soil systems

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Article

Biotic Interactions in Experimental Antarctic Soil Microcosms Vary with Abiotic Stress

E. Ashley Shaw [1,*] and Diana H. Wall [2]

[1] Department of Biology, Colorado State University, Fort Collins, CO 80523-1878, USA
[2] Department of Biology and Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523-1878, USA
* Correspondence: ashleysh@uoregon.edu

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Abstract: Biotic interactions structure ecological communities but abiotic factors affect the strength of these relationships. These interactions are difficult to study in soils due to their vast biodiversity and the many environmental factors that affect soil species. The McMurdo Dry Valleys (MDV), Antarctica, are relatively simple soil ecosystems compared to temperate soils, making them an excellent study system for the trophic relationships of soil. Soil microbes and relatively few species of nematodes, rotifers, tardigrades, springtails, and mites are patchily distributed across the cold, dry landscape, which lacks vascular plants and terrestrial vertebrates. However, glacier and permafrost melt are expected to cause shifts in soil moisture and solutes across this ecosystem. To test how increased moisture and salinity affect soil invertebrates and their biotic interactions, we established a laboratory microcosm experiment (4 community × 2 moisture × 2 salinity treatments). Community treatments were: (1) Bacteria only (control), (2) Scottnema (*S. lindsayae* + bacteria), (3) Eudorylaimus (*E. antarcticus* + bacteria), and (4) Mixed (*S. lindsayae* + *E. antarcticus* + bacteria). Salinity and moisture treatments were control and high. High moisture reduced *S. lindsayae* adults, while high salinity reduced the total *S. lindsayae* population. We found that *S. lindsayae* exerted top-down control over soil bacteria populations, but this effect was dependent on salinity treatment. In the high salinity treatment, bacteria were released from top-down pressure as *S. lindsayae* declined. Ours was the first study to empirically demonstrate, although in lab microcosm conditions, top-down control in the MDV soil food web.

Keywords: nematode; bacteria; soil communities; trophic interactions; biological interactions; polar; desert; top-down effects

1. Introduction

They ways biological interactions affect communities is a key research theme in ecology. Biotic interactions are ubiquitous in most terrestrial ecosystems and interact with abiotic factors and dispersal to determine populations and community structure [1]. For example, studies have shown how biotic interactions affect plants [1], benthic invertebrates [2], and bird communities [3] under varying environmental conditions, but relatively few studies have empirically examined how biotic interactions affect soil community structure and function (but see: [4,5]). This is partly due to the vast biodiversity in soils, where relationships are further confounded by the difficulty of directly observing interactions among microscopic species along with many interacting factors, including plants and aboveground animals.

The McMurdo Dry Valleys (MDV) in Victoria Land, Antarctica, compose the largest ice-free area on the continent [6]. Among the world's harshest environments, they are a simple ecosystem with very limited diversity of eukaryotes [7,8] compared to temperate ecosystems, making them an excellent

system in which to study soil communities [9]. There are no vascular plants or vertebrates, and the metazoan diversity includes just a few species of nematodes, rotifers, tardigrades, collembolans, and mites [10,11], the most abundant of which is a nematode [7,12]. Low temperatures, low water, low organic carbon availability, and high salinity are factors known to constrain life in the MDV [12–14]. However, these factors are heterogeneous and shift across the landscape due to climate-induced changes [15]. For example, elevated solar radiation and episodic warming has altered liquid water availability through melted buried ice, higher stream flows, expanded stream margins, and the formation of shallow groundwater transports, e.g., water tracks [16]. When water reaches previously dry soils, it liberates and mobilizes soil nutrients and salts, weathers soil, and stimulates primary productivity, significantly altering soil properties that affect soil biota [17–19]. Greater hydrological connectivity through the formation of more abundant streams and water tracks is predicted for the future [20], and could alter soil habitats and their biodiversity landscape-wide.

Previous research has shown that abiotic factors explain much of the variation in invertebrate populations and community structure in the MDV. Along with other factors such as pH and carbon availability, moisture and salinity affect microbes and invertebrates from population to ecosystem (see Table 1; [13,14,21]). Dry soil habitat (~2 to 3% gravimetric water content) is dominated by an endemic, microbivore nematode, *Scottnema lindsayae*. This nematode co-occurs with other invertebrates such as the omnivore-predator nematode *Eudorylaimus antarcticus*, and sometimes with the microbivore nematode *Plectus murrayi* along with tardigrades and rotifers. However, *E. antarcticus*, *P. murrayi*, tardigrades, and rotifers prefer wet soil habitat and *S. lindsayae* is most frequently found in single-species communities in dry soils [8,13]. When soil moisture increases in dry soil, *E. antarcticus* and *P. murrayi* populations often increase, whereas *S. lindayae* populations decrease [7]. However, the long-term ecosystem response differs when wetting occurs as an extreme pulse event [22,23] or as a long-term press [24]. Andriuzzi et al. [23] showed that long-term climate-associated increases in soil moisture had detrimental effects on *S. lindsayae* and marginal positive effects on other taxa.

Salinity co-varies with soil moisture and these factors interact to affect invertebrates. For example, moisture facilitates the movement of solutes and thus, alters the salinity of habitats. In drier soils, elevated soil salinity reduces water availability and puts osmotic pressure on MDV biota [25]. In newly wetted areas, the magnitude of changes to soil moisture and salinity and their interaction can result in either an increase or a decrease in biological activity [19]. Soil salinity is a primary driver of nematode populations in the MDV and affects taxa differently [13,14,26]. Salinity causes physiological stress, especially via nitrogen toxicity, on nematodes [26]. Poage et al. (2008) found that *S. lindsayae* was more abundant in saline soils than *E. antarcticus* or *P. murrayi,* but the mortality of all species increased as salinity increased. However, *Scottnema lindsayae* is more tolerant of increased salinity than other species [26]. Besides mortality, the effect of high salinity on soil water potential may cause nematodes to become inactive [27]. Nematodes are known for their state of suspended animation, called anhydrobiosis, which they use as a desiccation survival strategy [28,29]. When they are in this anhydrobiotic state, they are decoupled from ecological processes because they are not using resources, creating waste or otherwise interacting with their environment. Treonis and Wall [27] showed that the proportion of the nematode community in anhydrobiosis was negatively correlated with increasing soil moisture and positively correlated with increasing soil salinity in the dry valleys. In addition to physiological stress on nematodes, soil salinity and moisture likely also have trophic effects on nematodes through food availability. For example, soil salinity is an important driver of microbial communities in the MDV [30] and other ecosystems [31,32] where salinity can be toxic to microbial metabolism through extracellular enzyme denaturation or changes to cell ion balance [33]. The effects of salinity and moisture on soil invertebrates are likely twofold: (1) physiological and (2) trophic. Previous research provides evidence for the direct physiological effects of water and salt on soil invertebrates and microbes (Table 1), but it is also plausible that any effect on microbes could indirectly affect their invertebrate consumers and vice versa. Yet, evidence for biotic interactions' roles in community structure and/or ecosystem functions have not been documented in the MDV

and patterns of invertebrate co-occurrence could be coincidental due to shared basic requirements for life [34]. Recent evidence showed that *E. antarcticus* occupied the omnivore-predator trophic level in Taylor Valley [35]; the presence of multiple trophic levels—from microbes and microbivores to omnivore-predators—indicates that biotic interactions existed in the MDV. Whether or not these biotic interactions are significant drivers of nematode community structure or how these interactions change under varying environmental conditions is still undetermined.

Table 1. Effects of salinity and moisture on soil taxa from organism to ecosystem.

Taxa	Effects	Citations
Populations		
Microbes	-Gene expression of AOA or AOB changes in more saline, drier valleys [1] -Moisture was positively correlated to fungi abundance while salinity was negatively correlated [2]	[1] Magalhães, et al. [36] [2] Arenz and Blanchette [37]
Invertebrates	-*Scottnema* and *Plectus* are both negatively affected by salt, but type and concentration matter [3] -Populations of *Scottnema*, *Eudorylaimus*, and *Plectus* are negatively related to salinity [4]	[3] Nkem, Virginia, Barrett, Wall and Li [26] [4] Powers, et al. [38]
Communities		
Microbes	-Composition shifts with elevated salinity from Actinobacteria to Firmicutes dominated [1,5] -Greater community diversity in drier soils [2] -Salinity drives community composition in 4 valleys [3] -Alpha diversity of communities declines with salinity [4] -Soil moisture is a significant predictor of bacterial community diversity at genus level [5]	[1] Van Horn, et al. [30] [2] Takacs-Vesbach, et al. [39] [3] Lee, et al. [40] [4] Okie, et al. [41] [5] Geyer, et al. [42]
Invertebrates	-Greater community diversity in less saline soils [6,7,8] -Greater community diversity in wetter soils [6,7,8] -Nematodes *Plectus* and *Eudorylaimus* are associated with wetter soils, *Scottnema* with drier [6,7,8,9]	[6] Nielsen, et al. [43] [7] Ayres, et al. [11] [8] Treonis, et al. [8] [9] Powers, et al. [38]
Ecosystem		
Microbes	-Water tracks alter respiration rates, depending on soil chemistry [1] -Lower microbial biomass in saltier, drier valleys [2] -Moisture addition did not affect microbial biomass in field experiment [3] -Along with pH and organic C, salinity was a predictor of microbial activity in lake and stream margins [4]	[1] Ball and Virginia [19] [2] Tamppari, et al. [44] [3] Ball, et al. [45] [4] Zeglin, et al. [46]
Invertebrates	-Water tracks affect soil invertebrate habitats, via soil chemistry changes [5], and have lower invertebrate abundance, associated with higher salinity [6] -Salinity and moisture are drivers of habitat suitability for invertebrates, *S. lindsayae* found in saltier, drier soils than other nematodes [7]	[5] Ball and Virginia [19] [6] Smith, et al. [47] [7] Courtright, et al. [13]

We asked (1) How does soil moisture and salinity affect populations of bacteria, *S. lindsayae*, and *E. antarcticus*? (2) Does the strength of their biotic interactions shift with abiotic stress? To test these questions we designed a full-factorial laboratory microcosm experiment (4 community × 2 moisture × 2 salinity treatments) to test the effects of soil salinity, moisture, and their interaction on bacteria, *S. lindsayae*, and *E. antarcticus* at four levels of community diversity (bacteria only, bacteria + *S. lindsayae* only, bacteria + *E. antarcticus* only, and bacteria + both nematode species). We hypothesized that (1) elevated moisture would have a positive effect on soil bacteria and *E. antarcticus*, but a negative effect on *S. lindsayae*, (2) elevated salinity would negatively impact all biota, and (3) the magnitude of

responses would vary by community. Specifically, we expected bacterial abundance to be negatively related to total nematode abundance, and the response of *S. lindsayae* to depend on the response of *E. antarcticus* in the community treatment with both nematode species because *S. lindsayae* is a potential prey for *E. antarcticus*.

2. Materials and Methods

Community (4 levels), moisture (2 levels), and salinity treatments (2 levels) were applied in a full factorial design with 5 replicates ($4 \times 2 \times 2 \times 5 = 80$). Community treatments were Bacteria only (control), Scottnema (bacteria + *S. lindsayae*), Eudorylaimus (bacteria + *E. antarcticus*), and Mixed (bacteria + *S. lindsayae* and *E. antarcticus*). Moisture treatments were high moisture (~8% g/g soil moisture) and control moisture (~3% g/g soil moisture). Salinity treatments were high salinity (~600 uS/cm) and control salinity (~100 uS/cm). For soil moisture treatments, 8% gravimetric soil moisture level was chosen as 'high moisture' treatment because it is representative of soil moisture levels in stream and lake margins and in water tracks [8,11,19]. We considered 3% soil moisture (gravimetric) as control soil moisture because soils were 2.94 ± 0.63 % moisture at collection. Additionally, we wanted nematodes to be active in both our control and wet treatments, and activity decline as nematodes enter anhydrobiosis at <2% moisture [27]. We chose 600 uS/cm electrical conductivity as 'high salinity' because models show that this level negatively affects both *S. lindsayae* and *E. antarcticus* populations but does not cause complete mortality [14]. Soil collected for microcosms had a background electrical conductivity of 107.51 ± 1.03 uS/cm and we considered this control salinity. We aimed to inoculate Scottnema microcosms with *S. lindsayae* at abundances of 1000 to 2000 per kg dry soil (125 to 250 per microcosm) based on mean abundances found in Taylor Valley soils [8]. For treatments with *E. antarcticus*, we aimed for 300 to 500 per kg dry soil (38 to 63 per microcosm). We chose the top of the range of *E. antarcticus* abundance found in previous studies [8,11] to allow for detectable treatment effects.

The 25 bulk soil samples from Many Glaciers Pond were mixed and homogenized and used for microcosm set-up in March 2017 (2 kg soil was reserved for nematode extraction). A total of 10 kg of bulk soil was combined in large aluminum trays and defaunated by heating soil at 65 °C for 48 h [48]. Next, 125 g of soil was added to 80 pre-autoclaved glass mason jars (1 Pint size). After soil was added, microcosms were chilled for 24 h (4 °C). Then, all microcosms were inoculated with bacteria using a soil slurry method (e.g., [49]). Briefly, 150 g of fresh soil was mixed with 800 mL of cold (4 °C) sterile deionized water in a pre-sterilized 1000 mL beaker on a stir plate for 45 min. This water was passed through a 25-micron (500 mesh) sieve to remove any nematodes but allow bacteria through. Next, 7 mL of bacterial inoculant was added to each jar with a sterile pipette. Microcosms were placed into a 4 °C incubator for 2 weeks to allow bacteria to establish before moisture, salinity, and community treatments were added.

For community treatments, *S. lindsayae* was extracted from twenty replicates of 100 g soil from the bulk soil collected at Many Glaciers Pond via cold sugar centrifugation method [50] and counted under an inverted microscope (Olympus CKX41). Approximately 8500 *S. lindsayae* was available for inoculation of 40 microcosms. These nematodes were pooled in a 50 mL centrifuge tube, allowed to settle for 1 h, and the total volume was reduced to 20 mL with an aspirator. The supernatant was reserved in a separate centrifuge tube and examined under a microscope to ensure no nematodes were present. Using a vortex on the lowest setting, nematodes were gently mixed and 0.5 mL of water + nematodes was pipetted into to each Scottnema and Mixed treatment microcosm. During inoculation, five samples were counted at random, where the 0.5 mL inoculant was pipetted directly onto a counting dish. An average of 180 ± 10.5 live *S. lindsayae* was present in the inoculant. Then, 0.5 mL of the reserved nematode-free supernatant was added to Eudorylaimus and Bacteria only treatment microcosms to account for any bacteria or nutrients present in the water.

Since very few *E. antarcticus* were present in the Many Glacier Pond soil, ten bulk soil samples collected from moss beds in 2015 at Hjorth Hill were used for the collection of *E. antarcticus* for the Eudorylaimus treatment. Ten replicates of 100 g of soil were extracted and counted under the inverted

microscope. Many *E. antarcticus* were present, along with *Plectus murrayi*, rotifers, and tardigrades. Due to the biodiversity in these samples, *E. antarcticus* were picked by hand using an eyelash tool (Superfine eyelash with handle, Prod no. 113, Ted Pella, Inc., Redding, CA, USA). Approximately 2100 live *E. antarcticus* were hand-picked into a single falcon tube with water. Then, Eudorylaimus and Mixed treatments were established the same way as Scottnema treatments (described above). Five test samples were counted during inoculation and each contained an average of 52 ± 4.3 live *E. antarcticus* per microcosm. Again, 0.5 mL of the reserved supernatant containing no nematodes was added to the remaining microcosms (Scottnema and Bacteria only community treatments).

After nematode treatments were applied to microcosms, salinity and moisture treatments were added. We added 50 mg NaCl to the high salinity treatments to bring the soil electrical conductivity from ~100 uS/cm up to ~600 uS/cm. Microcosms that did not receive NaCl (e.g., Control) were removed from the incubator for the same amount of time to account for any effects of movement or brief temperature changes. We added 2 mL of sterile, deionized water to the high moisture treatments to bring gravimetric soil moisture up to ~8% (g water/g dry soil). Control moisture treatments were weighed and placed in a dessicator inside the incubator until moisture levels were ~3% (g water/g dry soil). Microcosms were weighed every 2 weeks to check moisture levels and sterile deionized water was added as needed.

Microcosms were incubated at 8 °C for three months, approximately the length of one active season [6]. Then, microcosms were harvested, and soil subsamples were taken in the following quantities for analyses: 5 g for bacteria extraction, 100 g for nematode extraction, 10 g for soil moisture, and 10 g for electrical conductivity. All extra soil was placed in sterile whirlpac bags and frozen (−20°C). Direct counts of bacteria cells were assessed via epi-fluorescent microscopy [51,52]. Nematodes were extracted via sugar centrifugation method [50] and then nematode abundance was assessed via bright-field microscopy (Olympus CKX41). Nematodes were identified to species, sex, and life stage (e.g., adult or juvenile).

Nematode counts were standardized to soil mass and expressed as the number of individuals kg dry soil^{-1}. Bacterial cells were calculated to the number of cells g dry soil^{-1}. A three-way ANOVA was used to test the effects of moisture, salinity, and community treatments on nematode and bacteria populations. Specifically, bacterial abundance, *S. lindsayae* total abundance, *E. antarcticus* total abundance, and *S. lindsayae* juveniles, *S. lindsayae* adults, *S. lindsayae* females, and *S. lindsayae* males were assessed with *F* tests, followed by post hoc tests (Tukey HSD), to confirm significant effects ($p < 0.05$). Residuals were tested for normality of distributions and homogeneity of variance, and data were logges (x + 1) or square root transformed if they failed (rejected when Shapiro–Wilks $p < 0.05$). Specifically, square-root transformation was chosen for bacterial cells, and log (x + 1) was chosen for *E. antarcticus* abundance. *Scottnema lindsayae* abundances met assumptions of normality and were not transformed. To understand biotic interactions in changing environments, we built separate linear models to test bacteria's response to *S. lindsayae* and *S. lindsayae*'s response to *E. antarcticus*. All analyses were carried out using R 3.1.3 (R Core Development Team 2013). All experiment data were archived and available from the Mendeley Data repository, http://dx.doi.org/10.17632/2s9ppdngkm.1 [53].

3. Results

3.1. Effect of Treatments on Bacteria

There were significant overall effects of community treatments and a significant interaction between community and salinity treatment on total bacteria (Table 2). Moisture treatment did not significantly affect bacterial abundance (LSMeans, $p = 0.827$). There were significantly less bacterial cells in Bacteria only, Scottnema, and Mixed community treatments compared to the Eudorylaimus community treatment in control salinity microcosms (LSMeans, $p < 0.05$), but this effect was diminished under high salinity (Figure 1). Furthermore, there were significantly more bacterial cells present in the

high salinity treatment for Scottnema and Mixed community treatments compared to Scottnema and Mixed communities with control salinity (Figure 1; LSMeans, $p < 0.05$).

Table 2. Results of three-way ANOVA. Effects of community (C), moisture (M), and salinity (S) treatments on *Eudorylaimus* total abundance, *Scottnema* total abundance, *Scottnema* adults, *Scottnema* juveniles, *Scottnema* females, *Scottnema* males, and total bacterial cells (d.f. = degrees of freedom). Bold font indicates significant effects.

Effect	d.f.	F	*p*	Effect	d.f.	F	*p*
Eudorylaimus total abundance				*Scottnema* total abundance			
C	**1.32**	**19.89**	**<0.0001**	C	1.31	2.02	0.165
M	1.32	0.29	0.597	M	1.31	1.57	0.220
S	1.32	0.10	0.759	**S**	**1.31**	**6.25**	**0.018**
C*M	1.32	0.033	0.858	C*M	1.31	0.62	0.437
M*S	1.32	1.252	0.271	M*S	1.31	0.48	0.494
C*S	1.32	0.164	0.688	C*S	1.31	0.10	0.758
C*M*S	1.32	3.362	0.076	C*M*S	1.31	3.21	0.083
Scottnema adults				*Scottnema* juveniles			
C	1.35	0.82	0.371	C	1.31	1.46	0.237
M	**1.35**	**4.82**	**0.035**	M	1.31	0.38	0.544
S	**1.35**	**8.47**	**0.007**	**S**	**1.31**	**4.24**	**0.048**
C*M	1.35	0.195	0.662	C*M	1.31	0.38	0.541
M*S	1.35	0.49	0.489	M*S	1.31	0.039	0.844
C*S	1.35	0.11	0.73	C*S	1.31	0.226	0.638
C*M*S	**1.35**	**5.75**	**0.023**	C*M*S	1.31	1.675	0.205
Scottnema females				*Scottnema* males			
C	1.31	3.60	0.067	C	1.31	0.35	0.5574
M	1.31	2.01	0.167	M	1.31	3.239	0.0817
S	**1.31**	**9.81**	**0.004**	S	1.31	2.023	0.1649
C*M	1.31	0.72	0.403	C*M	1.31	0.448	0.5085
M*S	1.31	1.49	0.232	M*S	1.31	0.677	0.4169
C*S	1.31	0.37	0.548	C*S	1.31	0.599	0.4450
C*M*S	1.31	3.38	0.076	C*M*S	1.31	3.436	0.0733
Bacteria cells							
C	**3.53**	**7.34**	**0.0003**				
M	1.53	0.35	0.557				
S	1.53	3.51	0.067				
C*M	3.53	0.75	0.527				
M*S	1.53	0.03	0.870				
C*S	**3.53**	**16.41**	**<0.0001**				
C*M*S	3.53	0.68	0.568				

3.2. Effect of Treatments on Nematodes

There were significant overall effects of salinity treatment, but not moisture or nematode treatment on *S. lindsayae* abundance (Table 2). Total *S. lindsayae* abundance was significantly lower in the high compared to control salinity treatment (LSMeans, $p = 0.02$; Figure 2a). This effect was particularly evident for juveniles and females, but not for males (Figure 2a). While there was no significant moisture treatment effect on total population (Table 2), there were significantly less adult *S. lindsayae* in the high compared to the control moisture treatment (LSMeans, $p = 0.041$), but this effect differed by nematode and salinity treatment (Figure 2b). The moisture treatment did not affect the total abundance of juveniles (LSMeans, $p = 0.498$). *Scottnema lindsayae* was added to two community treatments: Mixed and Scottnema. There were no differences in the total abundance of *S. lindsayae* between these two treatments (LSMeans, $p = 0.198$).

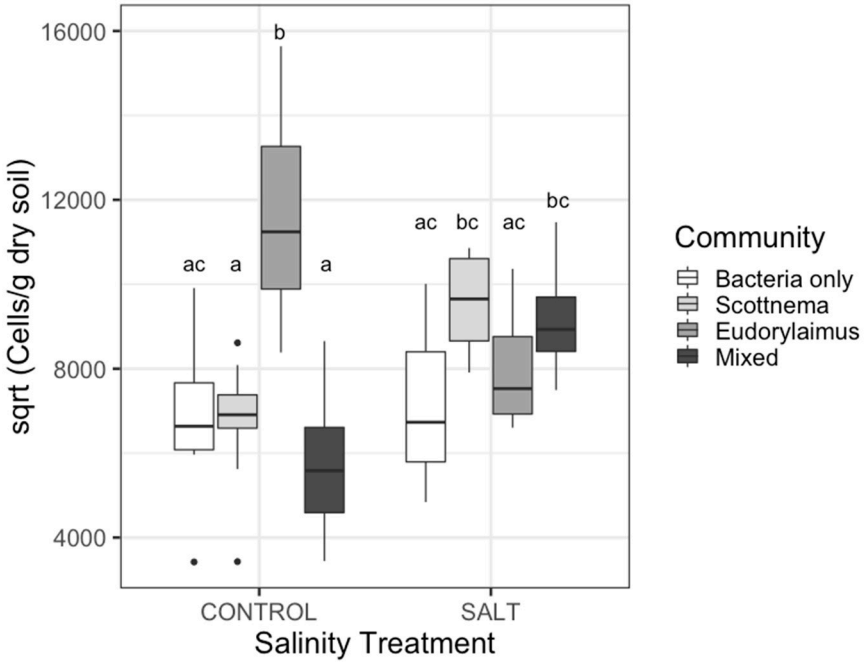

Figure 1. Community and salinity treatment effects on total bacterial cells. Different letters denote community treatments with significant differences ($p < 0.05$, LSMeans).

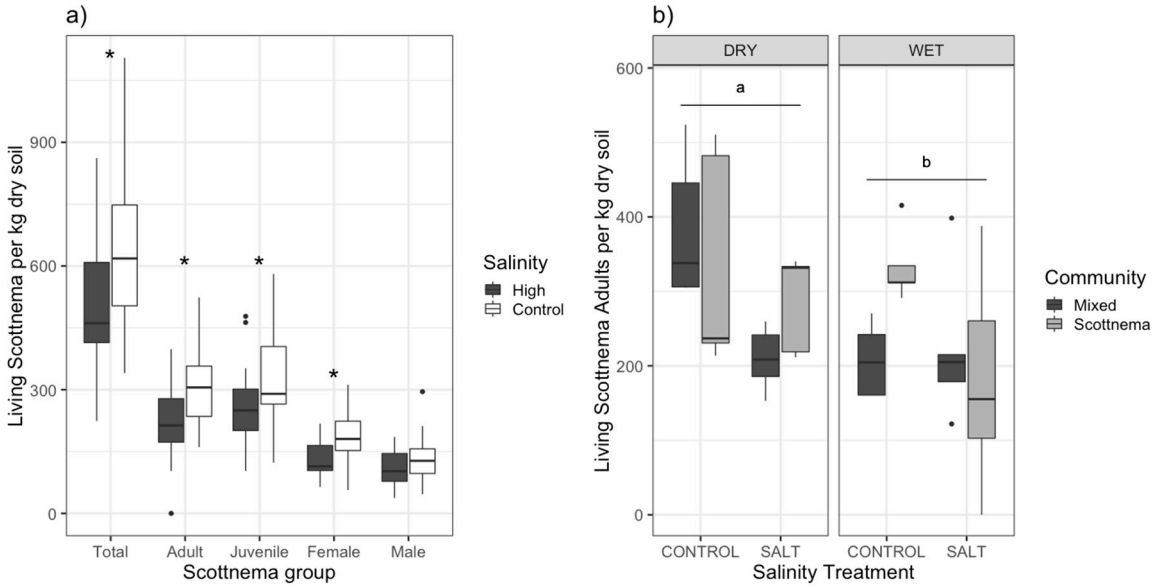

Figure 2. Salinity treatment effects on: (**a**) total living, adult, juveniles, female, and male *S. lindayae*. The asterisks denote significant differences ($p < 0.05$) between salinity treatments; (**b**) Community*Salinity*Moisture effects on *S. lindsayae* adults. The different letters denote significant differences ($p < 0.05$) between moisture treatments.

Eudorylaimus antarcticus did not survive in most microcosms (<1%). One single microcosm had 3 living *E. antarcticus*, this was the greatest survival of *E. antarcticus* (both nematode, control salinity, wet moisture). Eleven other microcosms had 1 or 2 living *E. antarcticus*, and all but two of these were the Mixed nematode treatment; the community treatment was the only significant effect on *E. antarcticus* abundance (Table 2).

3.3. Biotic Interactions

Due to the significant interaction between community and salinity treatments on bacterial abundance (Table 2), we built a multiple regression linear model to test the relationship between *S. lindsayae* and bacteria and its interaction with the salinity treatment (for Scottnema and Mixed community treatments only). Together, *S. lindsayae* abundance and salinity treatment explained 66% of the variation in bacterial abundance (Figure 3). When *S. lindsayae* abundance was held constant, there was an average of 1.07×10^8 fewer bacterial cells per g soil in control compared to high salinity (Figure 3). The relationship between bacteria and *S. lindsayae* abundance depended on the salinity treatment (interaction between *S. lindsayae* abundance and salinity treatment, $p = 0.048$). For high salinity, the number of bacterial cells per g soil declined by 9.68×10^4 per every additional *S. lindsayae* present per kg soil (Figure 3). When tested separately by salinity treatment, *S. lindsayae* abundance was significantly correlated to bacterial abundance under high, but not control salinity.

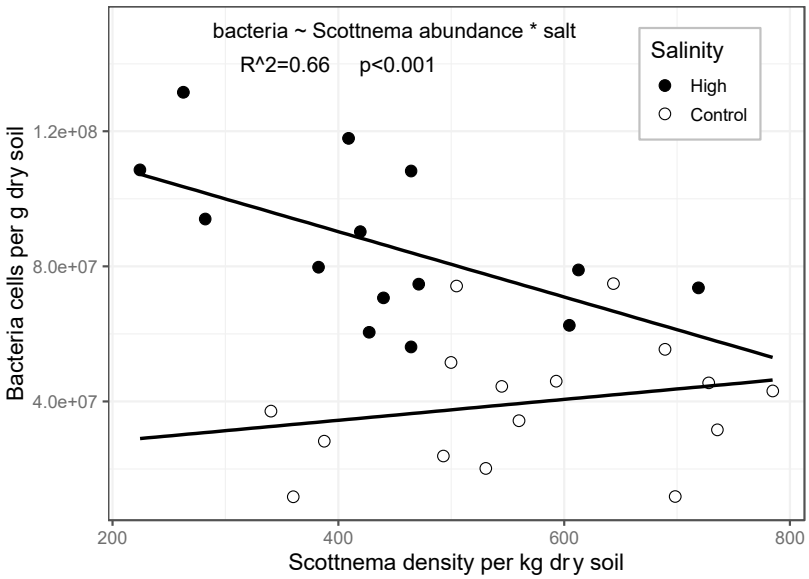

Figure 3. Linear relationship between bacteria and *S. lindsayae* abundance by salinity treatment (Control and High).

Due to the low survival of *E. antarcticus* in our microcosms, we did not test the response of *S. lindsayae* to *E. antarcticus* abundance.

4. Discussion

We hypothesized that elevated soil moisture would have a positive effect on soil microbes and *E. antarcticus* and a negative effect on *S. lindsayae*, but we found that moisture had no effect on bacteria and a negative effect on *S. lindsayae* adults only. A decline in adult nematodes could have lag effects through reduced reproduction, which negatively impacts future populations. Since our microcosm study mimicked the length of one 'active' season, and *S. lindsayae* generation time likely occurs over multiple seasons [54], it likely would have taken longer to see a moisture effect on the total *S. lindsayae* population. Moisture has been shown to have a negative impact in field studies on dry-soil adapted *S. lindsayae* (e.g., [22,23,55]), but these effects either occurred with an extreme flooding event when soil became saturated [22] or over a decade, when populations declined steadily [23].

The relative high soil salinity treatment had significant negative effects on total S. *lindsayae* abundance, as well as juveniles and females (Figure 2a), which could impact long-term populations through an effect on reproduction and recruitment to adulthood (e.g., see [56]). In other studies, not only nematode survival but also nematode activity was affected by soil salinity. Treonis and Wall [27] found a significant relationship between the number of nematodes in anhydrobiosis (a form

of cryptobiosis) and soil water potential, which is affected by the interaction between soil water and salt content. Furthermore, the response of *S. lindsayae* to elevated soil salinity in situ depends on the type and composition of salts, which are often more toxic than NaCl alone [26].

We hypothesized that the magnitude of responses would vary by community, driven by biotic interactions. We expected bacterial abundance to be negatively related to the abundance of total nematodes. Previous studies have shown that nematodes stimulate bacteria populations, whereby both their activity and populations may be increased in the presence of consumers [57]. This bacterial response to consumers often depends on the C and nutrient availability in the soil [58]. At <1% of soil content, soil organic carbon availability is extremely low in MDV soils [59]. Carbon availability may be a significant limitation for bacterial biomass even when other conditions are favorable (low salinity, suitable moisture). As Van Horn [30] found, microbes quickly take advantage of a new carbon source when it becomes available, especially in areas of low salinity. In the Eudorylaimus community treatment, effectively all ~50 nematodes added died during the experiment and were presumably decomposed by bacteria. We found that total bacterial abundance did not differ between bacteria-only and Scottnema treatments but was significantly higher in the Eudorylaimus treatment over the other three community treatments in control salinity (Figure 1). When no nematodes, and thus, no new carbon sources were added in the bacteria-only treatment, the bacteria densities were lower (Figure 1). Additionally, there was lower bacterial density for the Mixed compared to the Eudorylaimus treatment, despite high *E. antarcticus* mortality in both, suggesting that *S. lindsayae* grazing inhibits bacterial response to the elevated carbon provided by dead *E. antarcticus*. There were no overall differences in bacteria abundance between the bacteria-only and Scottnema community treatments, suggesting that either resource availability or top down control may have been in play (Figure 1). To further investigate this relationship, we performed multiple linear regression on bacterial densities within treatments containing nematodes and found that the relationship between bacteria and *S. lindsayae* abundance differed by salinity level (Figure 3). Despite the greater bacterial abundance in Eudorylaimus compared to Scottnema treatments within control salinity (Figure 1), there was no relationship between bacterial cells and *S. lindsayae* abundance when considered within treatments containing nematodes, suggesting a stable bacteria population or a constant rate of *S. lindsayae* activity (Figure 3). However, in high salinity, bacterial abundance was significantly correlated to *S. lindsayae* abundance (Figure 3), with bacterial abundance increasing as *S. lindsayae* declined, suggesting either a release of top-down pressure on bacteria or more C available to bacteria from dead nematodes. Recent research suggested top-down effects of *S. lindsayae* on soil bacteria abundance [60]. Furthermore, not only have recent climate changes altered nematode populations and community structure (e.g., [23,24]), but the long-term effects of these changes are predicted to impact trophic structure and strength of biotic interactions in the MDV [43].

We expected the response of *S. lindsayae* to depend on *E. antarcticus* in the Mixed community treatment with both nematode species present. We did not find evidence for our last hypothesis, likely due to the low survival of *E. antarcticus*. This could be due to a number of stresses, including the storage of soils, transferring the nematodes from Hjorth Hill soils to Many Glaciers Pond soil, the stress of picking the nematodes by hand with the eyelash tool, or providing insufficient food sources. Similarly, labs that culture other Antarctic nematodes have been unable to keep *E. antarcticus* alive in culture (C. Tomasel personal communication, B. Adams personal communication). Porazinska, Wall and Virginia [55] found a negative relationship between *S. lindsayae* and *E. antarcticus* in a field survey and suggested that this could be due either to a biological interaction or differing habitat requirements. Testing the biotic relationship between *S. lindsayae* and *E. antarcticus* and how this relationship is affected by environmental factors will require additional studies.

5. Conclusions

Biotic interactions—including competition, predation, and facilitation—are among the primary drivers of ecological community structure in ecosystems worldwide. The relative importance of these drivers differs across various ecosystems and depends on a suite of factors that influence the

strength of interactions. Antarctica's McMurdo Dry Valleys are among the coldest and driest terrestrial ecosystems in the world and one of the harshest to support life. Previously, little evidence of biotic interactions was found [34] and prior research has focused primarily on the relationship between abiotic factors and soil biodiversity. Ours study suggests, although in lab microcosm conditions, top-down control in the MDV soil food web through *S. lindsayae*'s effects on bacterial abundance, since bacterial abundances were lower in the Mixed (containing *S. lindsayae*) compared to the Eudorylaimus community treatment, even though all the *E. antarcticus* effectively died and were decomposed in both. Furthermore, within treatments containing nematodes, our results suggest that biotic interactions were significantly altered by abiotic stress, specifically salinity. While more studies are required to test the relationships between MDV bacteria and their consumers, especially where the abundance of consumers is explicitly manipulated, our study has implications for MDV biodiversity, where in the future, this ecosystem is expected to experience a new distribution of soil solutes and altered C availability with changing hydrological connectivity.

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References

1. Maestre, F.T.; Bowker, M.A.; Escolar, C.; Puche, M.D.; Soliveres, S.; Maltez-Mouro, S.; Garcia-Palacios, P.; Castillo-Monroy, A.P.; Martínez, I.; Escudero, A. Do biotic interactions modulate ecosystem functioning along stress gradients? Insights from semi-arid plant and biological soil crust communities. *Philos. Trans. R. Soc. B Biol. Sci.* **2010**, *365*, 2057–2070. [CrossRef] [PubMed]
2. Kolar, C.S.; Rahel, F.J. Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. *Oecologia* **1993**, *95*, 210–219. [CrossRef] [PubMed]
3. Heikkinen, R.K.; Luoto, M.; Virkkala, R.; Pearson, R.G.; Körber, J.H. Biotic interactions improve prediction of boreal bird distributions at macro-scales. *Glob. Ecol. Biogeogr.* **2007**, *16*, 754–763. [CrossRef]
4. Coleman, D.C.; Anderson, R.V.; Cole, C.V.; Elliott, E.T.; Woods, L.; Campion, M.K. Trophic interactions in soils as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon. *Microb. Ecol.* **1977**, *4*, 373–380. [CrossRef] [PubMed]
5. Wall, D.H.; Moore, J.C. Interactions underground: Soil biodiversity, mutualism, and ecosystem processes. *BioScience* **1999**, *49*, 109–117. [CrossRef]
6. Fountain, A.G.; Lyons, W.B.; Burkins, M.B.; Dana, G.L.; Doran, P.T.; Lewis, K.J.; McKnight, D.M.; Moorhead, D.L.; Parsons, A.N.; Priscu, J.C.; et al. Physical Controls on the Taylor Valley Ecosystem, Antarctica. *Bioscience* **1999**, *49*, 961–971. [CrossRef]
7. Freckman, D.W.; Virginia, R.A. Low-diversity Antarctic soil nematode communities: Distribution and response to disturbance. *Ecology* **1997**, *78*, 363–369. [CrossRef]
8. Treonis, A.M.; Wall, D.H.; Virginia, R.A. Invertebrate Biodiversity in Antarctic Dry Valley Soils and Sediments. *Ecosystems* **1999**, *2*, 482–492. [CrossRef]
9. Wall, D.H. Global change tipping points: Above- and below-ground biotic interactions in a low diversity ecosystem. *Philos. Trans. R. Soc. B Biol. Sci.* **2007**, *362*, 2291–2306. [CrossRef]

10. Adams, B.J.; Wall, D.H.; Virginia, R.A.; Broos, E.; Knox, M.A. Ecological Biogeography of the Terrestrial Nematodes of Victoria Land, Antarctica. *ZooKeys* **2014**, *419*, 29–71. [CrossRef]
11. Ayres, E.; Wall, D.H.; Adams, B.J.; Barrett, J.E.; Virginia, R.A. Unique similarity of faunal communities across aquatic–terrestrial interfaces in a polar desert ecosystem: Soil–sediment boundaries and faunal community. *Ecosystems* **2007**, *10*, 523–535. [CrossRef]
12. Wall, D.H.; Virginia, R.A. Controls on soil biodiversity: Insights from extreme environments. *Appl. Soil Ecol.* **1999**, *13*, 137–150. [CrossRef]
13. Courtright, E.M.; Wall, D.H.; Virginia, R.A. Determining habitat suitability for soil invertebrates in an extreme environment: The McMurdo Dry Valleys, Antarctica. *Antarct. Sci.* **2001**, *13*, 9–17. [CrossRef]
14. Poage, M.A.; Barrett, J.E.; Virginia, R.A.; Wall, D.H. The Influence of Soil Geochemistry on Nematode Distribution, Mcmurdo Dry Valleys, Antarctica. *Arct. Antarct. Alp. Res.* **2008**, *40*, 119–128. [CrossRef]
15. Fountain, A.G.; Levy, J.S.; Gooseff, M.N.; Van Horn, D. The McMurdo Dry Valleys: A landscape on the threshold of change. *Geomorphology* **2014**, *225*, 25–35. [CrossRef]
16. Fountain, A.G.; Saba, G.; Adams, B.; Doran, P.; Fraser, W.; Gooseff, M.; Obryk, M.; Priscu, J.C.; Stammerjohn, S.; Virginia, R.A. the Impact of a Large-Scale Climate Event on Antarctic Ecosystem Processes. *Bioscience* **2016**, *66*, 848–863. [CrossRef]
17. Ball, B.A.; Barrett, J.; Gooseff, M.N.; Virginia, R.A.; Wall, D.H.; Becky, A. Implications of meltwater pulse events for soil biology and biogeochemical cycling in a polar desert. *Polar Res.* **2011**, *30*, 14555. [CrossRef]
18. Ball, B.A.; Levy, J. The role of water tracks in altering biotic and abiotic soil properties and processes in a polar desert in Antarctica. *J. Geophys. Res. Biogeosci.* **2015**, *120*, 270–279. [CrossRef]
19. Ball, B.A.; Virginia, R.A. Meltwater seep patches increase heterogeneity of soil geochemistry and therefore habitat suitability. *Geoderma* **2012**, *189*, 652–660. [CrossRef]
20. Gooseff, M.N.; Wlostowski, A.; McKnight, D.M.; Jaros, C. Hydrologic connectivity and implications for ecosystem processes—Lessons from naked watersheds. *Geomorphology* **2017**, *277*, 63–71. [CrossRef]
21. Barrett, J.; Virginia, R.; Wall, D.; Cary, S.; Adams, B.; Hacker, A.; Aislabie, J. Co-variation in soil biodiversity and biogeochemistry in northern and southern Victoria Land, Antarctica. *Antarct. Sci.* **2006**, *18*, 535–548. [CrossRef]
22. Nielsen, U.N.; Wall, D.H.; Adams, B.J.; Virginia, R.A.; Ball, B.A.; Gooseff, M.N.; McKnight, D.M. The ecology of pulse events: Insights from an extreme climatic event in a polar desert ecosystem. *Ecosphere* **2012**, *3*, 1–15. [CrossRef]
23. Andriuzzi, W.S.; Adams, B.J.; Barrett, J.E.; Virginia, R.A.; Wall, D.H. Observed trends of soil fauna in the Antarctic Dry Valleys: Early signs of shifts predicted under climate change. *Ecology* **2018**, *99*, 312–321. [CrossRef]
24. Gooseff, M.N.; Barrett, J.E.; Adams, B.J.; Doran, P.T.; Fountain, A.G.; Lyons, W.B.; McKnight, D.M.; Priscu, J.C.; Sokol, E.R.; Takacs-Vesbach, C.; et al. Decadal ecosystem response to an anomalous melt season in a polar desert in Antarctica. *Nat. Ecol. Evol.* **2017**, *1*, 1334–1338. [CrossRef]
25. Wynn-Williams, D.D. Cyanobacteria in Deserts. In *The Ecology of Cyanobacteria*; Whitton, B.A., Potts, M., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; pp. 341–366.
26. Nkem, J.N.; Virginia, R.A.; Barrett, J.E.; Wall, D.H.; Li, G. Salt tolerance and survival thresholds for two species of Antarctic soil nematodes. *Polar Biol.* **2006**, *29*, 643–651. [CrossRef]
27. Treonis, A.M.; Wall, D.H. Soil Nematodes and Desiccation Survival in the Extreme Arid Environment of the Antarctic Dry Valleys. *Integr. Comp. Biol.* **2005**, *45*, 741–750. [CrossRef]
28. Crowe, J.H.; Madin, K.A.C. Anhydrobiosis in nematodes: Evaporative water loss and survival. *J. Exp. Zool.* **1975**, *193*, 323–333. [CrossRef]
29. Freckman, D.W.; Womersley, C. Physiological Adaptations of Nematodes in Chihuahuan Desert Soil. In *New Trends in Soil Biology*; Lebrun, P., André, H.M., de Medts, A., Grégoire-Wibo, C., Wathy, G.., Eds.; Dieu-Brichard Publishers: Louvain-LaNeuve, Belgium, 1983; pp. 396–404.
30. Van Horn, D.J.; Okie, J.G.; Buelow, H.N.; Gooseff, M.N.; Barrett, J.E.; Takacs-Vesbach, C.D. Soil Microbial Responses to Increased Moisture and Organic Resources along a Salinity Gradient in a Polar Desert. *Appl. Environ. Microbiol.* **2014**, *80*, 3034–3043. [CrossRef]
31. Lozupone, C.A.; Knight, R. Global patterns in bacterial diversity. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11436–11440. [CrossRef]

32. Wang, J.; Yang, D.; Zhang, Y.; Shen, J.; Van Der Gast, C.; Hahn, M.W.; Wu, Q. Do Patterns of Bacterial Diversity along Salinity Gradients Differ from Those Observed for Macroorganisms? *PLoS ONE* **2011**, *6*, e27597. [CrossRef]
33. Zahran, H.H. Diversity, adaptation and activity of the bacterial flora in saline environments. *Biol. Fertil. Soils* **1997**, *25*, 211–223. [CrossRef]
34. Hogg, I.D.; Cary, S.C.; Convey, P.; Newsham, K.K.; O'Donnell, A.G.; Adams, B.J.; Aislabie, J.; Frati, F.; Stevens, M.I.; Wall, D.H. Biotic interactions in Antarctic terrestrial ecosystems: Are they a factor? *Soil Biol. Biochem.* **2006**, *38*, 3035–3040. [CrossRef]
35. Shaw, E.A.; Adams, B.J.; Barrett, J.E.; Lyons, W.B.; Virginia, R.A.; Wall, D.H. Stable C and N isotope ratios reveal soil food web structure and identify the nematode Eudorylaimus antarcticus as an omnivore–predator in Taylor Valley, Antarctica. *Polar Biol.* **2018**, *41*, 1013–1018. [CrossRef]
36. Magalhães, C.M.; Machado, A.; Frank-Fahle, B.; Lee, C.K.; Cary, S.C. The ecological dichotomy of ammonia-oxidizing archaea and bacteria in the hyper-arid soils of the Antarctic Dry Valleys. *Front. Microbiol.* **2014**, *5*, 515. [CrossRef]
37. Arenz, B.; Blanchette, R. Distribution and abundance of soil fungi in Antarctica at sites on the Peninsula, Ross Sea Region and McMurdo Dry Valleys. *Soil Biol. Biochem.* **2011**, *43*, 308–315. [CrossRef]
38. Powers, L.E.; Ho, M.; Freckman, D.W.; Virginia, R.A. Distribution, Community Structure, and Microhabitats of Soil Invertebrates along an Elevational Gradient in Taylor Valley, Antarctica. *Arct. Alp. Res.* **1998**, *30*, 133–141. [CrossRef]
39. Takacs-Vesbach, C.; Zeglin, L.; Barrett, J.; Gooseff, M.N.; Priscu, J.C. Factors Promoting Microbial Diversity in the McMurdo Dry Valleys, Antarctica. In *Life in Antarctic Deserts and Other Cold Dry Environments*; Cambridge University Press (CUP): Cambridge, UK, 2010; pp. 221–257.
40. Lee, C.K.; Barbier, B.A.; Bottos, E.M.; McDonald, I.R.; Cary, S.C. The Inter-Valley Soil Comparative Survey: The ecology of Dry Valley edaphic microbial communities. *ISME J.* **2011**, *6*, 1046–1057. [CrossRef]
41. Okie, J.G.; Van Horn, D.J.; Storch, D.; Barrett, J.E.; Gooseff, M.N.; Kopsova, L.; Takacs-Vesbach, C.D. Niche and metabolic principles explain patterns of diversity and distribution: Theory and a case study with soil bacterial communities. *Proc. R. Soc. B Biol. Sci.* **2015**, *282*, 20142630. [CrossRef]
42. Altrichter, A.E.; Takacs-Vesbach, C.D.; Van Horn, D.J.; Barrett, J.E.; Geyer, K.M.; Gooseff, M.N. Bacterial community composition of divergent soil habitats in a polar desert. *FEMS Microbiol. Ecol.* **2014**, *89*, 490–494.
43. Nielsen, U.N.; Wall, D.H.; Adams, B.J.; Virginia, R.A. Antarctic nematode communities: Observed and predicted responses to climate change. *Polar Biol.* **2011**, *34*, 1701–1711. [CrossRef]
44. Tamppari, L.; Anderson, R.; Archer, P.; Douglas, S.; Kounaves, S.; McKay, C.; Ming, D.; Moore, Q.; Quinn, J.; Smith, P.; et al. Effects of extreme cold and aridity on soils and habitability: McMurdo Dry Valleys as an analogue for the Mars Phoenix landing site. *Antarct. Sci.* **2012**, *24*, 211–228. [CrossRef]
45. Ball, B.A.; Adams, B.J.; Barrett, J.; Wall, D.H.; Virginia, R.A. Soil biological responses to C, N and P fertilization in a polar desert of Antarctica. *Soil Biol. Biochem.* **2018**, *122*, 7–18. [CrossRef]
46. Zeglin, L.H.; Sinsabaugh, R.L.; Barrett, J.E.; Gooseff, M.N.; Takacs-Vesbach, C.D. Landscape Distribution of Microbial Activity in the McMurdo Dry Valleys: Linked Biotic Processes, Hydrology, and Geochemistry in a Cold Desert Ecosystem. *Ecosystems* **2009**, *12*, 562–573. [CrossRef]
47. Smith, T.; Wall, D.; Hogg, I.D.; Adams, B.; Nielsen, U.N.; Virginia, R. Thawing permafrost alters nematode populations and soil habitat characteristics in an Antarctic polar desert ecosystem. *Pedobiologia* **2012**, *55*, 75–81. [CrossRef]
48. Franco, A.L.; Knox, M.A.; Andriuzzi, W.S.; De Tomasel, C.M.; Sala, O.E.; Wall, D.H. Nematode exclusion and recovery in experimental soil microcosms. *Soil Biol. Biochem.* **2017**, *108*, 78–83. [CrossRef]
49. Bouwman, L.A.; Bloem, J.; Van den Boogert, P.H.; Bremer, F.; Hoenderboom, G.H.; De Ruiter, P.C. Short-term and long-term effects of bacterivorous nematodes and nematophagous fungi on carbon and nitrogen mineralization in microcosms. *Biol. Fertil. Soils* **1994**, *17*, 249–256. [CrossRef]
50. Freckman, D.; Virginia, R. Extraction of nematodes from Dry Valley Antarctic soils. *Polar Biol.* **1993**, *13*, 483–487. [CrossRef]
51. Frey, S.; Elliott, E.; Paustian, K. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biol. Biochem.* **1999**, *31*, 573–585. [CrossRef]
52. Sistla, S.A.; Moore, J.C.; Simpson, R.T.; Gough, L.; Shaver, G.R.; Schimel, J.P. Long-term warming restructures Arctic tundra without changing net soil carbon storage. *Nature* **2013**, *497*, 615–618. [CrossRef]

53. Shaw, E.A.; Wall, D.H. *MDV Lab Microcosm Data*; Mendeley Data; Elsevier: Amsterdam, The Netherlands, 2018.
54. Overhoff, A.; Freckman, D.W.; Virginia, R.A. Life cycle of the microbivorous Antarctic Dry Valley nematode Scottnema lindsayae (Timm 1971). *Polar Biol.* **1993**, *13*, 151–156. [CrossRef]
55. Porazinska, D.L.; Wall, D.H.; Virginia, R.A. Population Age Structure of Nematodes in the Antarctic Dry Valleys: Perspectives on Time, Space, and Habitat Suitability. *Arctic, Antarct. Alp. Res.* **2002**, *34*, 159–168. [CrossRef]
56. Knox, M.A.; Wall, D.H.; Virginia, R.A.; Vandegehuchte, M.L.; Gil, I.S.; Adams, B.J. Impact of diurnal freeze–thaw cycles on the soil nematode Scottnema lindsayae in Taylor Valley, Antarctica. *Polar Biol.* **2015**, *39*, 583–592. [CrossRef]
57. Ingham, R.E.; Trofymow, J.; Ingham, E.R.; Coleman, D.C. Interactions of bacteria, fungi, and their nematode grazers: Effects on nutrient cycling and plant growth. *Ecol. Monogr.* **1985**, *55*, 119–140. [CrossRef]
58. Anderson, R.V.; Elliott, E.T.; McClellan, J.F.; Coleman, D.C.; Cole, C.V.; Hunt, H.W. Trophic interactions in soils as they affect energy and nutrient dynamics. III. Biotic interactions of bacteria, amoebae and nematodes. *Microb. Ecol.* **1978**, *4*, 361–371. [CrossRef]
59. Burkins, M.B.; Virginia, R.A.; Chamberlain, C.P.; Wall, D.H. Origin and Distribution of Soil Organic Matter in Taylor Valley, Antarctica. *Ecology* **2000**, *81*, 2377–2391. [CrossRef]
60. Knox, M.A.; Andriuzzi, W.S.; Buelow, H.N.; Takacs-Vesbach, C.; Wall, D.H.; Adams, B.J. Decoupled responses of soil bacteria and their invertebrate consumer to warming, but not freeze-thaw cycles, in the Antarctic Dry Valleys. *Ecol. Lett.* **2017**, *20*, 1242–1249. [CrossRef]