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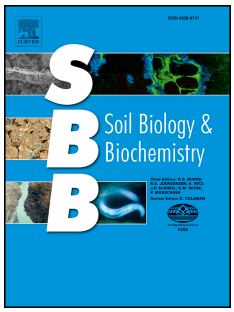

Microbial carbon and nitrogen cycling responses to drought and temperature in differently managed mountain grasslands

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

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

Lucia Fuchslueger[1,2*], Birgit Wild[1,3,4], Maria Mooshammer[1], Mounir Takriti[1,5], Sandra Kienzl[1], Anna Knoltsch[1], Florian Hofhansl[1,6], Michael Bahn[7], Andreas Richter[1,6]

Affiliations:

[1]Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria

[2]Faculty of Science, Department of Biology, Center of Excellence Plants and Ecosystems, University of Antwerp, Antwerp, Belgium

[3]Department of Environmental Science and Analytical Chemistry, Stockholm University, Stockholm, Sweden

[4]Bolin Centre for Climate Research Stockholm University, Stockholm, Sweden

[5]Lancaster Environment Centre, Lancaster University, Lancaster, UK

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

[7]Institute of Ecology, University of Innsbruck, Innsbruck, Austria

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

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Abstract

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

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

1. Introduction

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

Substrate stoichiometry and availability, as well as microbial nutrient demand affect the efficiency with which microbes convert available substrates into biomass, as opposed to the release of C or N as enzymes, exudates, or as CO_2 or inorganic N, i.e. the microbial C or N use efficiency (CUE or NUE, respectively). High microbial CUE denotes a greater potential for soil organic C storage, and lower losses of soil organic C through microbial respiration per unit of C processed (Manzoni et al., 2012; Mooshammer et al., 2014; Sinsabaugh et al., 2016) , and has been found to decrease with N deficiency (Keiblinger et al., 2010; Spohn et al., 2016). Likewise, high microbial NUE indicates efficient incorporation of N into microbial biomass, and concomitant low mineralization (i.e. release of inorganic N as NH_4^+ and NO_3^-) into the environment (Mooshammer et al., 2014). In addition, microbes can take up small organic N forms, such as amino acids; although their production and breakdown is considered a key step in soil N cycling in many systems, their role for soil N dynamics is often overlooked (Schimel and Bennett, 2004; Wild et al., 2013).

Soil C and N cycling is sensitive to changes in soil moisture (Moyano et al., 2013). Low water and osmotic potential and reduced substrate diffusion during drought can reduce microbial growth, increase microbial mortality, induce microbial dormancy and shifts in active microbial community composition (Blagodatskaya and Kuzyakov, 2013; Lennon and Jones, 2011; Moyano et al., 2013; Schimel et al., 2007). Simultaneously, drought reduces microbial activity indicated for example by reduced respiration (Moyano et al., 2013). Effects of drought on microbial N cycling are less clear: drought can reduce extracellular enzyme activity involved in protein depolymerization (Sanaullah et al., 2011). However, drought effects on gross amino acid uptake and production by microbes have rarely been determined. While drought can favor microbial strategies to conserve N, such as production of N-containing osmolyte compounds (Moyano et al., 2013; Schimel et al., 2007), drought effects on N mineralization, and nitrification seem to strongly depend on ecosystem type and land

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

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

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

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

2. Material and Methods

2.1 Site description and soil sampling

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

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

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

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

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

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

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

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

Microbial community composition was determined using phospholipid fatty acids according to the method described by Frostegård *et al.* (1991) with modifications described by (Fuchslueger et al., 2014a). Briefly, total lipids were extracted with a mixture of chloroform, methanol and 0.15 M citric acid buffer from frozen soils. Neutral lipids and phospholipids were separated on silica columns (Supelco, LC-Si SPE, Bellefonte, PE, USA) using chloroform, acetone and methanol as eluents. After addition of methyl-nonadecanoate (19:0) as an internal standard and the conversion of the phospholipids to fatty-acid methyl esters (FAMEs) by alkaline methanolysis, samples were dried and re-dissolved in isooctane and analyzed on a GC-FID (Trace GC Ultra, Thermo) using a DB23 column (Agilent 60 m x 0.25 mm x 0.25 µm). Bacterial and fungal FAME mixtures (bacterial acid methyl ester mix, Supelco, and 37 Comp. FAME Mix, Supelco) were used as qualitative standards. The internal standard 19:0 was used to calculate the concentration of FAMEs. As indicators for Gram-positive bacteria we used the i14:0, i15:0, a15:0, i16:0, a16:0, i17:0 and a17:0 fatty acids, while the markers 16:1ω7, 18:1ω7, cy17:0, and cy19:0 were used as indicators for Gram-negative bacteria. The sum of Gram-positive and Gram-negative markers together with 15:0, 17:0, 10Me18:0, 17:1ω7, and 18:1ω5 was used as a measure for total bacteria. The biomarkers 16:1ω5, 18:2ω6,9, 18:1ω9 and 18:3ω3,6,9 were used to assess the fungal contribution to the microbial community (Kaiser et al., 2010; Olsson, 2006; Zelles, 1997).

2.3 Microbial C cycling potential and microbial CUE

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

of 20, enriched at 10.4 atom%, for a detailed list of compounds see Takriti et al., (2018)). For the assay, 2 g of soil pre-incubated at 15°C or 25°C were placed into 250 ml glass bottles. Each subsample received dissolved C-substrate equaling 40 µg of C and the bottles were sealed with butyl rubber plugs. Immediately after ^{13}C label addition 12 ml gas samples were collected using a syringe and transferred to pre-evacuated Exetainer vials. The air removed from the bottles was replaced with air with known CO_2 concentration and ^{13}C composition. The samples were then again incubated for 24 h at 15°C and 25°C, respectively. At the end of the incubation further gas samples were taken as described above, and aliquots of soils were used to determine microbial biomass C (C_{mic}) by chloroform fumigation extraction as described in section 2.2. In K_2SO_4 extracts of both fumigated and non-fumigated soils $\delta^{13}C$ of EOC was determined by direct injection on an IC system (DX 3000, Dionex Corporation, Sunnyvale, CA, USA) without column and connected through a Finnigan LC IsoLink Interface (Thermo Fisher Scientific, Waltham, MA, USA) to a Finnigan Delta V Advantage Mass Spectrometer (Thermo Fisher, Bremen, Germany). Carbon substrate incorporation into microbial biomass was calculated as the difference between ^{13}C in EOC of chloroform-fumigated and non-fumigated samples. Gas samples were analyzed for their CO_2 concentrations and $\delta^{13}C$ signatures by a headspace gas sampler (GasBench II, Thermo Fisher, Bremen, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher, Bremen, Germany). Cumulative respiration (total microbial soil respiration) was calculated correcting for the air replaced at the start of the incubation. Substrate derived ^{13}C in CO_2 and EOC was corrected for mean natural abundance of soil by calculating atom percent excess. Microbial CUE was estimated as follows:

CUE=C substrate incorporation/(C substrate incorporation + C substrate respiration) **1)**

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

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

2.4 Microbial N cycling rates and microbial NUE

2.4.1 Microbial gross protein depolymerization and gross amino acid uptake

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

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

2.4.2 Gross N mineralization and NH_4^+ uptake, NO_3^- production and NO_3^- uptake

Gross rates of microbial N mineralization (N_{min}) and NH_4^+ uptake ($NH_4^+{}_{uptake}$) and of NO_3^- production ($NO_3^-{}_{prod}$) and uptake ($NO_3^-{}_{uptake}$) were determined using ^{15}N pool dilution assays (Kirkham and Bartholomew, 1954). For each assay pre-incubated aliquots of soil samples received in duplicates 500 µl $(NH_4)_2SO_4$ (0.125 mM; 10 atm% ^{15}N) or 500 µl KNO_3 (0.25 mM, 10 atm% ^{15}N). After ^{15}N-label additions samples were again incubated at 15°C or 25°C. From each assay one of the aliquots was extracted after 4 h, and the other after 24 h with 20 ml 2 M KCl. The extracts were stored frozen until further analyses. Gross N_{min} and $NH_4^+{}_{uptake}$ rates were determined by microdiffusion of NH_3 from KCl-extracts using acid traps, which were analyzed for total N concentrations and atom-percent excess of ^{15}N by EA-IRMS (EA 1110, CE Instruments, Italy coupled to a Finnigan MAT Delta Plus IRMS, Thermo Fisher Scientific, MA, USA). For analyzing gross NO_3^- production and uptake rates, NH_3 was removed from the extracts by adding MgO before converting NO_3^- to NH_3 by adding Devarda's Alloy, trapping NH_3 by microdiffusion and analysis as described before. Gross

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

Microbial NUE

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

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

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

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

2.5 Data analysis and statistics

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

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

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

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

3. Results

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

The managed grassland was characterized by a significantly lower total soil C and N concentrations, lower soil C:N ratio, as well as a significantly lower C_{mic} content compared to the abandoned grassland. At both sites the drought treatment significantly reduced soil moisture content and increased microbial C:N ratios, driven by a significant decrease in N_{mic} (Table 1). The managed grassland showed a significantly lower fungi:bacteria PLFA ratio, and a significantly higher Gram-positive:Gram-negative bacteria PLFA ratio than the 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 ±SE) and
360 abandoned grassland (0.69±0.03, mean ±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

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

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

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

In contrast to our hypothesis microbial N cycling showed a different, and less pronounced temperature sensitivity than microbial C cycling, independent of land management. In our experiment gross rates of protein depolymerization, N mineralization and NO_3^- production remained unchanged at higher temperatures. However, we detected an interactive effect of drought and temperature only on gross protein depolymerization rates, but not on inorganic N cycling and N turnover rates which is in contrast earlier studies (Auyeung et al., 2013; Wild et al., 2018). Microbial NUE did not change at higher temperatures (Table 2, Fig. 3). Although effects of higher temperatures on N turnover might be delayed in their response, several long term warming experiments also found no effect on soil N turnover (Niboyet et al., 2011; Schindlbacher et al., 2015). Our data suggests that soil microbial N turnover is less

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

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

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Tables and Figures:

Table 1: **a)** Soil parameters and soil microbial characteristics (0-10 cm) of control and drought treated plots of the managed and abandoned mountain grasslands (means, ±SE, *n=4*). SWC, gravimetric soil water content in % of fresh soil; Total C, total soil C; Total N, total soil N; Soil C:N, mass based soil C:N ratio; EOC, K_2SO_4 extractable organic C; EON, K_2SO_4 extractable organic N; TFAA, total free amino acids; C_{mic}, microbial biomass C; N_{mic}, microbial biomass N; Microbial C:N,mass based microbial biomass ratio) **b)** Effects of land management and drought treatment and their interactive effects on soil and microbial parameters were assessed by linear mixed effect models with plot identity as nested 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**								
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE		F	p	F	p	F	p
SWC (% fresh soil)	37.8	±0.8	25.1	±4.7	42.9	±1.6	31.3	±5.2		3.8	0.147	**17.7**	**0.006**	0.1	0.853
Total C (%)	6.8	±0.6	6.6	±0.6	10.5	±1.9	11.5	±1.8		**10.0**	**0.049**	0.0	0.925	0.2	0.683
Total N (%)	0.7	±0.1	0.7	±0.1	0.9	±0.2	1.0	±0.2		**5.5**	**0.037**	0.2	0.680	0.2	0.659
Soil C:N	9.9	±0.2	9.7	±0.1	11.6	±0.5	11.6	±0.7		**13.1**	**0.036**	3.9	0.097	0.0	0.853
EOC (µg C g^{-1} dw)	323.8	±42.1	304.2	±18.7	442.3	±55.1	371.6	±99.9		2.0	0.250	0.5	0.489	0.2	0.672
EON (µg N g^{-1} dw)	42.3	±4.4	46.0	±2.5	60.6	±6.4	49.9	±15.6		1.5	0.307	0.6	0.482	0.7	0.445
TFAA (µg N g^{-1} dw)	3.6	±0.8	3.7	±0.5	5.2	±1.1	3.9	±1.3		0.7	0.467	0.0	0.852	0.6	0.461
Ammonium (µg N g^{-1} dw)	11.2	±1.1	15.7	±1.7	18.6	±2.3	20.5	±9.6		1.5	0.314	5.0	0.068	0.1	0.806
Nitrate (µg N g^{-1} dw)	3.9	±1.7	3.4	±1.5	0.7	±0.2	0.7	±0.4		5.3	0.106	0.0	0.944	0.1	0.819
C_{mic} (mg C g^{-1} dw)	1.0	±0.1	0.8	±0.1	1.9	±0.5	1.9	±0.5		**7.6**	**0.017**	0.1	0.738	0.1	0.794
N_{mic} (mg N g^{-1} dw)	0.2	±0.02	0.1	±0.02	0.3	±0.1	0.2	±0.1		6.7	0.082	**15.4**	**0.008**	0.1	0.795
Microbial C:N	5.7	±0.2	9.1	±0.7	6.6	±0.7	8.6	±0.5		0.1	0821	**24.7**	**0.003**	1.3	0.297
Fungi:bacteria ratio	0.34	±0.01	0.34	±0.02	0.37	±0.02	0.39	±0.01		**9.9**	**0.008**	1.0	0.335	3.8	0.076
Gram-pos:Gram-neg ratio	0.46	±0.02	0.49	±0.03	0.32	±0.02	0.50	±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 µg C g^{-1} 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).

	Land man		**Drought**		**Temp**		**Land man x Drought**		**Land man x Temp**		**Drought x Temp**		**Land man x Drought x Temp**	
Processes	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 ±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 ±1.4	17.8 ±0.3	15.2 ±1.1	17.1 ±0.7	30.4 ±3.6	45.7 ±6.6	30.2 ±5.2	32.3 ±6.8
TFAA turnover (h)	2.1 ±0.5	2.1 ±0.2	2.1 ±0.3	2.1 ±0.4	2.7 ±1.2	0.7 ±0.3	2.3 ±0.9	1.4 ±0.7
NH_4^+ turnover (h)	20.3 ±3.0	20.3 ±3.9	8.8 ±2.4	9.1 ±3.5	19.4 ±3.4	18.1 ±6.5	20.4 ±2.2	24.6 ±11.4
NO_3^- turnover (h)	6.2 ±3.5	na	14.5 ±8.7	17.4 ±8.4	0.7 ±0.3	1.8 ±0.6	1.4 ±0.5	1.1 ±0.4

824

Figure captions:

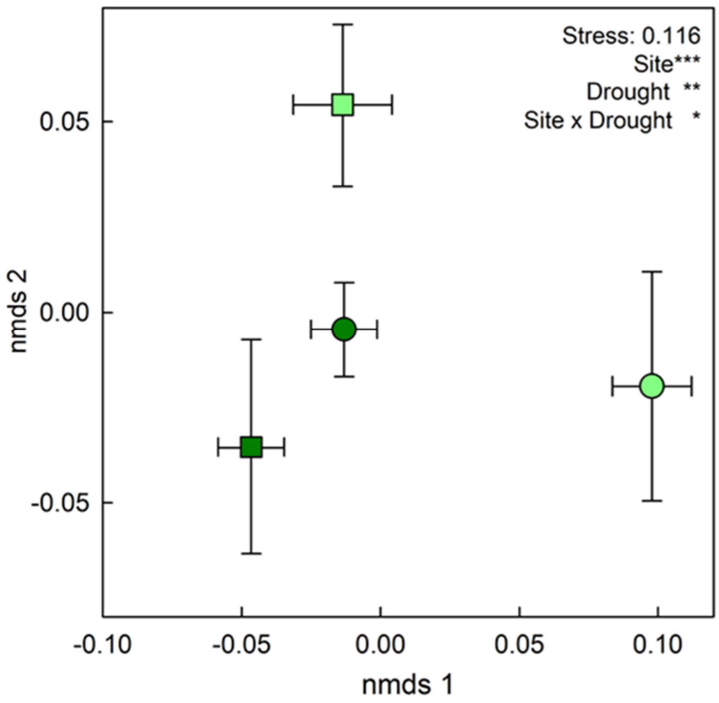
Figure 1: Effects of drought treatment on microbial community composition under ambient temperature conditions displayed as non-metric multidimensional scaling (nmds) plot based on a Bray-Curtis similarity matrix of relative PLFA abundances in control (light green) and drought (dark green) treated soil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

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

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

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

854 **Figure 1:**



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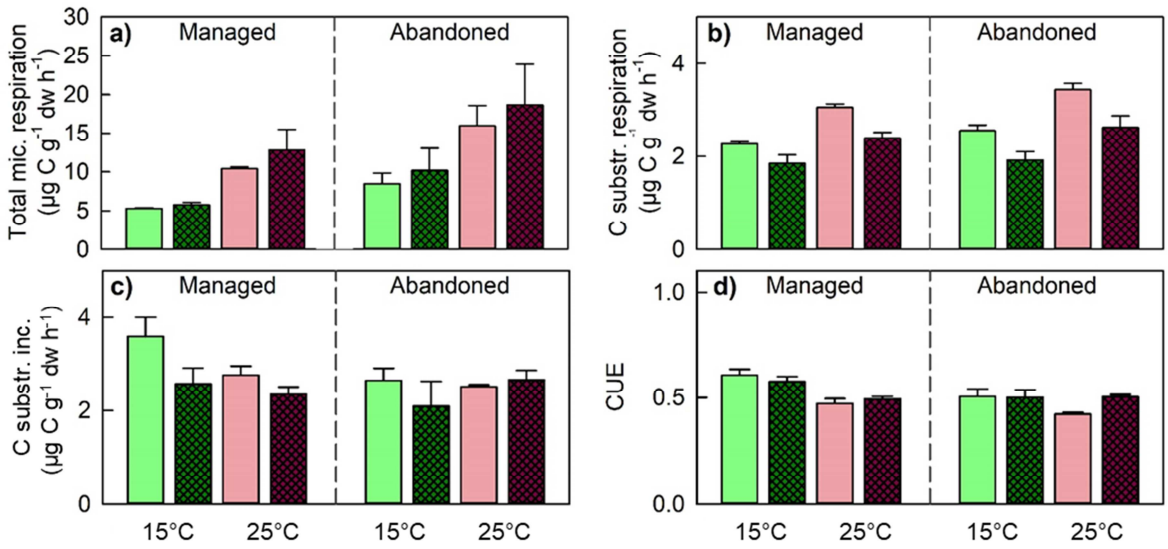

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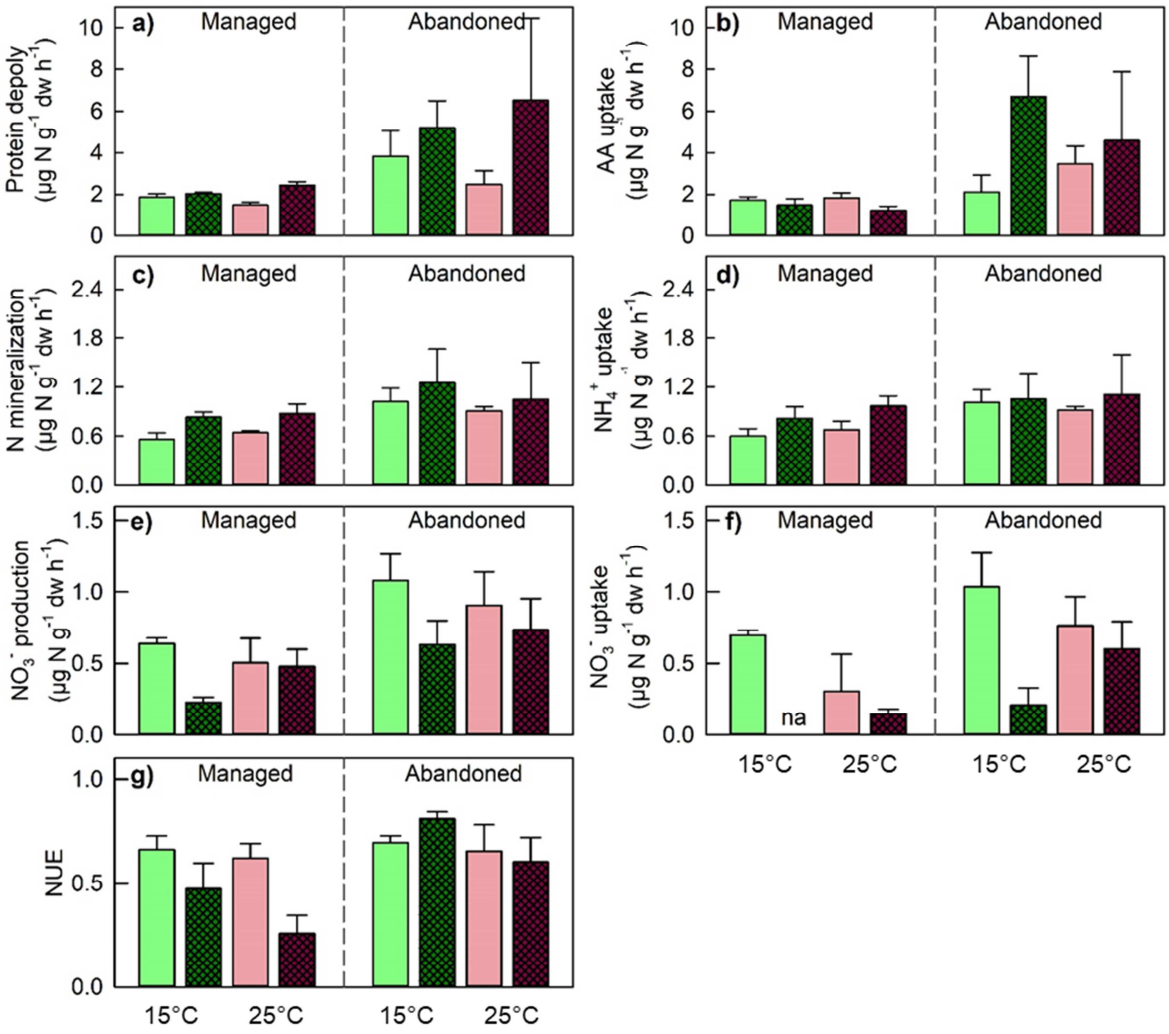

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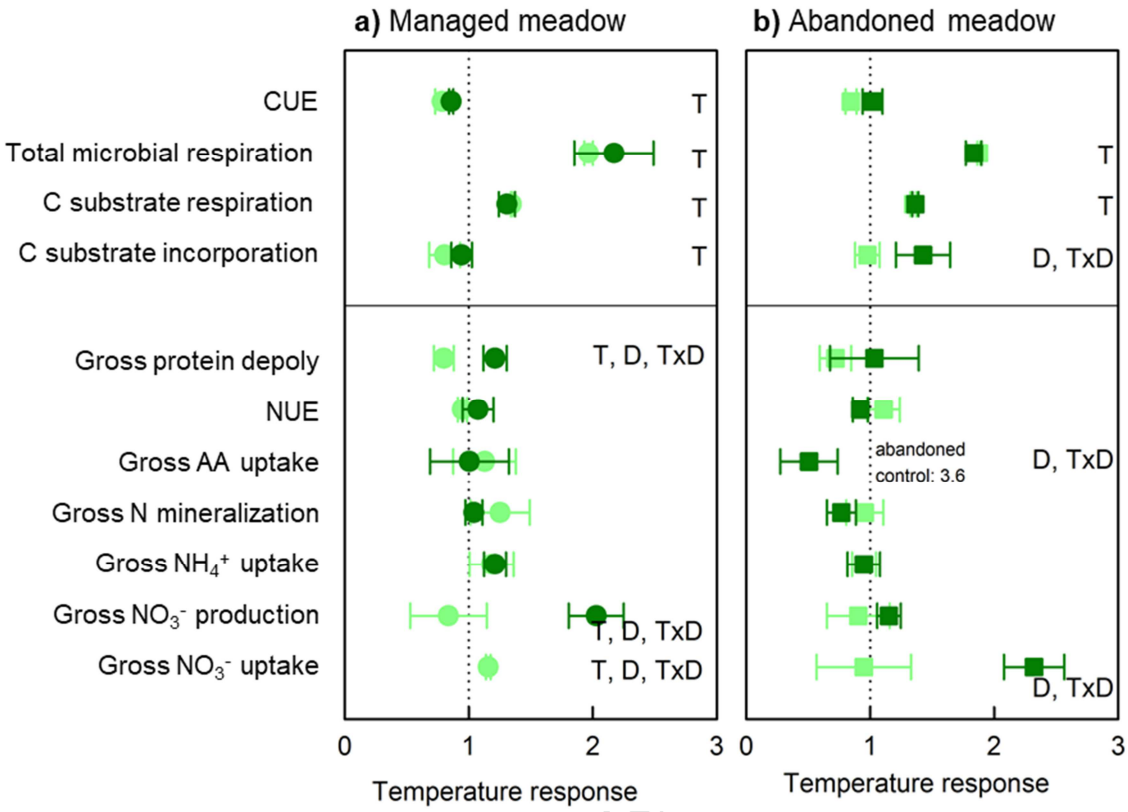

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Highlights:

- 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.