

Supplementary Text

Soil inoculation

Each soil microcosm contained 15g (dry mass) of artificial soil, inoculated with a 3ml microbe-media blend to reach a water holding capacity (WHC) of 70%. The blend, containing microbes (D0-D3), was mixed at a 1:1 ratio of media to inoculum to target ~ 1mg C⁻¹ g soil per week of incubation. Inoculation of the microcosms was conducted in a biosafety cabinet to avoid contamination, and the flask opening was covered with a parafilm layer immediately after inoculation. Thereafter, they were stored at three samples each in a jar in the incubator with the respective temperature (Figure 1). Furthermore, six non-inoculated controls, injected with 3ml TSB, were established to control for contamination. Respiration measurements were recorded over the 23 days incubation period.

In preliminary experiments, various media types were assessed, including the media recommended by the German Collection of Microorganisms and Cell Cultures (DSMZ), from which the bacterial strain was procured. For the experiment the tryptic soy broth (TSB) was chosen, due to its compatibility with *Streptomyces sp.* and the microbial communities extracted from the natural soil. For the liquid media 30g TSB was mixed with 1000 ml distilled water and autoclaved in a wet-cycle. This liquid media was used to grow *Streptomyces sp.* (DSM 687) to a previously determined optical density. For inoculation into the artificial soil a 2.5x more concentrated TSB media was used which was complemented with 100g glucose to reach a C content of 0.9mg per g of soil per week.

Strain Preparation

The *Streptomyces species* (DSM 687) ordered at DSMZ was inoculated as a single species and represents the least diverse treatment. It arrived freeze dried as a small pellet in a glass vial. The pellet was soaked with 500µl of the recommended Gym Streptomyces Media and from that stage plated with a loop on agar plates and suspended several times in 100ml of liquid media. Optical density (OD) was measured at 600nm over the period of 5 days in order to obtain a growth curve. Freezer stocks were created at OD 1.6 which lies within the exponential growth phase.

In preliminary experiments we evaluated that the *Streptomyces sp.* (DSM 687) grows well in the model soil under all the temperatures. Its preferred temperature in liquids lies at 28°C. Regarding dominant bacteria in natural soils, Delgado-Baquerizo *et al.* (2018) conducted a global analysis of bacterial communities in surface soils, where they identified the dominant bacterial phylotypes from 237 locations. They identified that the phyla Actinobacteria and Acidobacteria are one of the most abundant bacteria occurring in these soils. Furthermore, *Streptomyces sp.* which belong to the phylum Actinobacteria are important regarding carbon cycling (Barka *et al.*, 2016). Regarding the more diverse microcosms, the microbes were extracted from a natural agricultural soil, collected from the TwinWin experiment located at the Helsinki University, Finland (60°13'N/25°01'E). More detailed information regarding the soil properties can be found in the supplementary table 4.

Serial dilutions were made in order to create different diversity as previously (Diaz et al., 2003).

Respiration measurements

Soil respiration was used as an indicator for microbial activity and measured by gas chromatography (GC). For the respiration measurement the microcosms were placed in air-tight glass jars (volume = 900mL) with lids equipped with a valve allowing gas sampling with a gauge needle attached to a 60mL syringe. All jars were distributed randomly on a table and ventilated for several minutes in order for the CO₂ concentration to be the same in all jars. Two room controls were closed at the same time to determine the ambient air and subtract that initial value from all the measurements taken at that time. All jars were closed airtight within two minutes and put back to the respective incubator. One and two hours after closing the jars, air samples were extracted. For this purpose, the jars were brought back to room temperature for approximately 10 minutes. For each sample 25mL air was extracted from the headspace of the jar after flushing the syringe 2 times at 5mL to mix the air in the jar. The 25mL sample was then released into a 12mL vacuum-sealed glass vial. Finally, the jars were opened to prevent the air from becoming anoxic and returned to their respective incubator.

Following the sampling period, the glass vials were analyzed for their CO₂ concentration in ppm with a gas chromatograph (Agilent Technologies 7890A) fitted with an automatic sample handler (Gilson GX-271). The samples were calibrated against four CO₂ standards of 1050.1, 734.2, 408.4 and 18.2 ppm. For each measurement, a linear regression line was established, considering three points: t_0 = room sample (a control jar closed when other samples were sealed), t_1 = measurement after 1h, and t_2 = measurement after 2hrs. This approach aimed to identify and account for any variability arising from potential measurement errors in the gas chromatograph. All measurement points with an R² value exceeding 0.9 were retained which resulted in a removal of 16 measurement points. This threshold was selected because many points fell within the 0.9-1 R² value range. The values of the deleted points were notably lower than those 90% and would have confounded the data.

Addition of ¹⁸O-H₂O and stable isotope analysis for CUE measurements

This method used the incorporation of ¹⁸O-labeled water into DNA to measure gross microbial growth (Spohn 2016b). One day prior to ¹⁸O-H₂O addition, 0.6g of soil was collected from the 15g microcosm, weighed and put into microfuge tubes. Leaving the tubes open overnight in the biosafety cabinet allowed some water to evaporate. The weight was measured the following day, and ¹⁸O-H₂O diluted with unlabeled de-ionized water was added to reach 20 at% concentration of ¹⁸O-H₂O. Both microcosms (0.6g and 14.4g) were stored together in a single jar for respiration measurements. This decision was made because the 0.6g microcosm might have yielded CO₂ values relatively small to be accurately measured. The jars were closed air-tight immediately after water addition and put into the respective incubator.

After 48h of incubation, respiration measurements were taken as previously described. Immediately after air sampling, the soil samples were stored in a -80°C freezer until DNA extraction. DNA extracts were dried overnight in silver encapsulation tins at 60°C. Diluted salmon sperm DNA (5.000 ng) was spiked into samples to bring total oxygen mass within the detectable range. Prepared samples were sent to the stable isotope ecology lab in Basel (Switzerland), where $\delta^{18}\text{O}$ analyses were conducted using a Flash IRMS elemental analyzer operated in pyrolysis mode coupled to a Delta V Plus isotope ratio mass spectrometer (IRMS) (Thermo Fisher Scientific, Waltham, MA, USA). Measured values were normalized to Vienna Standard Mean Ocean Water (VSMOW) using calibrated in-house standards. Long term instrumental precision for the lab for non- ^{18}O -enriched analyses is 0.2 ‰. To characterize measurement precision for the current study using artificial ^{18}O -labeled materials, a quality control sample was prepared in a similar manner to the actual samples that were analyzed for this work, and aliquots of this were analyzed repeatedly throughout the course of the analyses. The analytical precision based on this assessment was 1.57 ‰ (n = 6).

Calculations of the CUE were based on Spohn *et al.* (2016a) and were previously recorded by Geyer *et al.* (2019). Measured $\delta^{18}\text{O}$ values were converted to atom %, and soil DNA oxygen ($\text{at}\% O_{\text{soil}}$) was found using the following calculation:

$$\text{at}\% O_{\text{soil}} = \frac{[(\text{at}\% O_{\text{soil}+ss} \times O_{\text{soil}+ss}) - (\text{at}\% O_{ss} \times O_{ss})]}{O_{\text{soil}}} \quad (1)$$

Where O_{soil} and O_{ss} represent the atom % and oxygen mass (μg) of extract soil DNA and salmon sperm DNA pools.

Atom % excess of soil DNA oxygen ($\text{APE } O_{\text{soil}}$) was calculated as the difference between atom % of oxygen in the presence of $^{18}\text{O}\text{-H}_2\text{O}$ ($\text{at}\% O_{\text{soil } t}$) and paired control samples in the presence of natural abundance water ($\text{at}\% O_{\text{soil } c}$):

$$\text{APE } O_{\text{soil}} = \text{at}\% O_{\text{soil } t} - \text{at}\% O_{\text{soil } c} \quad (2)$$

Total microbial growth during the incubation (^{18}O -increase; $\mu\text{g O}$) was estimated as the product of soil DNA oxygen content (O_{soil} ; $\mu\text{g O}$) and $\text{APE } O_{\text{soil}}$. At% Total, the final soil water enrichment after amendment, provides a correction for the diluting effect of native soil moisture:

$$^{18}\text{O}_{\text{increase}} = (O_{\text{soil}} \times \text{APE } O_{\text{soil}} \div 100) \times \frac{100}{\text{at}\% \text{ Total}} \quad (3)$$

Microbial growth was scaled to C (MBC; $\mu\text{g C g}^{-1}$ soil) by applying the conversion factors of 0.31 (oxygen:DNA mass ratio) and the average ratio of MBC:DNA measured by Spohn *et al.*, (2016a) in various samples of 11.9, assuming that only water-derived oxygen is used in biosynthesis.

$$^{18}MBC = ^{18}O \times \frac{1}{0.31} \times \frac{MBC_{soil}}{DNA_{soil}} \times \frac{1}{soil\ mass} \quad (4)$$

CUE was then calculated as follows:

$$CUE = \frac{^{18}MBC}{^{18}MBC + R} \quad (5)$$

Where R is the cumulative respiration ($\mu\text{g CO}_2\text{-C g}^{-1}$ soil) measured at the time of harvest.

DNA Extraction

Genomic DNA was extracted from 0.6g soil using the QIAGEN DNeasy PowerSoil HTP 96 Kit (384) following the protocol of the manufacturer in duplicate extractions. The resulting DNA concentration was quantified fluorometrically using ThermoFisher Scientific, Qubit 4 Fluorometer. The DNA samples were dried in a silver capsule with a 7.5ml salmon sperm DNA solution and sent to the stable isotope ecology lab in Basel (Switzerland) that analyzed the $\delta^{18}\text{O}$ values of the samples.

Respiration and CUE calculations

The respiration rate [ppm] was converted to $\text{CO}_2\text{-C}$ equivalent for each jar. From the measured CO_2 in the headspace, a room control was subtracted. For conversion from ppm to mL the amount of substance n was derived from the ideal gas law:

$$pV = nRT \quad (6)$$

Where p is the pressure [atm], V is the volume [mL] of the measurement taken from the headspace, n is the amount of substance in the gas, R is the ideal gas constant which equals 82.06 and T is the temperature [K].

In a next step the $\text{CO}_2\text{-C}_{\text{Equivalent}}$ [$\text{ml g}^{-1} \text{h}^{-1}$] was calculated by the following equation:

$$CO_2\text{-C}_{\text{Equivalent}} = \frac{CO_2\text{ Respiration} \times n \times M}{m \times t} \quad (7)$$

Where $CO_2_{Respiration}$ is the CO_2 respiration measurement [ppm], n is the amount of substance, M is the molecular weight of carbon which equals 12, m is the weight of total soil contributing to CO_2 respiration and t is the incubation time in the closed airtight jar.

In order to calculate the cumulative respiration of the 23 days incubation period, the following equation was used:

$$Cumulative_{Respiration} = \frac{\sum_{t_1}^{t_{10}} CO_2 - C_{Equivalent}}{10 \times t} \quad (8)$$

Where t_1 to t_{10} are the ten timepoints where respiration measurements were taken [$ml\ g^{-1}\ h^{-1}$], divided by 10 to get the average respiration rate per sample, times t which is the total incubation time [h]. In order to make the respiration between the fluctuating and the constant temperature treatment more comparable, a subset of three timepoints (t_2 , t_5 , t_8) were chosen where all incubation temperatures were at 23°C. The same calculation (4) was used, except that 10 was replaced with 3 because the number of timepoints changed.

The calculations of the microbial carbon use efficiency (CUE) were based on Spohn *et al.* (2016a) and Geyer *et al.* (2019). The amount of DNA produced during the 48hrs of incubation for each labeled sample is based on the abundance of ^{18}O . In a first step the microbial biomass carbon (MBC) was calculated:

$$MBC_{Soil} = DNA_{Soil} \times 11.9 \quad (9)$$

Where DNA_{Soil} is the measured DNA concentration [$ng\ \mu l^{-1}$] and 11.9 is the conversion factor provided by Spohn *et al.* (2016b).

In a second step MBC produced during the 48hrs of heavy-water inoculation was calculated:

$$C_{Growth} = {}^{18}MBC = {}^{18}O \times \frac{1}{0.31} \times \frac{MBC_{Soil}}{DNA_{Soil}} \times \frac{1}{m} \quad (10)$$

Where ${}^{18}O$ [μg] is the oxygen incorporated into microbial DNA during the incubation period and m is the mass of the soil [g]. C_{Growth} was derived from the MBC (eq. 5).

CUE was calculated as follows:

$$CUE = \frac{{}^{18}MBC}{({}^{18}MBC + C_{Respiration})} = \frac{C_{Growth}}{(C_{Growth} + C_{Respiration})} \times 100\% \quad (11)$$

where C_{Growth} is the flux of microbial C allocated to biomass production (growth) [C g^{-1} dry soil h^{-1}] and $C_{\text{Respiration}}$ is the flux of C allocated to the production of CO_2 (respiration) which was converted beforehand to $\text{CO}_2\text{-C}_{\text{Equivalent}}$ [C g^{-1} dry soil h^{-1}].

In order to calculate the temperature sensitivity (Q_{10}) of CO_2 respiration, growth and CUE I calculated the Q_{10} value for each sample during the $^{18}\text{O}\text{-H}_2\text{O}$ incubation using the following formula, as suggested by Fang and Moncrieff, (2001):

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{10/(T_2-T_1)} \quad (12)$$

Where Q_{10} refers to the change in the response variable with a 10°C change in temperature and R_1 and R_2 indicate respiration rates (or growth rates, CUE) separately measured at T_1 and T_2 . As the temperature was chosen with a 10°C difference, the superposed term in the formula equals 1. In a second step the mean Q_{10} values for the temperatures $13^\circ\text{C}\text{-}23^\circ\text{C}$ and $23^\circ\text{C}\text{-}33^\circ\text{C}$ were calculated in order to get just one Q_{10} value per dilution and temperature treatment.

The 95% Confidence Interval (CI) of the Q_{10} value was calculated as follows:

$$95\% \text{ CI} = \underline{x} \pm z \times \sigma/\sqrt{n} \quad (13)$$

where \underline{x} refers to the mean of the samples, z represents the confidence level 1.96, σ is the standard deviation of the population and n is the sample size which was 4 or 3 whenever outliers had to be removed beforehand.

Soil organic matter quality and quantity

We used ramped thermal rock-eval® pyrolysis (RE) to evaluate SOM quality (Soucémariadin *et al.*, 2018) after the pre-incubation phase but before the additional substrate addition (Supplementary figure 1). During RE, carbon oxides are quantified as they come off a soil sample subject to increasing temperatures, thereby providing a metric of SOM thermal stability. Compounds with high thermal stability include aromatic and phenolic non-lignin compounds, while lipids and polysaccharides tend to have lower thermal stability (Sanderman & Grandy, 2020). Soils were dried at 65°C and crushed to a fine powder in a mortar and pestle. Between 50 and 70 mg soil was pyrolyzed over a temperature ramp from 200 to 650°C , followed by combustion to 850°C using a rock-eval 6 pyrolyzer (Vinci technologies) at the Institute of Earth Sciences of the University of Lausanne (Switzerland).

Data analysis

Statistical analyses were performed in R software version 4.0.2 (R Development Core Team, 2020). Normality of each variable was tested, and log transformed to meet assumptions of normality of the distribution of residuals and variance homoscedasticity. Outliers were detected and cross-validated with three different methods and only removed if at least two coincided. The methods consisted of a visual inspection of the boxplots, inspection of the residuals vs. leverage plot and if a value was outside of the 1.5x interquartile range for the first and the third quartiles.

To determine the effect of the dilution treatment, temperature regime treatment and the incubation temperature on the C-cycling processes cumulative respiration, DNA yield and 95% CI of the Q_{10} values of growth, respiration and CUE, a two-way ANOVA was conducted. Whenever the p-value of the ANOVA indicated that at least one of the treatment groups differed from the others ($p < 0.05$) Tukey's Multiple Comparisons of Means post-hoc test was used to determine which dilution levels were significantly different from each other. For statistical significance we assumed an α -level of 0.05. Within that context, the dilution treatment, the temperature treatments (fluctuating and constant) and the incubation temperature (13°C, 23°C, 33°C) are the independent variables. The dependent variables were respiration, growth, CUE, respiration- Q_{10} , growth- Q_{10} , CUE- Q_{10} . In order to find the best-fit model, the lowest Akaike Information Criterion (AIC) was determined and diagnostic plots were looked at and compared among several models. In order to quantify the effect of incubation temperature on growth, respiration and CUE a linear-mixed effects model was used. Furthermore t-tests were applied for the two-level factor temperature treatment (constant and fluctuating). Model parameters and outputs are reported on Supplementary Tables 1, 2, 3 and 4.

Supplementary References

- Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.P., Clément, C., Ouhdouch, Y. & van Wezel, G.P. Taxonomy, Physiology, and Natural Products of Actinobacteria. *Microbiology and Molecular Biology Reviews*. 80(1), 1–43 (2016).
- Delgado-Baquerizo, M. Oliverio, A. M., Brewer, T. E., Benavent-González, A., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., Singh, B. K. & Fierer, N. A global atlas of the dominant bacteria found in soil. *Science*. 359(6373), 320–325 (2018).

- Diaz S., Symstad, A.J., Chapin, F. S., Wardle, D.A. & Huenneke, L.F. Functional diversity revealed by removal experiments. *Trends Ecology Evolution*, 18(3), 140–146 (2003).
- Fang, C., & Moncrieff, J. B. The dependence of soil CO₂ efflux on temperature. *Soil Biology and Biochemistry*, 33(2), 155–165 (2001).
- Geyer, K. M., Dijkstra, P., Sinsabaugh, R., & Frey, S. D. Clarifying the interpretation of carbon use efficiency in soil through methods comparison. *Soil Biology and Biochemistry*, 128, 79–88 (2019).
- Sanderman, J., & Grandy, A. S. Ramped thermal analysis for isolating biologically meaningful soil organic matter fractions with distinct residence times. *The Soil*, 6(1), 131–144 (2020).
- Soucémariadin, L., Cécillon, L., Chenu, C., Baudin, F., Nicolas, M., Girardin, C., & Barré, P. Is Rock-Eval 6 thermal analysis a good indicator of soil organic carbon lability? A method-comparison study in forest soils. *Soil Biology and Biochemistry*, 117, 108–116 (2018).
- Spohn, M., Pötsch, Erich M., Eichorst, S.A., Woebken, D., Wanek, W., & Richter, A. Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. *Soil Biology and Biochemistry*, 97, 168–175 (2016a).
- Spohn, M., Klaus, K., Wanek, W. & Richter, A. Microbial carbon use efficiency and biomass turnover times depending on soil depth—Implications for carbon cycling. *Soil Biology and Biochemistry*, 96, 74–81 (2016b).