Microbial carbon use efficiency of litter with distinct C/N ratios in soil at different temperatures, including microbial necromass as growth component

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Abstract
An incubation study was carried out to investigate the effects of litter quality, i.e. 15 N-labelled maize (C/N of 25.5) and Rhodes grass (C/N of 57.8) leaf litter on microbial carbon use efficiency (CUE) and priming effects in a moderate alkaline soil at two different temperatures (15 and 25 °C). CUE values were calculated from the isotopic composition of the particulate organic matter (POM) recovered as an index for the amount of non-decomposed litter. This approach allows the inclusion of microbial necromass growth components in the calculation of CUE values. Additionally, the soil was incubated for 10, 20, and 30 days to determine the optimum incubation period. Soil microbial CUE values of maize and Rhodes grass leaf litter, including microbial necromass C in the calculation of CUE, varied around 0.61, regardless of litter type, temperature, and incubation period. However, the optimum incubation time is between 20 and 30 days, depending on temperature. The strong priming effect on autochthonous soil organic carbon (SOC) mineralization was apparently not caused by N mining, as it was similar for both litter qualities. It most likely resulted from SOC being used by microbial co-metabolism. The litter-induced true priming effect was accompanied by a significant increase in autochthonous POM. The current approach, including microbial necromass as growth component, has been shown to be a strong tool for investigating CUE values and priming effects after application of litter and harvest residues to soil, probably under all environmental conditions.

Keywords Microbial biomass · 15 N/14 N ratio · 13C/12C ratio · CO2 mineralization · Particulate organic matter · Microbial necromass

Introduction
Soil microorganisms and soil organic matter (SOM) are central components determining soil fertility (Joergensen 2010), i.e. the ability to maintain key ecological soil functions, such as decomposition of plant residues and provision of nutrients for plant growth, and mediation of soil organic carbon (SOC) sequestration. Soil microorganisms maintain most enzymatic processes in soil and store energy and nutrients in their biomass (Jenkinson and Ladd 1981). The turnover of the soil microbial biomass is controlled by temperature (Joergensen et al. 1990). Consequently, the global change-induced rise in temperature will increase this turnover (Hagerty et al. 2014), which may have consequences for SOM stocks throughout the world (Zhang et al. 2020). SOC sequestration can be promoted by increasing C input or by decreasing microbial turnover, which is the product of maintenance coefficient × C use efficiency (CUE).

CUE values are often calculated as MBC growth / MBC uptake (Manzoni et al. 2012; Geyer et al. 2019). In this approach, MBC growth is usually measured as the increase in isotopically labelled substrate-derived C incorporated into the microbial biomass, while MBC uptake is the sum of substrate-derived MBC and CO2C (Manzoni et al. 2012). It should be noted in this context that CUE values are only valid for an active microbial community (Blagodatskaya and Kuzyakov 2013), growing on freshly added substrate. Thus, CUE values are not an appropriate index for the vast majority of dormant, i.e. non-growing microbial communities surviving on the use of humified SOM without growth.

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(Joergensen and Wichern 2018). Consequently, CUE determination for the microbial use of SOC as proposed by Spohn et al. (2016a, 2016b) may not be valid.

The approach of Manzoni et al. (2012) for determining CUE has already been questioned by Joergensen and Wichern (2018), who asked for all microbial metabolites to be included in the CUE calculation. Research has been concentrated on CUE of $^{13}$C and $^{14}$C labelled sugars, mainly glucose (Bardgett and Saggar 1994; Bremer and Kuikman 1994), and other simple organic components (Jones et al. 2018), which are completely metabolized within a short incubation time after addition (Joergensen and Wichern 2018). Less information is available on the CUE of complex plant residues (Muhammad et al. 2006; Rottmann et al. 2010; Sauvadet et al. 2018), which can be determined by the recovery of added substrate as particulate organic matter (Magid and Kjærgaard 2001). This approach is often combined with the difference in $\delta^{13}$C of C4 plants and SOC mainly originating from C3 plants (Ryan and Aravena 1994; Balesdent and Mariotti 1996; Faust et al. 2019).

CUE values calculated according to Manzoni et al. (2012) increase with clay content (Li et al. 2021) and decrease with temperature (Öquist et al. 2017; Li et al. 2019; Qiao et al. 2019; Ye et al. 2019). In contrast, the C/N ratio of the substrate added had inconsistent effects on CUE values (Oliver et al. 2021), calculated according to Manzoni et al. (2012). Positive (Oliver et al. 2021; Soares and Rousk 2019) and negative relationships (Manzoni et al. 2017; Xiao et al. 2021) have been reported between the litter C/N ratio and CUE values. However, Lukas et al. (2019) and Schroeder et al. (2020) did not find any effect of soil type on the CUE of maize ($Zea mays$ L.) and finger millet ($Eleusine coracana$ Gaertn.) litter, respectively, using the POM recovery approach (Joergensen and Wichern 2018). Schroeder et al. (2020) also observed no effect of N fertilization on CUE, but a strong true priming effect on SOM (Kuzyakov et al. 2000), apparently not caused by N mining, as N fertilization affected neither CUE nor SOM priming. This might be different for plant residues differing in their C/N ratio, especially at different temperatures, which has not been tested by the POM recovery approach (Joergensen and Wichern 2018) until now. The amendment of soil with N poor organic matter repeatedly reduced CUE and increased true SOM priming (Sauvadet et al. 2018; Xiao et al. 2021), whereas increasing nutrient inputs increased microbial CUE (Mo et al. 2021).

The central objective of the current incubation study was to investigate the following hypotheses: (1) The CUE of maize and Rhodes grass litter is higher at 15 °C than at 25 °C. (2) The CUE of N-rich maize litter (C/N = 25.5) is higher than that of N-poor Rhodes grass litter (C/N = 57.8). (3) N-rich maize litter caused a smaller true priming effect than N-poor Rhodes grass litter. Schroeder et al. (2020) also did not find any effect of incubation time, although the metabolization of added complex substrates is usually incomplete to different extents at specific sampling dates (Faust et al. 2019). However, incubation times of 7 and 42 days used by Schroeder et al. (2020) may still not be the optimum for estimating CUE values of plant residues, which requires additional experimental efforts.

### Materials and methods

#### Sites, soils, and litter

The soil used for the experiment was taken as a field moist bulk sample at 0–20 cm depth on 21 April 2020 from an arable site cropped with pumpkins ($Curcubita$ sp. L.) in the floodplain of the river Werra (North Hessia, Germany) and recently limed. The site was located in Ellershausen/Bad Sooden-Allendorf (150 m asl, 51° 17′ N, 9° 59′ E). The long-term annual mean temperature is 8.3 °C and the annual mean precipitation is 550 mm. The site had been cropped with different organic field vegetables since 2012 (Simon Schöne and Jürgen Reulein), after long-term arable management according to “Bioland” regulations since 1981 (Scheller and Joergensen 2008). Tillage was carried out with a mouldboard plough at 0–20 cm depth after harvest followed by a shallow stubble treatment with a harrow cultivator. The soil can be classified as Eutric Fluvisol (IUSS Working Group WRB 2015).

The soil was sieved (<2 mm), pre-incubated at room temperature for 2 weeks, adjusted to 50% water holding capacity, homogenized, and stored in polyethylene bags at 4 °C until the experiment started. A sub-sample of dried soil was finely ground for chemical analysis. Soil pH was measured electrochemically at a soil to water-ratio of 1 to 2.5. Water holding capacity was determined according to Wilke (2005) as described by Schroeder et al. (2020). Total C and total N were determined in soil and litter by gas chromatography after combustion using a Vario Max CN analyser (Elementar, Hanau, Germany). SOC was calculated as the difference of total C minus carbonate C (Table 1). Soil texture, carbonate C, and soil organic C (SOC) were measured as described by Muhammad et al. (2006).

$^{15}$N labelled maize ($Zea mays$ L.) leaf litter was obtained from an experimental field belonging to the University of Kassel in Neu Eichenberg, North Hessia (Table 2). Plants were labelled after emerging of the 5th leaf with $^{15}$NH$_4$NO$_3$ (10%) applied at a rate of 130 kg N ha$^{-1}$ (Wachendorf et al. 2020). Above-ground maize biomass was harvested at the beginning of tassel emergence 62 days after sowing. $^{15}$N labelled Rhodes grass (Chloris gayana Kunth) litter was produced by foliar application of $^{15}$N labelled urea at the Agricultural Experiment Station of Sultan Qaboos University.
The aboveground grass biomass was harvested 35 days after sowing and sun dried for two days.

### Experimental treatments

The study was designed as a two-factorial experiment with the following factors in quadruplicate: (1) litter addition (maize leaf litter and Rhodes grass litter), and non-amended control, and (2) temperature [15 and 25 °C]. For each treatment, 100-g soil at 50% water holding capacity were weighed in 1.0 L incubation vessels and incubated for 30 days in the dark. The litter (< 2 mm) was thoroughly mixed with the soil in the amendment treatments immediately before the incubation was started. For measuring particulate organic matter (POM), moist soil of 100 g per replicate was mixed with 1 mg litter C g⁻¹ soil and transferred into another 1-L plastic incubation vessel, incubated along with the other vessel.

### CO₂ evolution

The CO₂ evolved was trapped during the incubation in 0.25 M NaOH solution, which was changed after 2, 5, 7, 10, 20, and 30 days. The trapped CO₂ was precipitated with 5 ml of a saturated SrCl₂ solution and stored under CO₂ free atmosphere. Then, the NaOH not consumed was back titrated with 0.25 M HCl, using a TITRONIC 500 system (Xylem Analytics, Weilheim, Germany) to the transition point of phenolphthalein at a pH of 8.3. For the determination of δ¹³C values, SrCO₃ samples from the titration events day 2, 5, 10, and 30 were centrifuged (3000 g for 10 min at 20 °C), washed three times with H₂O to remove excess ions and freeze dried before analysis. The δ¹³C values of the titration events day 7 and 10 were estimated by linear interpolation between the neighbouring sampling days.

### Microbial biomass

Microbial biomass C (MBC) and N (MBN) were determined in soil by fumigation extraction (Brookes et al. 1985; Vance et al. 1987) at days 10, 20, and 30. For reducing inorganic N background, 15-g moist soil were pre-extracted for 30 min by oscillating shaking at 200 rev min⁻¹ with 40 ml 0.05 M K₂SO₄ (Widmer et al. 1989; Mueller et al. 1992). Then, non-fumigated and fumigated 5-g portions were extracted for 30 min by oscillation shaking at 200 rev min⁻¹ with 20 ml 0.05 M K₂SO₄ (Potthoff et al. 2003), centrifuged (3000 g for 10 min at 10 °C), filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany), and stored at -18 °C before analysis. Organic C and total N in the extracts were determined using a Multi N/C 2100S analyser (Analytik Jena, Germany). MBC was \( \frac{E_C}{k_{EC}} \), where \( E_C = \text{(organic C extracted from fumigated soils)} \) – (organic C extracted from non-fumigated soils) and \( k_{EC} = 0.45 \) (Wu et al. 2018).

| Table 1 | Soil properties of control treatment at day 0 of the incubation experiment; mean ± standard deviation (n = 4) |
|---------|--------------------------------------------------------------------------------------------------|
| Soil pH (H₂O) | 8.48 ± 0.09 |
| SOC (mg g⁻¹ soil) | 8.43 ± 1.93 |
| Carbonate-C (mg g⁻¹ soil) | 1.55 ± 0.13 |
| N_TOT (mg g⁻¹ soil) | 0.80 ± 0.20 |
| SOC/N_TOT | 12.0 ± 0.8 |
| Sand (%) | 57.5 ± 5.5 |
| Silt (%) | 28.1 ± 7.1 |
| Clay (%) | 14.4 ± 1.7 |
| δ¹³C without carbonate (‰) | -26.44 ± 0.38 |
| δ¹⁵N (atom%) | 0.369 ± 0.0002 |
| MBC (µg g⁻¹ soil at day 0) | 164 ± 25 |
| MBN (µg g⁻¹ soil at day 0) | 33.9 ± 6.7 |
| MBAS-C/N | 4.8 ± 0.3 |
| POM (µg g⁻¹ soil at day 0) | 241 ± 10 |
| POMC (mg g⁻¹ dry matter) | 297.3 ± 28.3 |
| POMN (mg g⁻¹ dry matter) | 13.7 ± 1.5 |
| POM/C/N | 21.9 ± 2.3 |
| POM-δ¹³C (‰) | -29.49 ± 0.46 |
| POM-δ¹⁵N (atom%) | 0.369 ± 0.0003 |
| POMC (µg g⁻¹ soil at day 0) | 73.2 ± 11.9 |
| POMN (µg g⁻¹ soil at day 0) | 3.4 ± 0.6 |

| Table 2 | Properties of maize and Rhodes grass litter added to soil and of soil and POM of both treatments at day 0; mean ± standard deviation (n = 4) |
|---------|-----------------------------------------------------------|
| Maize | Rhodes grass |
| C_TOT (mg g⁻¹ dry matter) | 416.3 ± 4.3 | 433.5 ± 2.8 |
| N_TOT (mg g⁻¹ dry matter) | 16.6 ± 2.2 | 7.5 ± 0.3 |
| C/N_TOT | 25.4 ± 3.6 | 57.8 ± 2.7 |
| δ¹³C (‰) | -12.48 ± 0.19 | -13.80 ± 0.02 |
| δ¹⁵N (atom%) | 5.052 ± 0.309 | 0.388 ± 0.028 |
| MBC_LD (µg g⁻¹ soil at day 0) | 30.3 ± 16.6 | 21.2 ± 10.2 |
| MBN_LD (µg g⁻¹ soil at day 0) | 5.5 ± 4.3 | NA |
| POMC (% added at day 0) | 99.8 ± 17.1 | 93.2 ± 10.3 |
| POMN (% added at day 0) | 59.6 ± 7.7 | 74.4 ± 7.0 |
| POMC/TOT (mg g⁻¹ dry matter) | 328.2 ± 29.0 | 403.5 ± 26.1 |
| POMN/TOT (mg g⁻¹ dry matter) | 5.4 ± 0.5 | 6.4 ± 0.5 |
| POM-C/N_TOT | 61.1 ± 2.3 | 63.6 ± 6.0 |
| POM-δ¹⁵N (atom%) | 3.161 ± 0.392 | 0.383 ± 0.001 |
| POMN_LD (µg g⁻¹ soil at day 0) | 13.3 ± 3.2 | 11.5 ± 0.9 |
| POM-C/N_LD | 76.7 ± 9.1 | 82.4 ± 14.6 |

| MB, microbial biomass; LD, litter-derived; POM, particulate organic matter; NA, not applicable |
MBN was \( E_N / k_{EN} \), where \( E_N = (\text{total N extracted from fumigated soils}) - (\text{total N extracted from non-fumigated soils}) \) and \( k_{EN} = 0.54 \) (Brookes et al. 1985). About 14 ml of the extracts was freeze-dried for \( \delta^{13}C \) and \( \delta^{15}N \) analysis (Alpha 1–4 LD plus, Christ, Osterode, Germany).

### Particulate organic matter

POM was recovered as described by Magid and Jørggaard (2001) at day 0, 10, 20, and 30 of incubation. Briefly, 100-g moist soil were dispersed in 400 ml 5% NaCl solution, stirred by hand and allowed to stand overnight (Muhammad et al. 2006). Samples were transferred onto a 400-μm sieve and washed with tap water. Soil aggregates were pushed through the sieve during the washing process. A 5% NaCl solution was added to the washed soil and the procedure was repeated until organic particles were no longer visible in the mineral fraction and the washing water was clear. Finally, POM was transferred into crucibles, dried at 60 °C, and weighed.

### C and N analyses and calculations

For analyses of total C, \( \delta^{13}C \), total N, and \( \delta^{15}N \), samples were dried for 24 h at 105 °C (soil) and 60 °C (POM and litter), respectively, and ball milled. Carbonate in the soil sample was removed by addition of 1 M HCl, which was washed out before \( \delta^{13}C \) measurement. The \( \delta^{13}C \) and \( \delta^{15}N \) in \( K_2SO_4 \) extracts as well as \( \delta^{13}C \) of SrCO\(_3\) were analysed in freeze-dried samples. Isotopes were measured by elemental analyser – isotope ratio mass spectrometry. The fraction of litter-derived C in the \( K_2SO_4 \) extracts of fumigated and non-fumigated samples, in \( CO_2\) as well as in POMC, was calculated for each individual replicate of all treatments from the \( \delta^{13}C \) data according to a two pool-mixing model (Balesdent and Mariotti 1996) using the following equation:

\[
\text{Litter – derived C fraction} = \left( \frac{\delta^{13}C_{\text{sample}} - \delta^{13}C_{\text{control}}}{\delta^{13}C_{\text{litter}} - \delta^{13}C_{\text{control}}} \right),
\]

where \( \delta^{13}C_{\text{sample}} \) represents the litter treatments, \( \delta^{13}C_{\text{control}} \) the non-amended treatments at the respective sampling days 10, 20, and 30. The fraction of litter-derived \( ^{15}N \) in POMN as well as in the \( K_2SO_4 \) extracts of fumigated and non-fumigated samples was calculated using the following equation (Dijkstra et al. 2006; Zareitalabad et al. 2010):

\[
\text{Litter-derived N fraction (%)} = \left( \frac{^{15}N(\text{atom}\%_{\text{sample}}) - ^{15}N(\text{atom}\%_{\text{control}})}{^{15}N(\text{atom}\%) - ^{15}N(\text{atom}\%_{\text{control}})} \right) \times 100.
\]

\( \delta^{15}N_{\text{sample}} \) represents the litter treatments, \( \delta^{15}N_{\text{control}} \) the non-amended control treatment at the respective sampling days in \( \delta^{15}N \).

### CUE calculations

CUE values of maize and Rhodes litter were calculated at sampling days 10, 20, and 30 according to Joergensen and Wichern (2018) and Schroeder et al. (2020), considering all microbial metabolites, i.e. litter-derived (LD) microbial necromass C (\( MNC_{LD} \)):

\[
\text{CUE} = \left( MBC_{LD} + MNC_{LD} \right) / \left( 100 - POMC_{LD} \right)
\]

\[
MNC_{LD} = 100 - POMC_{LD} - CO_2C_{LD} - MBC_{LD}
\]

In this case:

\[
\text{CUE} = \left( 100 - POMC_{LD} - CO_2C_{LD} \right) / \left( 100 - POMC_{LD} \right)
\]

Litter-derived C in MBC, microbial necromass, POM, and \( CO_2\) are abbreviated as \( MBC_{LD} \), \( MNCLD \), \( POMC_{LD} \), and \( CO_2C_{LD} \). Microbial necromass C (Liang et al. 2020) or microbial residues (Joergensen and Wichern 2018) embraces all freshly synthesized microbial non-biomass metabolites that leave the cells, such as exo-enzymes (Burns et al. 2013), extracellular polymeric substances (Redmile-Gordon et al. 2014), e.g. the glomalin-related soil protein (Wright and Upadhyaya 1996; Spohn and Giani 2011; Li et al. 2012), other secondary metabolites, e.g. antibiotics (Boruta 2018; Keller 2019), and dead tissue remains (Joergensen and Wichern 2018). In addition, CUE of litter at sampling days 10, 20, and 30 was calculated in the way proposed by Manzoni et al. (2012). This calculation approach solely considers the incorporation of litter-derived C into the microbial biomass and is thus abbreviated as \( \text{CUE}_{MB} \):

\[
\text{CUE}_{MB} = MBC_{LD} / \left( CO_2C_{LD} + MBC_{LD} \right)
\]

All litter-derived fractions were considered in the calculations as % of the added substrate.

### Statistical analysis

Data are presented as arithmetic means on an oven-dry weight basis. All data used for ANOVA analysis exhibited normality of residuals and homogeneity of variances according to the Shapiro–Wilks test and Levene test, respectively. The significance of temperature and litter effects was tested using a 2-way ANOVA. The significance of changes between days 10, 20, and 30 was tested using repeated measures. All statistical analyses were carried out using SigmaPlot 13.0 (Systat, San José, USA).
Results

Litter mineralization and incorporation into the microbial biomass

Litter was mineralized at a constant CO₂ evolution rate of 5.1 µg C g⁻¹ soil d⁻¹ over 30 days’ incubation at 15 °C, without significant differences between maize and Rhodes grass litter (Table 3). In contrast, the respective average litter-derived CO₂ evolution rate at 25 °C declined from 12.1 µg C g⁻¹ soil d⁻¹ during the first 10 days to 5.2 µg C g⁻¹ soil d⁻¹ from day 20 to 30 (data not shown). Temperature increased cumulative litter-derived CO₂C, whereas litter type had no effect (Table 3).

Litter-derived MBC varied around 48 µg  g⁻¹ soil during the incubation, approximately 85% higher than the mean initial values at day 0 (Table 2). The differences between litter type, temperature, and sampling day were all insignificant, neglecting the significant litter × temperature interaction at day 30 (Table 3). Rhodes grass derived MBN could not be calculated throughout the incubation. Maize litter-derived MBN was 6.0 µg  g⁻¹ soil at day 10 and significantly (P < 0.05) declined to 2.0 µg  g⁻¹ soil at day 30, without clear temperature effects (data not shown).

The mean recovery of maize and Rhodes grass litter-derived POMC was 63% at 15 °C and 50% at 25 °C at the end of the experiment. Higher temperature led to a decrease in POMC recovery, the effect being greater for Rhodes grass at day 30. The recovery of litter-derived POMN varied around 10.7 µg  g⁻¹ soil for maize and around 8.8 µg  g⁻¹ soil for Rhodes grass litter (data not shown). In contrast to POMC, the recovery of litter-derived POMN was not affected by temperature and not clearly influenced by incubation period.

Litter effects on dynamics of autochthonous SOM

The CO₂ evolution was 54% higher at 25 than at 15 °C throughout the 30 days’ incubation in the non-amended control treatment (Table 4). Litter application led to a mean increase of 2.5 µg autochthonous SOM-derived CO₂C g⁻¹ soil d⁻¹ at 15 °C and of 2.7 µg CO₂C g⁻¹ soil d⁻¹ at 25 °C. Neither the difference in litter quality between maize and Rhodes grass nor the 10 °C increase in temperature had strong effects on the additional release of autochthonous SOM-derived CO₂C.

MBCAS varied around 155 µg  g⁻¹ soil at day 10 and 187 µg  g⁻¹ soil at days 20 and 30, without any litter or temperature effects. The C/N ratio of the whole microbial biomass continuously increased from 5.6 at day 10, to 6.7 at day 20 and finally 7.5 at day 30, again without any litter effect throughout the incubation and only a small temperature effect at day 10.

Litter application increased the content of autochthonous SOM-derived POM by 58 µg  g⁻¹ soil, averaging all sampling dates. In contrast, POM in the control treatment increased later by 28 µg  g⁻¹ soil until day 30 (Fig. 1a). No litter type or temperature effects were observed on this increase, excluding the control from statistical analysis. The POM-C/N ratio constantly varied in the control treatment around 22 (Fig. 1b). The total POM-C/N ratio in the sum of autochthonous SOM-derived POM and litter-derived POM decreased from 47 at day 10 to 36 at day 30 in the maize treatment and from 60 at day 10 to 42 at day 30 in the Rhodes grass treatment. This decline

Table 3 Cumulative litter-derived ΣCO₂C, litter-derived MBC, and recovery of litter-derived POMC in soils amended with maize and Rhodes grass litter after 10, 20, and 30 days of incubation at 15 or 25 °C, probability values of a two-way ANOVA, using litter and temperature as factors

| Treatment          | ΣCO₂CLD (µg g⁻¹ soil) | MBCLD (µg g⁻¹ soil) | POMCLD (µg g⁻¹ soil) |
|--------------------|----------------------|---------------------|----------------------|
|                    | Day 10 | Day 20 | Day 30 | Day 10 | Day 20 | Day 30 | Day 10 | Day 20 | Day 30 |
| 15 °C maize        |        |        |        |        |        |        |        |        |        |
|                    | 54     | 102    | 140    | 42     | 53     | 59     | 870    | 720    | 600    |
| 25 °C maize        | 133    | 188    | 216    | 44     | 47     | 23     | 680    | 580    | 560    |
| 15 °C Rhodes grass | 45     | 109    | 163    | 59     | 62     | 47     | 870    | 710    | 660    |
| 25 °C Rhodes grass | 109    | 167    | 216    | 24     | 76     | 67     | 630    | 560    | 440    |
| Probability values |        |        |        |        |        |        |        |        |        |
| Litter type        | NS     | NS     | NS     | NS     | NS     | NS     | NS     | NS     | NS     |
| Temperature        | <0.01  | NS     | NS     | NS     | NS     | <0.01  | NS     | NS     | NS     |
| Litter × temperature| NS    | NS     | NS     | NS     | <0.01  | NS     | NS     | NS     | <0.01  |
| CV (± %)           | 26     | 22     | 29     | 49     | 38     | 26     | 10     | 18     | 10     |

CV, mean coefficient of variation between replicates (n = 4); NS, not significant; LD, litter-derived; MB, microbial biomass
was significantly stronger at 25 °C with a mean of 50 than at 15 °C with a mean of 45 in the litter treatments.

**Carbon use efficiency**

The CUE values varied around 0.61 and were not significantly affected by litter quality, temperature, and sampling day (Table 5). In contrast, the classical CUE_{MB} approach declined with increasing incubation time from 0.50 to 0.26 at 15 °C and with increasing temperature from a mean of 0.37 at 15 °C to a mean of 0.22 at 25 °C. However, the differences between incubation times were less pronounced at 25 °C, leading to a significant temperature × day interaction.

**Discussion**

**CUE calculations**

A mean CUE of 0.61 for maize and Rhodes grass litter is in the range of 0.55 and 0.63 obtained by Lukas et al. (2019) in the field and by Schroeder et al. (2020) in the laboratory for soils with pH values of 8.2 and 6.7, respectively, considering microbial necromass C as a microbial growth component. This is another strong indication that soil microorganisms are able to use organic substrates that enter soil nearly as efficiently as glucose (Joergensen and Wichern 2018). The absence of significant differences in CUE values of litter between day 10, 20, and 30 is remarkable. From day 10 to 20, MBC increased slightly, whereas microbial necromass C showed a strong linear increase with time, as similarly observed by Geyer et al. (2020). CUE values of soil microorganisms are apparently not strongly affected by temperature or C/N ratio of the litter, as shown by the current data, but also not by N fertilization or soil type, as demonstrated by Schroeder et al. (2020). The most likely reason for this observation during litter decomposition is the transfer of microbial biomass to necromass (Geyer et al. 2020), demonstrating the importance of these components for CUE calculations.

In agreement with our current data, the metabolic tracer probing method (Dijkstra et al. 2011a, 2011b) did not reveal temperature effects on CUE of glucose and pyruvate amendments, which varied around 0.72, in an even larger temperature range from 5 to 20 °C (Hagerty et al. 2014). However, the possibility of strong effects of serious nutrient and micro-nutrient limitations on CUE of plant residues cannot be excluded (Hemkemeyer et al. 2021), as suggested by experiments with substrates free of N and P (Joergensen and Raubuch 2002; Hartmann and Richardson 2013). Also, extremely low soil pH might reduce CUE of plant residues, using the current POM recovery approach, especially in combination with aluminium toxicity (Jones et al. 2019).

**Methodological remarks**

The temperature increase from 15 to 25 °C did not affect CUE, but increased decomposition rate of the litter added. Consequently, a 10-day incubation period at 15 °C might be too short for soils with low turnover rates amended with recalcitrant and N-poor plant residues or plant residues that need more time to be colonized by decomposers (Eck et al. 2015). In this case, it is possible that not enough POMC was lost within 10 days to obtain a significant decline in comparison with initial values. Another problem is that only small amounts of microbial necromass C were formed.

Table 4 Cumulative \( \Sigma \)CO₂CAS, MBCAS, and the MB_{TOT}-C/N ratio in soils amended with maize and Rhodes grass litter after 10, 20, and 30 days of incubation at 15 or 25 °C; probability values of a two-way ANOVA, using litter and temperature as factors including the control

| Treatment          | \( \Sigma \)CO₂CAS (µg g⁻¹ soil) | MBCAS (µg g⁻¹ soil) | MB_{TOT}-C/N |
|--------------------|----------------------------------|---------------------|--------------|
|                    | Day 10  | Day 20  | Day 30  | Day 10  | Day 20  | Day 30  | Day 10  | Day 20  | Day 30  |
| 15 °C control      | 60 101  | 139    |         | 172 181 | 186    |         | 5.9 6.9 | 7.6    |
| 25 °C control      | 91 156  | 215    |         | 144 173 | 179    |         | 5.6 5.7 | 7.2    |
| 15 °C maize        | 79 143  | 206    |         | 143 185 | 185    |         | 5.8 6.8 | 7.8    |
| 25 °C maize        | 140 223 | 304    |         | 163 199 | 182    |         | 5.4 6.6 | 7.4    |
| 15 °C Rhodes grass | 85 145  | 201    |         | 154 190 | 198    |         | 5.6 7.0 | 7.1    |
| 25 °C Rhodes grass | 152 235 | 335    |         | 153 203 | 182    |         | 5.3 7.2 | 7.1    |

Probability values

| Litter type        | <0.01 | <0.01 | <0.01 | NS  | NS  | NS  | NS  | NS  | NS  |
|--------------------|-------|-------|-------|-----|-----|-----|-----|-----|-----|
| Temperature        | <0.01 | <0.01 | <0.01 | NS  | NS  | NS  | 0.05| NS  | NS  |
| Litter × temperature| 0.02  | NS    | NS    | NS  | NS  | NS  | NS  | NS  | NS  |
| CV (± %)           | 10 8.2| 12    | 10 17 | 8.5 | 7.1 | 6.0 | 7.4 |     |     |

CV, mean coefficient of variation between replicates (n=4); NS, not significant; AS, autochthonous soil organic matter-derived; MB, microbial biomass; TOT, total.
within 10 days. For these reasons, 20- or 30-day incubation periods are more appropriate for determining CUE values of complex plant residues in most cases.

The assumption that all litter added recovered as POMC is equivalent to non-decomposed plant residues is not fully true. The litter is already colonized by microorganisms during maturation of the plants in the field or greenhouse (Pothoff et al. 2008; Scheller and Joergensen 2008). Microbial colonization increases during incubation in soil (Rottmann et al. 2011). However, up to 2% microbial biomass attributed to POM might be counterbalanced by the small amounts of substrate lost during POM fractionation, as indicated by the high day-0 recovery of 95% and more observed by Schroeder et al. (2020) and in the current study. Also, the effects of the initial microbial colonization of plant surfaces on CUE and CUE calculations need further experimental evaluation.

In contrast to POMC, the recovery of litter-derived POMN cannot be used to determine N use efficiency, because roughly 30% of maize litter-derived N were lost during POM recovery, whereas the N labelling of the Rhodes grass was insufficient to measure incorporation into MBN. The litter N remaining after POM extraction remains virtually stable, possibly being incorporated into the decomposing microbial community. This is also indicated by the general decrease in the total POM-C/N ratio of the litter treatments (Fig. 1b). Such a decrease is typical for decomposing litter (Joergensen and Meyer 1990) and indicates an N transfer from soil to litter (Rottmann et al. 2010, 2011). The decrease in maize litter-derived MBN suggests also a reverse N transfer from litter to soil during incubation. A large percentage of newly formed MBN may have been transferred to the microbial necromass fraction after microbial colonization of the litter by soil microorganisms. It would be possible to detect this transfer to microbial necromass by increases in 13C- or 15 N-labelled amino sugars in SOM and POM (Joergensen 2018).

**Litter induced priming of SOM mineralization**

The current increase in CO₂C evolution derived from autochthonous SOM mineralization after maize and Rhodes grass litter addition is a strong true positive priming effect (Kuzyakov et al. 2000). This was apparently not caused by N mining, as the different C/N ratio of both litter types did not affect the priming response. An increase in autochthonous POMC has been observed in the current study after adding plant residues, which might be caused by adsorption of autochthonous SOM by molecular interactions, such as van der Waals-forces. A less likely reason might the transfer of autochthonous microbial biomass and metabolites during colonization of the freshly added maize and Rhodes grass litter. The increase in autochthonous POMC due to litter application cannot by fully explained by the current data and, thus, needs further experimental evaluation.

However, N mining might be still an important reason for true priming effects after adding simple low molecular weight substrates, such as glucose (Dijkstra et al. 2013; Mason-Jones et al. 2018; Tian et al. 2019), but not after addition of complex plant residues (Schroeder et al. 2020). Priming mechanisms strongly differ between simple amendments or complex plant residues (Wu et al. 1993; Finley et al. 2018; Hicks et al. 2019).
It has been described earlier that a large part of litter mineralization in soil is usually carried out by litter surface colonizing microorganisms (Flessa et al. 2002; Potthoff et al. 2008). In the current study, the strong positive priming effect suggests that the extracellular enzymes produced by litter decomposing saprotrophic fungi were most likely also able to break down SOM by co-metabolism (Scheller and Joergensen 2008; Maynard et al. 2017; Perveen et al. 2019).

Conclusions

Soil microbial carbon use efficiency (CUE) of maize and Rhodes grass leaf litter, including microbial necromass C, varied around 0.61, regardless of litter quality (C/N ratios of 25.5 and 57.8), temperature (15 and 25 °C), and incubation period (10, 20, or 30 days). However, the optimum incubation time is presumably between 20 and 30 days, depending on temperature. The strong priming effect on autochthonous SOC mineralization was apparently not caused by N mining but resulted from soil organic matter, being used as microbial co-metabolism after adding easily available maize and Rhodes grass leaf litter. Future research should also test the effects of plant residues on CUE values in acidic soils with serious nutrient and micro-nutrient limitations, especially also under field conditions. Also, the increase in autochthonous particulate organic matter C due to litter application needs further evaluation. The current approach, including microbial necromass as growth component, has been shown to be a strong tool for investigating CUE values and priming effects after application of litter and harvest residues to soil, probably under all environmental conditions.

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Declarations

Conflict of interest

The authors declare no competing interests.

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