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Growth explains microbial carbon use efficiency across soils differing in land use and geology

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Abstract

The ratio of carbon (C) that is invested into microbial growth to organic C taken up is known as microbial carbon use efficiency (CUE), which is influenced by environmental factors such as soil temperature and soil moisture. How microbes will physiologically react to short-term environmental changes is not well understood, primarily due to methodological restrictions. Here we report on two independent laboratory experiments to explore short-term temperature and soil moisture effects on soil microbial physiology (i.e. respiration, growth, CUE, and microbial biomass turnover): (i) a temperature experiment with 1-day pre-incubation at 5, 15 and 25 °C at 60% water holding capacity (WHC), and (ii) a soil moisture/oxygen (O_2) experiment with 7-day pre-incubation at 20 °C at 30%, 60% WHC (both at 21% O_2) and 90% WHC at 1% O_2. Experiments were conducted with soils from arable, pasture and forest sites derived from both silicate and limestone bedrocks. We found that microbial CUE responded heterogeneously though overall positively to short-term temperature changes, and decreased significantly under high moisture level (90% WHC)/suboxic conditions due to strong decreases in microbial growth. Microbial biomass turnover time decreased dramatically with increasing temperature, and increased significantly at high moisture level (90% WHC)/suboxic conditions. Our findings reveal that the responses of microbial CUE and microbial biomass turnover to short-term temperature and moisture/O_2 changes depended mainly on microbial growth responses and less on respiration responses to the environmental cues, which were consistent across soils differing in land use and geology.

Keywords

Carbon use efficiency; Microbial biomass turnover time; Temperature; Moisture; Short-term environmental effects

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Conflicts of interest
The authors declare no conflict of interest.

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1 Introduction

As the major organic carbon (C) reservoir in terrestrial ecosystems, soils comprise approximately 1500–2400 Pg C (IPCC, 2013). This large reservoir of organic C is mainly decomposed and transformed by soil microorganisms (Schimel and Schaeffer, 2012; Xu et al., 2013). Heterotrophic microbes consume organic C and eventually mineralize a part of it to CO_2, thereby contributing significantly to global CO_2 fluxes. A part of the C taken up is, however, transformed into microbial biomass and eventually necromass which becomes stabilized in soils (Liang et al., 2017; Six et al., 2006; Tucker et al., 2013). This partitioning of C between growth and respiration can be described by microbial carbon use efficiency (CUE), a critical synthetic representation of microbial community C metabolism in models. Low CUE implies a decreased potential for long-term C sequestration in soils (Sinsabaugh et al., 2013). Besides CUE, microbial growth and biomass turnover also determine C sequestration in soils, with faster growth and microbial necromass production (i.e. lower turnover times) promoting soil C accumulation (Hagerty et al., 2014). Microbial biomass C (MBC) only accounts for about 1.2% of total soil organic C (Xu et al., 2013), but is the most active portion in driving SOM decomposition and storage (Kaschuk et al., 2010). Additionally, MBC is an important indicator of soil quality since it is highly sensitive to environmental variation (Kaschuk et al., 2010). Thus, a sound understanding of the controls of microbial CUE, growth, and biomass turnover will greatly improve our knowledge of soil microbial C cycling.

Environmental factors such as soil temperature and moisture have been widely recognized as primary controls on microbial physiology (e.g. respiration and growth). In recent years, extensive studies have focused on investigating the responses of microbial metabolism to environmental changes (Dijkstra et al., 2011; Suseela et al., 2012; Frey et al., 2013; Hagerty et al., 2014). For example, previous studies generally reported decreasing microbial CUE in soils with increasing temperatures (Devêvre and Horwáth, 2000; Schindlbacher et al., 2015). Moreover, higher temperatures could lead to acclimation of microbial activity, depletion of readily accessible substrates and nutrient limitation (Kirschbaum, 2004), which can adversely affect microbial growth and CUE. Temperature could also influence microbial biomass turnover, for instance, microbial turnover might accelerate with soil warming (Hagerty et al., 2014). Changing soil moisture could shift microbial CUE (Manzoni et al., 2012) and affect microbial biomass turnover, e.g. declining soil moisture could trigger microbial water-stress responses (e.g. dormancy or the synthesis of stress metabolites) and consequently affect microbial respiration and growth. Bacterial growth was shown to increase with soil moisture, reaching a broad plateau between 30 and 70% WHC in soils while respiration peaked only at 60% WHC (Iovieno and Bååth, 2008) decreasing towards water saturation (Singh et al., 2017; Wang et al., 2010). High soil moisture could also decrease O_2 diffusivity and availability, which might consequently inhibit microbial activity and respiration (Picek et al., 2000), as well as shift the balance of aerobic towards anaerobic respiration and fermentation in soils (Rubol et al., 2013).

As naturally occurring environmental fluctuations directly result in rapid changes of soil temperature and soil moisture, a thorough exploration of microbial physiological responses to short-term fluctuating environmental conditions is essential for better understanding soil

microbial C metabolism. However, this understanding is impeded by methodological restrictions (Sinsabaugh et al., 2013) since in most studies soil microbial CUE was estimated with ^{13}C-labeled labile substrates such as glucose (Brant et al., 2006; Dijkstra et al., 2015) that tends to overestimate CUE (Sinsabaugh et al., 2013). Additionally, it is difficult to disentangle microbial physiological responses to environmental changes from those of microbial community adaptations (e.g. shifts in microbial community composition) over longer incubation periods (Bárcenas-Moreno et al., 2009; Bradford et al., 2008; Pettersson and Bååth, 2003). Here, we aimed to explore the effects of short-term (i.e., < 7 days) temperature and soil moisture changes on microbial physiology, particularly on growth, respiration, CUE and microbial biomass turnover time. We applied short incubation periods to avoid the confounding effects of changes in microbial community composition or acclimation of microbial physiology, which may occur after weeks of incubation (Bradford, 2013). We employed an ^{18}O technique that quantifies the incorporation of ^{18}O into newly formed microbial dsDNA to estimate microbial CUE as well as microbial biomass turnover time. We hypothesized (1) that short-term increases in soil temperature would decrease microbial CUE since the response of microbial respiration might outpace the response of microbial growth (Allison et al., 2010; Buesing and Gessner, 2006). We also hypothesized (2) that microbial CUE will increase at high soil moisture and suboxic conditions as we assumed that microbial respiration will be inhibited more strongly than growth when O_2 diffusivity becomes limiting (Bastviken et al., 2001; Iovieno and Bååth, 2008; Picek et al., 2000). We tested for the generality of these patterns by comparing soils from two bedrocks (limestone, silicate) under three management regimes (arable field, pasture and forest).

2 Materials and methods

2.1 Site characteristics and soil sampling

In June 2016, soils were sampled in the central Enns valley, Styria, Austria at two locations differing in geology: the LFZ Raumberg-Gumpenstein (47° 29′ N, 14° 6′ E, 690 m above sea level) with silicate bedrock (Gneiss) and the Moarhof in Trautenfels-Pürgg (47° 30′ N, 14° 4′ E, 708 m above sea level) with calcareous bedrock (limestone, dolomite). These two locations exhibit similar climate as they lie on opposing sides of the Enns valley, with 7.2 °C mean annual temperature and 980 mm mean annual precipitation. From both geologies, soils from three land uses were collected, including arable land (A), pasture (P) and forest (F). Soils were classified according to the World Reference Base (WRB) as Luvisols (limestone, L) and Cambisols (silicate, S). Details on site locations, land use, and vegetation can be found in Supplementary Table S1. From each site, quadruplicates of fresh mineral soils were sampled to a soil depth of 15 cm using a root corer (Eijkelkamp, Netherlands), after removal of litter and organic layers. Soil replicates were stored and treated separately for further analysis.

2.2 Sample preparation

After transferring the samples back to the laboratory, all soils were sieved (< 2 mm). Subsequently, 250 g of sieved soils were adjusted to 60% WHC and stored at 15 °C in individual polyethylene Zip-Lock bags before the start of the incubation experiments. The bags were aerated every two days and water replenished when necessary. To investigate

short-term temperature impacts on soil microbial C cycling processes, duplicates of each soil sample (0.4 g) were weighed into 2 mL screw cap vials to measure ^{18}O-incorporation and respiration, and meanwhile duplicates of 2 g of each soil sample were weighed into 20 mL scintillation vials to determine soil extractable organic C and MBC. All weighed soils were pre-incubated in parallel for 1 day at three different temperatures: 5 °C, 15 °C and 25 °C, at 60% WHC. Four weeks later, to study short-term effects of soil moisture/O_2 content on soil microbial C processes, duplicates of each soil sample were prepared as described above and pre-incubated at 20 °C for 7 days at three different levels of moisture (WHC %) at two O_2 contents: low (30% WHC, 21% O_2), intermediate (60% WHC, 21% O_2) and high moisture level (90% WHC, 1% O_2 achieved by incubation in a suboxic chamber) before start of measurements.

2.3 Soil properties

Soil water contents (SWC) were determined by weighing soils before and after drying in a ventilated drying oven at 80 °C for three days. Soil water holding capacity (WHC) was measured by repeatedly saturating soils (10 g fresh weight) with deionized water and draining in between for 2.5 h in a funnel with an ash-free cellulose filter paper. Soil pH was determined in Milli-Q water (Millipore, Germany) at a fresh soil to solution ratio of 1:2.5 (w: v) with an ISFET electrode (Sentron, Netherlands). Aliquots of oven-dried soils were ball milled (MM200, Retsch, Germany) for total organic C and N analyses by an Elemental Analyzer (Carlo Erba 1110, CE Instruments) coupled to a Delta^{Plus} Isotope Ratio Mass Spectrometer (Finnigan MAT, Germany) via a Conflo III interface (Thermo Fisher, Austria). Soil carbonates were removed by treatment with 2 M HCl and drying before elemental analysis. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were determined photometrically in 1 M KCl extracts (Hood-Nowotny et al., 2010). Parallel to the start of the incubation experiments, dissolved organic C (DOC) and total dissolved N (TDN) were extracted from non-fumigated fresh soils in 1 M KCl (1:7.5 (w: v)) for 1 h and filtered through ash-free cellulose filters and then measured by a TOC/TN analyzer (TOC-VCPH/TNM-1, Shimadzu, Austria). In parallel, soils were fumigated for 48 h with chloroform to estimate microbial biomass C (MBC) and microbial biomass N (MBN) by the chloroform fumigation extraction (CFE) method (Vance et al., 1987), and afterwards were extracted in 1 M KCl as described for non-fumigated samples above. MBC and MBN were both calculated as the difference in DOC and TDN between fumigated and non-fumigated soils, corrected with an extraction efficiency factor of 0.45. Soil total P (TP) and total inorganic P (TIP) were measured in 0.5 M H_2SO_4 extracts of ignited (450 °C, 4 h) and control soils (Kuo, 1996) by malachite green measurements of reactive phosphate (Robertson et al., 1999). Soil total organic P (TOP) was calculated as the difference between TP and TIP. Dissolved inorganic P (DIP) was determined using the malachite green method in 0.5 M $NaHCO_3$ (pH 8.5; 1:7.5 (w: v)) extracts. Acid persulfate digestion (Robertson et al., 1999) was applied to measure total dissolved P (TDP) and allowed calculating dissolved organic P (DOP). Microbial biomass P (MBP) was calculated as the difference of fumigated and non-fumigated soil TDP, corrected with an extraction efficiency factor of 0.4. Soil texture, effective cation-exchange capacity (CEC_{eff}), and base saturation were determined by the Soil Analysis Laboratory of the Federal Office for Food Safety (AGES, Vienna, Austria)

according to standard protocols (ÖNORM). Phospholipid fatty acids (PLFAs) were analyzed to determine soil microbial community composition (Hu et al., 2018).

2.4 Determination of soil microbial C metabolism and soil enzymes

After pre-incubation, MBC, basal respiration and ^{18}O incorporation into dsDNA were measured (Spohn et al., 2016) to determine microbial growth, respiration, growth normalized to MBC (qGrowth), the metabolic quotient (qCO_2, microbial respiration normalized to MBC), as well as C uptake normalized to MBC (qC_{uptake}), microbial CUE and microbial biomass turnover time. Double-stranded DNA (dsDNA) is only newly formed during replication. ^{18}O incorporation into dsDNA from $H_2{}^{18}O$ therefore only happens during cell division and the method hence provides estimates of gross growth rates. The method tends to slightly underestimate gross growth rates due to concurrent mortality of growing microbes which decreases the ^{18}O enrichment in dsDNA over 24 h.

The pre-weighed and pre-incubated soil aliquots (0.4 g in 2 mL screw cap vials) were each transferred to 50 mL glass serum bottles (Supelco, Sigma-Aldrich Chemie GmbH, Germany). In half of the soil replicates, the ^{18}O content of soil water was adjusted to 20.0 at % ^{18}O by addition of $H_2{}^{18}O$ (97.0 at%, Campro Scientific, Germany; diluted to lower ^{18}O enrichments where needed), limiting increases in soil moisture (WHC < 5%). Milli-Q water was added to the other half of the soil replicates in order to serve as natural ^{18}O abundance (NA) controls. Directly after adding the water, all serum bottles were sealed with a crimp cap and butyl rubber stoppers (Supelco, Sigma-Aldrich Chemie GmbH, USA) and 5 mL headspace gas were sampled immediately by a gas-tight syringe (time 0 h, t_0) into 3 mL glass exetainer vials previously flushed with He and evacuated. Blank serum bottles containing no soil were treated accordingly and processed through all steps to serve as negative controls for DNA analysis. Subsequently, 5 mL of air with known CO_2 concentration from a tank with compressed air was injected back into each incubation vial in order to keep a balanced atmospheric pressure inside. Afterwards, all ^{18}O labeled and NA samples were incubated for 24 h at the respective conditions as during the pre-incubation period. Gas samples were then collected from each incubation vial at the end of the incubation period (time 24 h, t_{24}) and CO_2 concentrations were determined in all gas samples with a Trace Gas Chromatograph (TRACE Ultra Gas Chromatograph, Thermo Scientific, Germany) equipped with a methanizer-FID. After stopping the incubation experiments, the soil aliquots (in the 2 mL plastic vials) were retrieved and closed with screw caps, immediately frozen in liquid nitrogen, and stored at −80 °C until DNA extraction. Total soil DNA was extracted with a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals, Germany) following the modified manufacturer's recommendations (Spohn et al., 2016). DNA concentrations were then quantified by the Picogreen fluorescence assay (Quant-iT™ PicoGreen® dsDNA Reagent, Thermo Fisher, Germany) using a Microplate spectrophotometer (Infinite® M200, Tecan, Austria). Afterwards, aliquots (50 μL) of the DNA extracts were pipetted into silver capsules (70 μL nominal volume; IVA Analysentechnik, Germany), and dried in a ventilated drying oven at 60 °C for two days. Finally, the silver capsules were folded and analyzed for oxygen isotope composition (^{18}O: ^{16}O) by a Thermochemical Elemental Analyzer (glassy carbon reactor

temperature at 1350 °C) coupled to an Isotope Ratio Mass Spectrometer (TC/EA-IRMS, Delta V Advantage, Thermo Fisher, Germany).

The potential extracellular oxidative (phenoloxidase (POX)) and hydrolytic enzyme (β-glucosidase (BG)) activities that are involved in soil microbial C metabolism were measured photometrically using a modified microplate assay (Kaiser et al., 2010) at the respective temperatures. The POX activities were measured by a modified method (Floch et al., 2007), using 0.4mM ABTS (2,2′-azinobis-(-3 ethylbenzothiazoline-6-sulfonic acid)) as the substrate for photometric analysis at an absorbance wavelength of 420 nm. A modified method (Robertson et al., 1999) using 5 mM p-nitrophenyl (pNP)-linked β-glucopyranoside as substrate was used to measure BG activities at an absorbance wavelength of 410 nm. POX and BG activities were then calculated as the increase in color during the incubation period.

2.5 Calculations and statistical analyses

The enrichment of ^{18}O in the final soil solution ($at\%_{label}$) was calculated by the following equation:

$$at\%_{label} = \frac{at\%_{added} * A + 0.2 * W}{W + A}$$

where $at\%_{added}$ and A are the at% and amount of the labeled water (mL) added to the soils, respectively. W is the soil water content (SWC, mL), and 0.2 is the natural ^{18}O abundance (NA).

Total dsDNA produced (μg) during the 24 h incubation period was calculated according to differences in ^{18}O measurements between labeled and unlabeled NA samples.

$$DNA_{produced} = O_{total} * \frac{at\%_{excess}}{100} * \frac{100}{at\%_{label}} * \frac{100}{31.21}$$

where O_{total} is the total O content (μg) of the dried DNA extract, $at\%_{excess}$ is the at% excess ^{18}O of the labeled sample compared to the mean at% ^{18}O of NA samples. The average weight% of O in DNA is 31.21 according to the average formula ($C_{39}H_{44}O_{24}N_{15}P_4$). Here it was assumed that all newly incorporated O in DNA derived from soil water. Moreover, the mortality of newly produced ^{18}O-labeled microbial cells is negligible in the experiments due to the short incubation time and slow turnover rates.

A conversion factor (f_{DNA}) was calculated to represent the ratio of soil MBC (μg g^{-1} DW) to soil DNA content (μg g^{-1} DW), which was measured and calculated for each individual soil sample, and is representative for the entire soil microbial community. The specific f_{DNA} values were then applied to each replicate individually that, multiplied by the DNA production rate, allows calculating microbial growth rates based on dry soil mass (C_{growth}, ng C g^{-1} h^{-1}).

$$C_{growth} = \frac{f_{DNA} * DNA_{produced} * 1000}{DW * t}$$

where DW is the dry mass of soil in gram and t is the incubation time in hours. Additionally, microbial basal respiration rate ($C_{respiration}$, ng C g^{-1} h^{-1}) was calculated by the following equation:

$$C_{respiration} = \frac{D_{CO2}}{DW * t} * \frac{p * n}{R * T} * V_{hs} * 1000$$

where t (h) is the incubation time, p is the atmosphere pressure (kPa), n is the molecular mass of the element C (12.01 g mol^{-1}), R is the ideal gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature of the gas (295.15 K). V_{hs} is the volume (L) of the head space vials. D_{CO2} (ppm) is the increase in CO_2 concentration during the 24 h incubation period that can be calculated as:

$$D_{CO2} = \frac{(V_{hs} - 0.005) * C_{t0} + 0.005 * C_k}{V_{hs}} - C_{t24}$$

where C_{t0} and C_{t24} are the CO_2 concentration (ppm) measured at the start (t_0) and end (t_{24}) of the incubation period, respectively. C_k and 0.005 are the CO_2 concentration (ppm) and air volume (L) that was injected back into the incubation vial at the start time (t_0), respectively.

Microbial CUE and microbial C_{uptake} (ng C g^{-1} h^{-1}) then could be calculated by the following equations (Sinsabaugh et al., 2013):

$$CUE = \frac{C_{growth}}{C_{growth} + C_{respiration}} = \frac{C_{growth}}{C_{uptake}}$$

When dividing C_{growth}, $C_{respiration}$ and C_{uptake} by the respective MBC content (μg g^{-1} DW), microbial biomass based growth rates ($qGrowth$; ng C (μg MBC)$^{-1}$ h^{-1}), metabolic quotients (qCO_2, ng C (μg MBC)$^{-1}$ h^{-1}) and microbial biomass based C uptake rates (qC_{uptake}, ng C (μg MBC)$^{-1}$ h^{-1}) can be estimated as follows:

$$qGrowth = \frac{C_{growth}}{MBC}$$

$$qCO_2 = \frac{C_{respiration}}{MBC}$$

$$qC_{uptake} = \frac{C_{uptake}}{MBC}$$

Finally, microbial biomass turnover time (d) was calculated as follows:

$$t_{turnover} = \frac{DNA_{amount}}{DNA_{produced} * t/24}$$

where DNA_{amount} (μg) is the DNA content in each soil quantified by Picogreen assay, and t is incubation time in hours.

The temperature sensitivity of qCO_2, qGrowth, microbial CUE, C_{uptake} and soil enzyme activities was determined based on a log-linear regression and calculated by the following modified equation (Janssens and Pilegard, 2003):

$$Ln(R) = \frac{Ln(Q_{10})}{10} * T + b$$

where R is the measured process rate (qCO_2, qGrowth, microbial CUE, C_{uptake}) or potential enzyme activity (POX, BG), Q_{10} is the temperature sensitivity of the measured process or enzyme, T is incubation temperature (°C) and b is a fitted coefficient. Q_{10} is the factor by which the measured process rate increases when the temperature increases by 10°C.

We also - for the first time - used a polynomial multiple regression model to estimate maintenance respiration rates of soil microbial communities, which partitioned total heterotrophic respiration in maintenance respiration and respiration linked to combined growth, uptake, and transport. The regression model is based on the strong covariation of qCO_2 and $qGrowth$ within and across sites (bedrock x management) and was used to extrapolate qCO_2 at $qGrowth = 0$ which represents maintenance respiration. The model is based on the following equation:

$$qCO_2 = a + b * qGrowth + c * T + d * T^2 + e$$

in which qCO_2 is the modeled microbial respiration rate normalized to MBC content (mean and standard error) measured at 5, 15 and 25 °C in the temperature experiment. $qGrowth$ is the measured microbial growth rate normalized to MBC content, and T is the incubation temperature. Afterwards, a polynomial regression was fitted and the fitted coefficients a, b, c, d, e were obtained, where a is the intercept of the equation and e is the effect of bedrock and management. When $qGrowth$ is zero, we can calculate microbial maintenance respiration ($qCO_{2\ maintenance}$) for a specific temperature as:

$$qCO_{2\ maintenance} = a + c * T + d * T^2 + e$$

Here in this study, we calculated $qCO_{2\ maintenance}$ at 5, 15 and 25 °C.

Error propagation was performed throughout all models by using online tools (http://julianibus.de/physik/propagation-of-uncertainty), to estimate the variance around the mean of $qCO_{2\ maintenance}$ for each soil type at the three set temperatures.

All statistical analyses were performed in R software version 3.4.2 (R Core Team, 2017). The data were transformed when necessary to improve normality and homogeneity. Two-way ANOVA and Tukey-HSD tests were applied to test for the effects of bedrock and land management on soil physicochemical and biological properties as well as on the microbial C processes, temperature sensitivities of processes and potential enzyme activities. Student's t-tests were applied to compare the temperature sensitivity of microbial growth and of microbial respiration. Regression analysis between soil physicochemical and biological parameters, process rates and potential enzyme activities as well as their temperature sensitivities were applied and expressed as Pearson coefficient (R). Three-way ANOVA and Tukey-HSD tests were applied to test for effects of temperature treatment, land use and bedrock in the temperature incubation experiment, and for effects of soil moisture/O_2 treatment, land use and bedrock in the soil moisture incubation experiment. Variance partitioning was performed based on three-way ANOVA results and was calculated as the fraction of the total sum of squares (SS) explained by each specific factor and factor combination. $P < 0.05$ was set as the threshold value for significance.

3 Results

3.1 The effects of soil physicochemical and biological properties on microbial C metabolism

Soils pre-incubated at 15 °C were measured at 60% WHC. Silicate soils exhibited lower pH values compared to calcareous soils, and arable soils showed the highest pH (Table 1). Silicate soils showed relatively lower SOC but land use showed no effect on SOC. Higher DOC contents were found in silicate soils and in forest soils. MBC was also lower in silicate soils compared to calcareous soils, and highest MBC was found in pasture soils. The potential activities of β-glucosidase and phenoloxidase normalized to MBC (qBG and qPOX) were higher in silicate soils. Microbial CUE ranged between 0.26 and 0.66, and the highest CUE was found in silicate and in pasture soils. Microbial CUE decreased with increasing soil pH, base saturation, CEC_{eff}, and silt content, and was positively affected by sand, DOC content, C_{growth} and qGrowth (Table S3). In contrast, microbial CUE was not correlated with respiration rates, PLFA-based microbial community metrics, MBC, extractable nutrient contents and soil C: N: P stoichiometry. qCO_2 declined with MBC and Gram-positive bacteria, and was lowest in pasture soils. Microbial growth normalized to MBC (qGrowth) increased with SOC, soil C: P, DOC, and fungal: bacterial PLFA ratios, and decreased with soil pH, base saturation, and was significantly affected by bedrock and its interaction with management. Microbial biomass turnover times were lower in silicate soils, decreased with sand and DOC content and increased with soil pH, base saturation, CEC_{eff}, silt and nitrate content. However, microbial communities with low turnover times i.e. fast turnover and qGrowth had higher CUE, pointing to fast-growing and active microbial communities having higher CUE than slow-growing communities. Detailed information and

statistical analyses of soil physicochemical and biological properties can be found in Tables S1–S3.

3.2 Short-term temperature effects on microbial C metabolism

For the temperature incubation experiment, the metabolic quotients (qCO_2) increased exponentially with increasing temperature in all soils and differed significantly between soils at different temperatures (Fig. 1A). The temperature sensitivity (Q_{10R}) of qCO_2 varied from 1.41 to 2.74 and was significantly different between land uses (Table 2), with arable soils exhibiting the lowest temperature sensitivities (Q_{10R}) followed by pasture and forest soils (Table S2). Q_{10R} decreased with soil pH, base saturation, silt and TOP content (Table S3). Microbial growth rates per unit MBC (qGrowth) also increased with temperature across all tested soils (Fig. 1B). The temperature sensitivity of qGrowth (Q_{10G}) varied from 2.05 to 3.15 (Table 2), and was overall significantly higher than Q_{10R} ($p < 0.01$, Student's t-test) but was not affected by bedrock and management and not related to soil physicochemistry and microbial community composition. Temperature had a significant positive effect on microbial CUE, but the response of CUE to temperature changes was heterogeneous depending on land use (Fig. 1C). Arable soils consistently showed an increase in CUE with temperature ($Q_{10\ CUE}$ = 1.69–1.83), pasture soils behaved neutral ($Q_{10\ CUE}$ = 1.09–1.24) while forest soils showed divergent trends ($Q_{10\ CUE}$ of 0.85 and 1.19). The temperature sensitivity of CUE ($Q_{10\ CUE}$) was strongly negatively related to Q_{10R} (Table S3) but not related to soil physicochemical and microbial community parameters. qC_{uptake} showed a strong increase with temperature (Fig. 1D), with $Q_{10\ qCuptake}$ values ranging between 1.70 and 2.83, with lowest values in arable soils (Table S2). We also found a strong positive correlation between CUE and qGrowth (Fig. 2A), but found no correlation between CUE and qCO_2 (Fig. 2B). To test for the effect of autocorrelation underlying the above relationship we generated 100 random numbers for qGrowth (range from 0.2 to 4) and qCO_2 (range from 0.2 to 4), and calculated CUE as qGrowth/(qGrowth + qCO_2). In this case, CUE was negatively correlated to qCO_2 and positively correlated to qGrowth. Here, in our study, CUE was not correlated to qCO_2 but positively correlated to qGrowth, highlighting that the relationship was not caused by autocorrelation. Based on three-way ANOVA analysis, approximately 54% of the variation in qCO_2, 57% of the variation in qGrowth and 63% of the variation in qC_{uptake}, but only 8% of the variation in CUE could be directly accounted for by temperature (Fig. 3A). Additionally, microbial biomass turnover time decreased from an average of 325 days to 41 days when temperature increased from 5 to 25 °C (Fig. S1A). The β-glucosidase activities normalized to MBC (qBG) increased from 5 to 25 °C (Fig. S1B) with average $Q_{10\ qBG}$ values of 1.81–2.16 (Table 2). Phenoloxidase activities normalized to MBC (qPOX) did not respond to temperature, with $Q_{10\ qPOX}$ values ranging between 0.96 and 1.15 (0.34 in arable soils on limestone). Moreover, $Q_{10\ qPOX}$ was strongly influenced by land use and bedrock. The modeled maintenance respiration rates ($qCO_{2\ maintenance}$) were found to be significantly higher at 25 °C compared to 5 °C, and were significantly higher in arable soils compared to forest and pasture soils (Fig. S2).

3.3 Short-term moisture/O_2 effects on microbial C metabolism

In terms of the soil moisture/O_2 incubation experiment, approximately 52% of the variation in qGrowth and in microbial biomass turnover time, 39% of the variation in CUE and 35%

variation in qC_{uptake} could be directly explained by soil moisture/O_2 (Fig. 3B) based on a three-way ANOVA analysis. The metabolic quotient (qCO_2) varied between soils and soil moisture treatments, but was not directly affected by treatment at 30, 60 and 90% WHC (Fig. 4A). Microbial growth rates per unit MBC (qGrowth) did not change between soils pre-incubated at low and intermediate moisture level (30 and 60% WHC), but strongly decreased at high soil moisture level (90% WHC) under suboxic (1% O_2) conditions compared to ambient O_2 conditions (21% O_2) (Fig. 4B). Microbial CUE and qC_{uptake} followed the pattern of qGrowth in that a significant decrease was observed at suboxic conditions at high soil moisture level (Fig. 4C and D). Microbial biomass turnover times across all tested soils increased from an average of 15 days at low and intermediate moisture level to 66 days at high moisture level (Fig. S4A).

4 Discussion

Our study revealed that the response of microbial CUE to short-term environmental changes more strongly depended on changes in microbial growth than on changes in microbial respiration.

4.1 Effects of short-term temperature changes on soil microbial C metabolism

Here by comparing microbial CUE of soils from three land uses and two bedrocks, we found a general increase, though a heterogeneous pattern, in microbial CUE in response to elevated temperature (Fig. 1C), which was in contrast to our expectation that soil microbial CUE would decrease with increasing temperature. Temperature was reported to exert negative or negligible effects on microbial growth and CUE (Dijkstra et al., 2011; Tucker et al., 2013; Hagerty et al., 2014), but these studies did not directly measure actual microbial growth, and it was already previously shown that methods based on ^{13}C-substrate addition can only estimate microbial substrate use efficiency (SUE) rather than microbial CUE (Sinsabaugh et al., 2013), which might be one of the reasons that we obtained contrasting results compared to these previous studies. ^{13}C-glucose taken up by microbes is not necessarily allocated to growth or respiration, but can be intermittently stored without being metabolized (Hill et al., 2008). We also found that microbial CUE increased in both arable soils, while pasture soils behaved neutral and microbial CUE behaved divergent in forest soils when temperature increased. Therefore, the response of microbial CUE to temperature ($Q_{10\ CUE}$) varied depending on land management, but was not affected by bedrock and its interaction with land use (Table 2). The overall effect of temperature on microbial CUE both via direct and indirect pathways therefore was relatively weak.

After 1 day pre-incubation, we found that the metabolic quotient (qCO_2) of all tested soils both increased exponentially with temperature, in accordance with another study showing the same trend in qCO_2 in the temperature range from 5 up to 35 °C (Xu et al., 2006). Increased qCO_2 at higher temperatures could partly be attributed to the increased maintenance respiration rate per biomass, since elevated temperatures could induce higher maintenance energy costs in microbes (Alvarez et al., 1995), as shown for the first time in soils in this study (Fig. S2) using our polynomial multiple regression model. Previous studies have evaluated microbial maintenance energy by adding specific substrates (e.g.

glucose) and quantifying the amount of C needed to prevent microbial C loss during incubation (Anderson and Domsch, 1985a, 1985b). By using the substrate addition approach, the maintenance energy demand of dormant soil microbial communities ranged between 0.03 and 0.3 mg glucose C (g MBC)$^{-1}$ h^{-1} (Anderson and Domsch, 1985b; Cheng, 2009), but between 2.8 and 14.4 mg glucose C (g MBC)$^{-1}$ h^{-1} in pure cultures (Tännler et al., 2008; Vos et al., 2016). Here by using the ^{18}O method, without adding organic substrates to soils, the mean maintenance qCO_2 was 1.2 mg C (g MBC)$^{-1}$ h^{-1}, which is higher than found for dormant soil microbial communities but much lower than for the pure microbial cultures that were assessed by the substrate addition approach. The metabolic quotient (qCO_2) is considered as a proxy of soil microbial community CUE in response to short-term environmental change (Anderson and Domsch, 1993). Specifically, high qCO_2 values have been used as a proxy for low microbial CUE, since more energy is demanded for microbial biomass maintenance (Cheng et al., 2013), which would result in less substrate C available for growth at a higher qCO_2 and result in a trade-off between microbial growth and CUE (Lipson, 2015; Lipson et al., 2009; Pfeiffer et al., 2001). However, the obvious problem with this approach is that it can only be applied, if constant microbial C uptake is assumed, which is unlikely at higher temperatures. We here clearly show that microbial CUE was more strongly positively related to qGrowth (Fig. 2A) across all temperatures, which means that we did not find a trade-off i.e. a negative relationship between growth and yield (CUE) in this study. This shows that slow-growing microbial communities had low CUE due to high maintenance energy demand while fast-growing communities had conversely high CUE (Lipson, 2015). Energy spilling or overflow metabolism is typically high when organic C is in excess or nutrients are strongly limiting (Russell and Cook, 1995), which did not happen here, but – if present - might cause a trade-off between microbial growth, respiration and CUE (Lipson, 2015). Therefore, here anabolism and catabolism were not decoupled, and microbial growth increased while contributions of maintenance respiration to C uptake decreased, causing increases in microbial CUE. We also showed that microbial CUE was not related to qCO_2 (Fig. 2B), therefore qCO_2 does not represent a valid surrogate of the partitioning between microbial anabolic and catabolic processes and for microbial CUE.

Microbial growth rates were reported to increase exponentially or following a quadratic term with temperature in the studies available (Díaz-Raviña et al., 1994; Pietikäinen et al., 2005; Sinsabaugh et al., 2016). We also found that microbial growth rates per unit biomass (qGrowth) increased with incubation temperature within the range from 5 to 25 °C (Fig. 1B), which agrees with increasing soil fungal and/or bacterial growth rates from 0 to around 25 °C (Díaz-Raviña et al., 1994; Pietikäinen et al., 2005; Bárcenas-Moreno et al., 2009; Rinnan et al., 2009). Elevated temperature could influence microbial growth by affecting substrate availability (Shiah et al., 2000). For example, labile substrates were shown to deplete under long-term warming conditions, whereas it did not happen in our short-term incubation experiment, and we did not find DOC concentrations to decrease during the pre-incubation period (Fig. S5). As qGrowth increased with temperature, the average turnover time of microbial biomass (which is inversely proportional to qGrowth) across all tested soils decreased from a mean of 325 days to 41 days (Fig. S1A), and turnover times were in a similar range as reported in pasture (197–322 days) and in forest soils (33–140 days) measured at 15 °C (Spohn et al., 2016). These declines in microbial biomass turnover time

with temperature were due to faster microbial growth rates under steady-state conditions where microbial biomass did not change.

To what extent microbial respiration and growth influenced microbial CUE in the short-term depended on their respective responses to elevated temperature, as expressed by their Q_{10} values. The temperature sensitivity of soil microbial respiration (Q_{10R}) is regarded as an important biogeochemical model parameter that reflects the feedback between soil C cycling and global warming (Luo, 2007). The average values of Q_{10R} for the temperature range of 5–25 °C measured here ranged from 1.41 to 2.74 (Table 2), which were in a similar range as reported for global patterns of Q_{10R} between 1.3 and 3.3 (Raich and Schlesinger, 1992). In this study, we observed significantly lower Q_{10R} in arable soils than in pasture and forest soils (Table S2). This might be attributed to lower substrate availabilities that are typically found in arable soils, which can depress the temperature sensitivity of microbial respiration due to lower Q_{10} of diffusion-limited than of enzyme-limited processes (Blagodatskaya et al., 2016; Steinweg et al., 2012). In contrast, here we found higher average Q_{10} values of potential β-glucosidase activities normalized to MBC ($Q_{10\ qBG}$) than of Q_{10R} in both arable soils (Table 2). The data therefore indicate that respiration (and growth) of soil microbial communities was rather substrate- or diffusion-limited than enzyme-limited. The temperature sensitivity of microbial growth or growth per unit biomass (Q_{10G}) has rarely been tested in soils, the studies typically showing greater Q_{10G} values at lower temperatures (0–10, 5–15 °C) than at higher temperatures (10–20, 15–25 °C) (Rinnan et al., 2009; Rousk and Bååth, 2011). Here, the average values of Q_{10G} ranged between 2.05 and 3.15 (Table 2), which agrees with published Q_{10G} values obtained at similar temperatures in previous studies, ranging between 1.9 and 2.6 (Rinnan et al., 2009), 2.2–3.0 (Díaz-Raviña et al., 1994) and 2.1–2.3 (Birgander et al., 2013). More importantly, we found that Q_{10G} values were generally higher than Q_{10R} values across all tested soils ($p < 0.01$), except for the limestone forest soil that exhibiting similar Q_{10G} and Q_{10R} values (Table 2). Therefore, the pattern of $Q_{10G} > Q_{10R}$ in most soils explains the widespread increase in microbial CUE with increasing temperature in the soils studied here. Therefore, in this study, microbial growth was more sensitive to short-term temperature changes than respiration and thus growth played a more important role in determining microbial CUE responses to short-term temperature changes.

4.2 Effects of short-term soil moisture/O_2 changes on soil microbial C metabolism

In contrast to the less than 1-day pre-incubation period in the warming experiment, in the moisture/O_2 experiment soils were pre-conditioned for seven days, which might involve changes in microbial community structure accompanying changes in their physiology. However, we here were only interested in the effects such changes have on microbial community metabolism, independent of changes in their structure and composition. Soil moisture and O_2 are supposed to affect microbial CUE as the change in water and O_2 availability may alter the balance between microbial growth and maintenance (Manzoni et al., 2012). However, the impact of soil moisture/O_2 on microbial CUE has never been empirically tested in soils, and thus we have a limited understanding of how short-term changes in soil moisture/O_2 affect microbial physiology. Here we found a decrease in microbial CUE at high moisture level (90% WHC)/suboxic conditions that was accompanied

by almost constant qCO_2 but strong declines in qGrowth (Fig. 4A and B & C), which contradicted our expectation. The observed significant effects on microbial CUE across all soil types indicate that short-term increases in soil moisture combined with decreases in O_2 availability have a profound impact on microbial CUE, especially when soil water contents were close to saturation. Given that we did not find a consistent positive effect of increasing soil moisture from low to intermediate soil moisture level at 21% O_2 but a dramatic decline in microbial CUE towards high moisture level under suboxic conditions, this strongly points towards declining soil O_2 having the greater effect than increasing soil moisture in this experiment. Only in lake sediments decreased microbial growth efficiency (= microbial CUE) was found under anoxic compared to oxic conditions, i.e. 0.19 and 0.48 respectively, triggered by stronger declines in bacterial growth (5.0-fold) than in bacterial respiration (1.3-fold; Bastviken et al., 2003).

Soil water availability is suggested to influence microbial respiration by limiting substrate diffusivity at low moisture content and the diffusion of O_2 at high moisture content (Davidson et al., 2006; Rubol et al., 2013). However, in this study, we observed no significant influence of soil moisture on microbial respiration, even under suboxic conditions (Fig. 4A). We expected microbial respiration under water-saturated/suboxic conditions to be limited mainly by low O_2 availability, as we expected that high water content would not limit substrate diffusivity but rather enhance it. At low O_2, however, we found that qCO_2 of soils from all sites did not exhibit significant differences relative to the results obtained at 21% O_2 concentration at low and intermediate moisture level. Under O_2 limitation soil microbes shift from aerobic respiration (O_2 as the terminal electron acceptor) to anaerobic respiration (alternative electron acceptors, such as nitrate, Mn^{4+}, Fe^{3+}, SO_4^{2-} or CO_2) and finally to fermentation, with strong declines in the energy yield (32 mol to about 2 mol of ATP per mole of glucose) of microbial catabolism (Boyd, 1995; Lipson et al., 2009; Pfeiffer et al., 2001). Less energy gain at the same catabolic rate for anaerobic respiration might cause an adjustment (upregulation) in biochemical rates, keeping CO_2 production constant, but at the expense of microbial growth and eventually CUE, which both declined here within the 7-day pre-incubation period. The shift from aerobic to anaerobic respiration was further accompanied by the disappearance of nitrate due to dissimilatory nitrate reduction processes coupled to SOC decomposition (Fig. S6C) while methanogenic decomposition pathways did not substantially contribute (data not shown).

The strong decrease in qGrowth under suboxic conditions relative to the oxic treatments (Fig. 4B) was not due to a decrease in substrate availability, since the amount of available DOC did not decline during the 7-day pre-incubation period (Fig. S6A) and high water content should also lead to increased substrate diffusivity and access. The data therefore highlight that C mineralization is uncoupled from energy yield while declines in free energy yield in anaerobic metabolism affects energy (and C) allocation in microbial metabolism, causing the strong declines in microbial growth as found here as well as in lake sediments (Bastviken et al., 2003). With the strong decrease in qGrowth, we also found that microbial biomass turnover times increased from an average value of 15 days at 21% O_2 to 66 days at 1% O_2 (Fig. S4A), implying a general slow-down of microbial C metabolism and of SOC turnover. Given the negligible moisture/O_2 effect on microbial respiration but the significant negative impact on microbial growth, this translated to marked declines in microbial C

uptake and microbial CUE under suboxic conditions. Under anoxia exudation of fermentation end products such as short chain fatty acids would need to be accounted for in the calculation of C uptake (growth, respiration plus exudation) (Manzoni et al., 2012) alongside decreases in energy yield of anoxic metabolism (Lipson et al., 2009; Pfeiffer et al., 2001). This would further decrease CUE relative to the current estimates; however, we did not find evidence for high rates of fermentation in the suboxic treatments (low CH_4 and organic acid release, data not shown). Therefore, similar to the short-term temperature effects on microbial CUE, the influence of short-term soil moisture/O_2 fluctuations on microbial CUE mainly depended on the responses of microbial growth rather than of microbial respiration. However, we need to highlight that in this experiment we could not separate moisture and oxygen effects but studied their interactive effect on microbial physiology.

4.3 Other controls on microbial C metabolism

While there is a dearth of information on controls of soil heterotrophic respiration and qCO_2, including temperature, moisture/O_2 effects, microbial community structure and soil physicochemistry (Graham et al., 2016; Xu et al., 2017; Zhou et al., 2016), much less is known on controls of microbial growth (Rousk and Bååth, 2011) and microbial CUE in soils (Manzoni et al., 2012). Beyond soil temperature and moisture/O_2 controls on soil microbial metabolism as demonstrated in this study, microbial growth and microbial CUE are also predicted to be influenced by factors such as substrate concentration and quality, soil pH, nutrient limitation, or microbial community composition (Manzoni et al., 2012). Bedrock explained 33% of the variation in microbial CUE (Table S2), and calcareous soils exhibited lower microbial CUE than silicate soils (Fig. 1C), causing a negative relationship between soil pH and microbial CUE. Soil pH was also a major regulator of bacterial and fungal growth in soils, where fungal growth decreased and bacterial growth strongly increased above pH 5 (Rousk et al., 2011). This might be due to pH effects on substrate and nutrient availability (Aciego Pietri and Brookes, 2008; Filep et al., 2003) or on microbial community composition (Lauber et al., 2009). Here, we observed a strong positive correlation between DOC and CUE (Table S3), caused by strong increases in qGrowth when DOC increased while respiration was non-responsive. Land management explained another 18% of the variation in CUE, likely caused by enhanced nutrient inputs in the intensively managed land. However, extractable as well as total nutrient contents did not explain a significant fraction of the variability in microbial growth and CUE. Substrate availability and elemental stoichiometry may affect microbial CUE by impacting microbial growth and respiration, as soil microbial biomass stoichiometry may force microbial communities to adapt their foraging strategies to accessible substrates (Sinsabaugh et al., 2013). Again we did not find strong controls of soil C: N: P stoichiometry on microbial CUE and only C: P was positively related to microbial growth. Additionally, it has been suggested that fungi have a higher CUE than bacteria (Keiblinger et al., 2010), which suggests soil microbial community composition may impact microbial community CUE. Neither fungal or bacterial PLFA biomarkers, nor microbial biomass were related to microbial CUE but the fungal: bacterial PLFA ratio was positively related to microbial growth, partially corroborating the findings cited above. Finally, soil texture influenced microbial CUE but not growth and respiration. For example, silt content was strongly negatively and sand strongly positively correlated to

microbial CUE (Table S3), which might be due to stronger sorption of organic matter on finer soil particle size fractions, further enhanced by high base saturation in calcareous soils (Rowley et al., 2018).

5 Conclusions

We here, for the first time, show that the response of microbial CUE (and microbial biomass turnover time) to short-term environmental changes and to soil physicochemical properties, are mainly determined by microbial growth, and less by respiration. Therefore, our data indicate that anabolic processes (microbial growth) play a more important role than catabolic processes (respiration) in promoting soil C storage, as recently suggested (Liang et al., 2017). Promoting soil C sequestration depends on an increase in microbial CUE (Sinsabaugh et al., 2017) and/or a decrease in microbial biomass turnover time (Hagerty et al., 2014). Here we found a strong positive relationship between microbial growth and microbial CUE and a negative relation to microbial turnover time. Therefore, factors enhancing microbial growth are likely to decrease microbial turnover time, fostering microbial necromass production and soil C sequestration, and at the same time come along with increases in microbial CUE which may also stimulate soil C sequestration. However, such microbial physiological changes in the short-term can be modified in the long-term and at the ecosystem level, due to changes in labile plant C inputs relative to decomposition, due to changes in labile C availability or due to adjustment in microbial community structure and physiology to the external driver.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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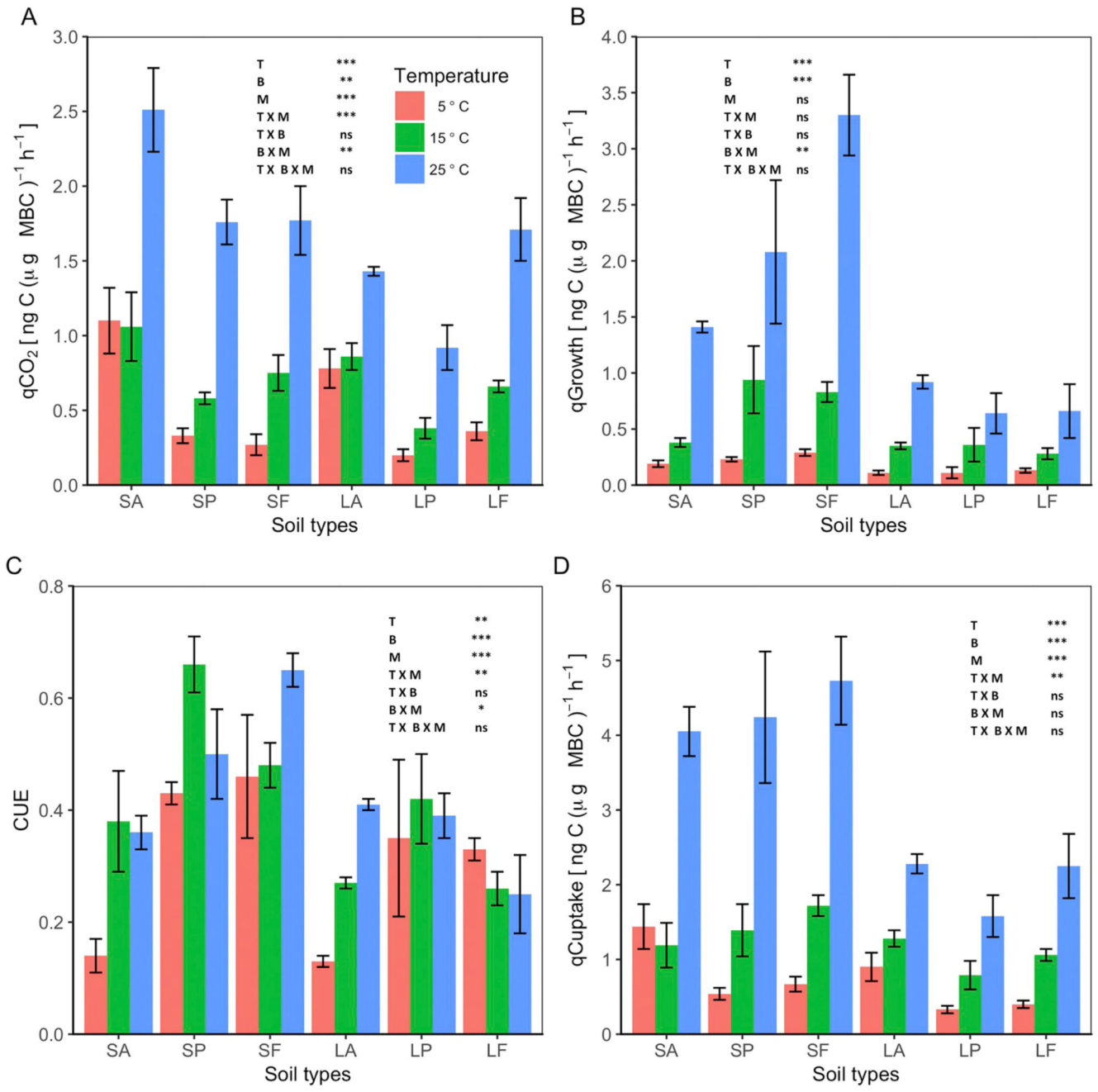

Fig. 1.

Temperature effects on microbial respiration per unit MBC (metabolic quotient, qCO_2; A), microbial growth rate per unit MBC (qGrowth; B), microbial CUE (C) and microbial organic C uptake per unit MBC (qC_{uptake}; D) in six soils from three land uses (A: arable; P: pasture and F: forest) on two bedrocks (S: silicate; L: limestone) measured in the temperature incubation experiment. Error bars represent standard errors. The three different colors represent process rates obtained at 60% WHC at three temperatures: red, 5 °C; green, 15 °C; and blue, 25 °C. Main effects of single factors and their interactive effects are

displayed for T: temperature; B: bedrock; M: management. Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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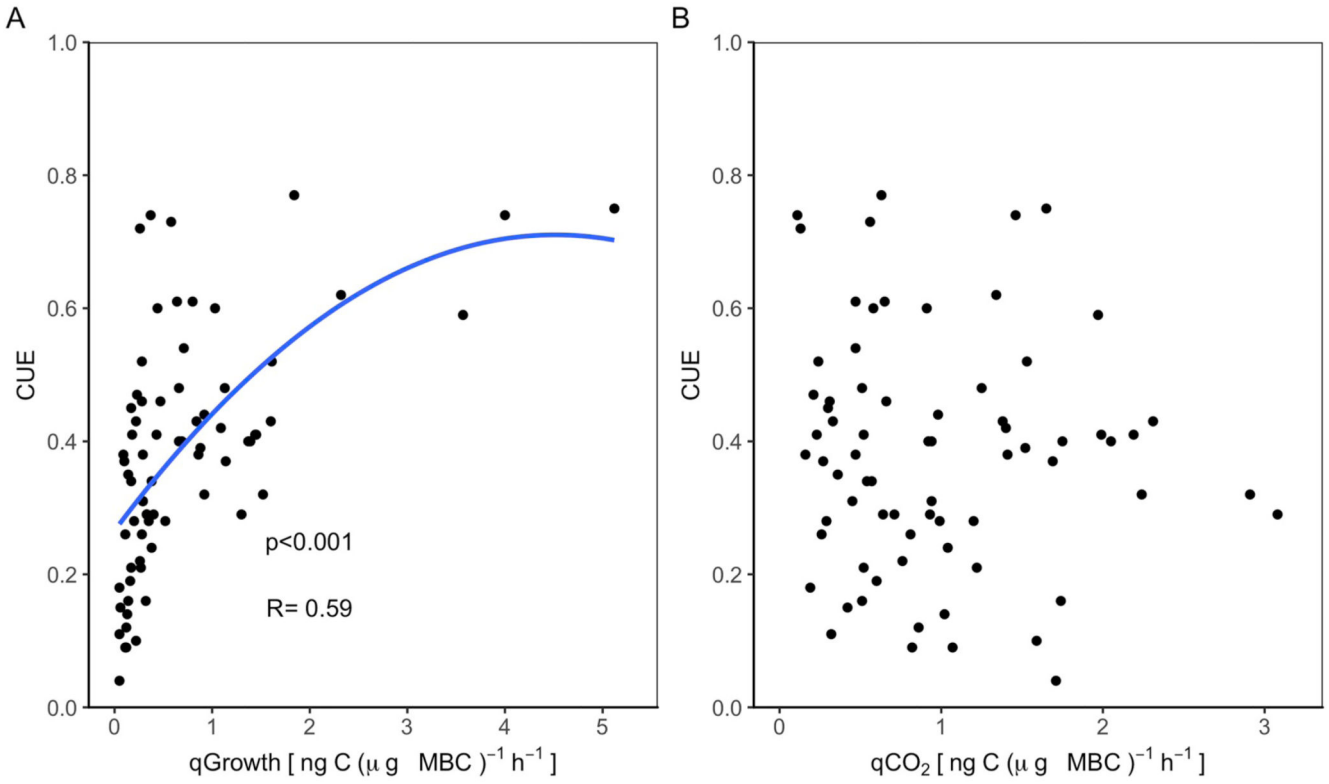

Fig. 2.

Correlation between microbial CUE and microbial growth rate per unit MBC (qGrowth, A), and between CUE and microbial respiration per unit MBC (metabolic quotient, qCO_2, B) in the temperature experiment.

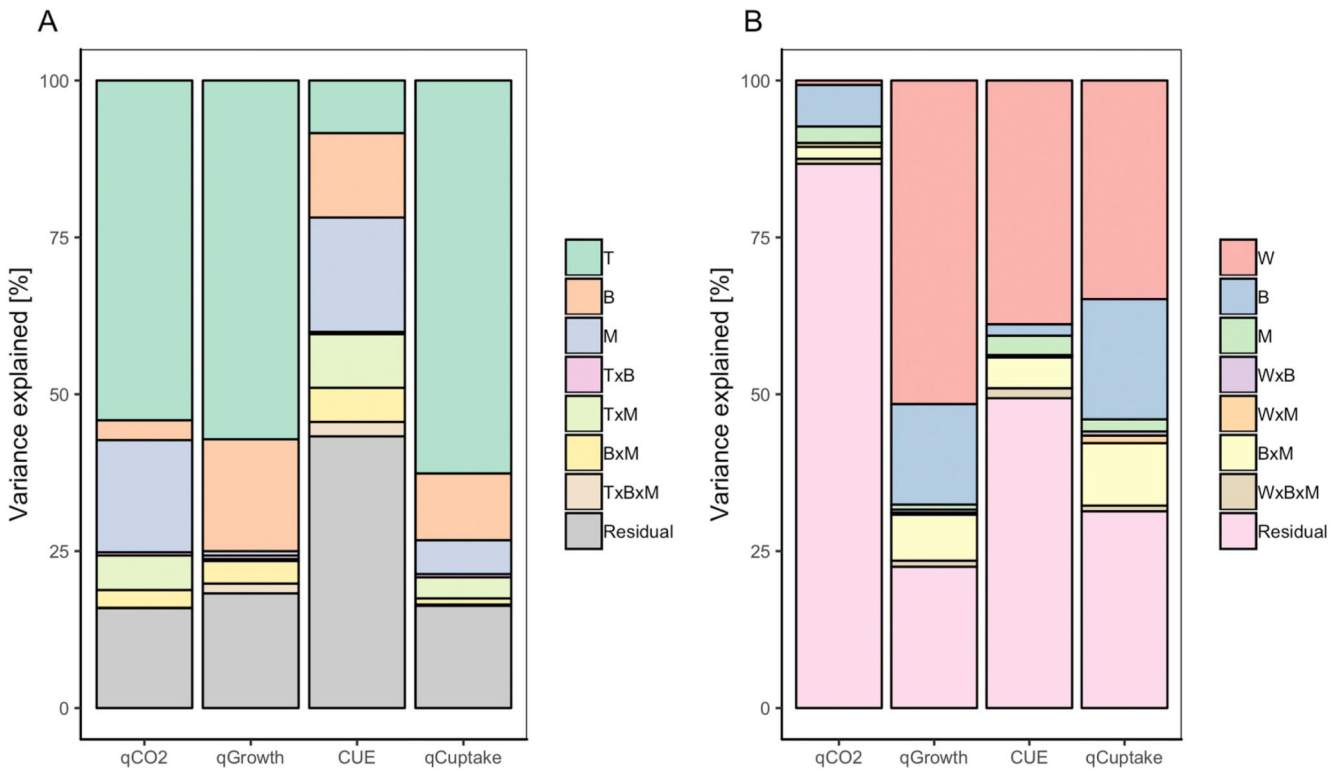

Fig. 3.

Total variation of microbial C metabolism i.e. microbial respiration per unit MBC (metabolic quotient, qCO_2), microbial growth rate per unit MBC (qGrowth), microbial CUE and microbial organic C uptake per unit MBC (qC_{uptake}) explained in three-way ANOVAs by different environmental factors and their interactive effects in the temperature incubation experiment (A) and in the moisture/O_2 incubation experiment (B). T: temperature; B: bedrock; M: management; W: moisture. Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

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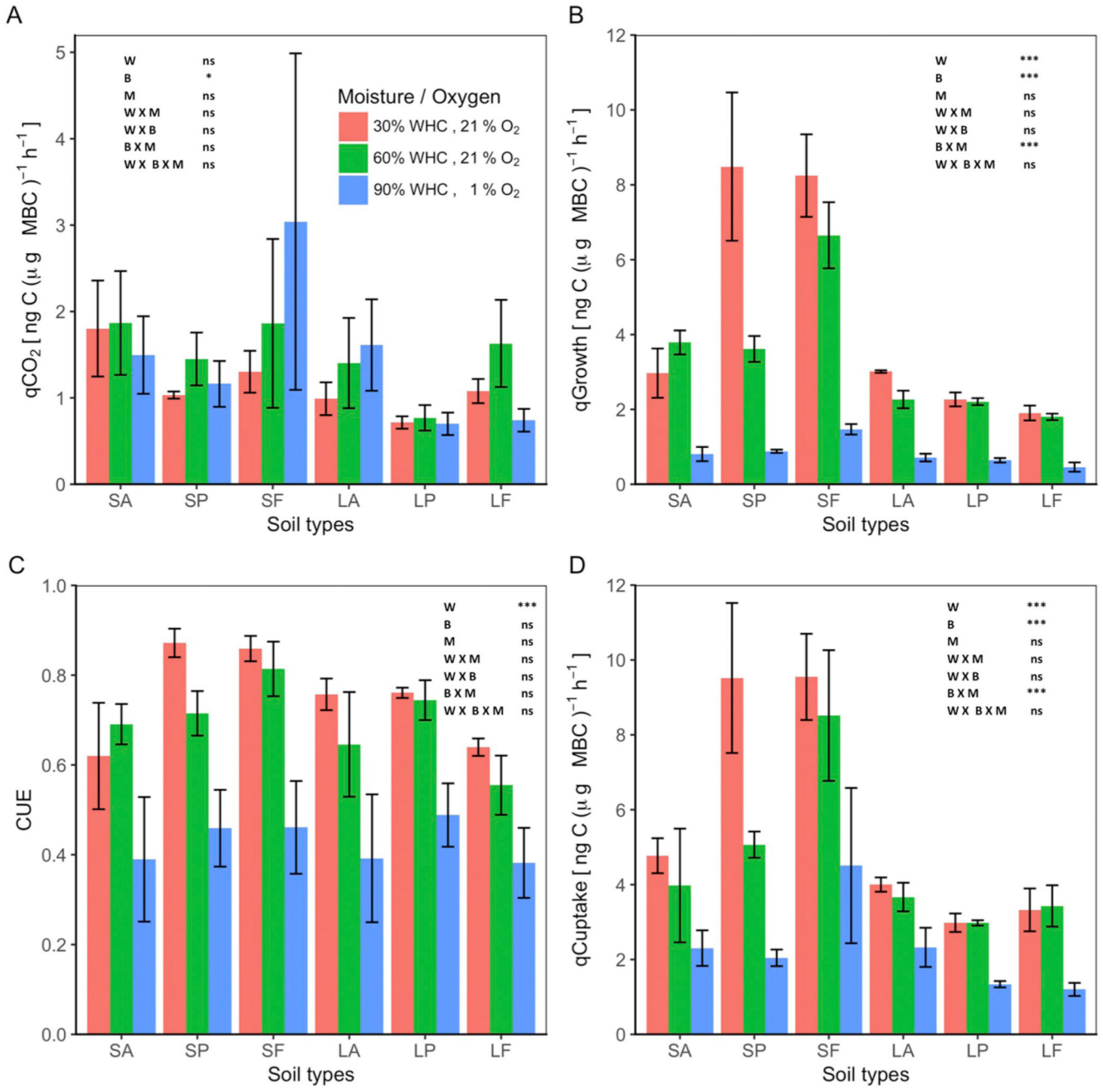

Fig. 4.

Soil moisture/O_2 effects on microbial respiration per unit MBC (metabolic quotient, qCO_2; A), microbial growth rate per unit MBC (qGrowth; B), microbial CUE (C) and microbial C uptake per unit MBC (qC_{uptake}; D) in six soils from three land uses (A: arable; P: pasture and F: forest) on two bedrocks (S: silicate; L: limestone) measured in the moisture/O_2 incubation experiment. Error bars represent standard errors. The three different colors represent process rates obtained at 20 °C, at three pre-incubation moisture levels: red, low moisture level (30% WHC, 21% O_2); green, intermediate moisture level (60% WHC, 21% O_2); and blue, high moisture level (90% WHC, 1% O_2). Main effects of single factors and

their interactive effects are displayed for W: moisture; B: bedrock; M: management. Significance levels: ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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Table 1

Selected initial soil physicochemical and biological properties (0–15 cm soil depth, means ± 1SE, n = 4) measured at 15 °C, 60% WHC.

Soil	SA	SP	SF	LA	LP	LF	P value		
Bedrock	**Silicate**	**Silicate**	**Silicate**	**Limestone**	**Limestone**	**Limestone**	**M**	**B**	**M x B**
Management	**Arable**	**Pasture**	**Forest**	**Arable**	**Pasture**	**Forest**			
pH (water)	5.90 ± 0.37	5.38 ± 0.18	4.05 ± 0.10	8.15 ± 0.22	6.43 ± 0.19	6.13 ± 0.08	***	***	*
SOC (mg g^{-1})	21.8 ± 1.1	26.7 ± 0.9	49.9 ± 7.6	47.0 ± 0.9	47.9 ± 7.6	36.8 ± 2.4	ns	**	***
DOC (μg g^{-1})	64.6 ± 3.6	85.6 ± 4.3	160.9 ± 13.1	52.5 ± 1.8	53.8 ± 12.6	53.4 ± 6.9	***	***	***
MBC (μg g^{-1})	292 ± 19	647 ± 42	581 ± 80	1441 ± 54	1809 ± 336	890 ± 16	**	***	*
DNA (μg g^{-1})	17.2 ± 1.8	14.9 ± 3.4	22.8 ± 2.1	40.4 ± 2.0	51.8 ± 8.4	40.3 ± 2.8	ns	***	ns
f_{DNA}	17.3 ± 1.3	60.2 ± 25.1	26.4 ± 4.8	35.7 ± 0.6	39.4 ± 12.5	22.4 ± 1.3	ns	***	*
qβ-glucosidase (nmol (μg MBC)$^{-1}h^{-1}$)	0.94 ± 0.06	0.62 ± 0.04	0.33 ± 0.03	0.29 ± 0.02	0.34 ± 0.07	0.38 ± 0.05	**	***	***
qPhenoloxidase (nmol (μg MBC)$^{-1}h^{-1}$)	8.37 ± 1.28	7.12 ± 0.39	16.72 ± 4.21	4.25 ± 0.47	6.42 ± 1.33	9.92 ± 0.59	**	*	ns

M: management; B: bedrock; M x B: interaction of management and bedrock. Significance levels: ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant.

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Table 2

Temperature sensitivities (Q_{10} values, 5–25 °C) of microbial C processes and extracellular enzyme activities (means ± 1SE, n = 4).

Soil	SA	SP	SF	LA	LP	LF	P value		
Bedrock	Silicate	Silicate	Silicate	Limestone	Limestone	Limestone	M	B	M x B
Management	Arable	Pasture	Forest	Arable	Pasture	Forest			
Q_{10R}	1.57 ± 0.14	2.39 ± 0.17	2.74 ± 0.35	1.41 ± 0.15	2.23 ± 0.33	2.27 ± 0.30	**	ns	ns
Q_{10G}	2.88 ± 0.28	3.14 ± 0.57	3.15 ± 0.29	3.05 ± 0.27	2.62 ± 0.19	2.05 ± 0.51	ns	ns	ns
$Q_{10\ CUE}$	1.69 ± 0.20	1.09 ± 0.08	1.19 ± 0.14	1.83 ± 0.11	1.24 ± 0.19	0.85 ± 0.18	***	ns	ns
$Q_{10\ qCuptake}$	1.73 ± 0.13	2.83 ± 0.30	2.69 ± 0.25	1.70 ± 0.24	2.21 ± 0.23	2.34 ± 0.19	**	ns	ns
$Q_{10\ qBG}$	1.81 ± 0.16	1.90 ± 0.04	2.13 ± 0.12	2.04 ± 0.02	2.10 ± 0.08	2.16 ± 0.21	ns	ns	ns
$Q_{10\ qPOX}$	1.10 ± 0.13	1.04 ± 0.04	1.15 ± 0.21	0.34 ± 0.08	0.97 ± 0.04	0.96 ± 0.08	*	**	*

M: management; B: bedrock; M x B: interaction of management and bedrock. Significance levels: ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, not significant.