

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

Conflict of interest: the authors declare no conflict of interest.

Keywords: Climate change, Global warming, Grassland, Microbial food web, Soil ecology, Trophic interactions.

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.

154 **Acknowledgement**

155 MBD thanks the DFG-funded Research Training Group RESPONSE (RTG 2010) for a short-term
156 Postdoc fellowship as well as the DFG for funding project BO 5559/1-1. AS acknowledges financial
157 support from the OeAD (Austrian agency for international mobility and cooperation in education,
158 science and research; Marietta-Blau-Fellowship). TU acknowledges the ESF and Ministry of
159 Education, Science and Culture of Mecklenburg-Western Pomerania project WETSCAPES (ESF/14-
160 BM-A55-0032/16). ATT acknowledges financial support from the Tromsø Research Foundation (Cells
161 in the Cold project 17_SG_ATT) and the Research Council of Norway FRIPRO Mobility Grant Project
162 Time and Energy (251027/RU, cofounded by ERC under Marie Curie Grant 606895). JP was funded by
163 the Spanish Government Grant TED2021-132627B-I00 of the MCIN AEI/10.13039/501100011033
164 and the European NextGenerationEU/PRTR, the Catalan government grant SGR2021-1333 and the

165 Fundación Ramón Areces grant CIVP20A6621. We thank Dr. Stefan Geisen for his highly valuable
166 contributions in the trophic classification of the Amoebozoa taxa.

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).

179 **References**

180 Adl SM. Motility and migration rate of protozoa in soil columns. *Soil Biol Biochem* 2007; **39**: 700–703.

181 Andriuzzi WS, Wall DH. Grazing and resource availability control soil nematode body size and
182 abundance–mass relationship in semi-arid grassland. *Journal of Animal Ecology* 2018; **87**: 1407–
183 1417.

184 Angel R, Claus P, Conrad R. Methanogenic archaea are globally ubiquitous in aerated soils and
185 become active under wet anoxic conditions. *ISME J* 2012; **6**: 847–862.

186 Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. Toward a metabolic theory of ecology. *Ecology*
187 2004; **85**: 1771–1789.

188 Cernava T, Erlacher A, Aschenbrenner IA, Krug L, Lassek C, Riedel K, *et al.* Deciphering functional
189 diversification within the lichen microbiota by meta-omics. *Microbiome* 2017; **5**: 82.

190 Clark K, Ainsworth M. A method of linking multivariate community structure to environmental
191 variables. *Mar Eco Prog Ser* 1993; **92**:205–219.

192 Coleman DC, Anderson R V., Cole C V., Elliott ET, Woods L, Champion MK. Trophic interactions in soils
193 as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon. *Microb Ecol*
194 1977; **4**: 373–380.

195 Conant RT, Ryan MG, Ågren GI, Birge HE, Davidson EA, Eliasson PE, *et al.* Temperature and soil
196 organic matter decomposition rates - synthesis of current knowledge and a way forward. *Glob Chang*
197 *Biol* 2011; **17**: 3392–3404.

198 Cragg RG, Bardgett RD. How changes in soil faunal diversity and composition within a trophic group
199 influence decomposition processes. *Soil Biol Biochem* 2001; **33**: 2073–2081.

200 Crowther TW, Todd-Brown KEO, Rowe CW, Wieder WR, Carey JC, Machmuller MB, *et al.* Quantifying
201 global soil carbon losses in response to warming. *Nature* 2016; **540**: 104–108.

202 Elliott ET, Anderson RV, Coleman DC, Cole CV. Habitable pore space and microbial trophic
203 interactions. *Oikos* 1980; **35**: 327.

204 Erktan A, Or D, Scheu S. The physical structure of soil: Determinant and consequence of trophic
205 interactions. *Soil Biol Biochem* 2020; **148**: 107876.

206 Gargallo-garriga A, Ayala-roque M, Sardans J, Bartrons M, Janssens IA, Peñuelas J. Impact of soil
207 warming on the plant metabolome of Icelandic grasslands. *Metabolites* 2017; **7**: 1–22.

208 Geisen S. The bacterial-fungal energy channel concept challenged by enormous functional versatility
209 of soil protists. *Soil Biol Biochem* 2016; **102**: 22–25.

210 Heisler C, Kaiser EA. Influence of agricultural traffic and crop management on collembola and
211 microbial biomass in arable soil. *Biol Fertil Soils* 1995; **19**: 159–165.

212 Holmstrup M, Ehlers BK, Slotsbo S, Ilieva-Makulec K, Sigurdsson BD, Leblans NIW, *et al.* Functional
213 diversity of Collembola is reduced in soils subjected to short-term, but not long-term, geothermal
214 warming. *Funct Ecol* 2018; **32**: 1304–1316.

215 Hulo C, de Castro E, Masson P, Bougueleret L, Bairoch A, Xenarios I, *et al.* ViralZone: A knowledge
216 resource to understand virus diversity. *Nucleic Acids Res* 2011; **39**: D576–D582.

217 Hungate BA, Marks JC, Power ME, Schwartz E, Groenigen J Van, Blazewicz SJ, *et al.* The functional
218 significance of bacterial predators. *mBio* 2021; **12**: e00466-21.

219 Hunt HW, Coleman DC, Ingham ER, Ingham RE, Elliott ET, Moore JC, *et al.* The detrital food web in a
220 shortgrass prairie. *Biol Fertil Soils* 1987; **3**: 57–68.

221 Huson D, Auch A, Qi J, Schuster S. MEGAN analysis of metagenome data. *Genome Res* 2007; **17**:
222 377–386.

223 Hünninghaus M, Koller R, Kramer S, Marhan S, Kandeler E, Bonkowski M. Changes in bacterial
224 community composition and soil respiration indicate rapid successions of protist grazers during
225 mineralization of maize crop residues. *Pedobiologia* 2017; **62**: 1–8.

226 Jenkinson DS, Adams D, Wild A. Model estimates of CO₂ emissions from soil in response to global
227 warming. *Nature* 1991; **351**: 304–306.

228 Jiang Y, Zhou H, Chen L, Yuan Y, Fang H, Luan L, et al. Nematodes and microorganisms interactively
229 stimulate soil organic carbon turnover in the macroaggregates. *Front Microbiol* 2018; **9**: 1–12.

230 Kim YC, Gao C, Zheng Y, He XH, Yang W, Chen L, et al. Arbuscular mycorrhizal fungal community
231 response to warming and nitrogen addition in a semiarid steppe ecosystem. *Mycorrhiza* 2015; **25**:
232 267–276.

233 Kopylova E, Noé L, Touzet H. SortMeRNA: Fast and accurate filtering of ribosomal RNAs in
234 metatranscriptomic data. *Bioinformatics* 2012; **28**: 3211–3217.

235 Kramer S, Dibbern D, Moll J, Huenninghaus M, Koller R, Krueger D, et al. Resource partitioning
236 between Bacteria, Fungi, and Protists in the detritusphere of an agricultural soil. *Front Microbiol*
237 2016; **7**: 1–12.

238 Lanzén A, Jørgensen SL, Huson DH, Gorfer M, Grindhaug SH, Jonassen I, et al. CREST - Classification
239 Resources for Environmental Sequence Tags. *PLoS One* 2012; **7**: e49334.

240 Lee S, Sieradzki ET, Nicolas AM, Walker RL, Firestone MK, Hazard C, et al. Methane-derived carbon
241 flows into host-virus networks at different trophic levels in soil. *PNAS* 2021; **118**: 1–8.

242 Marañón-Jiménez S, Soong JL, Leblans NIW, Sigurdsson BD, Peñuelas J, Richter A, et al. Geothermally
243 warmed soils reveal persistent increases in the respiratory costs of soil microbes contributing to
244 substantial C losses. *Biogeochemistry* 2018; **138**: 245–260.

245 Melillo JM, Frey SD, Deangelis KM, Werner WJ, Bernard MJ, Bowles FP, et al. Long-term pattern and
246 magnitude of soil carbon feedback to the climate system in a warming world. *Science* 2017; **358**:
247 101–105.

248 Naylor D, Sadler N, Bhattacharjee A, Graham EB, Anderton CR, McClure R, et al. Soil microbiomes
249 under climate change and implications for carbon cycling. *Annu Rev Environ Resour* 2020; **45**: 29–59.

250 Nielsen UN, Ayres E, Wall DH, Bardgett RD. Soil biodiversity and carbon cycling: a review and
251 synthesis of studies examining diversity – function relationships. *Eur J Soil Sci* 2011; **62**: 105–116.

252 Oksanen J, Blanchet G, Friendly M, Kindt R, Legendre P, McGlenn D, *et al.* Vegan: community ecology
253 package R version 2.6-2. 2021. <https://CRANR-project.org/package=vegan>.

254 Pausch J, Kramer S, Scharroba A, Scheunemann N, Butenschoen O, Kandeler E, *et al.* Small but active
255 – pool size does not matter for carbon incorporation in below-ground food webs. *Funct Ecol* 2016;
256 **30**: 479–489.

257 Petters S, Groß V, Söllinger A, Pichler M, Reinhard A, Bengtsson MM, *et al.* The soil microbial food
258 web revisited: Predatory myxobacteria as keystone taxa? *ISME Journal* 2021; **15**: 2665–2675.

259 Potapov AM, Guerra CA, van den Hoogen J, Babenko A, Bellini BC, Berg MP, Chown SL, Deharveng, L,
260 Kováč Ľ, Kuznetsova NA, Ponge J-F, Potapov MB, Russell D J, Alexandre D, Alatalo JM, Arbea JI,
261 Bandyopadhyaya I, Bernava V, Bokhorst S, ... Scheu S. Globally invariant metabolism but density-
262 diversity mismatch in springtails. *Nature Communications* 2023; **14**:674.

263 R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical
264 Computing. 2021. R Foundation for Statistical Computing Vienna, Austria. URL [https://www.R-](https://www.R-project.org/)
265 [project.org/](https://www.R-project.org/).

266 Rall BÖC, Vucic-Pestic O, Ehnes RB, Emmerson M, Brose U. Temperature, predator-prey interaction
267 strength and population stability. *Glob Chang Biol* 2010; **16**: 2145–2157.

268 Rutherford PM, Juma NG. Influence of soil texture on protozoa-induced mineralization of bacterial
269 carbon and nitrogen. *Can J Soil Sci* 1992; **72**: 183–200.

270 Rønn R, Thomsen IK, Jensen B. Naked amoebae, flagellates, and nematodes in soils of different
271 texture. *Eur J Soil Biol* 1995; **31**: 13–18.

272 Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics*
273 2011; **27**: 863–864.

274 Séneca J, Söllinger A, Herbold CW, Pjevac P, Prommer J, Verbruggen E, *et al.* Increased microbial
275 expression of organic nitrogen cycling genes in long-term warmed grassland soils. *ISME COMMUN*
276 2021; **1**, 69.

277 Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E. Microbial population structures in
278 soil particle size fractions of a long-term fertilizer field experiment. *Appl Environ Microbiol* 2001; **67**:
279 4215–4224.

280 Sigurdsson BD, Leblans NIW, Dauwe S, Gudmundsdóttir E, Gundersen P, Gunnarsdóttir GE, *et al.*
281 Geothermal ecosystems as natural climate change experiments: The ForHot research site in Iceland
282 as a case study. *Icel Agric Sci* 2016; **29**: 53–71.

283 Sokol NW, Slessarev E, Marschmann GL, Nicolas A, Blazewicz SJ, Brodie EL, *et al.* Life and death in the
284 soil microbiome: how ecological processes influence biogeochemistry. *Nat Rev Microbiol* 2022; **20**:
285 415–430.

286 Starr EP, Nuccio EE, Pett-Ridge J, Banfield JF, Firestone MK. Metatranscriptomic reconstruction
287 reveals RNA viruses with the potential to shape carbon cycling in soil. *PNAS* 2019; **116**: 25900–
288 25908.

289 Söllinger A, Séneca J, Borg Dahl M, Motleleng LL, Prommer J, Verbruggen E, *et al.* Down-regulation of
290 the bacterial protein biosynthesis machinery in response to weeks, years, and decades of soil
291 warming. *Sci Adv* 2022; **8**: eabm3230.

292 Söllinger A, Tveit AT, Poulsen M, Noel SJ, Bengtsson M, Bernhardt J, *et al.* Holistic assessment of
293 rumen microbiome dynamics through quantitative metatranscriptomics reveals multifunctional
294 redundancy during key steps of anaerobic feed degradation. *mSystems* 2018; **3**: 1–19.

295 Thakur MP, Sigurðsson BD, Sigurðsson P, Holmstrup M. Warming shifts the biomass distribution of
296 soil microarthropod communities. *Soil Biology and Biochemistry* 2023; **177**: 108897

297 Thakur MP, Geisen S. Trophic Regulations of the Soil Microbiome. *Trends Microbiol* 2019; **27**: 771–
298 780.

299 Trap J, Bonkowski M, Plassard C, Villenave C, Blanchart E. Ecological importance of soil bacterivores
300 for ecosystem functions. *Plant Soil* 2016; **398**: 1–24.

301 Tveit AT, Urich T, Svenning MM. Metatranscriptomic analysis of arctic peat soil microbiota. *Appl*
302 *Environ Microbiol* 2014; **80**: 5761–5772.

303 Urich T, Lanzén A, Qi J, Huson DH, Schleper C, Schuster SC. Simultaneous assessment of soil microbial
304 community structure and function through analysis of the meta-transcriptome. *PLoS One* 2008; **3**:
305 e2527.

306 van den Hoogen J, Geisen S, Routh D, Ferris H, Traunspurger W, Wardle DA, de Goede RGM, Adams
307 BJ, Ahmad W, Andriuzzi WS, Bardgett RD, Bonkowski M, Campos-Herrera R, Cares JE, Caruso T, de
308 Brito Caixeta L, Chen X, Costa SR, Creamer R, ... Crowther TW. Soil nematode abundance and
309 functional group composition at a global scale. *Nature* 2019; **572**:194–198.

310 Verbrigghe N, Leblans NIW, Sigurdsson BD, Vicca S, Fang C, Fuchslueger L, *et al.* Soil carbon loss in
311 warmed subarctic grasslands is rapid and restricted to topsoil. *Biogeosciences* 2022a; **19**: 3381–3393.

312 Verbrigghe N, Meeran K, Bahn M, Canarini A, Fransen E, Fuchslueger L, *et al.* Long-term warming
313 reduced microbial biomass but increased recent plant-derived C in microbes of a subarctic grassland.
314 *Soil Biol Biochem* 2022b; **167**: 108590.

315 Walker TWN, Kaiser C, Strasser F, Herbold CW, Leblans NIW, Woebken D, *et al.* Microbial
316 temperature sensitivity and biomass change explain soil carbon loss with warming. *Nat Clim Chang*
317 2018; **8**: 885–889.

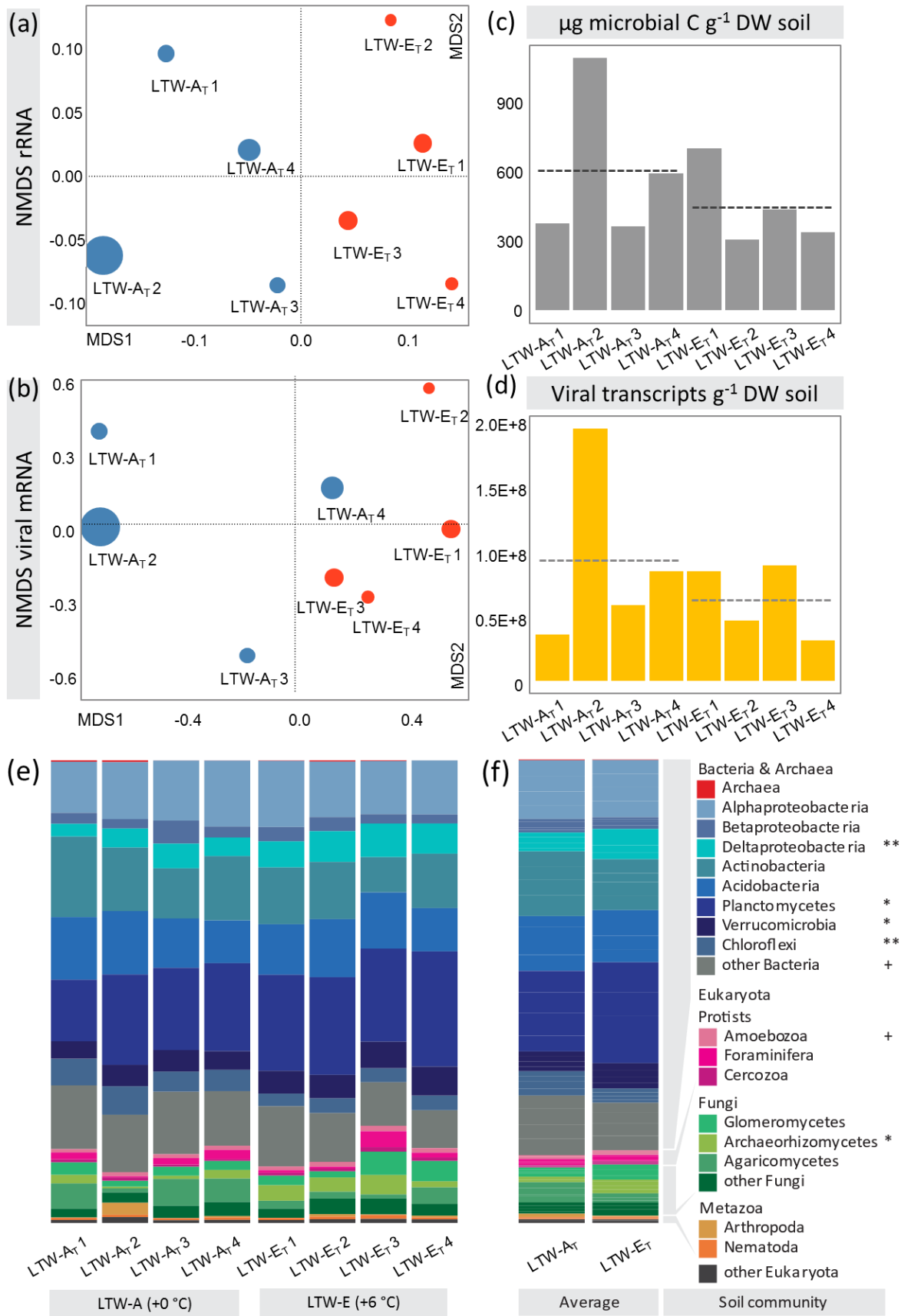
318 Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH. Ecological linkages
319 between aboveground and belowground biota. *Science* 2004; **304**: 1629–33.

320 Wardle DA, Verhoef HA, Clarholm M. Trophic relationships in the soil microfood-web: predicting the
321 responses to a changing global environment. *Glob Chang Biol* 1998; **4**: 713–727.

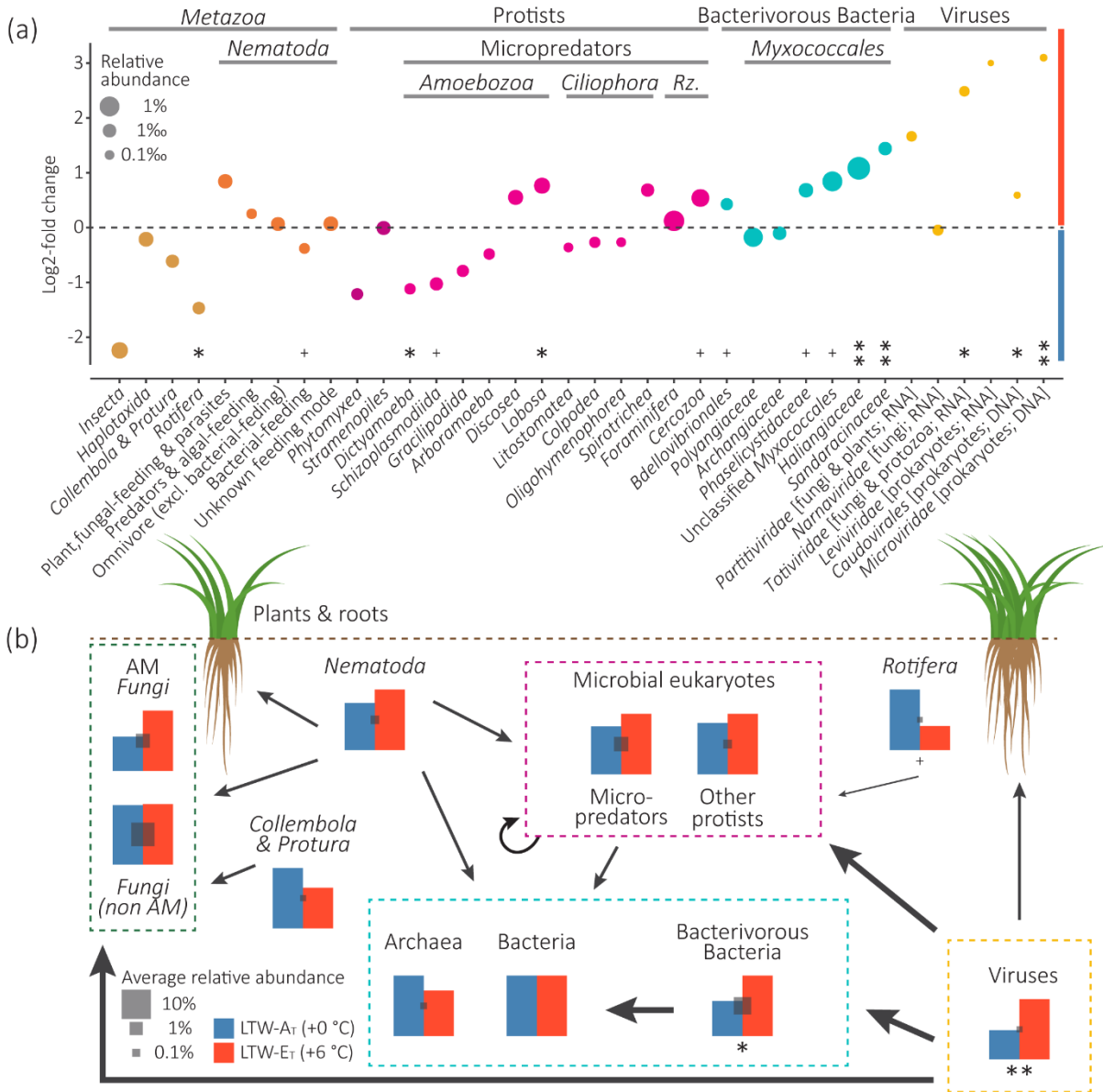
322 Wickham H. *ggplot2: Elegant graphics for data analysis*. 2010. Springer-Verlag, New York, 2009.

323 Wickham H. Reshaping Data with the reshape Package. *Journal of Statistical Software*. 2021, **21**: 1–
324 20.

325 Yin R, Siebert J, Eisenhauer N, Schädler M. Climate change and intensive land use reduce soil animal
326 biomass via dissimilar pathways. *Elife* 2020; **9**: 1–17.



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).