1 TITLE

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

3 in soils

5 Keywords: soil carbon cycling, microbial death, soil microbial processes, microbial temperature response,

6 microbial growth optimum

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17 ABSTRACT

18 Microbial respiration, growth and turnover are driving processes in the formation and decomposition of

19 soil organic matter. In contrast to respiration and growth, microbial turnover and death currently lack

20 distinct methods to be determined. Here we propose a new approach to determine microbial death rates

21 and to improve measurements of microbial growth. By combining sequential DNA extraction to

22 distinguish between intracellular and extracellular DNA and ^{18}O incorporation into DNA, we were able to

23 measure microbial death rates. We first evaluated methods to determine and extract intracellular and

24 extracellular DNA separately. We then tested the method by subjecting soil from a temperate agricultural

25 field and a deciduous beech forest to either 20 ℃, 30 ℃ or 45 ℃ for 24 h. Our results show, that while

26 mass specific respiration and gross growth either increased with temperature or remained stable,

27 microbial death rates strongly increased at 45 ℃ and caused a decrease in microbial biomass and thus in

28 microbial net growth. We further found that also extracellular DNA pools decreased at 45 ℃ compared to

29 lower temperatures, further indicating enhanced uptake and recycling of extracellular DNA along with

30 increased respiration, growth and death rates. Additional experiments including soils from more and

31 different ecosystems as well as testing the effects of factors other than temperature on microbial death

32 are certainly necessary to better understand the role of microbial death in soil C cycling. We are

33 nevertheless confident that this new approach to determine microbial death rates and dynamics of

34 intracellular and extracellular DNA separately will help to improve concepts and models of C dynamics in

35 soils in the future.

36

37 1 INTRODUCTION

38 Microorganisms are the driving force that sustains the 1450 Gt carbon (C) in soils globally (Liang et al.,

39 2017; Scharlemann et al., 2014). Active microorganisms take up and convert plant derived C and soil

40 organic C into microbial biomass and release C as CO_2 to the atmosphere via respiration. Upon cell death,

41 microbial C is released back to the soil solution and can be stabilized on mineral surfaces or in aggregates.

42 While causes for microbial death in soils can be numerous, ranging from osmotic shock and dehydration

43 to viral lysis and predation (Sokol et al., 2022), the relevance of this process and of the microbial

44 necromass pool for soil C cycling is undisputed. Since a large proportion of SOM is passing through the

45 microbial biomass pool (Kallenbach et al., 2016; Miltner et al., 2012), the process of microbial death might

46 be of equal importance as microbial growth for SOM formation.

47 Methodological developments in the last decades have made it possible to measure microbial C uptake

48 (Bååth, 2001; Frey et al., 2013; Rousk and Bååth, 2007). Substrate independent methods, that use [18]O

49 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

74 death rates. Besides providing insights into microbial death rates, observing changes in the iDNA as well

75 as eDNA pools holds potential information about microbial processes like microbial DNA uptake and

76 recycling.

77 In addition to evaluating extraction methods for eDNA and iDNA and evaluation of ^{18}O incorporation in

78 the two DNA pools over time, we have tested the method by subjecting soils to different temperatures.

79 We used 20 ℃, 30 ℃ and 45 ℃ assuming that these temperatures represent three distinct but relevant

80 temperatures for microbial activities in the investigated soils. The investigated soils were from two

81 contrasting temperate systems (an agricultural field and a deciduous forest) that regularly experience 20

82 ℃ and sometimes even 30 ℃ in the topsoil layers (Schnecker et al., 2022). Around 30 ℃ is the assumed

83 optimum temperature for microbial activity in many soils (Birgander et al., 2018; Nottingham et al., 2019;

84 Rousk et al., 2012). At 45 ℃microbial process rates are reduced in comparison to the temperature

85 optimum at 30 ℃ (Cruz-Paredes et al., 2021; Rousk et al., 2012). We expected, that (1) mass specific

86 respiration, would increase from 20 ℃ to 30 ℃ and further to 45 ℃. We further hypothesized that (2) a

87 previously shown decrease in microbial net growth above the temperature optimum at 30 ℃ would be

88 caused by increased microbial death and a net decrease in microbial biomass.

89

90 2 MATERIALS AND METHODS

91 2.1 Sampling sites

92 Soil samples were collected from an agricultural field site and a deciduous forest. The long-term

93 agricultural field experiment near Grabenegg, in Alpenvorland, Austria (48°12´N 15°15´E), was established

94 in 1986 and previously described in Spiegel et al. (2018). The soil is classified as gleyic Luvisol (Spiegel et

95 al., 2018) and has a silt loam texture (10 % sand, 73 % silt, and 17 % clay). Soil pH is 6.1 (Canarini et al.,

96 2020). The forest study site at the experimental forest Rosalia, Austria (47°42'N, 16°17'E) is dominated by

97 European beech (*Fagus sylvatica* L.). The soil at the site is a gleyic Cambisol (Leitner et al., 2016). Texture

98   is a sandy loam (55 % sand, 38 % silt, and 7 % clay), soil pH is 4.9 (Canarini et al., 2020). Soils were sampled

99   from 0-5cm depth with a soil corer with a diameter of 2 cm. At both sites, 10 soil cores per each of the

100   four replicate plots were combined to one sample resulting in four field replicates per site. At the

101   agricultural site, the four sampled plots were 7.5 m wide and 28 m long and at least 5 m apart from the

102   next plot. At the forest site, the 3 m by 3 m plots were at least 10 m apart from each other. All samples

103   were homogenized by sieving in the field through a 2 mm mesh before they were transported to the

104   laboratory.

105   2.2 Experimental setup

106   To evaluate the feasibility of eDNA extraction and determination of eDNA pool size, as well as the potential

107   for its use in conjunction with ^{18}O-based determination of microbial growth, we carried out three tests.

108   1) Comparing methods to collect or remove eDNA

109   2) Dynamics of eDNA over time at constant temperature

110   3) Temperature response of microbial biomass, DNA pools, microbial growth, death, and respiration

111   2.2.1   Comparing methods to collect or remove eDNA

112   To determine the contribution of eDNA to the total DNA pool, we compared two published methods. The

113   first method removes eDNA by addition of DNases (DNase method, (Lennon et al., 2018)), the second

114   method is based on a sequential DNA extraction (Ascher et al., 2009).

115   For this test, soil samples were collected in October 2021 and kept at 4 ℃ for one week before the

116   experiment. For the DNase method, 400 mg of field moist soil were weighed in two 2 mL plastic tubes

117   each. All tubes were then amended with 440 µL buffer consisting of 382.5 µL of ultrapure water, 5 µL of

118   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

119   two samples further received 40 µL DNase I solution (10U/µL), the other tube received 40 µL ultrapure

120   water and served as control. Both samples were incubated in an incubator at 37 ℃ for 1 h. Afterwards 25

121   µL 0.5M EDTA was added, and the tubes were transferred to an incubator at 75 ℃ to stop DNase activity.

122 After 15 min, the samples were centrifuged, the supernatant was discarded, and the remaining sample

123 was extracted using FastDNA™ SPIN Kit for Soil (MP Biomedicals).

124 For the sequential DNA extraction, we used the chemicals and materials provided in the FastDNA™ SPIN

125 Kit for Soil (MP Biomedicals). For this approach 400 mg of field moist soil were weighed in the 2 mL Lysing

126 Matrix E tubes from which the contents had been emptied and collected in a 2 mL plastic vial. We added

127 1100 µL sodium phosphate buffer to the soil in the lysing tube and shook the vials gently in a horizontal

128 position at 100 rpm at 4 °C for 20 minutes. After this, the vials were centrifuged at 12500 rpm for 2 min

129 and the supernatant was collected as the eDNA containing fraction. The original content of the Lysing

130 matrix E tubes was returned to the tubes and handled as described in the manufacturer instructions to

131 obtain the iDNA pool. To the eDNA-fraction we then added 250 µL Protein precipitation solution and

132 followed the MP bio instructions after this step, except for additional centrifugation steps for separating

133 binding matrix and the liquid solution. After DNA extraction and purification, DNA extracts were stored at

134 -80C until further use. In addition to these two approaches, the same soils were also extracted regularly

135 using the FastDNA™ SPIN Kit for Soil (MP Biomedicals) to determine the total extractable DNA pool. The

136 DNA concentration of all extracts was determined fluorometrically by a Picogreen assay using a kit (Quant-

137 iT™ PicoGreen® dsDNA Reagent, Life Technologies). Content of eDNA determined with the DNase method

138 was calculated by subtracting the DNA content of samples that received DNase I from samples that only

139 received water and served as control.

140

141 2.2.2   Dynamics of eDNA and iDNA over time at constant temperature

142 In this experiment, we explored the changes in eDNA and iDNA pools over time as well as the

143 incorporation of ^{18}O from added water into these two distinct DNA pools. Soils were sampled in August

144 2022 and the incubation was started one week later, where samples were stored at 20 °C. For the

145 experiment, 400 mg of field moist soil were weighed into empty lysing matrix E tubes and amended with

146 ^{18}O-water to achieve 60 % of the soils water holding capacity and a labelling of 20 atom percent (atm %)

147 of the total water in the soil. From each of the four field replicates, 7 vials were filled, labelled with ^{18}O

148 water and closed. Immediately after label addition and after 6 h, 12 h, 24 h, 48 h, 72 h and 168 h, eDNA

149 and iDNA was extracted with sequential DNA extraction as described above. DNA concentrations in all

150 DNA fractions were determined using the Picogreen assay. Subsequently, total oxygen content and ^{18}O

151 enrichment of the purified DNA fractions were measured following Spohn et al. (Spohn et al., 2016) and

152 Zheng et al. (Zheng et al., 2019) using a thermochemical elemental analyzer (TC/EA, Thermo Fisher)

153 coupled via a Conflo III open split system to an isotope ratio mass spectrometer (Delta V Advantage,

154 Thermo Fisher).

155

156 2.2.3   Temperature response of microbial biomass, DNA pools, microbial growth, death and respiration

157 In this experiment we subjected the samples to three different temperatures to test the response of

158 microbial communities. Soils were collected in August 2022 and stored at 20 ℃ for two days before the

159 start of the experiment.

160 For the incubation, around 400 mg of soil were weighed into empty lysing matrix E tubes. From each field

161 replicate, five lysing matrix E tubes were filled. Two sets of samples were amended with natural

162 abundance water and three sets were amended with ^{18}O-water to achieve 60 % water holding capacity

163 and 20 atm % ^{18}O in the final soil water, when ^{18}O-water was added. One set of samples that received

164 natural abundance water was extracted immediately using sequential DNA extraction. The second set of

165 natural abundance samples and one set of samples with ^{18}O-water were put in an incubator set to 20 ℃.

166 A second set was put in an incubator set to 30 ℃ and the third set of samples was incubated at 45 ℃.

167 After 24 h in the incubators, all samples were subjected to sequential DNA extraction to recover eDNA

168 and iDNA pools. All obtained DNA extracts were stored at -80 ℃ before DNA concentrations were

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

170   above.

171   In addition to the ^{18}O-incubation, we determined microbial respiration rates and microbial biomass C

172   following the descriptions in Schnecker et al. (Schnecker et al., 2023). For microbial respiration 400 mg of

173   soil were weighed in plastic vials, water was added to achieve 60 % WHC and the open plastic vials

174   containing the soil were inserted into 27 mL headspace vials. The headspace vials were sealed with a

175   rubber septum. This was done in three replicates for each soil sample, with one set being incubated at 20

176   °C, 30 °C and 45 °C respectively. In addition to the headspace vials containing soil samples, 5 empty glass

177   vials were sealed with rubber septa and added for each temperature. After 24 h, we measured the CO_2

178   concentration in the headspace vials by taking gas samples from a sealed headspace vial and measured it

179   directly with an infrared gas analyzer (EGM4, PP systems). Microbial respiration rate was then calculated

180   as the difference in CO_2 concentrations between the vials containing soil samples and empty glass vials,

181   which contained the air at the start of the incubation. The net increase in CO_2 was divided by the

182   incubation time.

183   Microbial biomass C (MBC) was determined following an approach based on (Brookes et al., 1985) and

184   described in Schnecker et al. (Schnecker et al., 2023) with parallel determinations for MBC at the three

185   temperatures. MBC was determined in 1M KCl and measured on a TOC/TN analyzer (TOC-L CPH/CPN,

186   Shimadzu). Measured MBC values were divided by 0.45 (Wu et al. 1990) to account for extraction

187   efficiency.

188

189   For each of the three temperatures, we calculated microbial gross growth rates , microbial net growth

190   rates , microbial gross death rates (DNA_{death}) and microbial carbon use efficiency (CUE).

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

192   iDNA produced:

193 $$iDNA_{produced} = O_{iDNA\,extr} * \frac{^{18}O\,at\%_{iDNA\,L} - {^{18}O\,at\%_{iDNA\,n.a.}}}{^{18}O\,at\%_{soil\,water}} * \frac{100}{31.21}$$

194 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

195 ^{18}O enrichment in the labeled DNA extracts from the different temperatures and unlabeled DNA extracts

196 respectively, and $^{18}O\,at\%_{soil\,water}$ is the ^{18}O enrichment of the soil water. The fraction at the end of the

197 formula accounts for the average oxygen content of DNA (31.21%, (Canarini et al., 2020; Zheng et al.,

198 2019)).

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

200 the respective sample.

201 Microbial net growth rate was calculated by subtracting the amount of iDNA in the samples that were

202 extracted immediately from the amount of iDNA at the end of the incubation divided by the incubation

203 time. Mass specific net growth rate (MSnG) was calculated by dividing microbial net growth rates by the

204 iDNA content at the end of the incubation. Microbial gross death rates were calculated by using the

205 following formula:

206 $$DNA_{death} = |\,\Delta iDNA \;\; - iDNA_{produced}\,|$$

207

208 Where microbial death rates (DNA_{death}) are determined by subtracting iDNA growth ($iDNA_{produced}$),

209 determined by ^{18}O incorporation into iDNA, from the net growth rate (ΔiDNA). Mass specific gross death

210 (MSD) was calculated by dividing DNA_{death} by the iDNA content.

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

212 $$CUE = \frac{C_{Growth}}{C_{Growth} + C_{Respiration}}$$

213 Where microbial biomass C produced (C_{Growth}) during the incubation was calculated as $iDNA_{produced}$ divided

214 by the total amount of iDNA in the sample and multiplied by MBC values. Microbial respiration ($C_{Respiration}$)

215 was calculated from the respiration measurements described above. Mass specific microbial respiration

216 (MSR) was calculated as $C_{Respiration}$ divided by MBC.

217

218 2.3 Statistics

219 All statistical analyses were performed in R 4.1.2 (R Development Core Team, 2013). To determine

220 whether eDNA or iDNA pools or ^{18}O atom percent access were different from timepoint 0 in Experiment

221 2.2.2 we used two sample comparison tests. We used either t-tests, Welch t-tests when variances were

222 not homogeneous or Wilcoxon rank sum tests when data were not normally distributed. We used Fit

223 Linear Model Using Generalized Least Squares (R function 'gls') and Linear Mixed-Effects Models ('lme'),

224 which are both contained in the R package 'nlme' (Pinheiro et al., 2021) and Estimated marginal means

225 ('emmeans') to determine effects of temperature on microbial processes and MBC and DNA pools

226 (Experiment 4) and differences in the extraction assays (Experiment 2.2.1). To account for non-normal

227 distributed residuals, we used log transformations where necessary. If residuals of the models were non-

228 homoscedastic, we introduced weights in the respective functions. We also introduced field plots as

229 random effects. Different models including weights and random effects were set up and compared with

230 the ANOVA('anova'). If models were statistically different, we chose the model with the lowest Akaike

231 information criterion (AIC). Statistical tests were assumed to be significant at $p<0.05$.

232

233 3 RESULTS and DISCUSSION

234 3.1 Comparing methods to collect or remove eDNA

235 To distinguish eDNA and iDNA, we tested two methods. First, eDNA digestion by DNase (Lennon et al.,

236 2018) and sequential extraction (Ascher et al., 2009). Compared to regular DNA extraction, sequential

237 extraction yielded on average 23.1 % less and the DNase method yielded on average 78.2 % less total DNA

238 (Table 1). The differences between regular extraction and sequential extraction were only statistically

239 significant in the agricultural soil but not in forest soil. Lower yields in total DNA extracted with sequential

240 extraction could be explained by the additional pipetting and cleaning steps used for this approach, which

241 can have an impact on extraction efficiency (Pold et al., 2020). The DNase digestion yielded significantly

242 less total DNA then the two other methods and also did not work as expected in two out of four replicates

243 at each site.

244 Table 1 Comparison of methods to estimate eDNA in soil samples from two soil systems. The column

245 "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), excluding negative values	10.60	6.061	15.14	2	-	6.053	5.082	7.024	2	-

246

247   Due to these findings and the fact, that the DNase method uses incubation temperatures of 35 ℃ and 75

248   ℃, which likely interfere with potential temperature treatments, we decided to use sequential extraction

249   for our further experiments. Sequential extraction also has the advantage that both eDNA and iDNA are

250   recovered and can be used for further analyses. The amounts of eDNA recovered with sequential DNA

251   extraction were on average 2.4 % of total DNA in agricultural soils and 6.5 % of total DNA in forest soils,

252   which is on the lower end of the range found in other studies (Carini et al., 2016; Lennon et al., 2018).

253   However, agricultural soils examined by Carini et al (2016) fell on the lower end of the wide range of eDNA

254   contents. Another reason for our low eDNA values could be that our soils were stored in the lab for at

255   least a couple of days. During this time eDNA might have already been degraded.

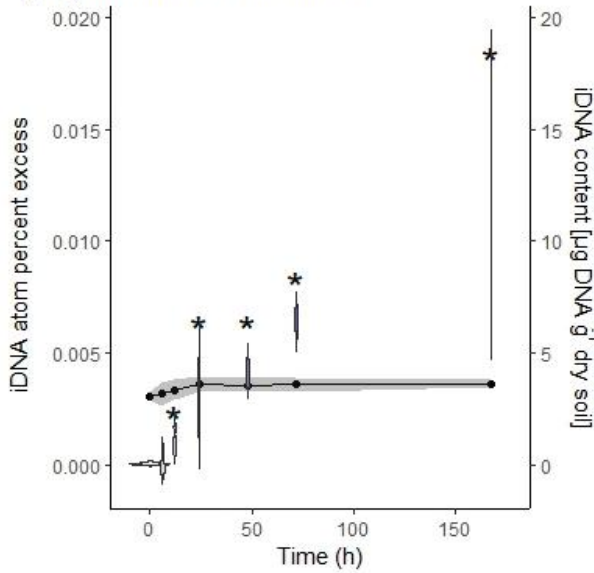
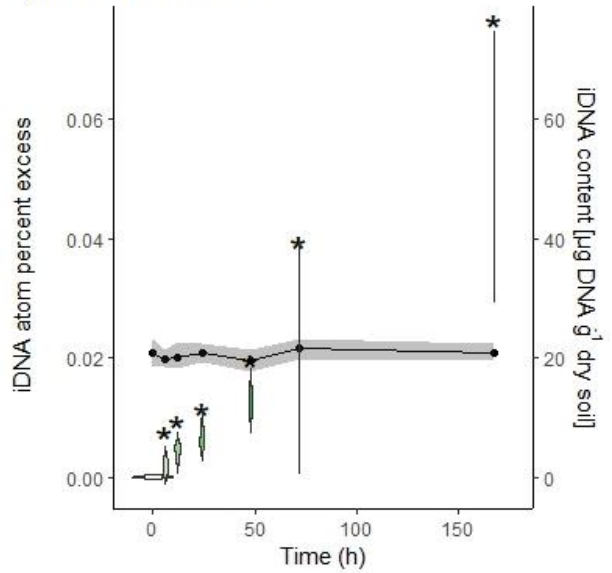
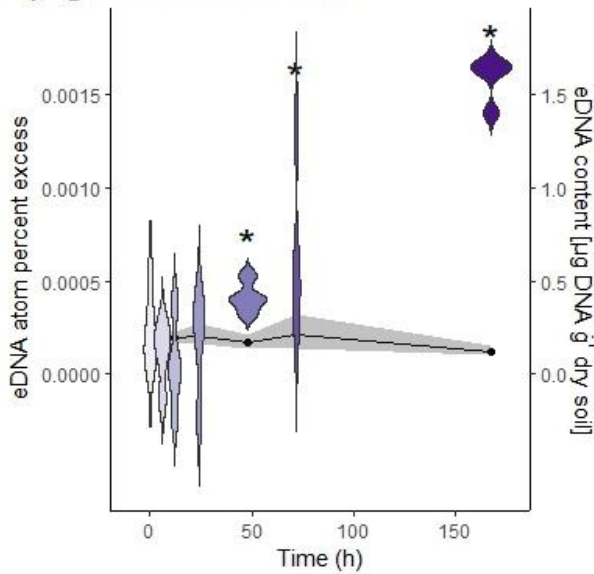
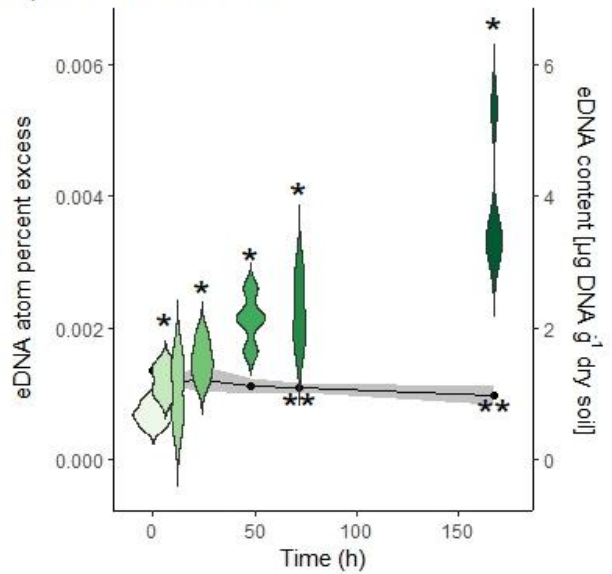



Figure 1. Temporal development of DNA pools and ^{18}O enrichment during incubation with ^{18}O-water. Upper panels depict iDNA pools and enrichment in a) agricultural soils and b) forest soils. Lower panels depict eDNA pools and enrichment in c) agricultural soils and d) forest soils. Violin plots represent ^{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.

263 3.2. Dynamics of eDNA and iDNA over time at constant temperature

264 We also determined the change in eDNA and iDNA content as well as the incorporation of ^{18}O from

265 amended ^{18}O-labelled water into these two DNA pools over time (Figure 1). We found that only the

266 amount of eDNA in forest soils slightly decreased over time and was significantly lower after 72 h and

267 after 168 h compared to the initial eDNA content (Figure 1d). In forest soils, the iDNA content and both

268 DNA pools in the agricultural soil did not change over time (Figure 1 a-c). The amended ^{18}O was

269 incorporated into both DNA pools at both sites over time, indicating production of iDNA and eDNA. While

270 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

271 the agricultural soil, increased ^{18}O values could only be found after 48 h in the eDNA pool of the

272 agricultural soil. This could indicate, that the eDNA pool in the agricultural soil might mainly be fed by

273 microbial death, and that the ^{18}O is thus first incorporated in iDNA and only when these newly formed

274 cells die, the label is released as eDNA. In the forest soil our findings indicate that eDNA is actively exuded

275 from the beginning on. It should be noted, that the detection of label in eDNA early on could also indicate

276 that freshly formed cells were lysed during the eDNA extraction. However, If eDNA is actively exuded as

277 e.g. part of microbial biofilm (Das et al., 2013; Nagler et al., 2018; Pietramellara et al., 2009) depends on

278 the present microorganisms (Cai et al., 2019). The amount of eDNA produced can also vary for different

279 microorganisms (Figure S1).

280

281 3.3. Temperature response of microbial biomass, DNA pools, microbial growth, death, and respiration

282 To test the combination of sequential DNA extraction and ^{18}O incorporation in DNA, we subjected soil

283 from the agricultural site and the forest site to three different temperatures. Microbial processes and

284 activity have been shown to strongly increase with temperature up to a temperature optimum (Rousk et

285 al., 2012). Above this temperature threshold conditions are adverse and have been shown to lead to a

286 reduction of the microbial biomass (Riah-Anglet et al., 2015). By subjecting the two investigated soil types

287 to 20 ℃, 30 ℃ and 45 ℃ we found that MBC was not affected by temperature (Figure 2 a,b). The content

288 of iDNA did not change from 20 ℃ to 30 ℃ and decreased significantly when soils were brought to 45 ℃

289 (Figure 2 c,d). The decrease in iDNA at 45 ℃ indicated that a part of the microbial community died because

290 of the high temperature and DNA might have been lost from within the microbial cells. In agricultural soils,

291 eDNA contents were significantly lower at 30 ℃ and 45 ℃ than at 20 ℃, while eDNA contents in forest

292 soils only dropped significantly in the 45 ℃ treatment (Figure 2 e-f). As we observed an increase in mass

293 specific death rate and a concomitant decrease in the eDNA pool we concluded that the efflux from the

294 eDNA pool must be increased. This could be eDNA uptake or degradation of eDNA. We thus suggest that

295 decreasing eDNA contents with temperature rather indicate a higher degradation and recycling of eDNA

296 than the reduction of eDNA release from microbial cells.

297

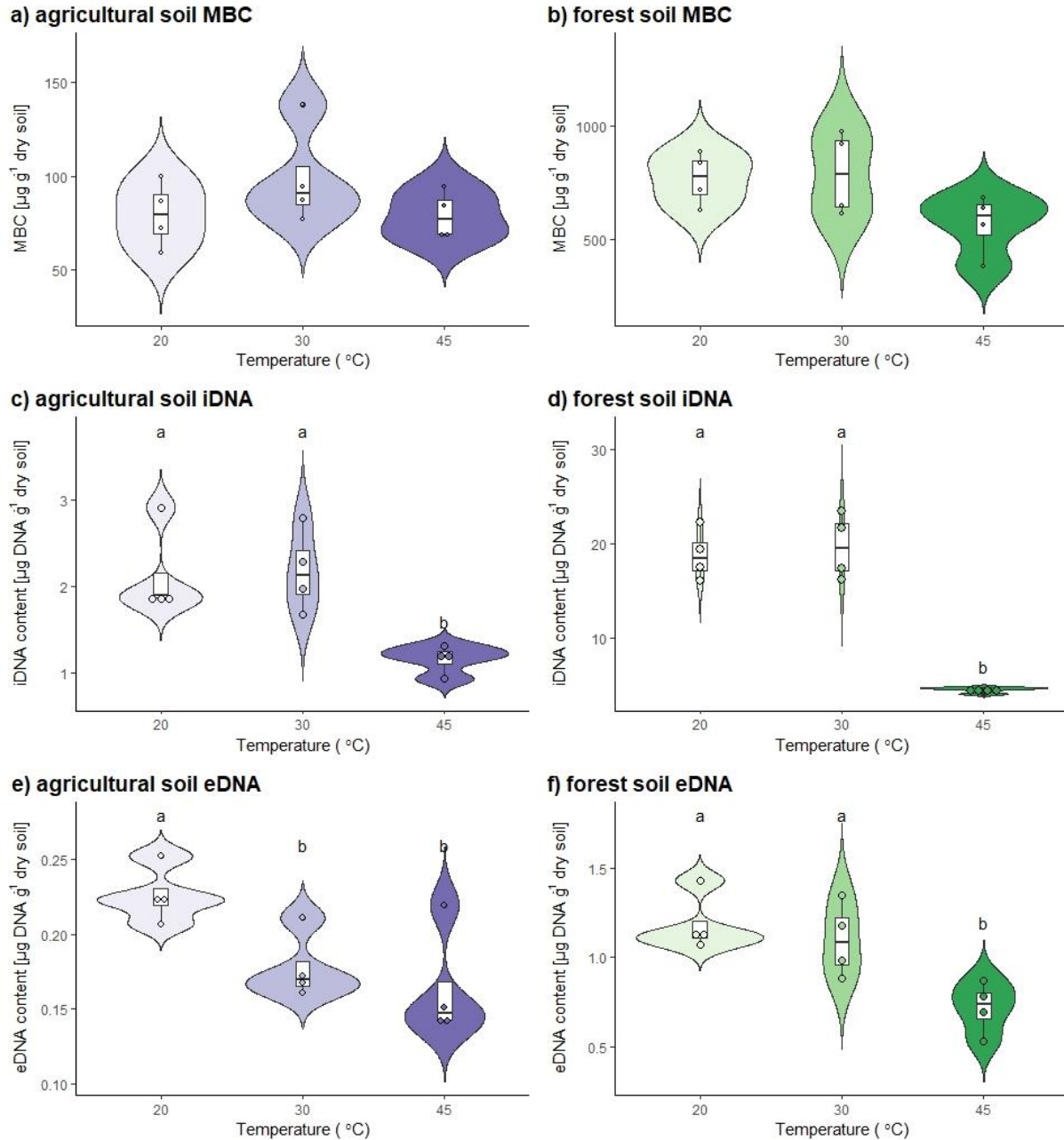



Figure 2. Microbial pool sizes in the two investigated soils after incubation at three different temperatures

for 24 h. Results for agricultural soils are shown on plots a, c and e. Forest soils are shown in plots b, d, f.

Microbial biomass C is shown in a) and b), iDNA contents are shown in c) and d) and eDNA contents are

shown in e) and f). Statistically significant differences between pool sizes at the three investigated

temperatures are marked with different letters above the violin plots.

303

304   Mass specific respiration increased in both soils from 20 ℃ over 30 ℃ to 45 ℃ (Figure 3 a) confirming

305   previous findings of other studies (Birgander et al., 2018; Cruz-Paredes et al., 2021; Rousk et al., 2012).

306   Mass specific gross growth did not change with temperature in agricultural soils but increased from 20 ℃

307   to 30 ℃ and even to 45 ℃ in forest soils (Figure 3 b). This is in contrast to previous studies (Birgander et

308   al., 2018; Cruz-Paredes et al., 2021; Rousk et al., 2012), which found that microbial uptake of leucine in

309   microbial biomass and acetate in fungal ergosterol, which was used as indicators of growth, showed a

310   clear temperature optimum around 30 ℃ and concomitant decrease at higher temperatures. These

311   studies however used other methods than we did under the assumption of no net decrease in microbial

312   biomass and equal rates of microbial growth or uptake and microbial death. While our data also show no

313   mass specific net change in microbial biomass from 20 ℃ to 30 ℃, a significant negative mass specific net

314   growth was observed at 45 ℃ in both soils (Figure 3 c). When we subtracted mass specific gross growth

315   from mass specific net growth the calculated microbial death rates were significantly higher at 45 ℃ than

316   at 20 ℃ and 30 ℃ in both soils (Figure 3 d).

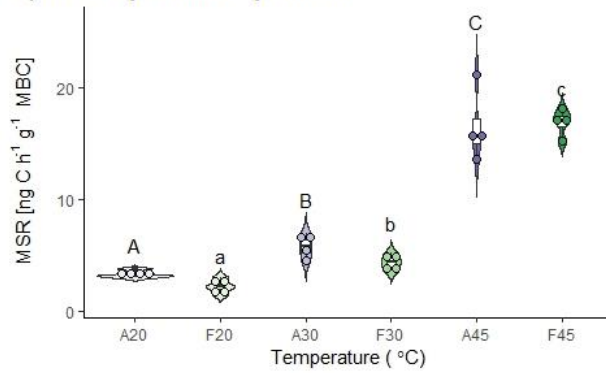
317   Carbon use efficiency decreased with increasing temperature in forest soil, while it stayed constant in

318   agricultural soils (Figure 3 e). This finding adds to an ever-growing list of ambiguous reactions of CUE to

319   soil temperature (e.g. (Hagerty et al., 2014; Schnecker et al., 2023; Simon et al., 2020; Walker et al., 2018))

320   and once again shows, that CUE should be used with caution to infer soil C cycling. As showcased in our

321   experiment, CUE was low at high temperatures in forest soils while growth as well as death rates were

322   high, thereby indicating fast microbial C cycling.

323

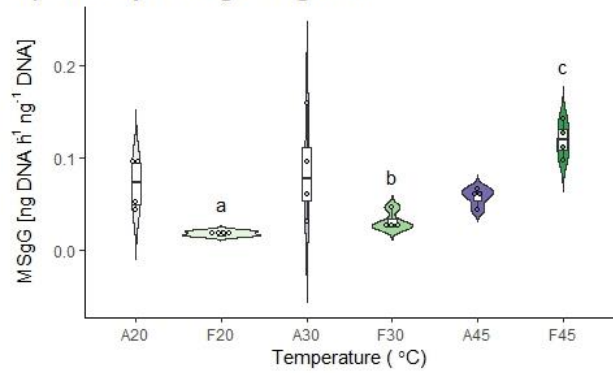
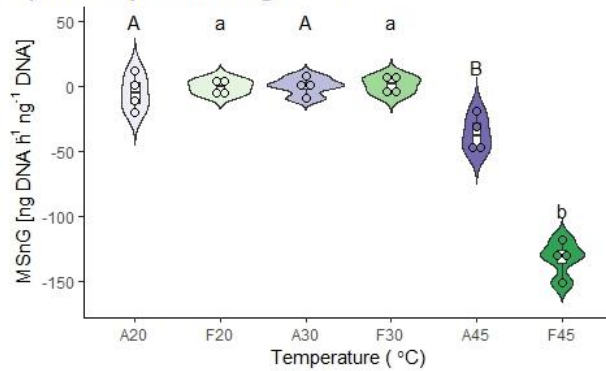
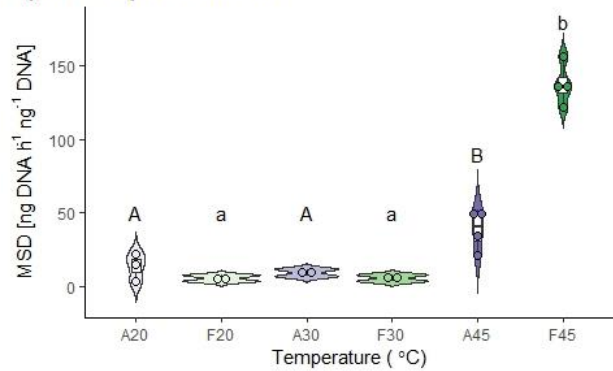
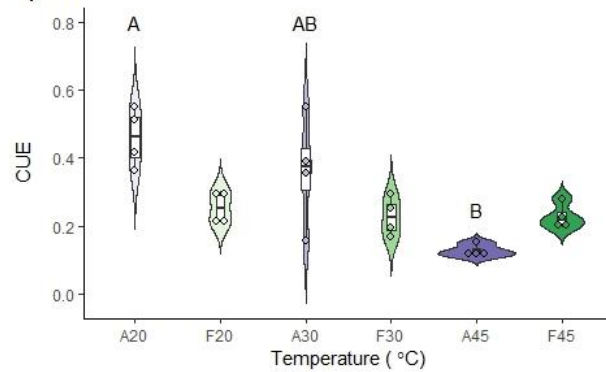



Figure 3. Mass specific microbial process rates and CUE in the two investigated soils after incubation at three different temperatures for 24 h. Results for agricultural soils are shown in purple hues and for forest soils are shown green hues. Statistically significant differences between pool sizes 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.

330   3.4. Caveats and potential of the approach

331   To our knowledge this is the first attempt to combine 18O-labelling of DNA to measure microbial growth

332   and sequential extraction to determine microbial death rates. Both of these methods have their individual

333   caveats and biases. Growth measurements depend on extractions of DNA and MBC. These extractions

334   have varying efficiencies, depending on e.g. soil type, fungal:bacteria ratios and much more (Pold et al.

335   2020). Sequential extraction methods to extract eDNA often have to use rather large amounts of soil to

336   capture the low amounts of eDNA. In out approach we had to accept tradeoffs between how practical our

337   method would be (e.g. using commercially available soil extraction kits) and how precise it is (e.g. using

338   less soil). And while there is room to improve both methods individually and also the combination of them,

339   we think that our approach provides a first step in doing this. We hope that our approach will be picked

340   up by the scientific community and will developed further to improve practicality and precision, especially

341   since we have only taken a first glimpse at its potential. Besides determination of growth and death, this

342   method could be used to study eDNA and iDNA dynamics separately which could help understand

343   exudation but also degradation and recycling of eDNA as well as the formation of microbial necromass as

344   well as extracellular polymeric substance in soils.

345   CONCLUSION

346   In conclusion we here present an approach to determine microbial death rates and turnover by accounting

347   for eDNA dynamics. To our knowledge, this is the first time microbial death rates were investigated in

348   addition to microbial growth rates and net changes in microbial iDNA. With this approach we could show

349   that microbial respiration and microbial growth in the two investigated soils increase with temperature

350   even up to 45 °C, a temperature, that is considered to be way beyond the temperature optimum of most

351   temperate microbial communities. The often observed drop in microbial growth or uptake at high

352   temperatures was however caused by the death of a significant part of the microbial community and

353   higher microbial death rates. While there is certainly room for improving the method and the necessity

354 to investigate its feasibility in other soil systems and under different environmental conditions, we think

355 that this approach will help to shed light on the role of microbial death in soil and a step forward to

356 understand soil C cycling.

357

358 AUTHOR CONTRIBUTION

359 **Jörg Schnecker:** Conceptualization (lead); investigation (supporting); methodology (supporting);

360 supervision (lead); formal analysis (lead); writing – original draft (lead) writing – review and editing (equal).

361 **Theresa Böckle:** investigation (equal); methodology (equal); writing – review and editing (equal). **Julia**

362 **Horak:** investigation (equal); methodology (equal); writing – review and editing (equal). **Victoria Martin:**

363 investigation (supporting); methodology (supporting); writing – review and editing (equal). **Taru Sandén:**

364 resources (equal); writing – review and editing (equal). **Heide Spiegel:** resources (equal); writing – review

365 and editing (equal).

366

367 COMPETING INTERESTS

368 The authors declare that they have no conflict of interest.

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