (x-axis) increases. Predictor variables are scaled such that the minimum is 0 and the maximum is 1. The predictor's relative importance in the model (i.e., magnitude of compositional change associated with the individual predictor) is indicated by the maximum height of the spline and reiterated in the keys

Nematode communities of different habitats were distinct in diversity and composition, indicating potential differences in the processes driving their assemblies across these habitats. With few

other studies with which to compare, our results of increasing diversity from lake to shoreline to prairie were in contrast to the only other study of nematodes in an inland water–terrestrial ecotone of Swedish Lakes in which the shore region was the most diverse in comparison to the surrounding purely aquatic or terrestrial habitats (Kreuzinger-Janik et al., 2021). These results indicate the habitat gradient probably structures nematode community diversity differently at a large landscape scale. Differences between our study and Kreuzinger-Janik et al. (2021) could indicate the role of other factors (e.g., stochasticity and abiotic conditions) shaping communities at a finer within-habitat scale. Surprisingly, nematode diversity in our study was negatively affected by alkalinity only in lakes. Other studies in alkaline lakes (e.g., lakes of the Tibetan Plateau) observed similar patterns (Liu et al., 2020; Wang et al., 2011; Xiong et al., 2012), corroborating our results. However, the lack of consistently similar relationships among all our habitats indicates that alkalinity as an abiotic factor may not play an equal role in the assembly of all nematode communities.

Compositionally, predatory Tobrilidae nematodes were dominant in the more alkaline lakes. Previous studies of eutrophic lakes (e.g., the Swedish Lake Limnaren) also observed high numbers of these nematodes alongside bacterial feeders such as *Eumonhystra* sp. and *Punctodora* sp. (Bert et al., 2007; Kazemi-Dinan et al., 2014; Traunspurger, 2000). The dominance of Tobrilidae across the entire alkalinity gradient can be attributed to their preference for living in truly aquatic environments (Tahseen, 2012; Traunspurger, 2000) and to their nature of feeding on larval forms of small, soft-bodied invertebrates and diatoms, which are often abundant in these environments (Zullini, 2006). Their almost exclusive presence in highly alkaline lakes and shorelines was surprising and indicates a unique adaptation to withstand extreme alkalinity. This flexibility to persist under a wide range of alkaline conditions perhaps points to their possibly lower sensitivity to abiotic factors including pH and a potentially stronger role of biotic interactions, for example via top-down control of other freshwater microorganisms (Kazemi-Dinan et al., 2014; Michiels & Traunspurger, 2004, 2005; Zullini, 2006). The highly diverse microbial communities in our lakes could be experiencing top-down controls. Experimental work under variable ranges of alkalinity and diversity of prey, in combination with more precise taxonomic resolution of Tobrilidae, is underway to allow more conclusive insights regarding their biotic interactions with other microorganisms.

In the shorelines, the presence of nematode taxa shared with lakes (i.e., *Tobrilus* spp.) and prairies (i.e., *Plectus* spp.) is indicative of ecological transitional zones or ecotones. Overall, shorelines supported more diverse communities than lakes, but they too were dominated by Tobrilidae although to a lesser extent. Among other abundant shoreline taxa were Tripylidae, a family commonly found in both terrestrial and aquatic habitats (Holovachov et al., 2015; Mulvey, 1969; Zullini, 2006). The differences between lakes and shorelines suggests different factors shape their communities. Although more evidence will be needed to assess it, stochasticity due to fluctuating water levels common in the area could be among these factors.

Consistent with previous studies in natural grasslands in North America (Biederman & Boutton, 2009; Cesarz et al., 2017; Neher et al., 1998; Todd et al., 2006), our prairies supported nematode communities with complex trophic structures, including abundant bacterivores, fungivores, plant parasites and omnivores, and hence were the most distinct from the other habitats. These communities were also most similar to each other regardless of alkalinity, suggesting that pH is not most influential in this habitat, or that innate heterogeneity of terrestrial microhabitats buffers diversity from the negative effects of pH or other abiotic factors (e.g., Na and K) that drive alkalinity in this environment.

4.2 | Abiotic and biotic factors influence nematode communities

Perhaps one of the most acknowledged abiotic factors playing a role in the assembly of belowground communities is pH (Lauber et al., 2009; Percent et al., 2008; Siles & Margesin, 2016; Xiong et al., 2012). At our study site, the pH of lakes and shorelines is historically driven by high concentrations of Na, K and Cl ions (Gosselin, 1997; Loope et al., 1995). High levels of these ions can be toxic in aquatic environments, limiting community diversity (Parker et al., 1997; Wang et al., 2018), consistent with our alpha diversity findings. Our GDM results somewhat confirmed this idea; concentrations of Na and Cl ions were among the better abiotic predictors of nematode community variability in the lakes and shorelines than in the prairies. This was particularly notable at low levels of ion variability, indicating that ion concentrations across the entire gradient present in these habitats can have drastic effects on nematode compositional dissimilarity. Likewise, high ion (Na/K) concentrations in the Tibetan Plateau lakes played a major role in the assembly of their microbial communities (Liu et al., 2020; Wang et al., 2011; Xiong et al., 2012). The overall stronger importance of biotic predictors in explaining lake (47.5%) and shoreline (27.4%) community variabilities was a major surprise even though in both habitats biotic predictors were most influential when predictor values were high. With diversity of bacteria, fungi and eukaryotes highest in the least alkaline lakes, we can predict that these factors are most important for nematode community assembly in neutral rather than alkaline lakes. These results begin to illustrate the dynamic importance of both abiotic and biotic processes at different degrees along the environmental gradient.

Prairie communities, experiencing the narrowest and the least harsh alkalinity gradient (pH 6.9–8.4), were poorly explained (17.9%) by both abiotic and biotic factors. While the role of soil moisture as the most important factor in shaping these communities was consistent with studies in the Antarctic desert (Adams et al., 2014; Caruso et al., 2019; Lee et al., 2018) and other grassland ecosystems (Nisa et al., 2021; Song et al., 2017; Sylvain et al., 2014; Todd et al., 1999), the general insignificance of biotic factors was rather unexpected.

Our results indicate that the amount of compositional variability explained by abiotic and biotic factors is context- (here habitat)

dependent. This was exemplified by the aquatic–terrestrial habitat gradient corresponding to a decrease in nematode compositional dissimilarity explained by both types of factors, with most dissimilarity explained in the lakes (~59.2%), followed by shorelines (~32.4%) and prairies (~17.9%). The large proportion of unexplained variation in the shorelines probably results from stochastic processes being particularly influential along shorelines of lakes and oceans (Chen et al., 2019; Graham et al., 2017; Tang et al., 2020). Within the Sandhills, lakes are often subject to rapid fluctuating water levels (Hayford & Baker, 2012) leading to potentially high degrees of species turnover alongside a high occurrence of species reseeding (Ma et al., 2018; Weise et al., 2016; Zohary & Ostrovsky, 2011). The proportion of variation explained by geography may hint at shoreline location, driven by these stochastic factors, influencing nematode communities. In the prairies, interactions with plants (de Deyn et al., 1995; Dietrich et al., 2021; Eisenhauer et al., 2011), not considered here, and stochasticity may account for large proportions of the unexplained variation. However, another factor that should be considered is nematode functional and taxonomic diversity. Could taxonomic and functional homogeneity of lake communities be underpinning their high predictability? Could the complexity of feeding and lifestyle traits of prairie communities undermine their overall predictability? To answer these questions, we reran the GDMs for all prairie trophic groups separately. Interestingly, the overall predictability of individual trophic groups generally increased (49.2% for bacterial feeders, 42.6% for omnivores, 20.2% for predators and 20.0% for fungal feeders). More importantly, the most significant factors shaping the different trophic groups also varied. While soil moisture was consistently important to all except plant parasites, the effects of biotic factors were trophic group-specific, probably diminishing their role in the overall nematode community analysis. The differential role of abiotic and biotic factors among trophic groups is not unique to our study (Porazinska et al., 2021) and suggests that analyses of nematode communities should consider not only differences of feeding traits, but perhaps other functional traits (e.g., life strategies and body size) (Fry et al., 2018). Finally, although nematode–nematode interactions were not included in the models, they may also be important in all three habitats, as shown in Antarctic nematode communities (Caruso et al., 2019). Future models that could include these interactions will provide a better understanding of the interplay of abiotic and biotic factors along environmental gradients.

5 | CONCLUSIONS

The alkaline lakes region of the Western Nebraska Sandhills has the potential to be a major site of ecological research. It is easily accessible and contains some of the most unique, extreme environments on the planet. In this study, we took the first steps to characterize nematode communities and determine some of the major predictive factors potentially playing a role in their assemblies. We found that the influence of different deterministic

processes was context-dependent, with the role of abiotic vs. biotic factors changing dynamically along the habitat gradient. Unexpectedly, biotic factors were significantly more important than abiotic factors in lake and shoreline nematode communities. The seemingly low importance of deterministic factors (either biotic or abiotic) in prairie communities was more evident at the trophic level of nematode community analysis. While all communities were driven to different degrees by deterministic processes, the high unexplained variation points to the importance of other processes including stochasticity, and plant–nematode and nematode–nematode interactions.

AUTHOR CONTRIBUTIONS

Kaitlin Gattoni, Dorota L. Porazinska and Thomas O. Powers conceived the research; Kaitlin Gattoni, Dorota L. Porazinska, Abigail Borgmeier, Kris Powers and Thomas O. Powers performed the fieldwork; Kaitlin Gattoni, Abigail Borgmeier, Kris Powers and Peter G. Mullin conducted laboratory work; Kaitlin Gattoni, Eli Michael Sokol Gendron, Dorota L. Porazinska and J. Parr McQueen performed data and statistical analyses. The manuscript was written by Kaitlin Gattoni, Dorota L. Porazinska and Eli Michael Sokol Gendron with input from all authors.

ACKNOWLEDGEMENTS

This research was funded in part by the University of Nebraska Collaborative Initiative Seed Grant Program (Sandhills Alkaline Lakes Collaborative Initiative) and the University of Florida. We thank the SALCI team (David Dunigan, Ethan Freese, Jayadra Ghosh, Roger Carlson, Mirae Guenther, Emma Brinley Buckley and Mary Harner) for their support.

DATA AVAILABILITY STATEMENT

Raw sequence data have been uploaded to the Sequence Read Archive (SRA) of NCBI under BioProject no. PRJNA740183. The entire code is available at <https://www.wormsetal.com/biotic-factors-shape-nematode-communities> (or on GitHub at <https://github.com/WormsEtAl/Biotic-Factors-Shape-Nematode-Communities>).

ORCID

Kaitlin Gattoni  <https://orcid.org/0000-0002-3368-6673>

Eli Michael Sokol Gendron  <https://orcid.org/0000-0002-2552-4658>

J. Parr McQueen  <https://orcid.org/0000-0002-7873-0850>

Dorota L. Porazinska  <https://orcid.org/0000-0002-0493-9926>

REFERENCES

- Adams, B. J., Wall, D. H., Virginia, R. A., Broos, E., & Knox, M. A. (2014). Ecological biogeography of the terrestrial nematodes of Victoria land, Antarctica. *ZooKeys*, 419, 29–71. <https://doi.org/10.3897/zookeys.419.7180>
- Afzal, S., Nesar, H., Imran, Z., & Ahmad, W. (2021). Altitudinal gradient affect abundance, diversity and metabolic footprint of soil nematodes in Banihal-pass of Pir-Panjial mountain range. *Scientific Reports*, 11, 16214. <https://doi.org/10.1038/s41598-021-95651-x>

- Aitchison, J., Barceló-Vidal, C., Martín-Fernández, J. A., & Pawłowsky-Glahn, V. (2000). Logratio analysis and compositional distance. *Mathematical Geology*, 32(3), 271–275. <https://doi.org/10.1023/A:1007529726302>
- Amaral-Zettler, L. A. (2012). Eukaryotic diversity at pH extremes. *Frontiers in Microbiology*, 3, 1–17. <https://doi.org/10.3389/fmicb.2012.00441>
- Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS One*, 4(7), 1–9. <https://doi.org/10.1371/journal.pone.0006372>
- Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, 75(2), 129–137. <https://doi.org/10.3354/ame01753>
- Baermann, G. (1917). Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) larven in Erdproben. *Geneeskundig Tijdschrift voor Nederlandsch Indië*, 57, 131–137.
- Bert, W., Messiaen, M., Hendrickx, F., Manhout, J., de Bie, T., & Borgonie, G. (2007). Nematode communities of small farmland ponds. *Hydrobiologia*, 583(1), 91–105. <https://doi.org/10.1007/s10750-006-0485-5>
- Biederman, L. A., & Boutton, T. W. (2009). Biodiversity and trophic structure of soil nematode communities are altered following woody plant invasion of grassland. *Soil Biology and Biochemistry*, 41(9), 1943–1950. <https://doi.org/10.1016/j.soilbio.2009.06.019>
- Bokulich, N., Subramanian, S., Faith, J., Gevers, D., Gordon, J. I., Knight, R., Mills, D. A., & Caporaso, J. G. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods*, 10, 57–59. <https://doi.org/10.1038/nmeth.2276>
- Bonaglia, S., Nascimento, F. J. A., Bartoli, M., Klawonn, I., & Brüchert, V. (2014). Meiofauna increases bacterial denitrification in marine sediments. *Nature Communications*, 5, 1–9. <https://doi.org/10.1038/NCOMMS6133>
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal*, 6(8), 1621–1624. <https://doi.org/10.1038/ismej.2012.8>
- Caruso, T., Chan, Y., Lacap, D. C., Lau, M. C. Y., McKay, C. P., & Pointing, S. B. (2011). Stochastic and deterministic processes interact in the assembly of desert microbial communities on a global scale. *ISME Journal*, 5(9), 1406–1413. <https://doi.org/10.1038/ismej.2011.21>
- Caruso, T., Hogg, I. D., Nielsen, U. N., Bottos, E. M., Lee, C. K., Hopkins, D. W., Cary, S. C., Barrett, J. E., Greem, T. G. A., Storey, B. C., Wall, D. H., & Adams, B. J. (2019). Nematodes in a polar desert reveal the relative role of biotic interactions in the coexistence of soil animals. *Communications Biology*, 2(63), 1–9. <https://doi.org/10.1038/s42003-018-0260-y>
- Cesarz, S., Ciobanu, M., Wright, A. J., Ebeling, A., Vogel, A., Weisser, W. W., & Eisenhauer, N. (2017). Plant species richness sustains higher trophic levels of soil nematode communities after consecutive environmental perturbations. *Oecologia*, 184(3), 715–728. <https://doi.org/10.1007/s00442-017-3893-5>
- Chase, J., & Meyer, J. (2011). Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366, 2351–2363. <https://doi.org/10.1098/rstb.2011.0063>
- Chave, J. (2004). Neutral theory and community ecology. *Ecology Letters*, 7, 241–253. <https://doi.org/10.1111/j.1461-0248.2003.00566.x>
- Chen, C., Chen, H. Y. H., Chen, X., & Huang, Z. (2019). Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nature Communications*, 10(1), 1–10. <https://doi.org/10.1038/s41467-019-09258-y>
- Creamer, R. E., Hannula, S. E., Van Leeuwen, J. P., Stone, D., Rutgers, M., Schmelz, R. M., de Ruiter, P. C., Bohse Hendrikson, N., Bolger, T., Bouffadud, M. L., Buee, M., Carvalho, F., Costa, D., Dirilgen, T., Francisco, R., Griffiths, B. S., Griffiths, R., Martin, F., ... Lemanceau, P. (2016). Ecological network analysis reveals the inter-connection between soil biodiversity and ecosystem function as affected by land use across Europe. *Applied Soil Ecology*, 97, 112–124. <https://doi.org/10.1016/j.apsoil.2015.08.006>
- de Deyn, G. B., Raaijmakers, C. E., van Ruijven, J., Berendse, F., & van der Putten, W. H. (1995). Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. *Oikos*, 106(3), 576–586. <https://doi.org/10.1111/j.0030-1299.2004.13265.x>
- de Mesel, I., Derycke, S., Moens, T., van der Gucht, K., Vincx, M., & Swings, J. (2004). Top-down impact of bacterivorous nematodes on the bacterial community structure: A microcosm study. *Environmental Microbiology*, 6(7), 733–744. <https://doi.org/10.1111/j.1462-2920.2004.00610.x>
- Dietrich, P., Cesarz, S., Liu, T., Roscher, C., & Eisenhauer, N. (2021). Effects of plant species diversity on nematode community composition and diversity in a long-term biodiversity experiment. *Oecologia*, 197, 297–311. <https://doi.org/10.1007/s00442-021-04956-1>
- Dini-Andreote, F., Stegen, J. C., van Elsas, J. D., & Salles, J. F. (2015). Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. *PNAS*, 112(11), E1326–E1332. <https://doi.org/10.1073/pnas.1414261112>
- Eisenhauer, N., Migunova, V. D., Ackermann, M., Ruess, L., & Scheu, S. (2011). Changes in plant species richness induce functional shifts in soil nematode communities in experimental grassland. *PLoS One*, 6(9), 1–9. <https://doi.org/10.1371/journal.pone.0024087>
- Fargione, J., Brown, C. S., & Tilman, D. (2003). Community assembly and invasion: An experimental test of neutral versus niche processes. *PNAS*, 100(15), 8916–8920. <https://doi.org/10.1073/pnas.1033107100>
- Ferrier, S., Manion, G., Elith, J., & Richardson, K. (2007). Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. *Diversity and Distributions*, 13(3), 252–264. <https://doi.org/10.1111/j.1472-4642.2007.00341.x>
- Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A., & Cleveland, C. C. (2009). Global patterns in belowground communities. *Ecology Letters*, 12(11), 1238–1249. <https://doi.org/10.1111/j.1461-0248.2009.01360.x>
- Fiore, N. A., Dunigan, D. D., Shaffer, J. J., Roberts, R., Antony-Babu, S., Plantz, B. A., Nickerson, K. W., Benson, A. K., & Weber, K. A. (2019). Microbial Community of Saline, Alkaline Lakes in the Nebraska sandhills based on 16S rRNA gene amplicon sequence data. *Microbiology Resource Announcements*, 8(12), 1–3. <https://doi.org/10.1128/MRA.00063-19>
- Fitzpatrick, M. A. C., Mokany, K., Manion, G., Lisk, M., Ferrier, S., & Nieto-Lugilde, D. C. (2021). Package “gdm.”
- Fry, E. L., De Long, J. R., Álvarez Garrido, L., Alvarez, N., Carrillo, Y., Castañeda-Gómez, L., Chomel, M., Dondini, M., Drake, J. E., Hasegawa, S., & Hortal, S. (2018). Using plant, microbe, and soil fauna traits to improve the predictive power of biogeochemical models. *Methods in Ecology and Evolution*, 10(1), 146–157. <https://doi.org/10.1111/2041-210X.13092>
- Gerlach, S. A. (1978). Oecologia in stimulating bacterial productivity. *Oecologia*, 69, 55–69. <https://doi.org/10.1007/BF00376996>
- Gosselin, D. C. (1997). Major-ion chemistry of compositionally diverse lakes, Western Nebraska, U.S.A.: Implications for paleoclimatic interpretations. *Journal of Paleolimnology*, 17(1), 33–49. <https://doi.org/10.1023/A:1007908909148>
- Goodey, T., & Goodey, J. B. (1963). *Soil and freshwater nematodes*. Meuthen.

- Graham, E. B., Crump, A. R., Resch, C. T., Fansler, S., Arntzen, E., Kennedy, D. W., Fredrickson, J. K., & Stegen, J. C. (2017). Deterministic influences exceed dispersal effects on hydrologically-connected microbiomes. *Environmental Microbiology*, 19(4), 1552–1567. <https://doi.org/10.1111/1462-2920.13720>
- Hayford, B. L., & Baker, D. (2012). Lakes of the Nebraska Sandhills. *Lakeview*, 31(4), 26–30.
- Heidemann, K., Hennies, A., Schakowske, J., Blumenberg, L., Ruess, L., Scheu, S., & Maraun, M. (2014). Free-living nematodes as prey for higher trophic levels of forest soil food webs. *Oikos*, 123(10), 1199–1211. <https://doi.org/10.1111/j.1600-0706.2013.00872.x>
- Holovachov, O., Camp, L., & Nadler, S. A. (2015). Sensitivity of ribosomal RNA character sampling in the phylogeny of Rhabditida. *Journal of Nematology*, 47(4), 337–355.
- Hubbell, S. P. (2005). Neutral theory in community ecology and the hypothesis of functional equivalence. *Functional Ecology*, 19, 166–172. <https://doi.org/10.1111/j.0269-8463.2005.00965.x>
- Jenkins, W. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reports*, 48, 692. <https://doi.org/10.1111/j.1365-3180.1974.tb01084.x>
- Kazemi-Dinan, A., Schroeder, F., Peters, L., Majdi, N., & Traunspurger, W. (2014). The effect of trophic state and depth on periphytic nematode communities in lakes. *Limnologia*, 44, 49–57. <https://doi.org/10.1016/j.limno.2013.05.011>
- Keeler, K. H., Harrison, A. T., & Vescio, L. S. (1980). The Flora and Sandhills prairie communities of Arapaho prairie. *Arthur County, Nebraska. Prairie Naturalist*, 12(3&4), 65–78.
- Kreuzinger-Janik, B., Majdi, N., & Traunspurger, W. (2021). Distribution and diversity of meiofauna along an aquatic-terrestrial moss ecotone. *Nematology*, 0, 1–20. <https://doi.org/10.1163/15685411-bja10070>
- Lauber, C. L., Hamady, M., Knight, R., & Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, 75(15), 5111–5120. <https://doi.org/10.1128/AEM.00335-09>
- Lee, K. C., Caruso, T., Archer, S. D. J., Gillman, L. N., Lau, M. C. Y., Craig Cary, S., Lee, C. K., & Pointing, S. B. (2018). Stochastic and deterministic effects of a moisture gradient on soil microbial communities in the McMurdo dry valleys of Antarctica. *Frontiers in Microbiology*, 9(2619), 1–12. <https://doi.org/10.3389/fmicb.2018.02619>
- Liu, K., Liu, Y., Hu, A., Wang, F., Chen, Y., Gu, Z., Anslan, S., & Hou, J. (2020). Different community assembly mechanisms underlie similar biogeography of bacteria and microeukaryotes in Tibetan lakes. *FEMS Microbiology Ecology*, 96(6), 1–10. <https://doi.org/10.1093/femsec/fiaa071>
- Loope, D. B., Swinehart, J. B., & Mason, J. P. (1995). Dune-dammed palaeovalleys of the Nebraska Sand Hills: Intrinsic versus climatic controls on the accumulation of lake and marsh sediments. *Geological Society of America Bulletin*, 107(4), 396–406. [https://doi.org/10.1130/0016-7606\(1995\)107<0396:DDPOTN>2.3.CO;2](https://doi.org/10.1130/0016-7606(1995)107<0396:DDPOTN>2.3.CO;2)
- Ma, Y., Li, J., Wu, J., Kong, Z., Feinstein, L. M., Ding, X., Ge, G., & Wu, L. (2018). Bacterial and fungal community composition and functional activity associated with lake wetland water level gradients. *Scientific Reports*, 8(1), 1–12. <https://doi.org/10.1038/s41598-018-19153-z>
- Majdi, N., & Traunspurger, W. (2015). Free-living nematodes in the freshwater food web: A review. *Journal of Nematology*, 47(1), 28–44.
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, 17, 10–12.
- McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, 8(4), 1–11. <https://doi.org/10.1371/journal.pone.0061217>
- Michiels, I. C., & Traunspurger, W. (2004). A three year study of seasonal dynamics of a zoobenthos community in a eutrophic lake. *Nematology*, 6(5), 655–669. <https://doi.org/10.1163/1568541042843568>
- Michiels, I. C., & Traunspurger, W. (2005). Impact of resource availability on species composition and diversity in freshwater nematodes. *Oecologia*, 142(1), 98–103. <https://doi.org/10.1007/s00442-004-1700-6>
- Mokany, K., Ware, C., Woolley, S. N. C., Ferrier, S., & Fitzpatrick, M. C. (2021). A working guide to harnessing generalized dissimilarity modelling for biodiversity analysis and conservation assessment. *Global Ecology and Biogeography*, 31, 802–821. <https://doi.org/10.1111/geb.13459>
- Mulvey, R. H. (1969). Soil-inhabiting nematodes of the orders Araeolairnida, Chromadorida, Enoplida, and Monhysterida from the Canadian high Arctic. *Canadian Journal of Zoology*, 47(3), 365–382. <https://doi.org/10.1139/z69-070>
- Neher, D. A., Easterling, K. N., Fiscus, D., & Campbell, C. L. (1998). Comparison of nematode communities in agricultural soils of North Carolina and Nebraska. *Ecological Applications*, 8(1), 213–223. [https://doi.org/10.1890/1051-0761\(1998\)008\[0213:CONCIA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1998)008[0213:CONCIA]2.0.CO;2)
- Nemergut, D. R., Schmidt, S. K., Fukami, T., O'Neill, S. P., Bilinski, T. M., Stanish, L. F., Knelman, J. E., Darcy, J. L., Lynch, R. C., Wickey, P., & Ferrenberg, S. (2013). Patterns and processes of microbial community assembly. *Microbiology and Molecular Biology Reviews*, 77(3), 342–356. <https://doi.org/10.1128/MMBR.00051-12>
- Nielsen, U. N., Wall, D. H., Adams, B. J., & Virginia, R. A. (2011). Antarctic nematode communities: Observed and predicted responses to climate change. *Polar Biology*, 34(11), 1701–1711. <https://doi.org/10.1007/s00300-011-1021-2>
- Nisa, R. U., Tantray, A. Y., Kouser, N., Allie, K. A., Wani, S. M., Alamri, S. A., Alyemeni, M. N., Wijaya, L., & Shah, A. A. (2021). Influence of ecological and edaphic factors on biodiversity of soil nematodes. *Saudi Journal of Biological Sciences*, 28(5), 3049–3059. <https://doi.org/10.1016/j.sjbs.2021.02.046>
- Oksanen, A. J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcginlin, O'Hara R.B., Simpson G. L., Solymos P., Stevens H.H. D., Szoecs, E., & Wagner H. (2012). Package 'vegan.' (December 2016), 0–291.
- Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, 18(5), 1403–1414. <https://doi.org/10.1111/1462-2920.13023>
- Parker, D. L., Kumar, H. D., Rai, L. C., & Singh, J. B. (1997). Potassium salts inhibit growth of the cyanobacteria *Microcystis* spp. in pond water and defined media: Implications for control of microcystin-producing aquatic blooms. *Applied and Environmental Microbiology*, 63(6), 2324–2329.
- Pascal, P. Y., Dupuy, C., Richard, P., Rzeznik-Orignac, J., & Niquil, N. (2008). Bacterivory of a mudflat nematode community under different environmental conditions. *Marine Biology*, 154(4), 671–682. <https://doi.org/10.1007/s00227-008-0960-9>
- Percent, S. F., Frischer, M. E., Vescio, P. A., Duffy, E. B., Milano, V., McLellan, M., Stevens, B. M., Boylen, C. W., & Nierzwicki-Bauer, S. A. (2008). Bacterial community structure of acid-impacted lakes: What controls diversity? *Applied and Environmental Microbiology*, 74(6), 1856–1868. <https://doi.org/10.1128/AEM.01719-07>
- Porazinska, D. L., Giblin-Davis, R. M., Faller, L., Farmerie, W., Kanzaki, N., Morris, K., Powers, T. O., Tucker, A. E., Sung, W., & Thomas, W. K. (2009). Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. *Molecular Ecology Resources*, 9(6), 1439–1450. <https://doi.org/10.1111/j.1755-0998.2009.02611.x>
- Porazinska, D. L., Giblin-Davis, R. M., Sung, W., & Thomas, W. K. (2010). Linking operational clustered taxonomic units (OCTUs) from

- parallel ultra sequencing (PUS) to nematode species. *Zootaxa*, 2427, 55–63 <https://doi.org/10.11646/zootaxa.2427.1.6>
- Porazinska, D. L., Farrer, E. C., Spasojevic, M. J., Bueno De Mesquita, C. P., Sartwell, S. A., Smith, J. G., White, C. J., Suding, K. N., & Schmidt, S. K. (2018). Plant diversity and density predict belowground diversity and function in an early successional alpine ecosystem. *Ecology*, 99(9), 1942–1952. <https://doi.org/10.1002/ecy.2420>
- Porazinska, D. L., Bueno de Mesquita, C. P., Farrer, E. C., Spasojevic, M. J., Suding, K. N., & Schmidt, S. K. (2021). Nematode community diversity and function across an alpine landscape undergoing plant colonization of previously unvegetated soils. *Soil Biology and Biochemistry*, 161, 108380. <https://doi.org/10.1016/j.soilbio.2021.108380>
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Yarza, P., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, 41(D1), 590–596. <https://doi.org/10.1093/nar/gks1219>
- R Core Team (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from <https://www.R-project.org/>
- Reiss, J., Forster, J., Cassio, F., Pascoal, C., Stewart, R., & Hirst, A. (2010). When microscopic organisms inform general ecological theory. *Advances in Ecological Research*, 5, 43, 45–85. <https://doi.org/10.1016/B978-0-12-385005-8.00002-2>
- Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, 10, 1–22. <https://doi.org/10.7717/peerj.2584>
- Schenk, J., Kleinbölting, N., & Traunspurger, W. (2020). Comparison of morphological, DNA barcoding, and metabarcoding characterizations of freshwater nematode communities. *Ecology and Evolution*, 10(6), 2885–2899. <https://doi.org/10.1002/ece3.6104>
- Schratzberger, M., Holterman, M., van Oevelen, D., & Helder, J. (2019). A Worm's world: Ecological flexibility pays off for free-living nematodes in sediments and soils. *Bioscience*, 69(11), 867–876. <https://doi.org/10.1093/biosci/biz120>
- Shen, C., Shi, Y., Fan, K., He, J. S., Adams, J. M., Ge, Y., & Chu, H. (2019). Soil pH dominates elevational diversity pattern for bacteria in high elevation alkaline soils on the Tibetan plateau. *FEMS Microbiology Ecology*, 95(2), 1–9. <https://doi.org/10.1093/femsec/fiz003>
- Siles, J. A., & Margesin, R. (2016). Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in alpine forest soils: What are the driving factors? *Microbial Ecology*, 72(1), 207–220. <https://doi.org/10.1007/s00248-016-0748-2>
- Sommers, P., Porazinska, D. L., Darcy, J. L., Gendron, E. M. S., Vimercati, L., Solon, A. J., & Schmidt, S. K. (2020). Microbial species–area relationships in Antarctic cryoconite holes depend on productivity. *Microorganisms*, 8(11), 1747. <https://doi.org/10.3390/microorganisms8111747>
- Song, D., Pan, K., Tariq, A., Sun, F., Li, Z., Sun, X., Zhang, L., Olusanya, O. A., & Wu, X. (2017). Large-scale patterns of distribution and diversity of terrestrial nematodes. *Applied Soil Ecology*, 114, 161–169. <https://doi.org/10.1016/j.apsoil.2017.02.013>
- Stegen, J. C., Lin, X., Konopka, A. E., & Fredrickson, J. K. (2012). Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME Journal*, 6, 1653–1664. <https://doi.org/10.1038/ismej.2012.22>
- Sylvain, Z. A., Wall, D. H., Cherwin, K. L., Peters, D. P. C., Reichmann, L. G., & Sala, O. E. (2014). Soil animal responses to moisture availability are largely scale, not ecosystem dependent: Insight from a cross-site study. *Global Change Biology*, 20(8), 2631–2643. <https://doi.org/10.1111/gcb.12522>
- Szilagy, J., Zlotnik, V. A., Gates, J. B., & Jozsa, J. (2011). Mapping mean annual groundwater recharge in the Nebraska Sand Hills, USA. *Hydrogeology Journal*, 19(8), 1503–1513. <https://doi.org/10.1007/s10040-011-0769-3>
- Tahseen, Q. (2012). Nematodes in aquatic environments: Adaptations and survival strategies. *Biodiversity Journal*, 3(1), 13–40.
- Tang, X., Xie, G., Shao, K., Hu, Y., Cai, J., Bai, C., Gong, Y., & Gao, G. (2020). Erratum: Contrast diversity patterns and processes of microbial community assembly in a river-lake continuum across a catchment scale in northwestern China. *Environmental Microbiomes*, 15(1), 1–17. <https://doi.org/10.1186/s40793-020-00360-z>
- Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., Prill, R. J., Tripathi, A., Gibbons, S. M., Ackermann, G., Navas-Molina, J. A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J. T., Mirarab, S., Xu, Z. Z., Jiang, L., ... The Earth Microbiome Project Consortium. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, 551, 467–463. <https://doi.org/10.1038/nature24621>
- Todd, T. C., Blair, J. M., & Milliken, G. A. (1999). Effects of altered soil-water availability on a tallgrass prairie nematode community. *Applied Soil Ecology*, 13, 45–55.
- Todd, T. C., Powers, T. O., & Mullin, P. G. (2006). Sentinel nematodes of land-use change and restoration in tallgrass prairie. *Journal of Nematology*, 38(1), 20–27.
- Trap, J., Bonkowski, M., Plassard, C., Villenave, C., & Blanchart, E. (2016). Ecological importance of soil bacterivores for ecosystem functions. *Plant and Soil*, 398(1–2), 1–24. <https://doi.org/10.1007/s11104-015-2671-6>
- Traunspurger, W. (2000). The biology and ecology of lotic nematodes. *Freshwater Biology*, 44(1), 63–91. <https://doi.org/10.1046/j.1365-2427.2000.00585.x>
- van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D. A., de Goede, R. G. M., Adams, B. J., Ahmad, W., Adruiz, W. S., Bardgett, R. D., Bonkowski, M., Campos-Herrera, R., Cares, J. E., Caruso, T., de Brito Caixeta, L., Chen, X., Costa, S. R., Creamer, R., ... Crowther, T. W. (2019). Soil nematode abundance and functional group composition at a global scale. *Nature*, 572(7768), 194–198. <https://doi.org/10.1038/s41586-019-1418-6>
- Vellend, M. (2010). Conceptual synthesis in community ecology. *Quarterly Review of Biology*, 85(2), 183–206. <https://doi.org/10.1086/652373>
- Wang, J., Yang, D., Zhang, Y., Shen, J., van der Gast, C., Hahn, M. W., & Wu, Q. (2011). Do patterns of bacterial diversity along salinity gradients differ from those observed for macroorganisms? *PLoS One*, 6(11), 1–8. <https://doi.org/10.1371/journal.pone.0027597>
- Wang, N., Ivey, C. D., Dorman, R. A., Ingersoll, C. G., Steevens, J., Hammer, E. J., Bauer, C. R., & Mount, D. R. (2018). Acute toxicity of sodium chloride and potassium chloride to a unionid mussel (*Lampsilis siliquoidea*) in water exposures. *Environmental Toxicology and Chemistry*, 37(12), 3041–3049. <https://doi.org/10.1002/etc.4206>
- Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., & Zemla, J. (2017). R package “corrplot”: Visualization of a correlation matrix. *Stat*, 56, 316–324. Retrieved from <https://github.com/taiyun/corrplot%0Ahttps://github.com/taiyun/corrplot/issues>
- Weise, L., Ulrich, A., Moreano, M., Gessler, A., Kayler, Z. E., Steger, K., Zeller, B., Rudolph, K., Knezevic-Jaric, J., & Premke, K. (2016). Water level changes affect carbon turnover and microbial community composition in lake sediments. *FEMS Microbiology Ecology*, 92(5), 1–14. <https://doi.org/10.1093/femsec/fiw035>
- Wiesel, L., Daniell, T. J., King, D., & Neilson, R. (2015). Determination of the optimal soil sample size to accurately characterise nematode communities in soil. *Soil Biology and Biochemistry*, 80, 89–91. <https://doi.org/10.1016/j.soilbio.2014.09.026>
- Wlostowski, A. N., Gooseff, M. N., & Adams, B. J. (2018). Soil moisture controls the thermal habitat of active layer soils in the McMurdo dry valleys. *Antarctica. Journal of Geophysical Research: Biogeosciences*, 123(1), 46–59. <https://doi.org/10.1002/2017JG004018>
- Wu, J., Chen, H., & Zhang, Y. (2016). Latitudinal variation in nematode diversity and ecological roles along the Chinese coast. *Ecology and Evolution*, 6(22), 8018–8027. <https://doi.org/10.1002/ece3.2538>

- Wu, T., Ayres, E., Bardgett, R. D., Wall, D. H., & Garey, J. R. (2011). Molecular study of worldwide distribution and diversity of soil animals. *PNAS*, 108(43), 17720–17725. <https://doi.org/10.1073/pnas.1103824108>
- Xiong, J., Liu, Y., Lin, X., Zhang, H., Zeng, J., Hou, J., Yang, Y., Yao, T., Knight, R., & Chu, H. (2012). Geographic distance and pH drive bacterial distribution in alkaline lake sediments across Tibetan plateau. *Environmental Microbiology*, 14(9), 2457–2466. <https://doi.org/10.1111/j.1462-2920.2012.02799.x>
- Yeates, G. W., Bongers, T., de Goede, R. G. M., Freckman, D. W., & Georgieva, S. S. (1993). Feeding habits in soil nematode families and genera—an outline for soil ecologists. *Journal of Nematology*, 25(3), 315–331.
- Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., & Glöckner, F. O. (2014). The SILVA and “all-species living tree project (LTP)” taxonomic frameworks. *Nucleic Acids Research*, 42(D1), 643–648. <https://doi.org/10.1093/nar/gkt1209>
- Zhang, L., Fang, J., & Joeckel, R. M. (2013). Microbial biomass and community structure in alkaline lakes of the Nebraska Sand Hills, USA. *Chemical Geology*, 356, 171–180. <https://doi.org/10.1016/j.chemgeo.2013.08.017>
- Zhao, P., Bao, J., Wang, X., Liu, Y., Li, C., & Chai, B. (2019). Deterministic processes dominate soil microbial community assembly in subalpine coniferous forests on the loess plateau. *PeerJ*, 7, e6746. <https://doi.org/10.7717/peerj.6746>
- Zhou, J., & Ning, D. (2017). Stochastic community assembly: Does it matter in microbial ecology? *Microbiology and Molecular Biology Reviews*, 81(4), 1–32. <https://doi.org/10.1128/MMBR.00002-17>
- Zohary, T., & Ostrovsky, I. (2011). Ecological impacts of excessive water level fluctuations in stratified freshwater lakes. *Inland Waters*, 1(1), 47–59. <https://doi.org/10.5268/IW-1.1.406>
- Zullini, A. (2006). Order Triplonchida. In E. Abebe, W. Traunspurger, & I. Andrassy (Eds.), *Freshwater nematodes: Ecology and taxonomy* (pp. 293–325). CABI Publishing.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Gattoni, K., Gendron, E. M. S., Borgmeier, A., McQueen, J. P., Mullin, P. G., Powers, K., Powers, T. O., & Porazinska, D. L. (2022). Context-dependent role of abiotic and biotic factors structuring nematode communities along two environmental gradients. *Molecular Ecology*, 00, 1–14. <https://doi.org/10.1111/mec.16541>