

Long-term warming-induced trophic downgrading in the soil microbial food web

Dahl Mathilde Borg^{1*}, Söllinger Andrea², Sigurðsson Páll³, Janssens Ivan⁴, Peñuelas Josep^{5,6}, Sigurdsson Bjarni D.³, Richter Andreas⁷, Tveit Alexander² and Urich Tim^{1*}

¹Institute of Microbiology, University of Greifswald, Greifswald, Germany

²Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Tromsø, Norway

³Agricultural University of Iceland, Hvanneyri, Iceland

⁴Department of Biology, University of Antwerp, Antwerp, Belgium

⁵CSIC, Global Ecology Unit CREAF-CSIC-UAB, Bellaterra 08193, Catalonia, Spain.

⁶CREAF, Cerdanyola del Vallès 08193, Catalonia, Spain.

⁷Centre for Microbiology and Environmental Systems Science, University of Vienna, Vienna, Austria

*Corresponding author: dahlm@uni-greifswald.de and tim.urich@uni-greifswald.de

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1 **Abstract**

2 Climatic warming has been hypothesized to accelerate organic matter decomposition by soil
3 microorganisms and thereby enhance carbon release to the atmosphere. However, the long-term
4 consequences of soil warming on belowground biota interactions are poorly understood. Here we
5 investigate how geothermal warming by 6 °C for more than 50 years affects soil microbiota. Using
6 metatranscriptomics we obtained comprehensive profiles of the prokaryotic, eukaryotic and viral
7 players of the soil microbial food web. When compared to ambient soil temperature conditions, we
8 found pronounced differences in taxa abundances within and between trophic modules of the soil
9 food web. Specifically, we observed a ‘trophic downgrading’ at elevated temperature, with soil fauna
10 decreasing in abundance, while predatory bacteria and viruses became relatively more abundant.
11 We propose that the drivers for this shift are previously observed decreases in microbial biomass
12 and soil organic carbon, and the increase in soil bulk density (decrease in soil porosity) at elevated
13 temperature. We conclude that a trophic downgrading may have important implications for soil
14 carbon sequestration and nutrient dynamics in a warming world.

15 **Main text**

16 The majority of soil organic carbon (SOC) is found in arctic and subarctic regions, where low soil
17 temperatures constrain mineralization and are considered the most important abiotic driver of
18 carbon (C) release to the atmosphere (Conant et al. 2011; Crowther et al., 2016). Microbially driven
19 processes are responsible for most of the SOC mineralisation (Nielsen et al., 2011). Warmer
20 conditions promote microbial activity, leading to the prediction that global warming will accelerate
21 SOC decomposition and enhance C release to the atmosphere (Jenkinson et al., 1991; Melillo et al.,
22 2017).

23 Traditionally, fast mineralization of easily degradable C has been considered primarily bacterial,
24 while slow mineralization of recalcitrant substrates was considered to be dominated by fungi (Hunt
25 et al., 1987; Wardle et al., 2004). In addition, the involvement of higher-trophic level taxa in
26 controlling mineralization rates has been evident for decades (Coleman et al., 1977; Wardle et al.,
27 1998; Cragg and Bardgett, 2001). However, due to the high spatial heterogeneity of the soil
28 environment and temporal variation in the community composition and activity, many aspects of
29 decomposition pathways remain unresolved (Nielsen et al., 2011; Kramer et al., 2016; Naylor et al.,
30 2020). Modern molecular biology techniques have allowed higher resolution and precision, and
31 recent studies have shed light on some of the complex dynamics of soil food webs (Thakur and
32 Geisen, 2019; Sokol et al., 2022). For example, Pausch et al. (2016) found fungi, not bacteria, to be
33 predominantly involved in utilizing easily degradable root exudates in arable soils, thus violating the

34 assumption mentioned above. Likewise, 'protists' have been demonstrated to act on multiple
35 trophic levels, feeding on both fungi and bacteria, but also on other 'protists' and even nematodes
36 (Geisen, 2016; Hünninghaus et al., 2017). Similarly, the influence of predatory bacteria on microbial
37 food web dynamics has recently received more attention (Petters et al., 2021; Hungate et al. 2021).
38 Finally, interactions between viruses and specific microbial processes in soil have been
39 demonstrated (Starr et al., 2019; Lee et al., 2021), however, the role of viruses and trophic
40 regulation in soil mineralization processes remains unresolved.

41 In this study we applied metatranscriptomics to analyse the small-subunit ribosomal RNA (SSU rRNA)
42 and viral RNA for a broad simultaneous and unbiased analysis of the soil microbial food web (Urich
43 et al., 2008), including, bacteria, archaea, fungi, 'protists', Metazoa and viruses. We studied a
44 grassland ecosystem in Iceland where geothermal activity has consistently warmed the soil for more
45 than 50 years, forming natural soil temperature gradients (the 'ForHot' site, see Sigurdsson et al.
46 2016 and supplement S1). We have compared non-heated (denoted *Long-Term Warming Ambient*
47 *soil Temperature*; LTW-A_T) and +6 °C above ambient temperature (*Long-Term Warming Elevated soil*
48 *Temperature*; LTW-E_T) soil conditions in four replicate soil samples collected at the peak of the
49 growing season in July 2016 (Séneca et al., 2021; Söllinger et al., 2022 and supplement S1).

50 The composition of belowground (micro-)biota differed significantly between LTW-A_T and LTW-E_T
51 (PERMANOVA, $p = 0.02$, $R^2 = 0.3$; Fig. 1a), a pattern also seen in the virome (PERMANOVA, $p = 0.03$,
52 $R^2 = 0.3$, Fig. 1b). Furthermore, the estimated viral load was strongly correlated with microbial
53 biomass (microbial carbon per g soil; $p < 0.01$, Pearson's $r = 0.94$ and when LTW-A_T2 was excluded as
54 an outlier; Pearson's $r = 0.75$, $p = 0.05$; Fig. 1c–d). We identified mainly RNA viruses, while a minor
55 viral fraction was DNA viruses, such as bacteriophages (*Caudovirales*; supplement S2 and Fig. 2a).
56 The reported virome composition was strikingly similar to the few available metatranscriptomics
57 studies on soil viromes (Starr et al., 2019), however the obtained annotations may be biased by the
58 still limited annotated public virome databases. The total amount of extracted RNA was on average
59 $3.0 \pm 3.2 \mu\text{g}$ per g dry weight soil, but the amount of total RNA and microbial biomass was not
60 significantly different between LTW-A_T and LTW-E_T likely due to large individual sample variation
61 (Fig. 1a–c), although a difference have been observed previously (with larger sample sets; Söllinger
62 et al., 2022; Walker et al., 2018). Likewise, the relative abundances of the main domains (i.e.,
63 bacteria, archaea and eukaryotes) did not significantly differ between LTW-A_T and LTW-E_T ($p > 0.1$,
64 supplement S3).

65 Within the domains, in contrast, several taxa did exhibit significantly different relative abundances
66 between LTW-A_T and LTW-E_T (Fig. 1e–f, supplement S4). Within bacteria, the *Chloroflexi* were

67 significantly less relative abundant in the warmed soils, while *Planctomycetes*, *Verrucomicrobia* and
68 especially *Deltaproteobacteria* were relatively more abundant. In addition, the root associated
69 fungal class *Archaeorhizomycetes* had a higher relative abundance under heated conditions. Some
70 differences in the relative abundance of both bacteria and fungi may be attributable to the response
71 of plants to warming; plants may increase their investment in symbiotic associations (e.g.,
72 mycorrhizae) in response to increased resource competition (Kim et al., 2015; Trap et al., 2016). In
73 accordance, an increased uptake of root exudates (¹³C-labeled) was observed for arbuscular
74 mycorrhizal fungi under elevated temperature conditions (Verbrigghe et al., 2022b). Furthermore,
75 biochemical differences in the litter of the dominant grass species (*Agrostis capillaris*; more
76 secondary metabolites; phenolic acids and terpenes, Gargallo-garriga et al., 2017) at LTW-E_T may
77 have favoured members of the bacterial phylum *Verrucomicrobia* e.g., '*Chthoniobacterales*', which
78 were recently reported as the primary contributor to phenolic acids degradation in microbiomes of
79 lichens (Cernava et al., 2017).

80 The significant taxonomic differences between LTW-A_T and LTW-E_T reflected relative differences in
81 the abundance within and between trophic modules of the soil food web (Fig. 2a–b). Generally, the
82 larger Metazoa (e.g., *Insecta*, *Haplotaxida*, *Collembola*, *Protura* and *Rotifera*) had a higher relative
83 abundance at LTW-A_T, although this pattern was only significant for the *Rotifera* ($p < 0.05$) and a
84 trend was seen for the bacterial-feeding nematodes ($p < 0.1$). On the contrary, the smallest
85 predators, the bacterivorous bacteria, had a significantly higher relative abundance at LTW-E_T.
86 Additionally, several of the most abundant viruses (of both pro- and eukaryotic hosts) had a
87 significantly higher relative abundance at LTW-E_T (Fig. 2a).

88 The composition of bacterivores can strongly affect microbially-mediated processes, such as
89 nitrogen (N) mineralization. For example, up to 20–40% less N was released during nematode
90 grazing than during ciliate grazing (Trap et al., 2016). A recent study based on the same
91 metatranscriptomes as used here reported an increase in the expression of genes for the
92 degradation of N-rich polymers, especially those in microbial necromass, suggesting that the
93 recycling of microbial residues is a key process at LTW-E_T (Séneca et al., 2021). Together with a
94 previously reported increased rate of mass-specific growth at LTW-E_T (Walker et al., 2018; Marañón-
95 Jiménez et al., 2018) and our findings, one might speculate that the shift towards dominance of
96 small-sized organisms and predators at LTW-E_T has shifted mineralisation pathways towards the
97 recycling of organic-N.

98 The consistent heating for at least 50 years has resulted in a depleted SOC stock at LTW-E_T (up to
99 27% SOC depletion compared to LTW-A_T; Walker et al., 2018; Verbrigghe et al., 2022a) and the

100 stability of soil aggregates has subsequently decreased, increasing bulk density and decreasing soil
101 porosity at LTW-E_T (Verbrigghe et al., 2022a). We propose that the differences in the relative
102 abundances of the trophic modules is driven by both reductions in resource availability (microbial
103 biomass and amount of SOC, Verbrigghe et al., 2022a) and physical constraints of the environment
104 (lower porosity, Verbrigghe et al., 2022a), parameters which could all be related to the variation in
105 community composition (Person's corr. coefficient > 0.5) although the effect was not statistically
106 significant likely given the limited number of samples (n=8, supplement S5).

107 A smaller community size (suggested by less total extracted RNA and reduced microbial biomass;
108 here non-significant, but previously reported up to 30% reduced; Walker et al., 2018; Verbrigghe et
109 al., 2022b) would be consistent with the strong substrate depletion at LTW-E_T. In addition, following
110 the fundamental relationship between metabolic rate and temperature an increased metabolic
111 demand is expected for the organisms at LTW-E_T (Brown et al., 2004). This was seen at community
112 scale (Walker et al., 2018; Séneca et al., 2021) and clearly evident from the mRNA transcripts pools
113 of the microbiota, especially for bacteria (Söllinger et al., 2022), where a down-regulation of the
114 cellular machinery for the biosynthesis of proteins (fewer ribosomes) was pointing to a direct
115 physiological response by the microbiota. For larger organisms, however, this development towards
116 less biomass and higher metabolic demand may lead to starvation (Rall et al., 2010). In accordance,
117 Holmstrup *et al.* (2018) found that Collembola species with smaller body masses became more
118 abundant at LTW-E_T than at LTW-A_T. The same authors found that the total biomass of mesofauna
119 declined at LTW-E_T compared to LTW-A_T (with seasonal variation, Thakur *et al.* 2023). These findings
120 are consistent with global patterns of Collembola density (increasing with latitude; Potapov et al.,
121 2023) and a recent study demonstrating how several microarthropods developed smaller bodies due
122 to climate change (increased warming and altered precipitation, Yin et al., 2020).

123 Besides basal resource availability (i.e., SOC and/or microbial biomass), the physical properties of the
124 soil matrix can influence the abundance and mobility of soil organisms (Rutherford and Yuma, 1992;
125 Adl, 2007). The role of physical constraints of the soil environment in shaping the microbial
126 community and as regulators of trophic interactions has recently received a renewed scientific focus
127 (Erktan et al., 2020). Soil pore size as a main driver for assembly has been shown for collembola
128 (Heisler and Kaiser, 1995) and nematodes in microcosm experiments (Rønn et al., 1995), and
129 agricultural field studies (Jiang et al., 2018), as well as on a global scale (van den Hoogen et al.,
130 2019), while Andriuzzi and Wall (2018) found nematode body shape and mass to be correlated with
131 soil pore size and SOC availability, respectively, in a grassland soil.

132 Also, similar soil structure effects have been reported for bacteria: Sessitsch *et al.* (2001) found that
133 particle size had a larger effect on bacterial composition than substrate amendments with various
134 organic materials, and suggested an indirect effect of the physical environment via trophic
135 interactions, i.e. that 'protist' grazing determined the bacterial composition in larger pores. Elliott *et al.* (1980) studied the interplay between the trophic interactions of nematodes, 'protists', bacteria
136 and pore size and demonstrated that 'protists' allocated resources up the food chain to nematodes
137 by feeding on bacteria living in pores that were inaccessible to nematodes. Similarly, a recent
138 metatranscriptomic study (Petters *et al.*, 2021) reported predatory 'myxobacteria' to be the
139 dominant micropredators in fine-textured mineral soils and suggested that the smaller pores
140 restricted access of the larger micropredators ('protists' and nematodes), yielding an advantage for
141 the – much smaller – bacterial predators. In the present study, these same 'myxobacteria' were
142 significantly more abundant at LTW-E_T compared to LTW-A_T (Fig. 2a), consistent with the increased
143 soil bulk density (supplement S5), suggesting that a similar dynamic is occurring in the soil food web
144 at LTW-E_T.
145

146 In summary, this study found that the soil food web under elevated temperatures was dominated by
147 smaller sized organisms; such trophic downgrading may have important implications for carbon
148 sequestration and nutrient dynamics in warming subarctic and arctic soils. We found strong
149 indications that differences in resource availability (e.g., amount of carbon content and microbial
150 biomass), quality (e.g., plant chemistry and changes in the ratio of plant versus microbial
151 necromass), inter-species competition (e.g., abundance of myxobacteria), as well as an increased
152 viral load (the consequences of which remain unknown) shaped the microbial community at
153 elevated temperatures.

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167 **Data availability**

168 All data needed to evaluate the conclusions in the paper are present in the paper and/or the
169 Supplementary Materials. The raw sequence data are available at the NCBI Sequence Read Archive
170 (SRA); BioProject ID: PRJNA663238, accession numbers SAMN16124403–SAMN16124422.

171 **Supplement**

172 Supplement S1 – Methods (.pdf)

173 Supplement S2 – Table of viral abundance (.xlsx).

174 Supplement S3 – Schematic overview of community profile (.pdf).

175 Supplement S4 – Table of abundances of SSU rRNA taxa (.xlsx).

176 Supplement S5 – Pearson’s correlation analysis on community composition and soil- and microbial
177 properties (pdf).

178 Supplement S6 – Analytical script (.txt).

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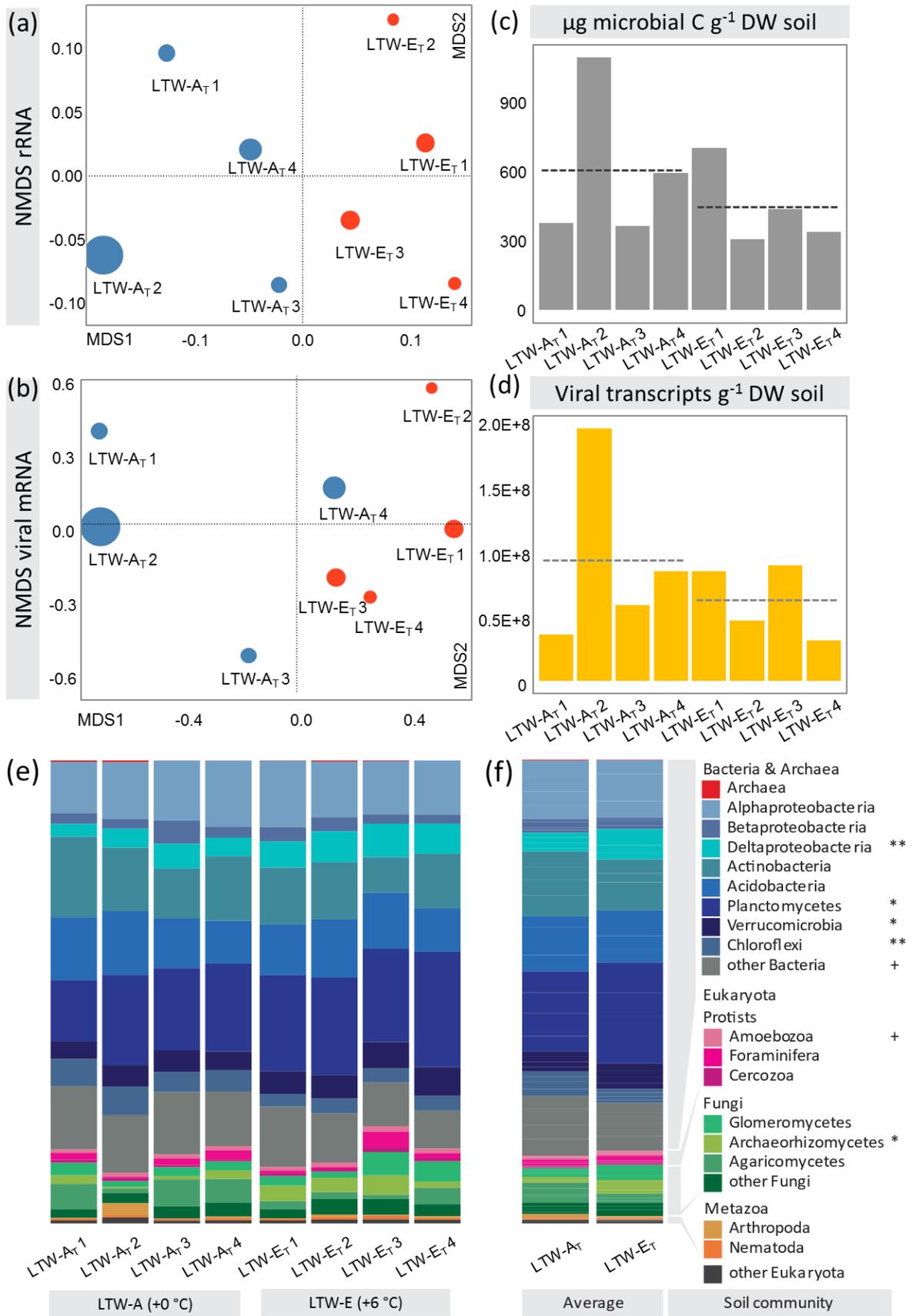
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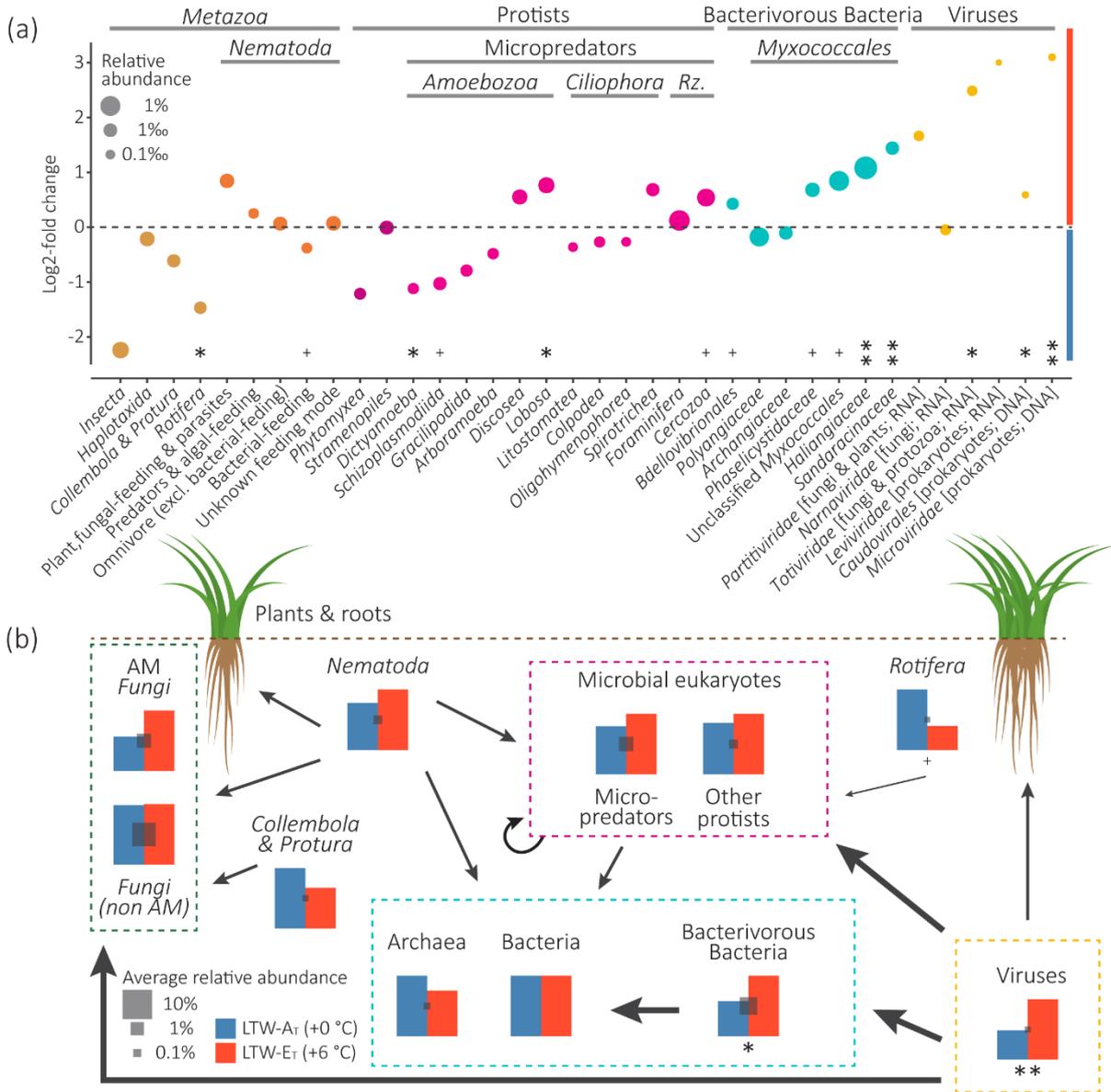
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329 **Figure 1 Soil (micro-)biota community similarity and composition under ambient and elevated**
330 **temperatures.** Non-metric multi-dimensional scaling (NMDS) ordination of the (micro-)biota community
331 profiles (stress = 0.05, i.e. how well the (dis)similarity between samples are represented in 2-dimensional
332 space; ≤ 0.05 is considered a very good fit, Clarke and Ainsworth, 1993) (a) and the viral community
333 composition (stress = 0.06) (b) for long-term warming ambient soil temperature (LTW-A_T; blue) and long-term
334 warming elevated soil temperature (LTW-E_T; red). Dots are scaled to the total RNA extract of the given sample
335 [ranging from 0.9 to 10.7 $\mu\text{g RNA g}^{-1}$ dry weight (DW) soil]. Total microbial carbon concentration (c), scaled
336 viral transcripts (d), and the relative abundances of the most dominant taxa in each domain shown for
337 individual samples (e) and summarised for each temperature (f). In (e) and (f) the bacteria are depicted at the
338 phylum level (*Proteobacteria* at the class level; bacterial phyla and *Proteobacteria* classes with low abundances
339 are grouped together as “other Bacteria”), fungi are depicted at the class level (classes with low abundances
340 are grouped together as “other Fungi”), and ‘protists’ and Metazoa are depicted at the phylum level (phyla
341 with low abundances are grouped together as “other Eukaryota”). *, $p < 0.05$; **, $p < 0.01$; +, $p < 0.1$ (t-test).
342



345 **Figure 2 Microbial food-web structure.** (a) The log₂-fold change in relative abundance to temperature
 346 condition (ambient; blue, elevated; red) for assumed predatory groups from each domain (see supplement
 347 S1). Eukaryota: shown at super-kingdom or phylum level (with mean relative abundance >0.05%), Rz.: *Rhizaria*;
 348 *Nematoda* are summarised by feeding mode (see supplement S1). Prokaryota: only bacterivorous bacteria are
 349 shown. Viruses: the three most abundant pro- and eukaryotic viruses are shown (based on their relative
 350 abundance within the mRNA datasets). (b) Structure of the soil food web under ambient (blue) and elevated
 351 (red) temperatures; arrow sizes indicate the amount of difference with increased temperature. The relative
 352 abundances of taxa are normalized to the size of the bacterial pool for each temperature. **, $p < 0.01$; *, $p <$
 353 0.05 ; +, $p < 0.1$ (t -test).