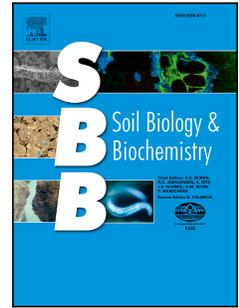


Accepted Manuscript

Microbial carbon and nitrogen cycling responses to drought and temperature in differently managed mountain grasslands

Lucia Fuchslueger, Birgit Wild, Maria Mooshammer, Mounir Takriti, Sandra Kienzl, Anna Knoltsch, Florian Hofhansl, Michael Bahn, Andreas Richter



PII: S0038-0717(19)30136-1

DOI: <https://doi.org/10.1016/j.soilbio.2019.05.002>

Reference: SBB 7481

To appear in: *Soil Biology and Biochemistry*

Received Date: 15 January 2019

Revised Date: 26 April 2019

Accepted Date: 1 May 2019

Please cite this article as: Fuchslueger, L., Wild, B., Mooshammer, M., Takriti, M., Kienzl, S., Knoltsch, A., Hofhansl, F., Bahn, M., Richter, A., Microbial carbon and nitrogen cycling responses to drought and temperature in differently managed mountain grasslands, *Soil Biology and Biochemistry* (2019), doi: <https://doi.org/10.1016/j.soilbio.2019.05.002>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Title: Microbial carbon and nitrogen cycling responses to drought and temperature in**
2 **differently managed mountain grasslands**

3 Lucia Fuchslueger^{1,2*}, Birgit Wild^{1,3,4}, Maria Mooshammer¹, Mounir Takriti^{1,5}, Sandra
4 Kienzl¹, Anna Knoltsch¹, Florian Hofhansl^{1,6}, Michael Bahn⁷, Andreas Richter^{1,6}

5
6 **Affiliations:**

7 ¹Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria

8 ²Faculty of Science, Department of Biology, Center of Excellence Plants and Ecosystems,
9 University of Antwerp, Antwerp, Belgium

10 ³Department of Environmental Science and Analytical Chemistry, Stockholm University,
11 Stockholm, Sweden

12 ⁴Bolin Centre for Climate Research Stockholm University, Stockholm, Sweden

13 ⁵Lancaster Environment Centre, Lancaster University, Lancaster, UK

14 ⁶Ecosystems Services and Management Program (ESM); International Institute for Applied
15 Systems Analysis (IIASA), Laxenburg, Austria;

16 ⁷Institute of Ecology, University of Innsbruck, Innsbruck, Austria

17
18 ***Corresponding author:** e-mail: lucia.fuchslueger@gmail.com, telephone: +32 3 2658831

19
20 **Key words:** microbial metabolism, microbial carbon use efficiency, microbial nitrogen use
21 efficiency, grassland, drought, temperature response

22 **Abstract**

23 Grassland management can modify soil microbial carbon (C) and nitrogen (N) cycling,
24 affecting the resistance to extreme weather events, which are predicted to increase in
25 frequency and magnitude in the near future. However, effects of grassland management on
26 microbial C and N cycling and their responses to extreme weather events, such as droughts
27 and heatwaves, have rarely been tested in a combined approach. We therefore investigated
28 whether grassland management affects microbial C and N cycling responses to drought and
29 temperature manipulation. We collected soils from *in situ* drought experiments conducted in
30 an extensively managed and an abandoned mountain grassland and incubated them at two
31 temperature levels. We measured microbial respiration and substrate incorporation, as well as
32 gross rates of organic and inorganic N cycling to estimate microbial C and N use efficiencies
33 (CUE and NUE). The managed grassland was characterized by lower microbial biomass,
34 lower fungi to bacteria ratio, and higher microbial CUE, but only slightly different microbial
35 NUE. At both sites drought induced a shift in microbial community composition driven by an
36 increase in Gram-positive bacterial abundance. Drought significantly reduced C substrate
37 respiration and incorporation by microbes at both sites, while microbial CUE remained
38 constant. In contrast, drought increased gross rates of N mineralization at both sites, whereas
39 gross amino acid uptake rates only marginally changed. We observed a significant direct, as
40 well as interactive effect between land management and drought on microbial NUE.
41 Increased temperatures significantly stimulated microbial respiration and reduced microbial
42 CUE independent of drought or land management. Although microbial N processing rates
43 showed no clear response, microbial NUE significantly decreased at higher temperatures. In
44 summary in our study, microbial CUE, in particular respiration, is more responsive to
45 temperature changes. Although N processing rates were stronger responding to drought than
46 to temperature microbial NUE was affected by both drought and temperature increase. We

47 conclude that direct effects of drought and heatwaves can induce different responses in soil
48 microbial C and N cycling similarly in the studied land management systems.

49

50 **1. Introduction**

51 Socioeconomic changes in mountain regions have altered grassland management and
52 increasing proportions of previously agriculturally managed grasslands have become
53 abandoned (Tappeiner et al., 2008). Land management change is affecting plant community
54 composition and associated plant traits (Fontana et al., 2017; Grigulis et al., 2013), net
55 ecosystem gas exchange (Harris et al., 2018; Schmitt et al., 2010), soil microbial community
56 composition (Fuchslueger et al., 2014b; Grigulis et al., 2013; Legay et al., 2016), as well as
57 soil C sequestration, soil structure, soil organic matter stocks (Meyer et al., 2012), and soil
58 microbial N turnover and related functional genes (Legay et al., 2016; Szukics et al., 2019).
59 Microbial C and N cycling in soil are tightly coupled and, amongst other factors, regulated by
60 environmental conditions (Allison et al., 2010; Creamer et al., 2015; Frey et al., 2013;
61 Hagerty et al., 2014; Keiblinger et al., 2010; Manzoni et al., 2012; Six et al., 2006;
62 Zechmeister-Boltenstern et al., 2015). Land management can strongly modify soil microbial
63 C and N cycling and influence the resistance and resilience to extreme weather events (De
64 Vries et al., 2012; Fuchslueger et al., 2014b; Ingrisch et al., 2017; Karlowsky et al., 2018),
65 which are projected to occur at higher intensity and frequency in mountain regions in the near
66 future (IPCC, 2012). An improved mechanistic understanding of soil microbial C and N
67 cycling (Wieder et al., 2015), as well as its interaction with land management is urgently
68 needed to accurately represent soil microbial feedbacks in ecosystem models to improve
69 predictions of grassland responses to projected climate change scenarios.

70 Substrate stoichiometry and availability, as well as microbial nutrient demand affect the
71 efficiency with which microbes convert available substrates into biomass, as opposed to the
72 release of C or N as enzymes, exudates, or as CO₂ or inorganic N, i.e. the microbial C or N
73 use efficiency (CUE or NUE, respectively). High microbial CUE denotes a greater potential
74 for soil organic C storage, and lower losses of soil organic C through microbial respiration
75 per unit of C processed (Manzoni et al., 2012; Mooshammer et al., 2014; Sinsabaugh et al.,
76 2016) , and has been found to decrease with N deficiency (Keiblinger et al., 2010; Spohn et
77 al., 2016). Likewise, high microbial NUE indicates efficient incorporation of N into microbial
78 biomass, and concomitant low mineralization (i.e. release of inorganic N as NH₄⁺ and NO₃⁻)
79 into the environment (Mooshammer et al., 2014). In addition, microbes can take up small
80 organic N forms, such as amino acids; although their production and breakdown is considered
81 a key step in soil N cycling in many systems, their role for soil N dynamics is often
82 overlooked (Schimel and Bennett, 2004; Wild et al., 2013).

83 Soil C and N cycling is sensitive to changes in soil moisture (Moyano et al., 2013). Low
84 water and osmotic potential and reduced substrate diffusion during drought can reduce
85 microbial growth, increase microbial mortality, induce microbial dormancy and shifts in
86 active microbial community composition (Blagodatskaya and Kuzyakov, 2013; Lennon and
87 Jones, 2011; Moyano et al., 2013; Schimel et al., 2007). Simultaneously, drought reduces
88 microbial activity indicated for example by reduced respiration (Moyano et al., 2013). Effects
89 of drought on microbial N cycling are less clear: drought can reduce extracellular enzyme
90 activity involved in protein depolymerization (Sanaullah et al., 2011). However, drought
91 effects on gross amino acid uptake and production by microbes have rarely been determined.

92 While drought can favor microbial strategies to conserve N, such as production of N-
93 containing osmolyte compounds (Moyano et al., 2013; Schimel et al., 2007), drought effects
94 on N mineralization, and nitrification seem to strongly depend on ecosystem type and land

95 management (Auyeung et al., 2013; Fuchslueger et al., 2014b; Hartmann et al., 2013;
96 Homyak et al., 2017; Larsen et al., 2011).

97 Since drought periods often coincide with heat waves, an understanding of water and
98 temperature interactions on soil C and N cycling is crucial (Auyeung et al., 2013; Bloor et al.,
99 2010). Temperature generally increases microbial activity (growth), but also maintenance
100 costs and microbial energy demand (Allison et al., 2010; Frey et al., 2013). If more C is
101 allocated to respiration as opposed to microbial biomass growth, microbial CUE is reduced
102 (Dijkstra et al., 2015; Manzoni et al., 2012), which can result in overall losses of soil C
103 (Davidson and Janssens, 2006; Melillo et al., 2017). Higher temperatures allow a
104 thermodynamically faster extracellular enzymatic breakdown of proteins into organic N
105 forms suitable for microbial uptake and thereby stimulate microbial growth (Wallenstein et
106 al., 2011), though they might also accelerate enzyme inactivation (Alvarez et al., 2018).
107 Microbial N mineralization and nitrification have been found to more strongly increase with
108 temperature than inorganic N uptake causing a net increase of inorganic N in soils (Larsen et
109 al., 2011; Niboyet et al., 2011; Shaw and Harte, 2001; Verburg et al., 1999). Overall, the
110 temperature response of microbial N cycling and consequently of microbial NUE remains
111 unclear.

112 Both microbial CUE and NUE are metrics attempting to integrate and characterize the
113 physiological potential of an established microbial community. Microbial CUE, which is
114 better studied than microbial NUE, can vary among ecosystems, land management systems
115 (Bölscher et al., 2016; Lee and Schmidt, 2014; Zheng et al., 2019), with climatic conditions
116 and incubation temperatures (Devêvre and Horwáth, 2000; Steinweg et al., 2008). However,
117 responses of microbial C and N cycling and CUE and NUE to extreme weather events have
118 to our knowledge never been tested in a combined approach. The aim of this study was
119 therefore to evaluate the responses of soil microbial C and N cycling to drought and to short

120 term temperature increases in two differently managed mountain grasslands. We assessed
121 microbial C cycling by measuring the partitioning of ^{13}C -labelled substrate into microbial
122 biomass and respired CO_2 and soil microbial N cycling by ^{15}N pool dilution approaches, in a
123 managed and an abandoned mountain grassland that were part of an *in situ* drought
124 experiment. We collected soil samples from controls and drought treated plots at peak
125 drought and tested the temperature response of soil C and N cycling rates under controlled
126 laboratory conditions. We hypothesized that (i) drought reduces microbial C and N uptake as
127 well as mineralization rates, and that microbial CUE and NUE consequently remain
128 unchanged. We further expected that (ii) short-term temperature increases stimulate
129 mineralization processes stronger than microbial growth, and thereby reduce microbial CUE
130 and NUE. As drought would reduce the temperature sensitivity of mineralization processes
131 (Suseela et al., 2012), we expected to find less pronounced temperature effects on CUE and
132 NUE in drought treated soil. Since the resistance of soil C and N cycling to extreme weather
133 events should decrease with increasing grassland management intensity (De Vries et al.,
134 2012; Karlowsky et al., 2018), we hypothesized (iii) that the drought and temperature
135 response of microbial C and N cycling will differ in managed and abandoned grassland.

136

137 **2. Material and Methods**

138 ***2.1 Site description and soil sampling***

139 Soil samples were collected from two grasslands with different land management histories
140 located in the Austrian Central Alps near Neustift, Stubai Valley ($47^{\circ}07'\text{N}$, $11^{\circ}19'\text{E}$). Both
141 grasslands are characterized by a temperate, seasonal cool, humid climate (mean annual
142 temperature of 3°C ; mean annual precipitation of 1097 mm); the predominant growing
143 (snow-free) season is from March/April to September. Samples were taken from a grassland
144 (referred to as 'managed grassland'; 1850 m a. s. l.), where total aboveground plant biomass

145 is cut and harvested once a year (Bahn et al., 2006), and from an abandoned grassland, where
146 all management activities were terminated in 1983 and which has since then undergone the
147 initial states of natural succession (referred to as ‘abandoned grassland’, 1900 m a.s.l.,
148 Schmitt *et al.*, 2010; Ingrisich *et al.*, 2017).

149 The grasslands differed in the amount of cumulative organic matter input. At the managed
150 grassland aboveground biomass is regularly cut and removed, and average soil organic matter
151 (SOM) content in the upper 10 cm of soil was 13.3 % ($\pm 0.8\%$ SE), while at the abandoned
152 grassland SOM was higher with 22.5 % ($\pm 1.5\%$ SE) (determined by loss on ignition at 550°C
153 (Fuchslueger et al., 2014b)). The plant community composition is described as *Trisetetum*
154 *flavescentis* at the managed, and as *Seslerio-Caricetum* at the abandoned grassland (Grigulis
155 et al., 2013; Schmitt et al., 2010). At both sites the soil has been characterized as Dystric
156 Cambisol (FAO classification) with a pH of 5.5 in the uppermost 10 cm (determined in
157 CaCl_2).

158 The two sites were part of a multi-year drought experiment in the CARBO-Extreme network.
159 Drought was simulated by excluding precipitation using rain-out shelters equipped with light-
160 and UV-B-permeable plastic foil (UV B Window; Folitec GmbH, Westerburg, Germany;
161 light permeability ca. 95%; UV-B permeability >70%). Each shelter covered an area of 3 m x
162 3.5 m. Shelters had been installed over a period of ten weeks during the growing season in
163 2011, as well as for four weeks before sample collection in June 2012. In both grasslands,
164 soil moisture significantly decreased by at least 30% during drought simulations (Table 1; for
165 a detailed experimental description see Fuchslueger et al., (2014b)). Soil samples were taken
166 from the center of each of the drought plots (called ‘drought’ hereafter), as well as from
167 control plots close to each rain-out shelter exposed to ambient weather conditions (called
168 ‘control’ hereafter, n=4 respectively). Per sample, two soil cores (5 cm x 7 cm) to a depth of
169 10 cm were pooled, sieved to 2 mm, and fine roots were manually removed. Samples were

170 stored cool and transferred to the lab on the same day. One set of soil aliquots was
171 immediately processed to determine soil C and N pools and microbial community
172 composition. The remaining soil was split into aliquots for incubations at two temperature
173 levels to test the temperature responses of microbial C and N cycling: 15°C was chosen as it
174 is close to field temperature conditions (ranging between 7.3 and 17.1°C in the week before
175 sampling), and 25°C was chosen to simulate a strong heatwave. All samples were pre-
176 incubated at the two temperature levels for 24 h before they were used for C and N cycling
177 measurements.

178

179 ***2.2 Soil parameters and soil C and N pools, microbial community composition***

180 Soil samples were analyzed as described in Fuchslueger et al., (2014b). Soil water content
181 (SWC) was determined gravimetrically by weighing 5 g of fresh soil and drying at 60°C for
182 48 h. Dried soil samples were ground and analyzed for total C and total N using an EA-IRMS
183 (EA 1110, CE Instruments, Italy, coupled to a Finnigan MAT Delta Plus IRMS; Thermo
184 Fisher Scientific, MA, USA). Microbial biomass C and N (C_{mic} , N_{mic}) was determined in
185 fresh soils using the chloroform fumigation extraction method (Vance et al., 1987).
186 Fumigated and non-fumigated soils (2 g respectively) were extracted with 20 ml of 0.5 M
187 K_2SO_4 and analyzed for extractable organic C (EOC) and total extractable N on a TOC/TN
188 Analyzer (TOC-V CPH E200V/TNM-122V; Shimadzu, Austria); no correction factor was
189 applied to values on C_{mic} and N_{mic} reported (Table 1). Total free amino acid concentrations
190 (TFAA) were analyzed in aliquots of K_2SO_4 extracts fluorimetrically as described by Jones *et*
191 *al.* (2002), modified by Prommer *et al.* (2014). Similarly, NH_4^+ concentrations were analyzed
192 photometrically in K_2SO_4 extract aliquots using a modified indophenol reaction method
193 (Kandeler and Gerber, 1988). Nitrate (NO_3^-) concentrations were determined in water
194 extracts (2 g of soil with 20 ml of MilliQ water) by chemically suppressed ion-

195 chromatography (DX500, Dionex, Austria) on a Dionex AS11 column. Extractable organic
196 nitrogen (EON) was calculated by subtracting inorganic N (NH_4^+ and NO_3^-) from total
197 extractable N.

198 Microbial community composition was determined using phospholipid fatty acids according
199 to the method described by Frostegård *et al.* (1991) with modifications described by
200 (Fuchslueger *et al.*, 2014a). Briefly, total lipids were extracted with a mixture of chloroform,
201 methanol and 0.15 M citric acid buffer from frozen soils. Neutral lipids and phospholipids
202 were separated on silica columns (Supelco, LC-Si SPE, Bellefonte, PE, USA) using
203 chloroform, acetone and methanol as eluents. After addition of methyl-nonadecanoate (19:0)
204 as an internal standard and the conversion of the phospholipids to fatty-acid methyl esters
205 (FAMES) by alkaline methanolysis, samples were dried and re-dissolved in isooctane and
206 analyzed on a GC-FID (Trace GC Ultra, Thermo) using a DB23 column (Agilent 60 m x 0.25
207 mm x 0.25 μm). Bacterial and fungal FAME mixtures (bacterial acid methyl ester mix,
208 Supelco, and 37 Comp. FAME Mix, Supelco) were used as qualitative standards. The internal
209 standard 19:0 was used to calculate the concentration of FAMES. As indicators for Gram-
210 positive bacteria we used the i14:0, i15:0, a15:0, i16:0, a16:0, i17:0 and a17:0 fatty acids,
211 while the markers 16:1 ω 7, 18:1 ω 7, cy17:0, and cy19:0 were used as indicators for Gram-
212 negative bacteria. The sum of Gram-positive and Gram-negative markers together with 15:0,
213 17:0, 10Me18:0, 17:1 ω 7, and 18:1 ω 5 was used as a measure for total bacteria. The
214 biomarkers 16:1 ω 5, 18:2 ω 6,9, 18:1 ω 9 and 18:3 ω 3,6,9 were used to assess the fungal
215 contribution to the microbial community (Kaiser *et al.*, 2010; Olsson, 2006; Zelles, 1997).

216

217 **2.3 Microbial C cycling potential and microbial CUE**

218 The microbial C cycling potential was estimated by incubating soil aliquots with a mixture of
219 ^{13}C -labelled substrates (sugars, amino sugars, organic acids and amino acids with a C:N ratio

220 of 20, enriched at 10.4 atom%, for a detailed list of compounds see Takriti et al., (2018)). For
221 the assay, 2 g of soil pre-incubated at 15°C or 25°C were placed into 250 ml glass bottles.
222 Each subsample received dissolved C-substrate equaling 40 µg of C and the bottles were
223 sealed with butyl rubber plugs. Immediately after ¹³C label addition 12 ml gas samples were
224 collected using a syringe and transferred to pre-evacuated Exetainer vials. The air removed
225 from the bottles was replaced with air with known CO₂ concentration and ¹³C composition.
226 The samples were then again incubated for 24 h at 15°C and 25°C, respectively. At the end of
227 the incubation further gas samples were taken as described above, and aliquots of soils were
228 used to determine microbial biomass C (C_{mic}) by chloroform fumigation extraction as
229 described in section 2.2. In K₂SO₄ extracts of both fumigated and non-fumigated soils δ¹³C of
230 EOC was determined by direct injection on an IC system (DX 3000, Dionex Corporation,
231 Sunnyvale, CA, USA) without column and connected through a Finnigan LC IsoLink
232 Interface (Thermo Fisher Scientific, Waltham, MA, USA) to a Finnigan Delta V Advantage
233 Mass Spectrometer (Thermo Fisher, Bremen, Germany). Carbon substrate incorporation into
234 microbial biomass was calculated as the difference between ¹³C in EOC of chloroform-
235 fumigated and non-fumigated samples. Gas samples were analyzed for their CO₂
236 concentrations and δ¹³C signatures by a headspace gas sampler (GasBench II, Thermo Fisher,
237 Bremen, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage,
238 Thermo Fisher, Bremen, Germany). Cumulative respiration (total microbial soil respiration)
239 was calculated correcting for the air replaced at the start of the incubation. Substrate derived
240 ¹³C in CO₂ and EOC was corrected for mean natural abundance of soil by calculating atom
241 percent excess. Microbial CUE was estimated as follows:

242

243
$$\text{CUE} = \frac{\text{C substrate incorporation}}{\text{C substrate incorporation} + \text{C substrate respiration}}$$

244 1)

245

246 where C substrate incorporation is the ^{13}C labelled substrate incorporated into biomass and C
247 substrate respiration is the CO_2 respired from labelled substrates during the incubation.
248 Microbial C turnover was calculated by dividing the total microbial biomass pool by the C
249 substrate incorporation rate:

250

$$251 \quad \text{C substrate turnover (days)} = \text{total } C_{\text{mic}} / \text{C substrate incorporation} \quad 2)$$

252

253 ***2.4 Microbial N cycling rates and microbial NUE***

254 *2.4.1 Microbial gross protein depolymerization and gross amino acid uptake*

255 Gross rates of protein depolymerization and microbial amino acid uptake ($\text{AA}_{\text{uptake}}$) were
256 determined following Wanek *et al.* (2010), with the modifications for soil samples described
257 by Wild *et al.* (2013). Briefly, 500 μl of a ^{15}N -labelled amino acid mixture (20 amino acids,
258 $0.25 \mu\text{g } \mu\text{l}^{-1}$, $>98 \text{ atm\% } ^{15}\text{N}$, Spectra and Cambridge Isotope Laboratories) were added to
259 duplicates of 2 g fresh, but pre-incubated soil. Samples were then further incubated at either
260 15°C or 25°C ; one of the duplicates was extracted after 10 min, the second after 30 min of
261 incubation with 20 ml 10 mM CaSO_4 containing 3.7% formaldehyde. Extracts were
262 centrifuged, filtered, and loaded on pre-cleaned cation exchange cartridges (OnGuard II H
263 1cc cartridges, Dionex). Amino acids were eluted from the cartridges using 10 ml 3 M NH_3 ,
264 amended with an internal standard (1 μg nor-valine, nor-leucine and para-chloro-
265 phenylalanine each, Sigma-Aldrich), dried under N_2 , re-dissolved in 20% ethanol and dried
266 again in a SpeedVac. Blanks and amino acid standards were processed with the samples
267 throughout the procedure. After derivatization with ethyl-chloroformate (Wanek *et al.*, 2010),
268 samples were analyzed with gas chromatography-mass spectrometry (Thermo Trace GC
269 Ultra and ISQ mass spectrometer, Agilent DB-5 column, PTV injection in splitless mode at

270 270°C, 1 ml min⁻¹ helium as carrier, temperature program: 60°C for 1.5 min, first ramp 5°C
271 min⁻¹ to 200°C, second ramp 15°C min⁻¹ to 300°C, 300°C for 4 min). We calculated
272 concentrations of alanine, glycine, isoleucine, leucine, phenylalanine, proline, serine, valine,
273 asparagine & aspartate, and glutamine & glutamate against external standards that were
274 measured interspersed with the samples, and ¹⁵N isotopic compositions of these amino acids
275 from the peak areas of fragments containing ¹⁴N or ¹⁵N as described by Wanek *et al.* (2010).
276 We finally calculated gross rates of amino acid consumption and protein depolymerization
277 based on the equations in Kirkham & Bartholomew (1954); a detailed description is reported
278 in Wild *et al.*, (2018)

279

280 2.4.2 Gross N mineralization and NH₄⁺ uptake, NO₃⁻ production and NO₃⁻ uptake

281 Gross rates of microbial N mineralization (N_{min}) and NH₄⁺ uptake (NH₄⁺_{uptake}) and of NO₃⁻
282 production (NO₃⁻_{prod}) and uptake (NO₃⁻_{uptake}) were determined using ¹⁵N pool dilution assays
283 (Kirkham and Bartholomew, 1954). For each assay pre-incubated aliquots of soil samples
284 received in duplicates 500 µl (NH₄)₂SO₄ (0.125 mM; 10 atm% ¹⁵N) or 500 µl KNO₃ (0.25
285 mM, 10 atm% ¹⁵N). After ¹⁵N-label additions samples were again incubated at 15°C or 25°C.
286 From each assay one of the aliquots was extracted after 4 h, and the other after 24 h with 20
287 ml 2 M KCl. The extracts were stored frozen until further analyses. Gross N_{min} and NH₄⁺_{uptake}
288 rates were determined by microdiffusion of NH₃ from KCl-extracts using acid traps, which
289 were analyzed for total N concentrations and atom-percent excess of ¹⁵N by EA-IRMS (EA
290 1110, CE Instruments, Italy coupled to a Finnigan MAT Delta Plus IRMS, Thermo Fisher
291 Scientific, MA, USA). For analyzing gross NO₃⁻ production and uptake rates, NH₃ was
292 removed from the extracts by adding MgO before converting NO₃⁻ to NH₃ by adding
293 Devarda's Alloy, trapping NH₃ by microdiffusion and analysis as described before. Gross

294 rates of N_{\min} and NH_4^+ uptake as well as of NO_3^- production and uptake were calculated as
 295 described by Kirkham & Bartholomew (1954).

296

297 *Microbial NUE*

298 Microbial NUE was calculated based on Wild et al., (2013)

299

$$300 \quad \text{NUE} = (AA_{\text{uptake}} - N_{\min}) / (AA_{\text{uptake}}) \quad 3)$$

301

302 where NUE is the ratio of the sum of N taken up by microbes as amino acids (AA_{uptake}) minus
 303 N mineralized (N_{\min} as NH_4^+) over the sum of N taken up by microbes. Since gross NO_3^-
 304 production was occurring in the same range as gross NH_4^+ uptake we could not separate the
 305 two processes and therefore did not consider inorganic N process rates for estimating
 306 microbial NUE. The turnover times of N pools (TFAA, NH_4^+ and NO_3^-) were calculated as
 307 follows:

308

$$309 \quad \text{N pool turnover (hours)} = \text{N pool} / ((\text{N-pool}_{\text{production}} + \text{N-pool}_{\text{uptake}}) / 2) \quad 4)$$

310

311 *2.5 Data analysis and statistics*

312 Effects of land management and drought treatment on soil parameters were assessed by linear
 313 mixed effect models with land management and drought treatment as fixed factors and plot
 314 identity nested within land management as random factor using the ‘nlme’ package in R
 315 (Pinheiro et al., 2019). The influence of land management and drought treatment on microbial
 316 community composition using relative PLFA abundances as a proxy was displayed as a non-
 317 metric multidimensional scaling plot based on a Bray-Curtis similarity matrix; significant
 318 effects were evaluated by permutation ANOVA using the ‘vegan’-package in R (Oksanen et

319 al., 2013). Effects of land management, drought treatment and incubation temperature as well
320 as their interactions on microbial CUE and NUE, and on the respective C- and N process
321 rates were also assessed applying linear mixed effect models with plot identity nested as
322 random factor within land management. Variables were tested for normal distribution of
323 residuals. Since many variables showed unequal variances between the two land management
324 systems we used the weights function to fix variance weights. For all process rates Q_{10} values
325 were calculated as follows:

$$326 \quad Q_{10} = (R_{25}/R_{15})^{(10/(25-15))} \quad 5)$$

327
328
329 where R_{25} and R_{15} are the rates measured in soil incubated at 25°C and 15°C, respectively,
330 and the drought and temperature were assessed with two-way ANOVA in each site
331 individually.

332 333 **3. Results**

334 *3.1 Are drought responses of soil microbial C and N cycling depending on land* 335 *management?*

336 The managed grassland was characterized by a significantly lower total soil C and N
337 concentrations, lower soil C:N ratio, as well as a significantly lower C_{mic} content compared to
338 the abandoned grassland. At both sites the drought treatment significantly reduced soil
339 moisture content and increased microbial C:N ratios, driven by a significant decrease in N_{mic}
340 (Table 1). The managed grassland showed a significantly lower fungi:bacteria PLFA ratio,
341 and a significantly higher Gram-positive:Gram-negative bacteria PLFA ratio than the
342 abandoned grassland. In both sites the drought treatment changed microbial community

343 composition driven by a significant increase of Gram-positive:Gram-negative bacteria (Fig.
344 1, Table 1).

345 Total soil microbial respiration (per g dry mass soil) was not significantly different between
346 the two sites, but specific respiration (i.e., respiration normalized to C_{mic}) was significantly
347 higher in the managed compared to the abandoned grassland (Fig 2, Fig. S1, Table 2, Table
348 S1). Neither microbial C substrate incorporation nor C substrate respiration differed
349 significantly between the two sites; yet the small differences resulted in significantly higher
350 microbial CUE of 0.61 (± 0.03) in the managed, compared to 0.51 (± 0.04) in the abandoned
351 grassland (Fig. 2, Table 2). Microbial C turnover occurred almost three times faster in the
352 managed (11.2 days) than in the abandoned grassland (30.4 days; Table 3). Drought did not
353 affect total microbial respiration rates in either grassland, neither on a dry mass soil basis, nor
354 when rates were normalized to C_{mic} (Fig. 2, Table 2, Fig. S1, Table S1). However, drought
355 significantly reduced both microbial C substrate incorporation and C substrate respiration,
356 which slowed down microbial C turnover, while microbial CUE remained constant (Fig. 2,
357 Tables 2 and 3).

358 The analyzed microbial gross N cycling rates did not significantly differ between the two
359 sites and also microbial NUE was similar in the managed (0.66 ± 0.06 , mean \pm SE) and
360 abandoned grassland (0.69 ± 0.03 , mean \pm SE) (Fig. 3, Table 2). Normalized to C_{mic} , also most
361 microbial gross N-processing rates were comparable. Only NO_3^- pool turnover was
362 significantly higher in the managed compared to the abandoned grassland (Table 3).

363 The drought treatment differently affected microbial N cycling rates mostly independent of
364 land management. Drought significantly increased gross protein depolymerization, gross N
365 mineralization and gross NH_4^+ uptake and significantly reduced gross NO_3^- production rates
366 at both sites, while gross AA uptake only showed small changes in either site (Fig. 3, Table
367 2.). The response of N cycling rates normalized to C_{mic} were less pronounced (Fig. S2, Table

368 S1). Nonetheless, the drought response of microbial NUE depended significantly with land
369 management and was reduced in the managed, but increased in the abandoned grassland (Fig.
370 3, Table 2).

371

372 ***3.2 Does drought affect the temperature response of microbial C and N cycling?***

373 The temperature increase significantly stimulated total microbial soil respiration (Q_{10} : 1.8-
374 2.2), and C substrate derived respiration (Q_{10} : 1.3-1.4), regardless of land management and
375 drought treatment (Fig. 2, Fig. 4, Table 2). Microbial C substrate incorporation was not
376 significantly affected by temperature, but its temperature response showed a trend to vary
377 with land management ($F=3.6$, $p=0.075$). Increased temperatures caused a reduction of
378 microbial CUE (Fig. 2, 4, Table 2), which also seemed to tend to interactively depend on
379 land management ($F=3.9$, $p=0.064$) and drought treatment ($F=3.7$, $p=0.070$, Fig. 2, Fig. 4,
380 Table 2).

381 Although increased temperatures did not significantly change the measured gross N cycling
382 rates, neither per dry mass nor normalized per C_{mic} (Table 2, Table S1, Fig. 3, Fig. S2),
383 microbial NUE was significantly reduced. Moreover, the temperature response of gross
384 protein depolymerization rates depended on the drought treatment and rates decreased in
385 control and increased in drought treated plots (Table 2, Fig. 3).

386

387 **4. Discussion**

388 Our study provides experimental evidence that drought and temperature pulses can induce
389 different responses of microbial C and N cycling in grassland soils, and in contrast to our
390 hypothesis independent of land management. Abandonment of agricultural grassland
391 management is known to introduce ecosystem wide changes, from reducing gross primary
392 production, ecosystem respiration and changing overall net ecosystem CO_2 exchange (Harris

393 et al., 2018; Schmitt et al., 2010) to altering plant litter inputs to the soil, reducing litter
394 quality (wider C/N ratio, increased lignin and lower N content) and labile C inputs into the
395 rhizosphere (Ingrisch et al., 2017; Karlowsky et al., 2018). In line with earlier findings
396 (Karlowsky et al., 2018; Legay et al., 2016), we found that land abandonment increased
397 microbial biomass C and induced a shift in microbial community composition, characterized
398 by an increase in the abundance of fungal PLFAs compared to the managed grassland and
399 shifted gram positive and gram negative PLFA composition.

400 Despite the difference in microbial community composition, total microbial respiration, C
401 substrate respiration and C substrate incorporation rates were comparable in the two
402 grasslands per dry soil (Table 2, Fig. 2). However, normalized to microbial biomass all C
403 cycling rates were higher, and C turnover occurred faster in the managed grassland indicating
404 a more active microbial community, or a higher proportion of active microbes compared to
405 the abandoned site (Table S1, Fig. S1). Microbial CUE was however significantly higher in
406 the managed compared grassland with lower fungi:bacteria ratio compared to the abandoned
407 grassland (Fig. 2, Table 2). Bacterial growth efficiency has been shown to increase from
408 forest to cropland soils with management intensity (Lee and Schmidt, 2014), and in
409 grasslands microbial CUE has been shown to increase with nutrient availability (Spohn et al.,
410 2016). In contrast, Bölscher et al., (2016) reported a higher CUE of microbial communities in
411 forest soils with higher fungal abundances and potential higher CUE of saprotrophic fungi
412 compared to microbial communities in arable land and grasslands.

413 Changes in land management can also strongly influence plant and soil N cycling, and the
414 gene abundance of microbial N cyclers and N cycling rates (Hartmann and Niklaus, 2012;
415 Legay et al., 2016; Szukics et al., 2019). Despite of significant lower gross NO_3^- production
416 rates at the managed compared to the abandoned grassland, all other measured gross N
417 cycling rates, both per dry weight and normalized by microbial biomass, as well as N

418 turnover times and microbial NUE were similar at the two sites (Table 2, Fig. 3g). Microbial
419 NUE was within the range of values reported for mineral soils (Mooshammer et al., 2014),
420 but lower than in temperate heathland soils (Wild et al., 2018).

421 We hypothesized that drought reduces microbial C (incorporation and respiration) and N
422 cycling (N uptake and mineralization), and that microbial CUE and NUE consequently
423 remain unchanged. Since earlier studies found that grassland management intensity can
424 modify the resistance of soil C and N cycling to extreme weather events (De Vries et al.,
425 2012; Karlowisky et al., 2018), we expected that the drought response differs in the managed
426 and abandoned grassland. However, independent of differences in soil properties and
427 microbial community composition, and in contrast to our hypothesis, the drought response of
428 microbes was similar at the two sites. The simulated drought induced shifts in microbial
429 community composition, characterized by an increase in fungal and Gram-positive PLFA
430 markers in line with earlier findings (Karlowisky et al., 2018). Microbial biomass C remained
431 stable, but our data indicated that the active proportion of the soil microbial community
432 incorporating and mineralizing C substrates, was reduced by drought (Fig. S1). One strategy
433 of microbes to cope with drought is to promote the accumulation of osmolytes within the
434 microbial biomass, which would increase microbial CUE in the short term (Manzoni et al.,
435 2012). However, microbial CUE was unaffected, indicating that microbes may have rather
436 switched to dormancy (Schimel, 2018), and that drought did not uncouple respiration from
437 growth independent of observed differences induced by land management.

438 We also show that the responses of microbial N cycling processes to drought were more
439 diverse than C cycling responses. Similarly as for drought effects on C cycling, the direction
440 of drought effects on inorganic microbial N cycling in this experiment was independent of
441 land management, which is in contrast to earlier studies (Fuchslueger et al., 2014b; Hartmann
442 et al., 2013). Drought reduced N concentrations in microbial biomass and increased microbial

443 C:N ratios in both grasslands. This response is in line with previous observations (Jensen et
444 al., 2003; Zeglin et al., 2013), and indicates that drought may have stronger effects on
445 microbial N than C cycling. Independent of land management, drought significantly
446 increased protein depolymerization rates, an extracellular process catalyzed by proteases
447 (Wanek et al., 2014), which is in contrast to dynamics observed in temperate heathland,
448 where protein depolymerization rates were unaffected by drought (Wild et al., 2018). During
449 drought organic compounds can concentrate in the remaining soil solution and may increase
450 substrate availability for enzymatic depolymerization (Fuchslueger et al., 2014b; Tiemann
451 and Billings, 2012). Moreover, extracellular enzymes may be longer active during dry
452 conditions than microbial cells (Steinweg et al., 2013). Drought reduced NO_3^- production
453 and increased NH_4^+ uptake. The reduction in N mineralization led to an overall reduction of
454 microbial NUE. The effects of drought on NUE depended on land management (Table 2),
455 mostly caused by small, but differential changes in gross amino acid uptake rates at the two
456 sites.

457 Drought periods are often accompanied by heat waves, where soil temperatures can quickly
458 rise above the normal range. In line with our hypothesis and earlier findings, microbial CUE
459 decreased with increased temperature (Allison, 2014; Bölscher et al., 2017; Devêvre and
460 Horwáth, 2000; Frey et al., 2013; Li et al., 2014; Steinweg et al., 2008; Walker et al., 2018),
461 with C substrate respiration increasing stronger than C incorporation (Table 2, Fig. 2). Higher
462 temperatures stimulate intracellular metabolic processes (e.g. several steps in glycolysis and
463 the Krebs cycle (Dijkstra *et al.*, 2011)) and stimulate microbial turnover compared to growth
464 efficiency (Hagerty et al., 2014). Moreover, can also stimulate extracellular enzyme rates
465 increasing SOM and substrate turnover (Steinweg et al., 2013). On ecosystem scale the strong
466 temperature dependency of heterotrophic soil respiration can account for large C losses
467 (Walker *et al.*, 2018; Mayer *et al.*, 2017). However, previous field experiments have shown

468 that total soil respiration (which includes autotrophic plant root respiration) exhibits lower
469 temperature-sensitivity under drier conditions (Davidson & Janssens, 2006; Suseela *et al.*,
470 2012). In contrary to our expectation, we found that the temperature response of CUE was
471 only marginally interactively affected by land management ($p=0.064$) or drought ($p=0.070$),
472 respectively (Table 2). This suggests that the differences in microbial community
473 composition between the two sites and induced by drought may not have been strong enough
474 (yet) to change the responses to increased temperatures. In contrast, the temperature response
475 of C turnover times were significantly interactively affected by both land management and
476 drought driven by only slightly different temperature responses in C substrate incorporation
477 (Fig. 2c). However, microbes can adapt over long times to higher temperatures (Bradford *et*
478 *al.*, 2008; Rousk *et al.*, 2012), thus the observed temperature sensitivity of microbial CUE
479 could represent a short-term stress response. On the other hand, some long term warming
480 studies showed that even after several years of warming microbes exhibited a high
481 temperature sensitivity (Frey *et al.*, 2013; Schindlbacher *et al.*, 2015; Walker *et al.*, 2018).

482 In contrast to our hypothesis microbial N cycling showed a different, and less pronounced
483 temperature sensitivity than microbial C cycling, independent of land management. In our
484 experiment gross rates of protein depolymerization, N mineralization and NO_3^- production
485 remained unchanged at higher temperatures. However, we detected an interactive effect of
486 drought and temperature only on gross protein depolymerization rates, but not on inorganic N
487 cycling and N turnover rates which is in contrast earlier studies (Auyeung *et al.*, 2013; Wild
488 *et al.*, 2018). Microbial NUE did not change at higher temperatures (Table 2, Fig. 3).

489 Although effects of higher temperatures on N turnover might be delayed in their response,
490 several long term warming experiments also found no effect on soil N turnover (Niboyet *et*
491 *al.*, 2011; Schindlbacher *et al.*, 2015). Our data suggests that soil microbial N turnover is less

492 sensitive to short-term temperature changes than C cycling, similar as shown by Koch *et al.*,
493 (2007).

494 Overall, we conclude that microbial C and N cycling processes respond differently to changes
495 in environmental conditions. Microbial C cycling was more sensitive to temperature changes,
496 whereas N cycling was more strongly controlled by water availability. Our results suggest
497 that alterations on soil N cycling induced by land management could modulate in particular
498 soil NUE in under future scenarios.

499

500 **Acknowledgements**

501 LF, AR, BW and MM designed the experiment; MB set up, maintained and provided access
502 to the drought experiment; LF, BW, MT, SK, AK and FH conducted the experiment, and
503 analyzed samples and data; all authors contributed to writing the manuscript. We want to
504 thank Margarete Watzka for her valuable help in analyzing samples and Dr. Alberto Canarini
505 for statistical advice. This experiment was supported by the Austrian Science Fund (FWF
506 P22214-B17) and by the European Community's Seventh Framework Program
507 (FP/2007.2013) under grant agreement no. 226701 (CARBO-Extreme). L.F. received a PhD
508 completion grant from the University of Vienna. The authors declare no conflict of interest.

509

510 **References**

511 Allison, S.D., 2014. Modeling adaptation of carbon use efficiency in microbial communities.

512 *Frontiers in Microbiology* 5, 1–9. doi:10.3389/fmicb.2014.00571

513 Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming

514 dependent on microbial physiology. *Nature Geoscience* 3, 336–340.

515 doi:10.1038/ngeo846

- 516 Alvarez, G., Shahzad, T., Andanson, L., Bahn, M., Wallenstein, M.D., Fontaine, S., 2018.
517 Catalytic power of enzymes decreases with temperature: New insights for understanding
518 soil C cycling and microbial ecology under warming. *Global Change Biology* 24, 4238–
519 4250. doi:10.1111/gcb.14281
- 520 Auyeung, D.S.N., Suseela, V., Dukes, J.S., 2013. Warming and drought reduce temperature
521 sensitivity of nitrogen transformations. *Global Change Biology* 19, 662–676.
522 doi:10.1111/gcb.12063
- 523 Bahn, M., Knapp, M., Garajova, Z., Pfahringer, N., Cernusca, A., 2006. Root respiration in
524 temperate mountain grasslands differing in land use. *Global Change Biology* 12, 995–
525 1006. doi:10.1111/j.1365-2486.2006.01144.x
- 526 Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: Critical review of
527 estimation criteria and approaches. *Soil Biology & Biochemistry* 67, 192–211.
528 doi:10.1016/j.soilbio.2013.08.024
- 529 Bloor, J., Pichon, P., Falcimagne, R., Leadley, P., Soussana, J.-F., 2010. Effects of Warming,
530 Summer Drought, and CO₂ Enrichment on Aboveground Biomass Production,
531 Flowering Phenology, and Community Structure in an Upland Grassland Ecosystem.
532 *Ecosystems* 13, 888–900.
- 533 Bölscher, T., Paterson, E., Freitag, T., Thornton, B., Herrmann, A.M., 2017. Temperature
534 sensitivity of substrate-use efficiency can result from altered microbial physiology
535 without change to community composition. *Soil Biology and Biochemistry* 109, 59–69.
536 doi:10.1016/j.soilbio.2017.02.005
- 537 Bölscher, T., Wadsö, L., Börjesson, G., Herrmann, A.M., 2016. Differences in substrate use
538 efficiency: impacts of microbial community composition, land use management, and
539 substrate complexity. *Biology and Fertility of Soils* 52, 547–559. doi:10.1007/s00374-
540 016-1097-5

- 541 Bradford, M.A., Davies, C.A., Frey, S.D., Maddox, T.R., Melillo, J.M., Mohan, J.E.,
542 Reynolds, J.F., Treseder, K.K., Wallenstein, M.D., 2008. Thermal adaptation of soil
543 microbial respiration to elevated temperature. *Ecology Letters* 11, 1316–1327.
544 doi:10.1111/j.1461-0248.2008.01251.x
- 545 Creamer, C.A., de Menezes, A.B., Krull, E.S., Sanderman, J., Newton-Walters, R., Farrell,
546 M., 2015. Microbial community structure mediates response of soil C decomposition to
547 litter addition and warming. *Soil Biology and Biochemistry* 80, 175–188.
548 doi:10.1016/j.soilbio.2014.10.008
- 549 Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition
550 and feedbacks to climate change. *Nature* 440, 165–173. doi:10.1038/nature04514
- 551 De Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M.,
552 Bardgett, R.D., 2012. Land use alters the resistance and resilience of soil food webs to
553 drought. *Nature Climate Change* 2, 276–280. doi:10.1038/nclimate1368
- 554 Devêvre, O.C., Horwáth, W.R., 2000. Decomposition of rice straw and microbial carbon use
555 efficiency under different soil temperatures and moistures. *Soil Biology and*
556 *Biochemistry* 32, 1773–1785.
- 557 Dijkstra, P., Salpas, E., Fairbanks, D., Miller, E.B., Hagerty, S.B., Jan, K., Groenigen, V.,
558 Hungate, B.A., Marks, J.C., Koch, G.W., Schwartz, E., 2015. High carbon use efficiency
559 in soil microbial communities is related to balanced growth, not storage compound
560 synthesis. *Soil Biology and Biochemistry* 89, 35–43. doi:10.1016/j.soilbio.2015.06.021
- 561 Dijkstra, P., Thomas, S.C., Heinrich, P.L., Koch, G.W., Schwartz, E., Hungate, B.A., 2011.
562 Effect of temperature on metabolic activity of intact microbial communities: Evidence
563 for altered metabolic pathway activity but not for increased maintenance respiration and
564 reduced carbon use efficiency. *Soil Biology and Biochemistry* 43, 2023–2031.
565 doi:10.1016/j.soilbio.2011.05.018

- 566 Fontana, V., Kohler, M., Niedrist, G., Bahn, M., Tappeiner, U., Frenck, G., 2017.
567 Decomposing the land-use specific response of plant functional traits along
568 environmental gradients. *Science of the Total Environment* 599–600, 750–759.
569 doi:10.1016/j.scitotenv.2017.04.245
- 570 Frey, S.D., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial
571 efficiency and its feedback to climate. *Nature Climate Change* 3, 395–398.
572 doi:10.1038/nclimate1796
- 573 Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid
574 phosphate in soils of different organic content. *Journal of Microbiological Methods* 14,
575 151–163.
- 576 Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., Richter, A., 2014a. Experimental drought
577 reduces the transfer of recently fixed plant carbon to soil microbes and alters the
578 bacterial community composition in a mountain meadow. *New Phytologist* 201, 916–
579 927.
- 580 Fuchslueger, L., Kastl, E.-M., Bauer, F., Kienzl, S., Hasibeder, R., Ladreiter-Knauss, T.,
581 Schmitt, M., Bahn, M., Schloter, M., Richter, A., Szukics, U., 2014b. Effects of drought
582 on nitrogen turnover and abundances of ammonia-oxidizers in mountain grassland.
583 *Biogeosciences* 11, 6003–6015. doi:10.5194/bg-11-6003-2014
- 584 Grigulis, K., Lavorel, S., Krainer, U., Legay, N., Baxendale, C., Dumont, M., Kastl, E.-M.,
585 Arnoldi, C., Bardgett, R.D., Poly, F., Pommier, T., Schloter, M., Tappeiner, U., Bahn,
586 M., Clément, J.-C., 2013. Relative contributions of plant traits and soil microbial
587 properties to mountain grassland ecosystem services. *Journal of Ecology* 101, 47–57.
- 588 Hagerty, S.B., Groenigen, K.J., Allison, S.D., Hungate, B. a., Schwartz, E., Koch, G.W.,
589 Kolka, all K., Dijkstra, P., Groenigen, K.J., Kolka, R.K., van Groenigen, K.J., Allison,
590 S.D., Hungate, B. a., Schwartz, E., Koch, G.W., Kolka, R.K., Dijkstra, P., 2014.

- 591 Accelerated microbial turnover but constant growth efficiency with warming in soil.
592 Nature Climate Change 4, 903–906. doi:10.1038/nclimate2361
- 593 Harris, E., Ladreiter-Knauss, T., Butterbach-Bahl, K., Wolf, B., Bahn, M., 2018. Land-use
594 and abandonment alters methane and nitrous oxide fluxes in mountain grasslands.
595 Science of the Total Environment 628–629, 997–1008.
596 doi:10.1016/j.scitotenv.2018.02.119
- 597 Hartmann, A., Barnard, R., Marhan, S., Niklaus, P., 2013. Effects of drought and N-
598 fertilization on N cycling in two grassland soils. Oecologia 1–13. doi:10.1007/s00442-
599 012-2578-3
- 600 Hartmann, A., Niklaus, P., 2012. Effects of simulated drought and nitrogen fertilizer on plant
601 productivity and nitrous oxide (N₂O) emissions of two pastures. Plant and Soil 361,
602 411–426.
- 603 Homyak, P.M., Allison, S.D., Huxman, T.E., Goulden, M.L., Treseder, K.K., 2017. Effects of
604 Drought Manipulation on Soil Nitrogen Cycling: A Meta-Analysis. Journal of
605 Geophysical Research: Biogeosciences 122, 3260–3272. doi:10.1002/2017JG004146
- 606 Ingrisch, J., Karlowsky, S., Anadon-Rosell, A., Hasibeder, R., König, A., Augusti, A.,
607 Gleixner, G., Bahn, M., 2017. Land Use Alters the Drought Responses of Productivity
608 and CO₂ Fluxes in Mountain Grassland. Ecosystems 1–15. doi:10.1007/s10021-017-
609 0178-0
- 610 IPCC, 2012, S., Field, C.B., Barros, V., Stocker, T., Qin, D., Dokken, D.J., Eb, I.K.L.,
611 Mastandrea, M.D., Mach, K.J., Plattner, G.-K., Allen, S.K., Tignor, M., Midgley, P.M.,
612 2012. IPCC, 2012: Managing the Risks of Extreme Events and Disasters to Advance
613 Climate Change Adaptation 1–594.
- 614 Jensen, K., Beier, C., Michelsen, A., Emmett, B., 2003. Effects of experimental drought on
615 microbial processes in two temperate heathlands at contrasting water conditions.

- 616 Applied Soil Ecology 24, 165–176.
- 617 Jones, D.L., Owen, a G., Farrar, J.F., 2002. Simple method to enable the high resolution
618 determination of total free amino acids in soil solutions and soil extracts. *Soil Biology &*
619 *Biochemistry* 34, 1893–1902. doi:10.1016/S0038-0717(02)00203-1
- 620 Kaiser, C., Frank, A., Wild, B., Koranda, M., Richter, A., 2010. Negligible contribution from
621 roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2??6,9 and 18:1??9.
622 *Soil Biology and Biochemistry* 42, 1650–1652. doi:10.1016/j.soilbio.2010.05.019
- 623 Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric
624 determination of ammonium. *Biology and Fertility of Soils* 6, 68–72.
- 625 Karlowsky, S., Augusti, A., Ingrisich, J., Hasibeder, R., Lange, M., Lavorel, S., Bahn, M.,
626 Gleixner, G., 2018. Land use in mountain grasslands alters drought response and
627 recovery of carbon allocation and plant-microbial interactions. *Journal of Ecology* 106,
628 1230–1243. doi:10.1111/1365-2745.12910
- 629 Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., Hämmerle, I., Ellersdorfer, G., Böck,
630 S., Strauss, J., Sterflinger, K., Richter, A., Zechmeister-Boltenstern, S., 2010. The effect
631 of resource quantity and resource stoichiometry on microbial carbon-use-efficiency.
632 *FEMS Microbiology Ecology* 73, 430–440. doi:10.1111/j.1574-6941.2010.00912.x
- 633 Kirkham, D., Bartholomew, W.V., 1954. Equations for Following Nutrient Transformations
634 in Soil, Utilizing Tracer Data. *Soil Science Society of America Journal* 18, 33–34.
635 doi:10.2136/sssaj1955.03615995001900020020x
- 636 Koch, O., Tschërko, D., Kandeler, E., 2007. Temperature sensitivity of microbial respiration,
637 nitrogen mineralization, and potential soil enzyme activities in organic alpine soils.
638 *Global Biogeochemical Cycles* 21, 1–11. doi:10.1029/2007GB002983
- 639 Larsen, K.S., Andresen, L.C., Beier, C., Jonasson, S., Albert, K.R., Ambus, P., Arndal, M.F.,
640 Carter, M.S., Christensen, S., Holmstrup, M., Ibrom, A., Kongstad, J., Van Der Linden,

- 641 L., Maraldo, K., Michelsen, A., Mikkelsen, T.N., Pilegaard, K., Priemé, A., Ro-Poulsen,
642 H., Schmidt, I.K., Selsted, M.B., Stevnbak, K., 2011. Reduced N cycling in response to
643 elevated CO₂, warming, and drought in a Danish heathland: Synthesizing results of the
644 CLIMAITE project after two years of treatments. *Global Change Biology* 17, 1884–
645 1899. doi:10.1111/j.1365-2486.2010.02351.x
- 646 Lee, Z.M., Schmidt, T.M., 2014. Bacterial growth efficiency varies in soils under different
647 land management practices. *Soil Biology and Biochemistry* 69, 282–290.
648 doi:10.1016/j.soilbio.2013.11.012
- 649 Legay, N., Lavorel, S., Baxendale, C., Krainer, U., Bahn, M., Binet, M.N., Cantare, A.A.M.,
650 Colace, M.P., Foulquier, A., Kastl, E.M., Grigulis, K., Mouhamadou, B., Poly, F.,
651 Pommier, T., Schloter, M., Clément, J.C., Bardgett, R.D., 2016. Influence of plant traits,
652 soil microbial properties, and abiotic parameters on nitrogen turnover of grassland
653 ecosystems. *Ecosphere* 7, 1–17. doi:10.1002/ecs2.1448
- 654 Lennon, J.T., Jones, S.E., 2011. Microbial seed banks: the ecological and evolutionary
655 implications of dormancy. *Nature Reviews Microbiology* 9, 119–130.
- 656 Li, P., Yang, Y., Han, W., Fang, J., 2014. Global patterns of soil microbial nitrogen and
657 phosphorus stoichiometry in forest ecosystems. *Global Ecology and Biogeography* 23,
658 979–987. doi:10.1111/geb.12190
- 659 Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and
660 stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist*
661 196, 79–91.
- 662 Mayer, M., Sandén, H., Rewald, B., Godbold, D.L., Katzensteiner, K., 2017. Increase in
663 heterotrophic soil respiration by temperature drives decline in soil organic carbon stocks
664 after forest windthrow in a mountainous ecosystem. *Functional Ecology* 31, 1163–1172.
665 doi:10.1111/1365-2435.12805

- 666 Melillo, J.M., Frey, S.D., Deangelis, K.M., Werner, W.J., Bernard, M.J., Bowles, F.P., Pold,
667 G., Knorr, M.A., Grandy, A.S., 2017. Long-term pattern and magnitude of soil carbon
668 feedback to the climate system in a warming world. *Science* 358, 101–105.
669 doi:10.1126/science.aan2874
- 670 Meyer, S., Leifeld, J., Bahn, M., Fuhrer, J., 2012. Free and protected soil organic carbon
671 dynamics respond differently to abandonment of mountain grassland. *Biogeosciences* 9,
672 853–865.
- 673 Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A.,
674 Schneckner, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K.M., Zechmeister-
675 Boltenstern, S., Richter, A., 2014. Adjustment of microbial nitrogen use efficiency to
676 carbon:nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications* 5,
677 3694. doi:10.1038/ncomms4694
- 678 Moyano, F.E., Manzoni, S., Chenu, C., 2013. Responses of soil heterotrophic respiration to
679 moisture availability: An exploration of processes and models. *Soil Biology &*
680 *Biochemistry* 59, 72–85. doi:10.1016/j.soilbio.2013.01.002
- 681 Niboyet, A., Le Roux, X., Dijkstra, P., Hungate, B.A., Barthes, L., Blankinship, J.C., Brown,
682 J.R., Field, C.B., Leadley, P.W., 2011. Testing interactive effects of global
683 environmental changes on soil nitrogen cycling. *Ecosphere* 2, art56. doi:10.1890/ES10-
684 00148.1
- 685 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson,
686 G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan.' R Package
687 Ver. 2.0–8.
- 688 Olsson, P.A., 2006. Signature fatty acids provide tools for determination of the distribution
689 and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology* 29, 303–310.
- 690 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, S., Core, T.R., 2019. nlme: Linear and Nonlinear

- 691 Mixed Effects Models. R package version 3.1-139, [https://CRAN.R-](https://CRAN.R-project.org/package=nlme)
692 [project.org/package=nlme](https://CRAN.R-project.org/package=nlme).
- 693 Prommer, J., Wanek, W., Hofhansl, F., Trojan, D., Offre, P., Urich, T., Schleper, C.,
694 Sassmann, S., Kitzler, B., Soja, G., Hood-Nowotny, R.C., 2014. Biochar decelerates soil
695 organic nitrogen cycling but stimulates soil nitrification in a temperate arable field trial.
696 *PloS One* 9, e86388. doi:10.1371/journal.pone.0086388
- 697 Rousk, J., Frey, S.D., Bååth, E., 2012. Temperature adaptation of bacterial communities in
698 experimentally warmed forest soils. *Global Change Biology* 18, 3252–3258.
699 doi:10.1111/j.1365-2486.2012.02764.x
- 700 Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2011. Drought
701 effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend
702 on plant community composition. *Applied Soil Ecology* 48, 38–44.
- 703 Schimel, J., Balsler, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its
704 implications for ecosystem function. *Ecology* 88, 1386–1394. doi:10.1890/06-0219
- 705 Schimel, J.P., 2018. Life in Dry Soils: Effects of Drought on Soil Microbial Communities
706 and Processes. *Annual Review of Ecology, Evolution, and Systematics* 49, annurev-
707 ecolsys-110617-062614. doi:10.1146/annurev-ecolsys-110617-062614
- 708 Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm.
709 *Ecology* 85, 591–602.
- 710 Schindlbacher, A., Schnecker, J., Takriti, M., Borken, W., Wanek, W., 2015. Microbial
711 physiology and soil CO₂ efflux after 9 years of soil warming in a temperate forest - no
712 indications for thermal adaptations. *Global Change Biology* 21, 4265–4277.
713 doi:10.1111/gcb.12996
- 714 Schmitt, M., Bahn, M., Wohlfahrt, G., Tappeiner, U., Cernusca, A., 2010. Land use affects
715 the net ecosystem CO₂ exchange and its components in mountain grasslands.

- 716 Biogeosciences 7, 2297–2309.
- 717 Shaw, M.R., Harte, J., 2001. Response of nitrogen cycling to simulated climate change:
718 Differential responses along a subalpine ecotone. *Global Change Biology* 7, 193–210.
719 doi:10.1046/j.1365-2486.2001.00390.x
- 720 Sinsabaugh, R.L., Turner, J.M., Talbot, J., Waring, B.G., Powers, J.S., Kuske, C.R.,
721 Moorhead, D.L., Follstad Shah, J.J., 2016. Stoichiometry of microbial carbon use
722 efficiency in soils. *Ecological Monographs* 86, 172–189.
723 doi:10.1017/CBO9781107415324.004
- 724 Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and Fungal Contributions to
725 Carbon Sequestration in Agroecosystems. *Soil Science Society of America Journal* 70,
726 555. doi:10.2136/sssaj2004.0347
- 727 Spohn, M., Pötsch, Erich, M., Eichorst, S.A., Wobken, D., Wanek, W., Richter, A., 2016.
728 Soil microbial carbon use efficiency and biomass turnover in a long- term fertilization
729 experiment in a temperate grassland. *Soil Biology & Biochemistry* 97, 168–175.
730 doi:10.1016/j.soilbio.2016.03.008
- 731 Steinweg, J.M., Dukes, J.S., Paul, E.A., Wallenstein, M.D., 2013. Microbial responses to
732 multi-factor climate change: Effects on soil enzymes. *Frontiers in Microbiology* 4, 1–11.
733 doi:10.3389/fmicb.2013.00146
- 734 Steinweg, J.M., Plante, A.F., Conant, R.T., Paul, E.A., Tanaka, D.L., 2008. Patterns of
735 substrate utilization during long-term incubations at different temperatures. *Soil Biology*
736 *and Biochemistry* 40, 2722–2728. doi:10.1016/j.soilbio.2008.07.002
- 737 Suseela, V., Conant, R.T., Wallenstein, M.D., Dukes, J.S., 2012. Effects of soil moisture on
738 the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field
739 climate change experiment. *Global Change Biology* 18, 336–348. doi:10.1111/j.1365-
740 2486.2011.02516.x

- 741 Szukics, U., Grigulis, K., Legay, N., Kastl, E.M., Baxendale, C., Bardgett, R.D., Clément,
742 J.C., Lavorel, S., Schloter, M., Bahn, M., 2019. Management versus site effects on the
743 abundance of nitrifiers and denitrifiers in European mountain grasslands. *Science of the*
744 *Total Environment*. doi:10.1016/j.scitotenv.2018.08.039
- 745 Takriti, M., Wild, B., Schnecker, J., Mooshammer, M., Knoltsch, A., Lashchinskiy, N., Eloy,
746 R.J., Gentsch, N., Gittel, A., Mikutta, R., Wanek, W., Richter, A., 2018. Soil organic
747 matter quality exerts a stronger control than stoichiometry on microbial substrate use e
748 ffi ciency along a latitudinal transect. *Soil Biology and Biochemistry* 121, 212–220.
749 doi:10.1016/j.soilbio.2018.02.022
- 750 Tappeiner, U., Tasser, E., Leitinger, G., Cernusca, A., Tappeiner, G., 2008. Effects of
751 Historical and Likely Future Scenarios of Land Use on Above- and Belowground
752 Vegetation Carbon Stocks of an Alpine Valley. *Ecosystems* 11, 1383–1400.
- 753 Tiemann, L.K., Billings, S.A., 2012. Tracking C and N flows through microbial biomass with
754 increased soil moisture variability. *Soil Biology and Biochemistry* 49, 11–22.
755 doi:10.1016/j.soilbio.2012.01.030
- 756 Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An Extraction Method for Measuring
757 Microbial Biomass C. *Soil Biology and Biochemistry* 19, 703–707. doi:10.1016/0038-
758 0717(87)90052-6
- 759 Verburg, P.S.J., van Loon, W.K.P., Lükewille, A., 1999. The CLIMEX soil heating
760 experiment: soil response after 2 years of treatment. *Biol.Fertil.Soil* 28, 271–276.
- 761 Walker, T.W.N., Kaiser, C., Strasser, F., Herbold, C.W., Leblans, N.I.W., Woebken, D.,
762 Janssens, I.A., Sigurdsson, B.D., Richter, A., 2018. Microbial temperature sensitivity
763 and biomass change explain soil carbon loss with warming. *Nature Climate Change*.
764 doi:10.1038/s41558-018-0259-x
- 765 Wallenstein, M., Allison, S.D., Ernakovich, J., Steinweg, J.M., Sinsabaugh, R., 2011.

- 766 Controls on the Temperature Sensitivity of Soil Enzymes: A Key Driver of In Situ
767 Enzyme Activity Rates, in: Shukla, G., Varma, A. (Eds.), *Soil Enzymology* Chapter 13.
768 p. 401. doi:10.1007/978-3-642-14225-3
- 769 Wanek, W., Mooshammer, M., Blöchl, A., Hanreich, A., Richter, A., 2010. Determination of
770 gross rates of amino acid production and immobilization in decomposing leaf litter by a
771 novel ¹⁵N isotope pool dilution technique. *Soil Biology & Biochemistry* 42, 1293–
772 1302.
- 773 Wieder, W.R., Allison, S.D., Davidson, E.A., Georgiou, K., Hararuk, O., He, Y., Hopkins, F.,
774 Luo, Y., Smith, M.J., Sulman, B., Todd-Brown, K., Wang, Y.P., Xia, J., Xu, X., 2015.
775 Explicitly representing soil microbial processes in Earth system models. *Global*
776 *Biogeochemical Cycles* 29, 1782–1800. doi:10.1002/2015GB005188
- 777 Wild, B., Ambus, P., Reinsch, S., Richter, A., 2018. Resistance of soil protein
778 depolymerization rates to eight years of elevated CO₂, warming, and summer drought in
779 a temperate heathland. *Biogeochemistry* 140, 255–267. doi:10.1007/s10533-018-0487-1
- 780 Wild, B., Schnecker, J., Bárta, J., Čapek, P., Guggenberger, G., Hofhansl, F., Kaiser, C.,
781 Lashchinsky, N., Mikutta, R., Mooshammer, M., Šantrůčková, H., Shibistova, O., Urich,
782 T., Zimov, S.A., Richter, A., 2013. Nitrogen dynamics in Turbic Cryosols from Siberia
783 and Greenland. *Soil Biology and Biochemistry* 67, 85–93.
784 doi:10.1016/j.soilbio.2013.08.004
- 785 Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Penuelas, J., Richter, A.,
786 Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant-
787 microbial-soil organic matter transformation. *Ecological Monographs* 85, 133–155.
788 doi:10.1890/14-0777.1
- 789 Zeglin, L.H., Bottomley, P.J., Jumpponen, A., Rice, C.W., Arango, M., Lindsley, A.,
790 McGowen, A., Mfombep, P., Myrold, D.D., 2013. Altered precipitation regime affects

- 791 the function and composition of soil microbial communities on multiple time scales.
792 Ecology 94, 2334–2345.
- 793 Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial
794 communities. Chemosphere 35, 275–294. doi:10.1016/S0045-6535(97)00155-0
- 795 Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Richter, A., Wanek, W., 2019. Growth
796 explains microbial carbon use efficiency across soils differing in land use and geology.
797 Soil Biology and Biochemistry. doi:10.1016/j.soilbio.2018.10.006

798 **Tables and Figures:**

799 **Table 1: a)** Soil parameters and soil microbial characteristics (0-10 cm) of control and drought treated plots of the managed and abandoned
800 mountain grasslands (means, \pm SE, $n=4$). SWC, gravimetric soil water content in % of fresh soil; Total C, total soil C; Total N, total soil N; Soil
801 C:N, mass based soil C:N ratio; EOC, K₂SO₄ extractable organic C; EON, K₂SO₄ extractable organic N; TFAA, total free amino acids; C_{mic},
802 microbial biomass C; N_{mic}, microbial biomass N; Microbial C:N, mass based microbial biomass ratio) **b)** Effects of land management and drought
803 treatment and their interactive effects on soil and microbial parameters were assessed by linear mixed effect models with plot identity as nested
804 random factor within land management. Significant differences are shown in bold.

	a) Managed meadow				Abandoned meadow				b) Land man		Drought		Land man x Drought	
	Control		Drought		Control		Drought		F	p	F	p	F	p
	Mean	\pm SE	Mean	\pm SE	Mean	\pm SE	Mean	\pm SE						
SWC (% fresh soil)	37.8	\pm 0.8	25.1	\pm 4.7	42.9	\pm 1.6	31.3	\pm 5.2	3.8	0.147	17.7	0.006	0.1	0.853
Total C (%)	6.8	\pm 0.6	6.6	\pm 0.6	10.5	\pm 1.9	11.5	\pm 1.8	10.0	0.049	0.0	0.925	0.2	0.003
Total N (%)	0.7	\pm 0.1	0.7	\pm 0.1	0.9	\pm 0.2	1.0	\pm 0.2	5.5	0.037	0.2	0.680	0.2	0.659
Soil C:N	9.9	\pm 0.2	9.7	\pm 0.1	11.6	\pm 0.5	11.6	\pm 0.7	13.1	0.036	3.9	0.097	0.0	0.853
EOC (μ g C g ⁻¹ dw)	323.8	\pm 42.1	304.2	\pm 18.7	442.3	\pm 55.1	371.6	\pm 99.9	2.0	0.250	0.5	0.489	0.2	0.672
EON (μ g N g ⁻¹ dw)	42.3	\pm 4.4	46.0	\pm 2.5	60.6	\pm 6.4	49.9	\pm 15.6	1.5	0.307	0.6	0.482	0.7	0.445
TFAA (μ g N g ⁻¹ dw)	3.6	\pm 0.8	3.7	\pm 0.5	5.2	\pm 1.1	3.9	\pm 1.3	0.7	0.467	0.0	0.852	0.6	0.461
Ammonium (μ g N g ⁻¹ dw)	11.2	\pm 1.1	15.7	\pm 1.7	18.6	\pm 2.3	20.5	\pm 9.6	1.5	0.314	5.0	0.068	0.1	0.806
Nitrate (μ g N g ⁻¹ dw)	3.9	\pm 1.7	3.4	\pm 1.5	0.7	\pm 0.2	0.7	\pm 0.4	5.3	0.106	0.0	0.944	0.1	0.819
C _{mic} (mg C g ⁻¹ dw)	1.0	\pm 0.1	0.8	\pm 0.1	1.9	\pm 0.5	1.9	\pm 0.5	7.6	0.017	0.1	0.738	0.1	0.794
N _{mic} (mg N g ⁻¹ dw)	0.2	\pm 0.02	0.1	\pm 0.02	0.3	\pm 0.1	0.2	\pm 0.1	6.7	0.082	15.4	0.008	0.1	0.795
Microbial C:N	5.7	\pm 0.2	9.1	\pm 0.7	6.6	\pm 0.7	8.6	\pm 0.5	0.1	0.821	24.7	0.003	1.3	0.297
Fungi:bacteria ratio	0.34	\pm 0.01	0.34	\pm 0.02	0.37	\pm 0.02	0.39	\pm 0.01	9.9	0.008	1.0	0.335	3.8	0.076
Gram-pos:Gram-neg ratio	0.46	\pm 0.02	0.49	\pm 0.03	0.32	\pm 0.02	0.50	\pm 0.02	14.5	0.003	19.8	0.004	22.1	0.003

809 **Table 2:** Effects of land management, drought treatment, and incubation temperature on microbial CUE (unitless), total and C substrate derived
 810 microbial respiration and C substrate incorporation by microbes (given in $\mu\text{g C g}^{-1} \text{dw soil h}^{-1}$), as well as on microbial NUE (unitless) and gross N
 811 processing rates, such as protein depolymerization, amino acid (AA) uptake, N (nitrogen) mineralization NH_4^+ uptake, NO_3^- production and NO_3^-
 812 uptake, and on C substrate turnover (days) and N pool turnover (hours) were assessed by linear mixed effects models using land management
 813 system, drought treatment and incubation temperature as fixed factor and accounting for paired control and drought plots as nested random effect
 814 within land management ($n=4$). Missing data for NO_3^- production does not allow to test for drought and drought interactions is marked as na (not
 815 available).

Processes	Land man		Drought		Temp		Land man x Drought		Land man x Temp		Drought x Temp		Land man x Drought x Temp	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
CUE	9.9	0.050	0.7	0.427	22.3	<0.001	1.6	0.222	3.9	0.064	3.7	0.070	0.2	0.631
Total microbial respiration	4.6	0.121	2.8	0.116	43.9	<0.001	0.1	0.732	0.8	0.388	0.6	0.439	0.1	0.832
C substrate respiration	5.0	0.111	51.7	<0.001	73.1	<0.001	0.9	0.369	0.4	0.516	2.0	0.175	0.1	0.876
C substrate incorporation	2.2	0.236	4.6	0.046	0.2	0.647	1.7	0.209	3.6	0.075	2.9	0.105	0.1	0.904
C substrate turnover (d)	20.8	0.020	20.0	<0.001	0.6	0.452	1.7	0.209	4.8	0.043	6.0	0.024	1.5	0.230
NUE	7.7	0.070	6.0	0.027	5.0	0.041	4.9	0.044	0.0	0.904	1.7	0.218	0.0	0.973
Gross protein depoly	5.6	0.099	29.6	<0.001	0.0	0.888	1.0	0.334	0.0	0.988	15.3	0.001	0.2	0.673
Gross AA uptake	7.0	0.078	2.7	0.115	0.1	0.747	2.7	0.12	0.0	0.881	0.8	0.388	0.6	0.446
Gross N mineralization	4.6	0.123	12.1	0.003	0.5	0.474	0.0	0.826	0.5	0.485	0.1	0.764	0.0	0.942
Gross NH_4^+ uptake	3.1	0.123	5.3	0.034	0.9	0.367	0.2	0.638	0.2	0.655	0.2	0.699	0.0	0.891
Gross NO_3^- production	14.0	0.033	7.4	0.014	0.1	0.721	0.2	0.668	0.2	0.653	3.9	0.064	0.1	0.778
Gross NO_3^- uptake	0.7	0.457	na		1.5	0.236	na		1.3	0.275			na	
TFAA turnover (h)	1.7	0.287	0.3	0.582	0.0	0.998	1.2	0.283	0.1	0.815	0.4	0.535	2.4	0.139
NH_4^+ turnover (h)	0.8	0.450	4.5	0.048	0.0	0.839	3.2	0.092	0.1	0.726	3.4	0.080	3.7	0.070
NO_3^- turnover (h)	0.7	0.402	0.5	0.482	0.0	0.974	3.1	0.092	0.1	0.729	6.7	0.016	3.6	0.070

816 **Table 3:** Turnover times of labile C substrate, organic N (TFAA), ammonium (NH_4^+) and
 817 nitrate (NO_3^-) by the microbial biomass given in days (d) and hours (h), respectively, in
 818 ambient controls, as well as in response to drought treatment and to temperature increase
 819 (means \pm SE, $n=4$). Microbial C substrate turnover time was calculated as the C_{mic} divided by
 820 microbial C substrate incorporation, the turnover times of TFAA, NH_4^+ and NO_3^- were
 821 calculated by dividing the N pools by the average of the respective microbial production and
 822 uptake rates. Due to analytical problems NO_3^- turnover times in the managed grassland during
 823 drought were not available (na).

	Managed grassland				Abandoned grassland			
	15°C		25°C		15°C		25°C	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
C substrate turnover (d)	11.9 \pm 1.4	17.8 \pm 0.3	15.2 \pm 1.1	17.1 \pm 0.7	30.4 \pm 3.6	45.7 \pm 6.6	30.2 \pm 5.2	32.3 \pm 6.8
TFAA turnover (h)	2.1 \pm 0.5	2.1 \pm 0.2	2.1 \pm 0.3	2.1 \pm 0.4	2.7 \pm 1.2	0.7 \pm 0.3	2.3 \pm 0.9	1.4 \pm 0.7
NH_4^+ turnover (h)	20.3 \pm 3.0	20.3 \pm 3.9	8.8 \pm 2.4	9.1 \pm 3.5	19.4 \pm 3.4	18.1 \pm 6.5	20.4 \pm 2.2	24.6 \pm 11.4
NO_3^- turnover (h)	6.2 \pm 3.5	na	14.5 \pm 8.7	17.4 \pm 8.4	0.7 \pm 0.3	1.8 \pm 0.6	1.4 \pm 0.5	1.1 \pm 0.4

824

825 **Figure captions:**

826 **Figure 1:** Effects of drought treatment on microbial community composition under ambient
827 temperature conditions displayed as non-metric multidimensional scaling (nmDS) plot based
828 on a Bray-Curtis similarity matrix of relative PLFA abundances in control (light green) and
829 drought (dark green) treated soil of a managed (circles) and abandoned grassland (squares).
830 Differences between sites and drought-preconditioning were computed by permutational
831 ANOVA; (mean, \pm SE, $n=4$)

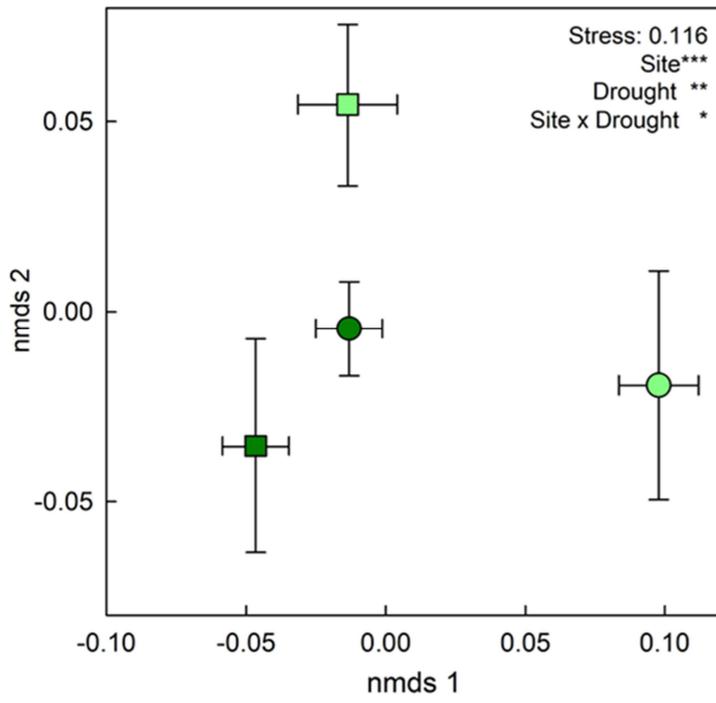
832 **Figure 2:** (a) Total microbial respiration, (b) C substrate derived respiration, (c) C substrate
833 incorporation into microbial biomass, and (d) microbial CUE in control (open bars) and
834 drought treated (hatched bars) soils of a managed and abandoned grassland incubated at
835 ambient temperatures (green bars) and 25°C (red bars); (means, error bars=SE; $n=4$).
836 Statistical details are given in Table 2.

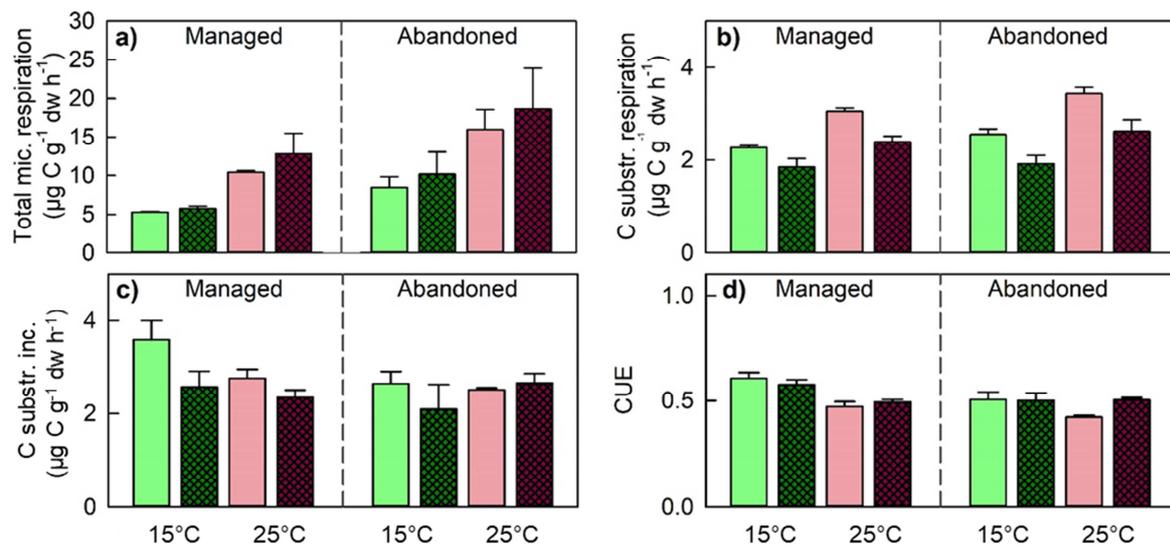
837 **Figure 3:** Gross rates of microbial N cycling and microbial NUE in differently managed
838 grasslands in response to drought and increased temperature; a) Protein deploy refers to gross
839 protein depolymerization, b) AA uptake refers to gross amino acid uptake, c) N mineralization
840 refers to gross N mineralization, d) NH_4^+ uptake refers to gross NH_4^+ uptake, e) NO_3^-
841 production shows gross NO_3^- production, and f) NO_3^- uptake shows gross NO_3^- uptake, as
842 well as g) microbial NUE in control (open bars) and drought treated (hatched bars) soils of a
843 managed and abandoned grassland incubated at ambient (green bars) and 25°C (red bars);
844 (means, error bars=SE; $n=4$; na: data not available). Results from a detailed statistical
845 analysis are shown in Table 2.

846 **Figure 4:** Temperature response (Q_{10}) of microbial C and N cycling rates and of microbial
847 CUE and NUE in control (light green) and drought treated soils (dark green) of the **a)**
848 managed and **b)** abandoned grassland. Values higher than 1 indicate an increase, values
849 smaller than 1 indicate a decrease in response to increased temperature. Letters indicate
850 significant temperature responses (T), drought effects (D), or interactive temperature and
851 drought effects (TxD) (two-way ANOVA, level of minimum significance $p<0.05$, $n=4$).

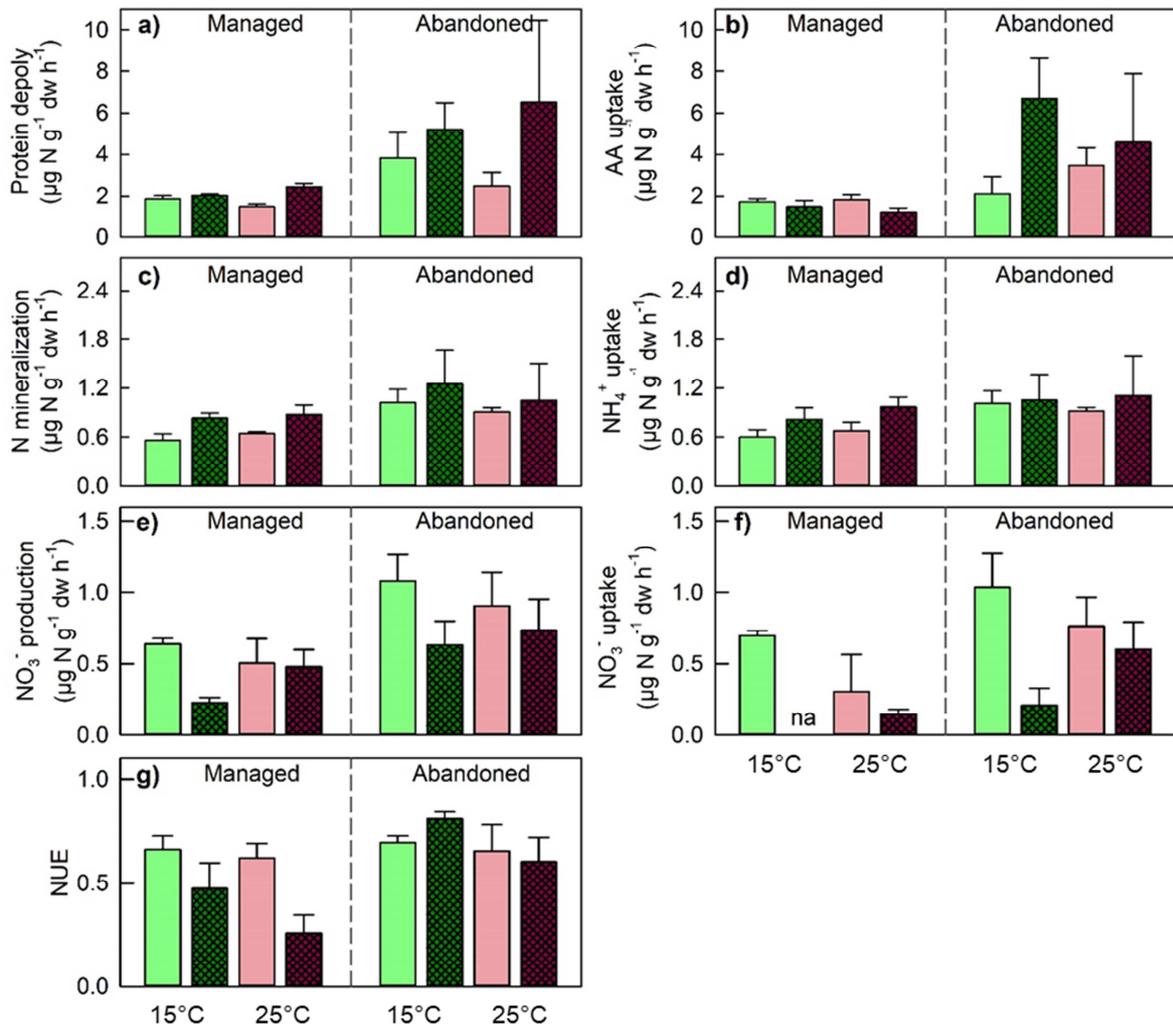
852

853

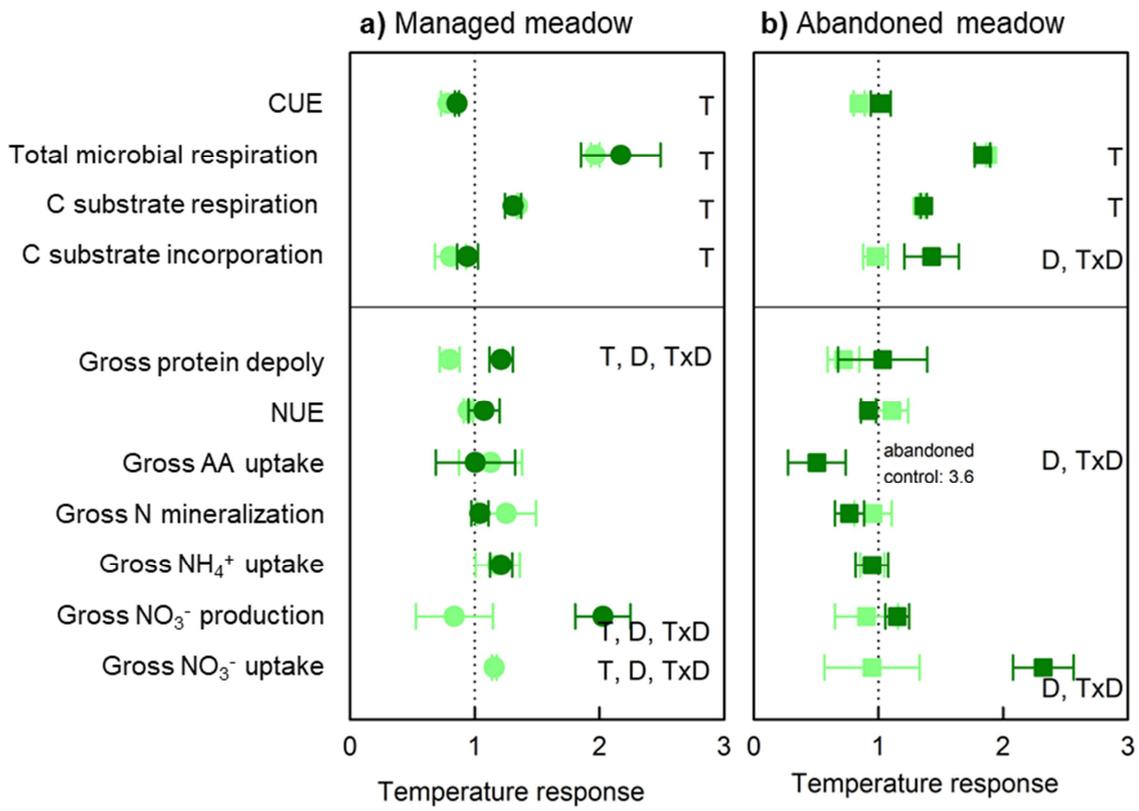
854 **Figure 1:**

856 **Figure 2:**

857

858 **Figure 3:**

859

860 **Figure 4:**

861

Highlights:

- Microbial CUE, but not NUE, was higher in managed compared to abandoned grasslands
- Drought reduced microbial C metabolism at constant CUE
- Drought increased gross N mineralization, but affected NUE interactively with land management
- Higher temperatures reduced CUE and NUE.
- Only minimal interactive effects of drought and temperature were observed.