TITLE

Improving measurements of microbial growth, death, and turnover by accounting for extracellular DNA in soils

Keywords: soil carbon cycling, microbial death, soil microbial processes, microbial temperature response, microbial growth optimum

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ABSTRACT

Microbial respiration, growth and turnover are driving processes in the formation and decomposition of soil organic matter. In contrast to respiration and growth, microbial turnover and death currently lack distinct methods to be determined. Here we propose a new approach to determine microbial death rates and to improve measurements of microbial growth. By combining sequential DNA extraction to distinguish between intracellular and extracellular DNA and ^{18}O incorporation into DNA, we were able to measure microbial death rates. We first evaluated methods to determine and extract intracellular and extracellular DNA separately. We then tested the method by subjecting soil from a temperate agricultural field and a deciduous beech forest to either 20 °C, 30 °C or 45 °C for 24 h. Our results show, that while

mass specific respiration and gross growth either increased with temperature or remained stable, microbial death rates strongly increased at 45 °C and caused a decrease in microbial biomass and thus in microbial net growth. We further found that also extracellular DNA pools decreased at 45 °C compared to lower temperatures, further indicating enhanced uptake and recycling of extracellular DNA along with increased respiration, growth and death rates. Additional experiments including soils from more and different ecosystems as well as testing the effects of factors other than temperature on microbial death are certainly necessary to better understand the role of microbial death in soil C cycling. We are nevertheless confident that this new approach to determine microbial death rates and dynamics of intracellular and extracellular DNA separately will help to improve concepts and models of C dynamics in soils in the future.

1 INTRODUCTION

Microorganisms are the driving force that sustains the 1450 Gt carbon (C) in soils globally (Liang et al., 2017; Scharlemann et al., 2014). Active microorganisms take up and convert plant derived C and soil organic C into microbial biomass and release C as CO_2 to the atmosphere via respiration. Upon cell death, microbial C is released back to the soil solution and can be stabilized on mineral surfaces or in aggregates. While causes for microbial death in soils can be numerous, ranging from osmotic shock and dehydration to viral lysis and predation (Sokol et al., 2022), the relevance of this process and of the microbial necromass pool for soil C cycling is undisputed. Since a large proportion of SOM is passing through the microbial biomass pool (Kallenbach et al., 2016; Miltner et al., 2012), the process of microbial death might be of equal importance as microbial growth for SOM formation.

Methodological developments in the last decades have made it possible to measure microbial C uptake (Bååth, 2001; Frey et al., 2013; Rousk and Bååth, 2007). Substrate independent methods, that use ^{18}O have enabled the measurement of growth of the whole soil microbial community and individual taxa

without changing substrate availability for microbes (Blazewicz and Schwartz, 2011; Hungate et al., 2015; Spohn et al., 2016). Recently developed methods even allow these measurements without changing soil water contents (Canarini et al., 2020; Metze et al., 2023). In contrast to uptake and growth, turnover and death rates of the microbial community have not seen a suitable method yet. Microbial turnover can be calculated using only growth rates and the microbial biomass pool (e.g., Prommer et al., 2020; Spohn et al., 2016). This is done under the assumption of a stabile state of the microbial community and no net changes in the living microbial biomass as well as death rates being the same as growth rates. An assumption that might not always be met under natural conditions.

A reason for the lack of methods to determine microbial death rates might be that DNA extractions used for ^{18}O-based methods do not account for extracellular DNA (eDNA). Extracellular DNA is DNA that persists outside of intact microbial cells (Pietramellara et al., 2009). The eDNA pool is on the one hand fed by disintegrated microbial cells (Ascher et al., 2009; Nagler et al., 2020), which could have died as consequence to chemical or physical stressors or lysis caused by predators or viruses (Sokol et al., 2022). On the other hand, it has been shown that DNA is actively exuded by microorganisms as an integral component of microbial biofilms in soils (Cai et al., 2019; Das et al., 2013). Pools of eDNA can be rather prominent in soils and has been shown to account for up to 80 % of the total DNA extracted (Carini et al., 2016). Such a large pool of DNA, irrespective of its origin has the capacity to mask subtle changes in the pool of DNA inside living microbial cells (iDNA) and to bias measurements of microbial growth that are based on the determination of DNA contents.

Here we propose a novel approach to assess microbial turnover rates. We suggest that separating the eDNA and iDNA pools upon the determination of microbial growth rates based on ^{18}O-water incorporation into DNA harbors several advantages over the conventional method. The adaptation provides more precise growth rate measurements as it also allows the calculation of only iDNA production rates. Accordingly, changes in the iDNA pool can be used to calculate gross DNA release rates, i.e. microbial

death rates. Besides providing insights into microbial death rates, observing changes in the iDNA as well as eDNA pools holds potential information about microbial processes like microbial DNA uptake and recycling.

In addition to evaluating extraction methods for eDNA and iDNA and evaluation of ^{18}O incorporation in the two DNA pools over time, we have tested the method by subjecting soils to different temperatures. We used 20 ℃, 30 ℃ and 45 ℃ assuming that these temperatures represent three distinct but relevant temperatures for microbial activities in the investigated soils. The investigated soils were from two contrasting temperate systems (an agricultural field and a deciduous forest) that regularly experience 20 ℃ and sometimes even 30 ℃ in the topsoil layers (Schnecker et al., 2022). Around 30 ℃ is the assumed optimum temperature for microbial activity ~~for microorganisms~~ in many soils (Birgander et al., 2018; Nottingham et al., 2019; Rousk et al., 2012). At ~~and~~ 45 ℃ ~~is a temperature, that has been shown to be beyond the temperature optimum where~~ microbial process rates are reduced in comparison to ~~under~~ the temperature optimum at 30 ℃ (Cruz-Paredes et al., 2021; Rousk et al., 2012). We expected, that (1) mass specific respiration, would increase from 20 ℃ to 30 ℃ and further to 45 ℃. We further hypothesized that (2) a previously shown decrease in microbial net growth above the temperature optimum at 30 ℃ would be caused by increased microbial death and a net decrease in microbial biomass.

2 MATERIALS AND METHODS

2.1 Sampling sites

Soil samples were collected from an agricultural field site and a deciduous forest. The long-term agricultural field experiment near Grabenegg, in Alpenvorland, Austria (48°12´N 15°15´E), was established in 1986 and previously described in Spiegel et al. (2018). The soil is classified as gleyic Luvisol (Spiegel et al., 2018) and has a silt loam texture (10 % sand, 73 % silt, and 17 % clay). Soil pH is 6.1 (Canarini et al., 2020). The forest study site at the experimental forest Rosalia, Austria (47°42'N, 16°17'E) is dominated by

European beech (*Fagus sylvatica* L.). The soil at the site is a gleyic Cambisol (Leitner et al., 2016). Texture is a sandy loam (55 % sand, 38 % silt, and 7 % clay), soil pH is 4.9 (Canarini et al., 2020). Soils were sampled from 0-5cm depth with a soil corer with a diameter of 2 cm. At both sites, 10 soil cores per each of the four replicate plots were combined to one sample resulting in four field replicates per site. At the agricultural site, the four sampled plots were 7.5 m wide and 28 m long and at least 5 m apart from the next plot. At the forest site, the 3 m by 3 m plots were at least 10 m apart from each other. All samples were homogenized by sieving in the field through a 2 mm mesh before they were transported to the laboratory.

2.2 Experimental setup

To evaluate the feasibility of eDNA extraction and determination of eDNA pool size, as well as the potential for its use in conjunction with ^{18}O-based determination of microbial growth, we carried out three tests.

1) Comparing methods to collect or remove eDNA
2) Dynamics of eDNA over time at constant temperature
3) Temperature response of microbial biomass, DNA pools, microbial growth, death, and respiration

2.2.1 Comparing methods to collect or remove eDNA

To determine the contribution of eDNA to the total DNA pool, we compared two published methods. The first method removes eDNA by addition of DNases (DNase method, (Lennon et al., 2018)), the second method is based on a sequential DNA extraction (Ascher et al., 2009).

For this test, soil samples were collected in October 2021 and kept at 4 ℃ for one week before the experiment. For the DNase method, 400 mg of field moist soil were weighed in two 2 mL plastic tubes each. All tubes were then amended with 440 µL buffer consisting of 382.5 µL of ultrapure water, 5 µL of 1 M $MgCl_2$, 2.5 µL of bovine serum albumin (10 mg/ml), and 120 µL of 0.5 M Tris-HCl (pH 7.5). One of the two samples further received 40 µL DNase I solution (10U/µL), the other tube received 40 µL ultrapure water and served as control. Both samples were incubated in an incubator at 37 ℃ for 1 h. Afterwards 25

µL 0.5M EDTA was added, and the tubes were transferred to an incubator at 75 ℃ to stop DNase activity. After 15 min, the samples were centrifuged, the supernatant was discarded, and the remaining sample was extracted using FastDNA™ SPIN Kit for Soil (MP Biomedicals).

For the sequential DNA extraction, we used the chemicals and materials provided in the FastDNA™ SPIN Kit for Soil (MP Biomedicals). For this approach 400 mg of field moist soil were weighed in the 2 mL Lysing Matrix E tubes from which the contents had been emptied and collected in a 2 mL plastic vial. We added 1100 µL sodium phosphate buffer to the soil in the lysing tube and shook the vials gently in a horizontal position at 100 rpm at 4 ℃ for 20 minutes. After this, the vials were centrifuged at 12500 rpm for 2 min and the supernatant was collected as the eDNA containing fraction. The original content of the Lysing matrix E tubes was returned to the tubes and handled as described in the manufacturer instructions to obtain the iDNA pool. To the eDNA-fraction we then added 250 µL Protein precipitation solution and followed the MP bio instructions after this step, except for additional centrifugation steps for separating binding matrix and the liquid solution. After DNA extraction and purification, DNA extracts were stored at -80C until further use. In addition to these two approaches, the same soils were also extracted regularly using the FastDNA™ SPIN Kit for Soil (MP Biomedicals) to determine the total extractable DNA pool. The DNA concentration of all extracts was determined fluorometrically by a Picogreen assay using a kit (Quant-iT™ PicoGreen® dsDNA Reagent, Life Technologies). Content of eDNA determined with the DNase method was calculated by subtracting the DNA content of samples that received DNase I from samples that only received water and served as control.

2.2.2 Dynamics of eDNA and iDNA over time at constant temperature

In this experiment, we explored the changes in eDNA and iDNA pools over time as well as the incorporation of ^{18}O from added water into these two distinct DNA pools. Soils were sampled in August 2022 and the incubation was started one week later, where samples were stored at 20 ℃. For the

146 experiment, 400 mg of field moist soil were weighed into empty lysing matrix E tubes and amended with
147 ^{18}O-water to achieve 60 % of the soils water holding capacity and a labelling of 20 atom percent (atm %)
148 of the total water in the soil. From each of the four field replicates, 7 vials were filled, labelled with ^{18}O
149 water and closed. Immediately after label addition and after 6 h, 12 h, 24 h, 48 h, 72 h and 168 h, eDNA
150 and iDNA was extracted with sequential DNA extraction as described above. DNA concentrations in all
151 DNA fractions were determined using the Picogreen assay. Subsequently, total oxygen content and ^{18}O
152 enrichment of the purified DNA fractions were measured following Spohn et al. (Spohn et al., 2016) and
153 Zheng et al. (Zheng et al., 2019) using a thermochemical elemental analyzer (TC/EA, Thermo Fisher)
154 coupled via a Conflo III open split system to an isotope ratio mass spectrometer (Delta V Advantage,
155 Thermo Fisher).
156
157 2.2.3 Temperature response of microbial biomass, DNA pools, microbial growth, death and respiration
158 In this experiment we subjected the samples to three different temperatures to test the response of
159 microbial communities. Soils were collected in August 2022 and stored at 20 ℃ for two days before the
160 start of the experiment.
161 For the incubation, around 400 mg of soil were weighed into empty lysing matrix E tubes. From each field
162 replicate, five lysing matrix E tubes were filled. Two sets of samples were amended with natural
163 abundance water and three sets were amended with ^{18}O-water to achieve 60 % water holding capacity
164 and 20 atm % ^{18}O in the final soil water, when ^{18}O-water was added. One set of samples that received
165 natural abundance water was extracted immediately using sequential DNA extraction. The second set of
166 natural abundance samples and one set of samples with ^{18}O-water were put in an incubator set to 20 ℃.
167 A second set was put in an incubator set to 30 ℃ and the third set of samples was incubated at 45 ℃.
168 After 24 h in the incubators, all samples were subjected to sequential DNA extraction to recover eDNA
169 and iDNA pools. All obtained DNA extracts were stored at -80 ℃ before DNA concentrations were

determined using Picogreen assay and oxygen content and ^{18}O enrichment were determined as described above.

In addition to the ^{18}O-incubation, we determined microbial respiration rates and microbial biomass C following the descriptions in Schnecker et al. (Schnecker et al., 2023). For microbial respiration 400 mg of soil were weighed in plastic vials, water was added to achieve 60 % WHC and the open plastic vials containing the soil were inserted into 27 mL headspace vials. The headspace vials were sealed with a rubber septum. This was done in three replicates for each soil sample, with one set being incubated at 20 ℃, 30 ℃ and 45 ℃ respectively. In addition to the headspace vials containing soil samples, 5 empty glass vials were sealed with rubber septa and added for each temperature. After 24 h, we measured the CO_2 concentration in the headspace vials by taking gas samples from a sealed headspace vial and measured it directly with an infrared gas analyzer (EGM4, PP systems). Microbial respiration rate was then calculated as the difference in CO_2 concentrations between the vials containing soil samples and empty glass vials, which contained the air at the start of the incubation. The net increase in CO_2 was divided by the incubation time.

Microbial biomass C (MBC) was determined following an approach based on (Brookes et al., 1985) and described in Schnecker et al. (Schnecker et al., 2023) with parallel determinations for MBC at the three temperatures. MBC was determined in 1M KCl and measured on a TOC/TN analyzer (TOC-L CPH/CPN, Shimadzu). Measured MBC values were divided by 0.45 (Wu et al. 1990) to account for extraction efficiency.

For each of the three temperatures, we calculated microbial gross growth rates ~~(gG)~~, microbial net growth rates ~~(nG)~~, microbial gross death rates (DNA_{death}) and microbial carbon use efficiency (CUE).

Microbial gross growth was calculated following Canarini et al (Canarini et al., 2020) as the amount of iDNA produced:

$$iDNA_{produced} = O_{iDNA\ extr} * \frac{^{18}O\ at\%_{iDNA\ L} - ^{18}O\ at\%_{iDNA\ n.a.}}{^{18}O\ at\%_{soil\ water}} * \frac{100}{31.21}$$

Where $O_{iDNA\ extr}$ is the total amount of oxygen in the iDNA extract, $^{18}O\ at\%_{iDNA\ L}$ and $^{18}O\ at\%_{iDNA\ n.a.}$ are the ^{18}O enrichment in the labeled DNA extracts from the different temperatures and unlabeled DNA extracts respectively, and $^{18}O\ at\%_{soil\ water}$ is the ^{18}O enrichment of the soil water. The fraction at the end of the formula accounts for the average oxygen content of DNA (31.21%, (Canarini et al., 2020; Zheng et al., 2019)).

Mass specific gross growth rate (MSgG) was calculated by dividing $iDNA_{produced}$ by the amount of iDNA in the respective sample.

Microbial net growth rate was calculated by subtracting the amount of iDNA in the samples that were extracted immediately from the amount of iDNA at the end of the incubation divided by the incubation time. Mass specific net growth rate (MSnG) was calculated by dividing ~~nG~~ microbial net growth rates by the iDNA content at the end of the incubation. Microbial gross death rates were calculated by using the following formula:

$$DNA_{death} = | \Delta iDNA - iDNA_{produced} |$$

Where microbial death rates (DNA_{death}) are determined by subtracting iDNA growth ($iDNA_{produced}$), determined by ^{18}O incorporation into iDNA, from the net growth rate (ΔiDNA). Mass specific gross death (MSD) was calculated by dividing DNA_{death} by the iDNA content.

Microbial CUE was calculated using the following equation (Manzoni et al., 2012):

$$CUE = \frac{C_{Growth}}{C_{Growth} + C_{Respiration}}$$

Where microbial biomass C produced (C_{Growth}) during the incubation was calculated as $iDNA_{produced}$ divided by the total amount of iDNA in the sample and multiplied by MBC values. Microbial respiration ($C_{Respiration}$)

was calculated from the respiration measurements described above. Mass specific microbial respiration (MSR) was calculated as $C_{Respiration}$ divided by MBC.

2.3 Statistics

All statistical analyses were performed in R 4.1.2 (R Development Core Team, 2013). To determine whether eDNA or iDNA pools or ^{18}O atom percent access were different from timepoint 0 in Experiment 2.2.2 we used two sample comparison tests. We used either t-tests, Welch t-tests when variances were not homogeneous or Wilcoxon rank sum tests when data were not normally distributed. We used Fit Linear Model Using Generalized Least Squares (R function 'gls') and Linear Mixed-Effects Models ('lme'), which are both contained in the R package 'nlme' (Pinheiro et al., 2021) and Estimated marginal means ('emmeans') to determine effects of temperature on microbial processes and MBC and DNA pools (Experiment 4) and differences in the extraction assays (Experiment 2.2.1). To account for non-normal distributed residuals, we used log transformations where necessary. If residuals of the models were non-homoscedastic, we introduced weights in the respective functions. We also introduced field plots as random effects. Different models including weights and random effects were set up and compared with the ANOVA('anova'). If models were statistically different, we chose the model with the lowest Akaike information criterion (AIC). Statistical tests were assumed to be significant at $p<0.05$.

3 RESULTS and DISCUSSION

3.1 Comparing methods to collect or remove eDNA

To distinguish eDNA and iDNA, we tested two methods. First, eDNA digestion by DNase (Lennon et al., 2018) and sequential extraction (Ascher et al., 2009). Compared to regular DNA extraction, sequential extraction yielded on average 23.1 % less and the DNase method yielded on average 78.2 % less total DNA (Table 1). The differences between regular extraction and sequential extraction were only statistically

significant in the agricultural soil but not in forest soil. Lower yields in total DNA extracted with sequential extraction could be explained by the additional pipetting and cleaning steps used for this approach, which can have an impact on extraction efficiency (Pold et al., 2020). The DNase digestion yielded significantly less total DNA then the two other methods and also did not work as expected in two out of four replicates at each site.

Table 1 Comparison of methods to estimate eDNA in soil samples from two soil systems. The column "stat" indicates statistical differences in total DNA amounts between the three extraction methods.

	agricultural soil					forest soil				
	mean	min.	max.	n	stat	mean	min.	max.	n	stat
regular DNA extraction, total DNA (µg DNA g^{-1} dry soil)	6.791	6.060	7.285	4	a	19.67	13.32	22.50	4	a
sequential DNA extraction, total DNA (µg DNA g^{-1} dry soil)	4.956	4.556	5.190	4	b	15.91	12.53	19.69	4	a
DNase method, total DNA (µg DNA g^{-1} dry soil)	0.756	0.712	0.805	4	c	6.388	5.460	6.830	4	b
Sequential DNA extraction, eDNA (% of total)	2.447	1.838	3.265	4	-	6.472	5.957	7.183	4	-
DNase method, eDNA (% of total DNA)	-7.063	-32.19	15.14	4	-	-6.917	-30.14	7.024	4	-
DNase method, eDNA (% of total DNA), ~~corrected for~~excluding negative values	10.60	6.061	15.14	2	-	6.053	5.082	7.024	2	-

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Due to these findings and the fact, that the DNase method uses incubation temperatures of 35 °C and 75 °C, which likely interfere with potential temperature treatments, we decided to use sequential extraction for our further experiments. Sequential extraction also has the advantage that both eDNA and iDNA are recovered and can be used for further analyses. The amounts of eDNA recovered with sequential DNA extraction were on average 2.4 % of total DNA in agricultural soils and 6.5 % of total DNA in forest soils, which is on the lower end of the range found in other studies (Carini et al., 2016; Lennon et al., 2018). However, agricultural soils examined by Carini et al (2016) fell on the lower end of the wide range of eDNA contents. Another reason for our low eDNA values could be that our soils were stored in the lab for at least a couple of days. During this time eDNA might have already been degraded.

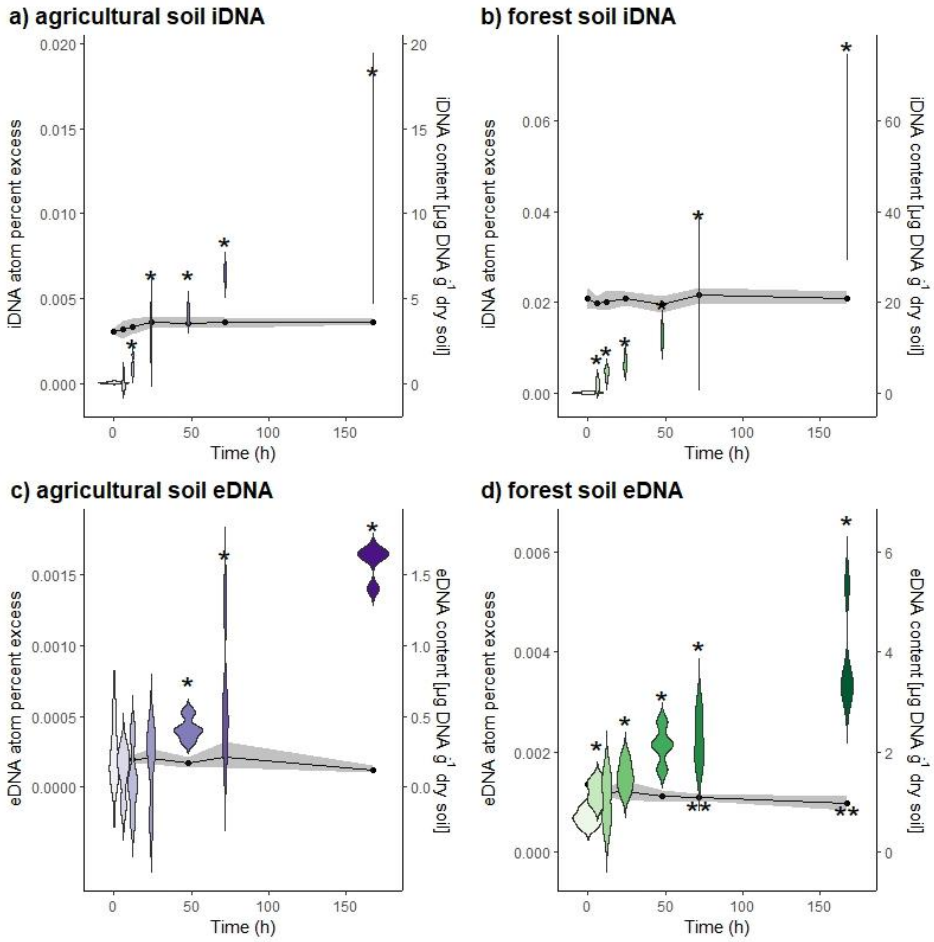


Figure 1. Temporal development of DNA pools and ^{18}O enrichment during incubation with ^{18}O-water. Upper panels depict iDNA pools and enrichment in a) agricultural soils and b) forest soils. Lower panels depict eDNA pools and enrichment in c) agricultural soils and d) forest soils. Violin plots represent ^{18}O enrichment of DNA pools (atom percent excess) and dot and line plots DNA pool sizes over time. Asterisks indicate significant differences (p-value < 0.05) from timepoint 0.

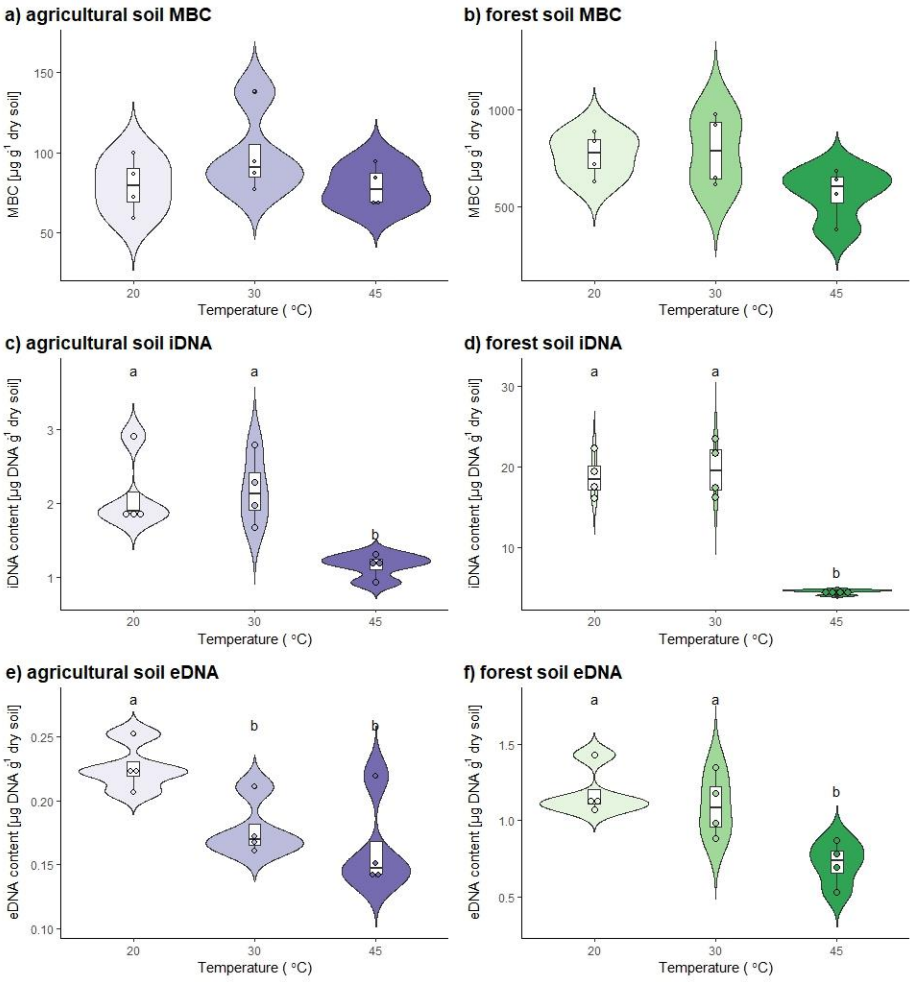
3.2. Dynamics of eDNA and iDNA over time at constant temperature

We also determined the change in eDNA and iDNA content as well as the incorporation of ^{18}O from amended ^{18}O-labelled water into these two DNA pools over time (Figure 1). We found that only the amount of eDNA in forest soils slightly decreased over time and was significantly lower after 72 h and after 168 h compared to the initial eDNA content (Figure 1d). In forest soils, the iDNA content and both DNA pools in the agricultural soil did not change over time (Figure 1 a-c). The amended ^{18}O was incorporated into both DNA pools at both sites over time, indicating production of iDNA and eDNA. While we could detect ^{18}O label at the latest after 12 h in both DNA pools of the forest soil and the iDNA pool of the agricultural soil, increased ^{18}O values could only be found after 48 h in the eDNA pool of the agricultural soil. This could indicate, that the eDNA pool in the agricultural soil might mainly be fed by microbial death, and that the ^{18}O is thus first incorporated in iDNA and only when these newly formed cells die, the label is released as eDNA. In the forest soil our findings indicate that eDNA is actively exuded from the beginning on. It should be noted, that detection of label in eDNA early on could also indicate that freshly formed cells were lysed during the eDNA extraction. However, If eDNA is actively exuded as e.g. part of microbial biofilm (Das et al., 2013; Nagler et al., 2018; Pietramellara et al., 2009) depends on the present microorganisms (Cai et al., 2019). The amount of eDNA produced can also vary for different microorganisms (Figure S1).

3.3~~2~~. Temperature response of microbial biomass, DNA pools, microbial growth, death, and respiration

To test the combination of sequential DNA extraction and ^{18}O incorporation in DNA, we subjected soil from the agricultural site and the forest site to three different temperatures. Microbial processes and activity have been shown to strongly increase with temperature up to a temperature optimum (Rousk et al., 2012). Above this temperature threshold conditions are adverse and have been shown to lead to a reduction of the microbial biomass (Riah-Anglet et al., 2015). By subjecting the two investigated soil types

to 20 ℃, 30 ℃ and 45 ℃ we found that MBC was not affected by temperature (Figure 2 a,b). The content of iDNA did not change from 20 ℃ to 30 ℃ and decreased significantly when soils were brought to 45 ℃ (Figure 2 c,d). The decrease in iDNA at 45 ℃ indicated that a part of the microbial community died because of the high temperature and DNA might have been lost from within the microbial cells. In agricultural soils, eDNA contents were significantly lower at 30 ℃ and 45 ℃ than at 20 ℃, while eDNA contents in forest soils only dropped significantly in the 45 ℃ treatment (Figure 2 e-f). As we observed an increase in mass specific death rate and a concomitant decrease in the eDNA pool we concluded that the efflux from the eDNA pool must be increased. This could be eDNA uptake or degradation of eDNA. We thus suggest that decreasing eDNA contents with temperature rather indicate a higher degradation and recycling of eDNA than the reduction of eDNA release from microbial cells.



299

300 Figure 2. Microbial pool sizes in the two investigated soils after incubation at three different temperatures
301 for 24 h. Results for agricultural soils are shown on plots a, c and e. Forest soils are shown in plots b, d, f.
302 Microbial biomass C is shown in a) and b), iDNA contents are shown in c) and d) and eDNA contents are
303 shown in e) and f). Statistically significant differences between pool sizes at the three investigated
304 temperatures are marked with different letters above the violin plots.

305

306 Mass specific respiration increased in both soils from 20 °C over 30 °C to 45 °C (Figure 3 a) confirming
307 previous findings of other studies (Birgander et al., 2018; Cruz-Paredes et al., 2021; Rousk et al., 2012).
308 Mass specific gross growth did not change with temperature in agricultural soils but increased from 20 °C
309 to 30 °C and even to 45 °C in forest soils (Figure 3 b). This is in contrast to previous studies (Birgander et
310 al., 2018; Cruz-Paredes et al., 2021; Rousk et al., 2012), which found that microbial uptake of leucine in
311 microbial biomass and acetate in fungal ergosterol, which was used as indicators of growth, showed a
312 clear temperature optimum around 30 °C and concomitant decrease at higher temperatures. These
313 studies however used other methods than we did under the assumption of no net decrease in microbial
314 biomass and equal rates of microbial growth or uptake and microbial death. While our data also show no
315 mass specific net change in microbial biomass from 20 °C to 30 °C, a significant negative mass specific net
316 growth was observed at 45 °C in both soils (Figure 3 c). When we ~~combine~~ subtracted mass specific gross
317 growth~~MSgG~~ ~~and~~ from mass specific net growth~~MSnG~~ the calculated microbial death rates were
318 significantly higher at 45 °C than at 20 °C and 30 °C in both soils (Figure 3 d).
319 Carbon use efficiency decreased with increasing temperature in forest soil, while it stayed constant in
320 agricultural soils (Figure 3 e). This finding adds to an ever-growing list of ambiguous reactions of CUE to
321 soil temperature (e.g. (Hagerty et al., 2014; Schnecker et al., 2023; Simon et al., 2020; Walker et al., 2018))
322 and once again shows, that CUE should be used with caution to infer soil C cycling. As showcased in our
323 experiment, CUE was low at high temperatures in forest soils while growth as well as death rates were
324 high, thereby indicating fast microbial C cycling.

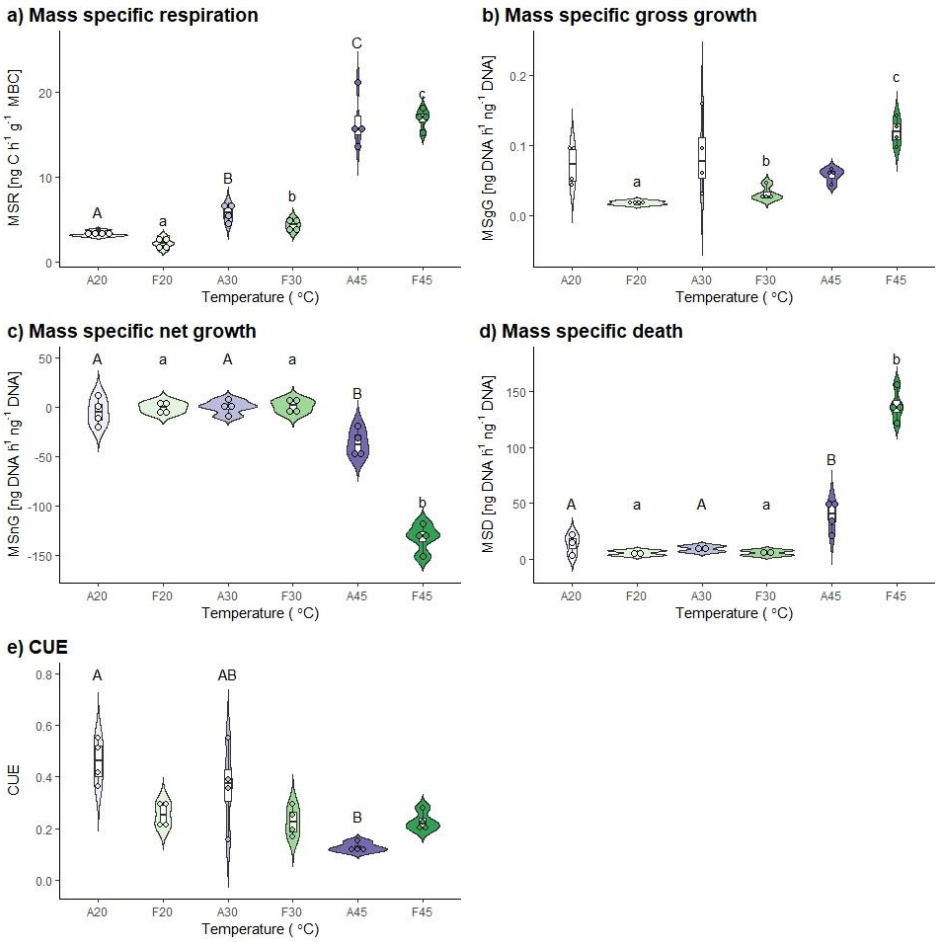


Figure 3. Mass specific microbial process rates and CUE in the two investigated soils after incubation at three different temperatures for 24 h. Results for agricultural soils are shown in purple hues and for forest soils are shown green hues. Statistically significant differences between pool sizes at the three investigated temperatures and respective soil are marked with different letters above the violin plots. Capital letters for differences between agricultural soils and lower-case letters are used to indicate differences for forest soil.

3.4. Caveats and potential of the approach

To our knowledge this is the first attempt to combine 18O-labelling of DNA to measure microbial growth and sequential extraction to determine microbial death rates. Both of these methods have their individual caveats and biases. Growth measurements depend on extractions of DNA and MBC. These extractions have varying efficiencies, depending on e.g. soil type, fungal:bacteria ratios and much more (Pold et al. 2020). Sequential extraction methods to extract eDNA often have to use rather large amounts of soil to capture the low amounts of eDNA. In out approach we had to accept tradeoffs between how practical our method would be (e.g. using commercially available soil extraction kits) and how precise it is (e.g. using less soil). And while there is room to improve both methods individually and also the combination of them, we think that our approach provides a first step in doing this. We hope that our approach will be picked up by the scientific community and will developed further to improve practicality and precision, especially since we have only taken a first glimpse at its potential. Besides determination of growth and death, this method could be used to study eDNA and iDNA dynamics separately which could help understand exudation but also degradation and recycling of eDNA as well as the formation of microbial necromass as well as extracellular polymeric substance in soils.

CONCLUSION

In conclusion we here present an approach to determine microbial death rates and turnover by accounting for eDNA dynamics. To our knowledge, this is the first time microbial death rates were investigated in addition to microbial growth rates and net changes in microbial iDNA. With this approach we could show that microbial respiration and microbial growth in the two investigated soils increase with temperature even up to 45 °C, a temperature, that is considered to be way beyond the temperature optimum of most temperate microbial communities. The often observed drop in microbial growth or uptake at high temperatures was however caused by the death of a significant part of the microbial community and

higher microbial death rates. While there is certainly room for improving the method and the necessity to investigate its feasibility in other soil systems and under different environmental conditions, we think that this approach will help to shed light on the role of microbial death in soil and a step forward to understand soil C cycling.

AUTHOR CONTRIBUTION

Jörg Schnecker: Conceptualization (lead); investigation (supporting); methodology (supporting); supervision (lead); formal analysis (lead); writing – original draft (lead) writing – review and editing (equal). **Theresa Böckle:** investigation (equal); methodology (equal); writing – review and editing (equal). **Julia Horak:** investigation (equal); methodology (equal); writing – review and editing (equal). **Victoria Martin:** investigation (supporting); methodology (supporting); writing – review and editing (equal). **Taru Sandén:** resources (equal); writing – review and editing (equal). **Heide Spiegel:** resources (equal); writing – review and editing (equal).

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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