Seasonal dynamics of soil microbial growth, respiration, biomass, and carbon use efficiency in temperate soils

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ABSTRACT

Soil microbial growth, respiration, and carbon (C) use efficiency (CUE) are essential parameters to understand, describe and model the soil carbon cycle. While seasonal dynamics of microbial respiration are well studied, little is known about how microbial growth and CUE change over the course of a year, especially outside the plant growing season. In this study, we measured soil microbial respiration, gross growth via ^{18}O incorporation into DNA, and biomass in an agricultural field and a deciduous forest 16 times over the course of two years. We sampled soils to a depth of 5 cm from plots at which harvest residues or leaf litter remained on the plot or was removed. We observed strong seasonal variations of microbial respiration, growth, and biomass. All these microbial parameters were significantly higher at the forest site, which contained 4.3 % organic C compared to the agricultural site with 0.9 % organic C. CUE also varied strongly (0.1 to 0.7) but was overall significantly higher at the agricultural site compared to the forest site. We found that microbial respiration and to a lesser extent microbial growth followed the seasonal dynamics of soil temperature. Microbial growth was further affected by the presence of plants in the agricultural system or foliage in the forest. At low temperatures in winter, both microbial respiration and gross growth showed the lowest rates, whereas CUE (calculated from both respiration and growth) showed amongst the highest values determined during the two years, due to the higher temperature sensitivity of microbial respiration. Microbial biomass C strongly increased in winter. Surprisingly, this winter peak was not connected to high microbial growth or an increase in DNA content. This suggests that microorganisms accumulated C and N, potentially in the form of osmo- or cryoprotectants or increased in cell size but did not divide. This microbial winter bloom and following decline, where C is released from microbial biomass and freely available, might constitute a highly dynamic time in the annual C cycle in temperate soil systems. Highly variable CUE, which was observed in our study, and the fact that CUE is calculated from independently controlled microbial respiration and microbial growth, ask for great caution when CUE is used to describe soil microbial physiology, soil C dynamics or C sequestration. Instead, microbial respiration, microbial growth, and microbial biomass C should be investigated individually in combination to better understand the soil C cycle.

1. Introduction

Soil microorganisms are at the center of the terrestrial carbon (C) cycle. They degrade and take up plant-derived organic matter, use it to produce energy, and convert it to microbial biomass. Upon microbial death, C in the form of microbial necromass can be stabilized on soil

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minerals or in soil aggregates (Miltner et al., 2011; Kallenbach et al., 2016; Liang et al., 2019). The interplay of microbial growth and respiration is described by microbial C use efficiency (CUE) which is calculated as the fraction of microbial growth divided by microbial C uptake (Manzoni et al., 2012). As CUE is potentially informative of the first step in microbe-driven C sequestration, it is regularly used as a key parameter in soil C models and concepts of soil C cycling (Cotrufo et al., 2013; Poeplau et al., 2019; Pold et al., 2019; Sokol et al., 2019).

Microbial respiration and growth and consequently CUE are dependent on numerous factors influencing microbial life in soil. Microbial respiration, which has been extensively studied in different soil systems has been shown to increase with short-term increasing temperature (Lloyd and Taylor, 1994; Fierer et al., 2006), water content (Davidson et al., 1998), C availability (Wang et al., 2003) and microbial biomass (Colman and Schimel, 2013). Similarly, growth increases with short-term increases in temperature (Pietikäinen et al., 2005; Apple et al., 2006; Cruz-Paredes et al., 2021) but CUE can decrease in the short-term at higher temperatures (Frey et al., 2013; Schindlbacher et al., 2015). Interestingly, most field studies find that warming has little effect on CUE while respiration and growth rates are strongly increased (Hagerty et al., 2014; Walker et al., 2018; Simon et al., 2020). Soil water content has been shown to be positively correlated with CUE (Zheng et al., 2019). Very high soil water contents can however lead to reductions in microbial activities due to oxygen limitation and drought has been shown to severely reduce microbial respiration, growth, and CUE (Canarini et al., 2020). As soil microorganisms are generally considered to be limited in C (Soong et al., 2020), substrate availability as well as quality are other factors that influence microbial respiration, growth, and CUE (Frey et al., 2013; Takriti et al., 2018). Carbon inputs can be used by C-limited microorganisms to fulfill their energy demand through increases in respiration which, as a consequence reduces CUE (Sinsabaugh et al., 2013). Since microorganisms cannot grow on C alone, the availability of other nutrients, in particular nitrogen (N) has an influence on microbial growth and CUE in the way that additional N can lead to an increase in growth and CUE (Sinsabaugh et al., 2013; Spohn et al., 2016b). Furthermore, microbial community composition and diversity have been related to CUE (Domeignoz-Horta et al., 2020). The importance, as well as the interactions, of these individual drivers of microbial respiration, growth, and CUE are still not well understood (Geyer et al., 2016).

As most of the above-mentioned factors, i.e. temperature, moisture, C and N quality and availability vary strongly in temperate soil systems over the course of a year, it is not surprising that microbial parameters also show strong seasonality. Seasonal differences have been shown for microbial respiration (Lloyd and Taylor, 1994; Davidson et al., 1998), microbial community composition (Bardgett et al., 1999; Lazzaro et al., 2015) and microbial enzyme activities (Kaiser et al., 2010). A recent study investigated microbial respiration, growth and CUE in a temperate grassland soil during the growing season covering spring, summer and fall (Simon et al., 2020). The study found a strong effect of season on microbial growth and respiration, which exceeded the response of respiration and growth to elevated temperature or elevated atmospheric CO_2. Combined effects of various potential drivers of microbial processes can lead to unexpected outcomes and often cancel each other out (Castro et al., 2010; Steinweg et al., 2013). This could also be the case for seasonal dynamics of microbial processes and their respective drivers in temperate soil systems. Increased microbial activity due to high temperatures in summer can be, on the one hand, counteracted by low water availability (Davidson et al., 1998) or on the other hand, further increased by high input of easily available root exudates during the peak of plant's photosynthetic activity (Franzluebbers et al., 1994; Curiel Yuste et al., 2007). Litterfall in deciduous forests has been shown to increase soil respiration despite decreasing temperatures (Raich and Tufekcioglu, 2000). In addition to seasonal effects on microbial processes and physiology, management practices, with the aim to optimize plant growth and crop yields, can strongly affect soil microbial activities

and their seasonal dynamics. For instance, the switch from vegetated soils to bare fields after harvest has been shown to decrease microbial biomass (Franzluebbers et al., 1994). Also, mechanical disruption of the soil through tillage is known to disturb microbial communities and alter soil microbial community composition (Jackson et al., 2003; Sandén et al., 2018) as well as microbial processes such as respiration (Elder and Lal, 2008). Interactions and complex seasonal changes of individual drivers of microbial physiology along with superimposed management practices make it difficult to predict the behavior of microbial respiration, microbial growth, and CUE over the course of a year.

The aim of the study presented here was to investigate seasonal dynamics of microbial respiration, growth, biomass, and CUE in a temperate agricultural and a forest soil system and to identify their potential drivers. To do so, we measured soil microbial respiration, gross growth via incorporation of ^{18}O from soil water into DNA, and biomass in an agricultural field and a deciduous forest 16 times over the course of two years. At the field sites, we differentiated between plots that either received harvest residues or leaf litter and plots where harvest residues or leaf litter was removed to investigate the influence of a major C input event in late summer and fall. (1) We hypothesized that microbial respiration, growth, and CUE would show differences over the seasons. In particular, we expected respiration and growth to increase with rising temperature and thus be high in summer and low in winter. (2) We further expected that harvest residue and litter removal would decrease microbial respiration, growth, and microbial biomass whereas CUE would not be affected when respiration and growth are similarly reduced.

2. Materials and methods

2.1. Study sites

Soil samples were collected from an agricultural field site and from a deciduous forest. Agricultural soils were sampled at a long-term agricultural field experiment near Wieselburg, in Alpenvorland, Austria (48°12′N 15°15′E). Mean annual temperature (MAT) at the site is 8.5 °C and mean annual precipitation (MAP) is around 840 mm. 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 was 6.1 and C content 0.9 % (Canarini et al., 2020). At the site, two field treatments, in four replicated plots each, had been established in 1986. In the 'control' treatment, harvest residues are left on the field after crop harvest, and incorporated into the upper soil layer during the next tilling event. In the 'removal' treatment harvest residues are removed from the field. The field crops were spring wheat (*Triticum aestivum* L.) in 2018 and winter barley (*Hordeum vulgare* L.) in 2019. After harvesting winter barley, the soil remained uncultivated until another summer crop was planted in 2020. The forest study site is located at the experimental forest Rosalia, Austria (47°42′N, 16°17′E) and is dominated by European beech (*Fagus sylvatica* L.). The site has a MAT of 6.5 °C and MAP of 800 mm. 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 and C content 4.3 % (Canarini et al., 2020). At the forest site four control plots and four litter removal plots, where litter was removed regularly during the period of main litter fall were established in May 2017.

We sampled soils from all field treatments at both sites 16 times over the course of two years from March 2018 to January 2020. Dates of sampling, harvest, fertilization, and tillage as well as litterfall at the forest site are listed in Table S1. As the topsoil horizon in forest soils was oly 5–10 cm deep, soil samples were taken with a soil corer with a diameter of 2 cm from 0 to 5 cm depth. At the forest site, 6 soil cores per plot (3 m by 3 m) were combined to one sample. At the agricultural site (plot size 7.5 m by 28 m), 10 cores were pooled. Soil temperature was measured during sampling by inserting a temperature probe 4–5 times per plot. All samples were homogenized by sieving through a 2 mm mesh and kept at the respective field temperature until further processing

within 48 h after sampling.

2.2. Water content, water holding capacity and pH

Water content was determined gravimetrically in sample aliquots that were dried at 60℃ for 24 h. Water holding capacity was measured by determining the water content after the saturation of soil samples and letting the excess water leach gravimetrically for two days while preventing evaporation (Reynolds and Topp, 2007). Soil pH was determined in a 1:5 w/v soil to water mixture using a pH meter (Si600, Sentron). Soil temperature was determined at the time of sampling using a soil thermometer.

2.3. Extractable organic carbon (EOC), total extractable N (TEN), microbial biomass carbon (MBC) and nitrogen (MBN)

Extractable organic carbon (EOC) and total extractable N (TEN) were measured in 1 M KCl extracts (1:7.5 w/v) using a TOC/TN analyzer (TOC-L CPH/CPN, Shimadzu). Microbial biomass carbon (MBC) and nitrogen (MBN) were determined by chloroform fumigation extraction (Brookes et al., 1985). Samples were fumigated in a desiccator under chloroform atmosphere for 24 h in the dark and subsequently extracted with 1 M KCl and measured on a TOC/TN analyzer. MBC and MBN were calculated as the difference in C and N between fumigated samples and fresh soil samples (EOC and TDN). Measured MBC values were divided by 0.45 (Wu et al. 1990).and MBN was divided by 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996) to account for extraction efficiency.

2.4. Respiration, microbial gross growth, and carbon use efficiency (CUE)

Measurements for microbial respiration, gross growth, and CUE were conducted at the respective field temperatures at the time of soil sampling (mean of the two treatments at each site) at the day of soil sampling. The minimum incubation temperature was 2 °C. We used the methods to determine microbial respiration, gross growth, and CUE described by Spohn et al. (2016a) and Zheng et al. (2019) with slight modifications. For this assay duplicate 400 mg fresh soil aliquots were either amended with ^{18}O enriched water, to reach a final enrichment of all water in the sample of 20 atom percent, or natural abundance water. We measured microbial respiration by taking gas samples from a sealed headspace vial, which contained the soil aliquot right after the addition of ^{18}O enriched water and 24 h (spring, summer, fall) or 48 h (winter) after the start of the incubation. Longer incubation times in winter were chosen to ensure sufficient accumulation of CO2 in the headspace and incorporation of 18O into DNA to be measurable. Gas samples were analyzed using an infrared gas analyzer (EGM4, PP systems). Microbial respiration was then calculated as the difference in CO_2 concentrations between those two time points and accounting for the replaced air, divided by the incubation time.

Microbial gross growth was determined based on the incorporation of ^{18}O from soil water into genomic DNA. DNA was extracted using a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MP Biomedicals) following the manufacturer's instructions. The DNA concentration of each extract was determined fluorimetrically by a Picogreen assay using a kit (Quant-iT™ PicoGreen® dsDNA Reagent, Life Technologies). Subsequently, the ^{18}O enrichment and the total O content of the purified DNA were measured using a Thermochemical elemental analyzer (TC/EA, Thermo Fisher) coupled via a Conflo III open split system to an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher).

The amount of DNA produced was calculated using the following formula:

$$DNA_{produced} = O_{DNAextr} * \frac{18Oat\%_{DNAL} - 18Oat\%_{DNAn.a.}}{18Oat\%_{soilwater}} * \frac{100}{31.21}$$

Where $O_{DNA\ extr}$ is the total amount of oxygen in the DNA extract, $^{18}O\ at\%_{DNA\ L}$ and $^{18}O\ at\%_{DNA\ n.a.}$ are the ^{18}O enrichment in the labeled 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 %, Zhang et al 2019; Canarini et al 2020). To calculate microbial biomass C produced (C_{Growth}) during the incubation, $DNA_{produced}$ was divided by the total amount of DNA in the sample and multiplied by MBC values. Microbial respiration ($C_{Respiration}$) was calculated from the respiration measurements described above.

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

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

Temperature coefficients for respiration and gross growth (Q_{10}) was calculated for both treatments at each site individually following the approach by Meyer et al. (2019): First an exponential regression was fitted to the data using the R function nls using microbial respiration ($C_{Respiration}$) and soil temperature (T).

$$C_{Respiration=a \times exp^{b \times T}}$$

Then, the exponential coefficient b was inserted in the following equation:

$$Q_{10} = exp^{10 \times b}$$

2.5. Statistics

All statistical analyses were performed in R 4.1.1 (R Development Core Team, 2013). To test for differences in microbial respiration, gross growth, CUE, and biomass between sites and effects of sampling timepoint and field treatments, we used the functions gls (Fit Linear Model Using Generalized Least Squares) and lme (Linear Mixed-Effects Models), which are both contained in the R package nlme (Pinheiro et al., 2021). As the treatments showed different seasonal patterns we explored them seperately. To account for non-normal distributed residuals, we used log and square root transformations where necessary. If residuals of the models were non-homoscedastic, we introduced weights in the respective functions. We also introduced plot and year as random effects (independent intercept). To find the most parsimonious model, different models including weights and random effects were set up and compared with the anova function. If models were statistically different, we chose the model with the lowest Akaike information criterion (AIC). Treatment effects at individual sampling timepoints and differences between two consecutive samplings were tested using either t-tests, Welch t-tests when variances were not homogeneous or Wilcoxon rank sum tests when data were not normally distributed. Correlations between microbial respiration and microbial gross growth with soil temperature were tested by fitting an exponential model nls. We further tested if the presence of plants/foliage which was determined visually in the field and had values of either present or not present, water content, EOC, TEN or MBC had an effect on microbial respiration or gross growth in addition to soil temperature as wll as interactive effects of soil temperature and the other factors. For the analysis, we again used the lme function and the following R-code: lme(Crespiration or Cgrowth ~ soil_T + plants*soil_T + WC*soil_T + DOC*soil_T + TDN*soil_T + MicC*soil_T,random=~ 1|plot,na.action = na.omit). Differences and correlations were assumed to be significant at p < 0.05.

3. Results

3.1. Differences between sites

All investigated soil microbial parameters i.e. microbial respiration,

gross growth, CUE, and MBC were significantly different between soils from the agricultural field and the forest (Table 1). At the forest site microbial biomass was on average more than five times higher than at the agricultural field site during the two years of investigation. Microbial respiration was more than 2.5 times and microbial growth more than two times higher in forest soil compared to agricultural soil. Microbial CUE was however lower in forest soil compared to agricultural soil.

3.2. Seasonality of soil temperature, soil water content and microbial carbon dynamics

Soil temperatures at both sites were high in summer with up to 25.4 ℃ in agricultural soils and 18.8 ℃ in forest soils and low in winter with −0.1 ℃ in both soils. Water content showed an inverse seasonal pattern and was high in winter with up to 31.2 % in agricultural soils and 36.8 % in forest soils and low in summer with down to 7.8 % in agricultural soils and 11.3 % in forest soils. -Microbial parameters, along with soil temperature and soil water content (Fig. 1) showed changes and seasonal dynamics during the two years of investigation at both sites (Fig. 2, Fig. 3, Table 2).

3.2.1. Microbial respiration

Microbial respiration from soils at the agricultural field site and the deciduous forest site showed strong seasonal variation (Fig. 2a, Fig. 3a, Table 2). At the agricultural field site respiration was low in winter, increased towards a peak in summer and decreased again during fall (Table S3). Respiration strongly followed the soil temperature and was highly correlated with temperature (Fig. 4). Examined across all sampling timepoints, the removal treatment at the agricultural field site did not have a significant effect on microbial respiration when tested with a linear mixed effects model (Table 2). In contrast, individual sampling timepoints showed significant differences of respiration between the field treatments. The observed differences were, however, small and did not follow a discernible clear pattern (Fig. 2a, Table S2). When we accounted for soil temperature and additional parameters in the linear mixed effects model, to test which other factors might potentially influence microbial respiration, we found MBC in control soils to have an significant effect on respiration rates and soil water content to have a statistically significant interactive effect with temperature (Table 3).

In forest soils, microbial respiration also closely followed seasonal dynamics of soil temperature (Fig. 4). Differences between control plots and litter removal plots became apparent only in fall and winter of the second year (Fig. 3a). During litter fall in November 2019, microbial respiration was significantly higher in control plots than in litter removal plots (Fig. 3a, Table S2). In December 2019 microbial

respiration was still marginally significantly (p-value < 0.1) increased and in January 2020 it was significantly increased in control plots (Fig. 3a). Besides the effect of temperature, microbial respiration decreased with increasing soil water content and was effected by EOC, TEN and MBC as well as an interactive effect of temperature and foliage presence (Table 3).

3.2.2. Microbial gross growth

Similar to microbial respiration, microbial gross growth showed seasonal fluctuations that were related to soil temperature (Fig. 2b, Fig. 3b, Table 2). However, soil temperature explained less of the variation in microbial growth than of the variation in respiration (Fig. 4). In agricultural soils, microbial gross growth increased until early summer of the first year but decreased strongly after crop harvest, although soil temperatures and microbial respiration remained high (Fig. 2b, Table S5). Following a further decrease after tillage, microbial gross growth slightly increased again in winter (Table S5). In spring of the second year, microbial gross growth increased only in soils from control plots (Fig. 2b, Table S5). After the crop harvest, microbial gross growth was down to the levels of the previous winter and further decreased in both field treatments towards the second winter. In contrast to the first winter where winter barley was grown, the field was bare during the second winter. Even though, microbial gross growth in soils from control plots that had received harvest residues, was still significantly higher than in the summer before (Fig. 2b, Table S2). Plant cover had a significant effect on growth in a linear mixed effects model including potential drivers (Table 3) in both agricultural field treatments. Additionally, we found significant influences on growth of EOC and MBC in the agricultural control treatment and of TEN in the residue removal treatment (Table 3). Microbial gross growth in forest soils increased in spring until July 2018 and decreased after that until December 2018 (Fig. 3b, Table S6). Soils from control plots showed an increase in microbial gross growth in spring and summer 2019, while microbial gross growth remained low in soils from litter removal plots and only increased in August 2019 (Table S6). Microbial gross growth decreased in fall and winter of 2019 under both treatments. Besides soil temperature (Fig. 4), microbial gross growth was only related to soil water content in control plots, but also to EOC, TEN and MBC in litter removal plots at the forest site (Table 3).

3.2.3. Microbial carbon use efficiency

Microbial carbon use efficiency (CUE) was significantly affected by the timepoint of sampling in agricultural soils (Table 2). CUE increased in the first spring, decreased, similar to microbial gross growth, after crop harvest and then strongly increased during the first winter in soils from both field treatments (Fig. 2c; Table S5). CUE values in December 2018, January 2019, and March 2019 were amongst the highest measured during this study and reached values higher than 0.7. In the second year, CUE remained rather high in soils from control plots during spring and summer and decreased only after the next crop harvest. In contrast, in soils where harvest residues were removed CUE decreased earlier from April onwards. CUE increased again in September 2019 and remained high during the second winter in control plots while it decreased again in removal plots (Fig, 2c, Table S5).

As in agricultural soils, microbial CUE in forest soils was affected by sampling timepoint (Table 2) and closely followed the dynamics of microbial gross growth during spring (Fig. 3b, c), summer and fall 2018. CUE increased in December 2018 and was highest in March 2019 where we also found significantly higher CUE in control plots than in litter removal plots (Fig. 3c, Table S2). In April 2019, CUE decreased and remained rather constant in control plots until it slightly decreased in January 2020. CUE decreased from March until June 2019 in litter removal plots after which CUE increased until November 2019 and slightly decreased afterwards (Table S6). Overall CUE was not affected by litter removal at the forest site (Table 2). However, when individual sampling timepoints were examined a significant treatment effect on

Table 1
Mean values and differences of microbial parameters between sites tested with linear mixed-effects models. Statistically significant effects are in bold.

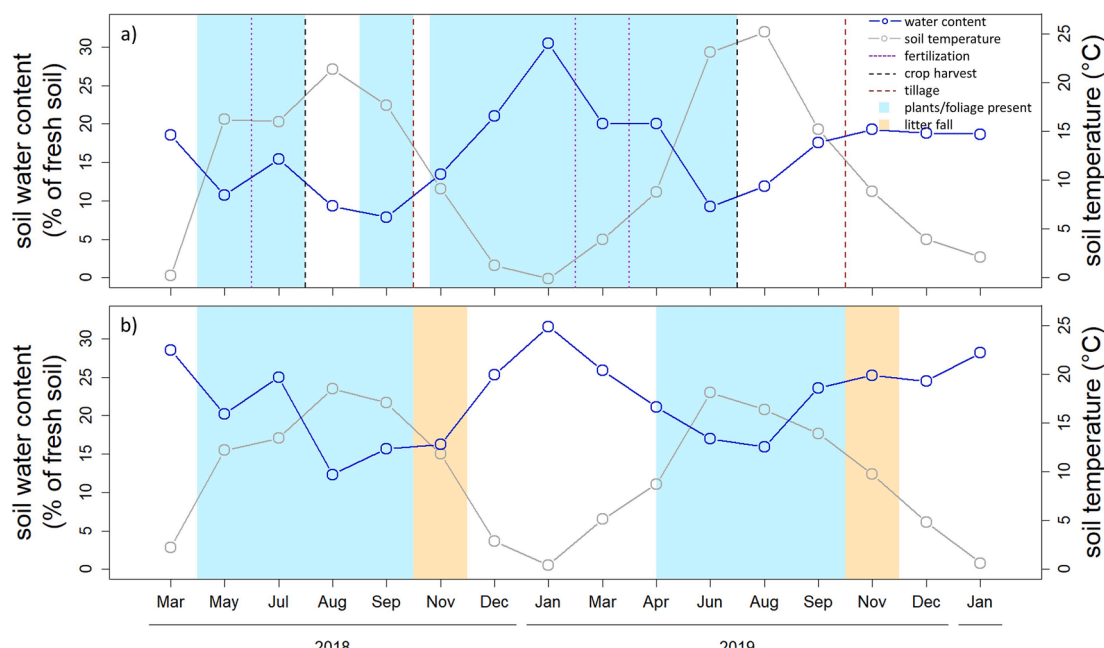|	mean ± standard error		F-value	p-value	R formula
	agricultural	forest			
microbial respiration (ng C h^{-1} g^{-1} dry soil)	431.2 ± 35.03	1118 ± 51.23	**122.2**	**<0.0001**	**gls(resp ~ site)**
microbial gross growth (ng C h^{-1} g^{-1} dry soil)	280.5 ± 23.00	653.7 ± 47.64	**47.19**	**<0.0001**	**gls (growth ~ site)**
CUE	0.420 ± 0.019	0.342 ± 0.014	**11.07**	**0.001**	**gls(CUE ~ site)**
microbial biomass C (µg C g^{-1} dry soil)	139.7 ± 5.722	788.9 ± 18.62	**1060**	**<0.0001**	**gls(MBC ~ site)**

Fig. 1. Seasonal dynamics of mean soil water content and mean soil temperature during soil sampling between March 2018 and January 2020. The blue line and symbols indicate mean soil water contents and the grey lines and symbols show mean soil temperatures of eight plots at the agricultural field site a) and the forest site b). In a) vertical lines indicate management events: purple dotted lines depict N fertilization events, dashed black lines are crop harvests and red dashed lines is the main tillage event. Green background indicates times with visible plant cover. In b) light brown background indicates litter fall.

CUE was found in March 2019 and June 2019 (Table S2) and marginal differences (p-value 0.057) between treatments were found in January 2020.

3.2.4. Microbial biomass carbon

Microbial biomass carbon (MBC) did not change strongly in agricultural soils in spring and summer of the first year (Fig. 2d). After crop harvest MBC was significantly increased in control plots compared to soils from removal plots (Fig. 2d, Table S2, Table S4). After tillage this difference could not be detected anymore. MBC was strongly increased in January 2019 in soils from both treatments although this increase was only significant in control plots (Table S5). However, this increase was not reflected by MBN or DNA contents (Table S3). Furthermore, MBC was in general neither significantly related to MBN nor to DNA content in agricultural soils (Table 4). After the winter peak in MBC, MBC decreased gradually towards June 2019 and remained low for the rest of the investigated time in soils where harvest residues were removed (Fig. 2d, Table S5). MBC in control soils decreased to a lesser extent after January 2019. MBC was significantly higher in control plots than in removal plots in April and June 2019. MBC strongly decreased after crop harvest 2019 and remained at a low level thereafter (Table S5). In contrast to the first winter, MBC did not peak in the second winter.

In forest soils MBC remained constant in spring, summer and fall in both investigated years (Fig. 3d). MBC was significantly elevated in January 2019 in control plots compared to litter removal plots and in March 2019 in litter removal plots compared to control plots (Table S5). From November 2019 on MBC strongly increased in control plots and remained high until January 2020 (Table S5). MBC increased less in litter removal plots compared to control plots during this time (Table S2). Other than at the agricultural field site MBC correlated significantly with MBN (p-value < 0.0001 in both treatments) but not with DNA content in forest soils (forest control p-value = 0.325, forest removal p-value = 0.086; Table S7).

4. Discussion

In our study, we found that microbial respiration, growth and CUE

are highly variable in an agricultural field and a deciduous forest over the course of a year (Figs. 2, 3). In both soil systems microbial respiration closely followed the seasonal dynamics of soil temperature with low values in winter and high values in summer. Microbial growth was similarly affected by soil temperature but was additionally dependent on soil water content and C availability and thus likely on plant carbon inputs at the agricultural site (Table 3). Surprisingly, amongst the highest values of CUE were found in winter, which may be explained by the higher temperature sensitivity of microbial respiration compared to microbial growth (Fig. 4). Microbial biomass C which is a potential source for stable soil organic matter (Miltner et al., 2012) strongly increased in the first winter, and when litter or harvest residues were present in the second winter. The strong seasonal changes as well as differences between the two investigated years of all measured microbial parameters question the usefulness of measurements of microbial processes and parameters at single timepoints to make general statements about soil C dynamics. This is especially true for CUE as it ranged from 0.1 to 0.7 in soils from the agricultural field site and from 0.1 to 0.6 at the forest site within one year. We argue that due to the high seasonal variability of CUE and high values of CUE in combination with low growth rates in winter caution should be taken to use microbial CUE alone as an indicator for soil C dynamics and soil C sequestration.

4.1. Differences between sites

Microbial respiration in soils at a regional scale and differences in respiration between ecosystems are often driven by soil microbial biomass C and soil C content (Colman and Schimel, 2013). The results of our study confirm this general pattern as not only microbial respiration, but also microbial growth and MBC were on average significantly lower in agricultural soils with 0.9 % SOC than in forest soils with 4.3 % SOC (Table 1). Interestingly, microbial CUE was on average higher in agricultural soils compared to forest soils. Most soils can be considered C-limited (Soong et al., 2020) which mainly represents a limitation in energy (Sinsabaugh et al., 2013). High availability of C, at the forest site might have allowed microorganisms to acquire energy through respiration which reduced CUE. The lower CUE in forest soils compared to
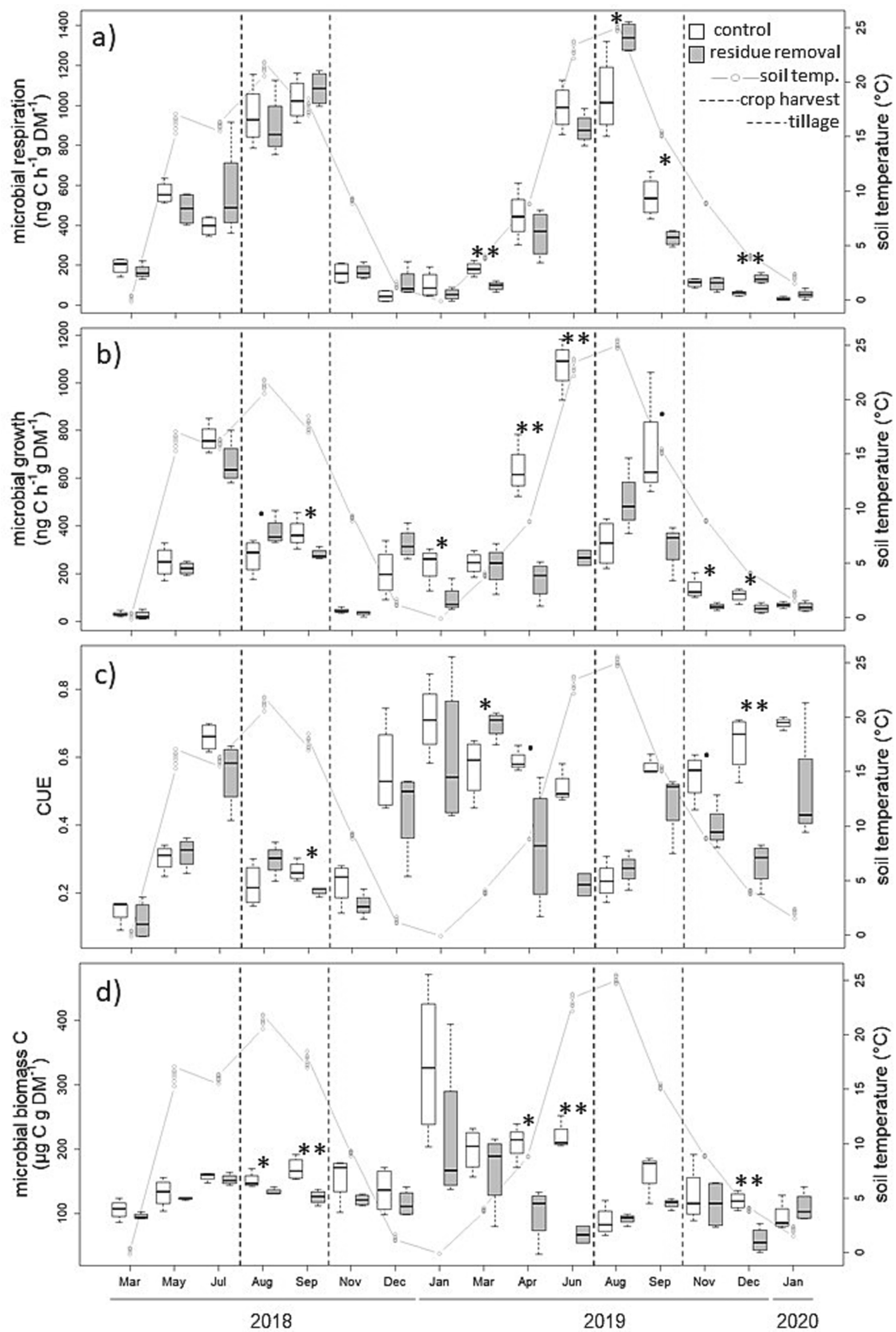
Fig. 2. Seasonal dynamics of microbial respiration a), microbial gross growth b), CUE c) and MBC d) at the agricultural field site. Grey lines and symbols in the background indicate the seasonality of soil temperature. Black dashed vertical lines show the timepoint of crop harvest and red dashed vertical lines indicate tillage. White boxes are mean values for the control plots that received harvest residues. Grey boxes are mean values for the plots where harvest residues were removed. Symbols and asterisks above the boxes indicate significant differences between the field treatments at the respective harvest with the following levels of significance:. p < 0.1, * p < 0.05, ** p < 0.01. Detailed statistical results can be found in Table 2 and Table S3.
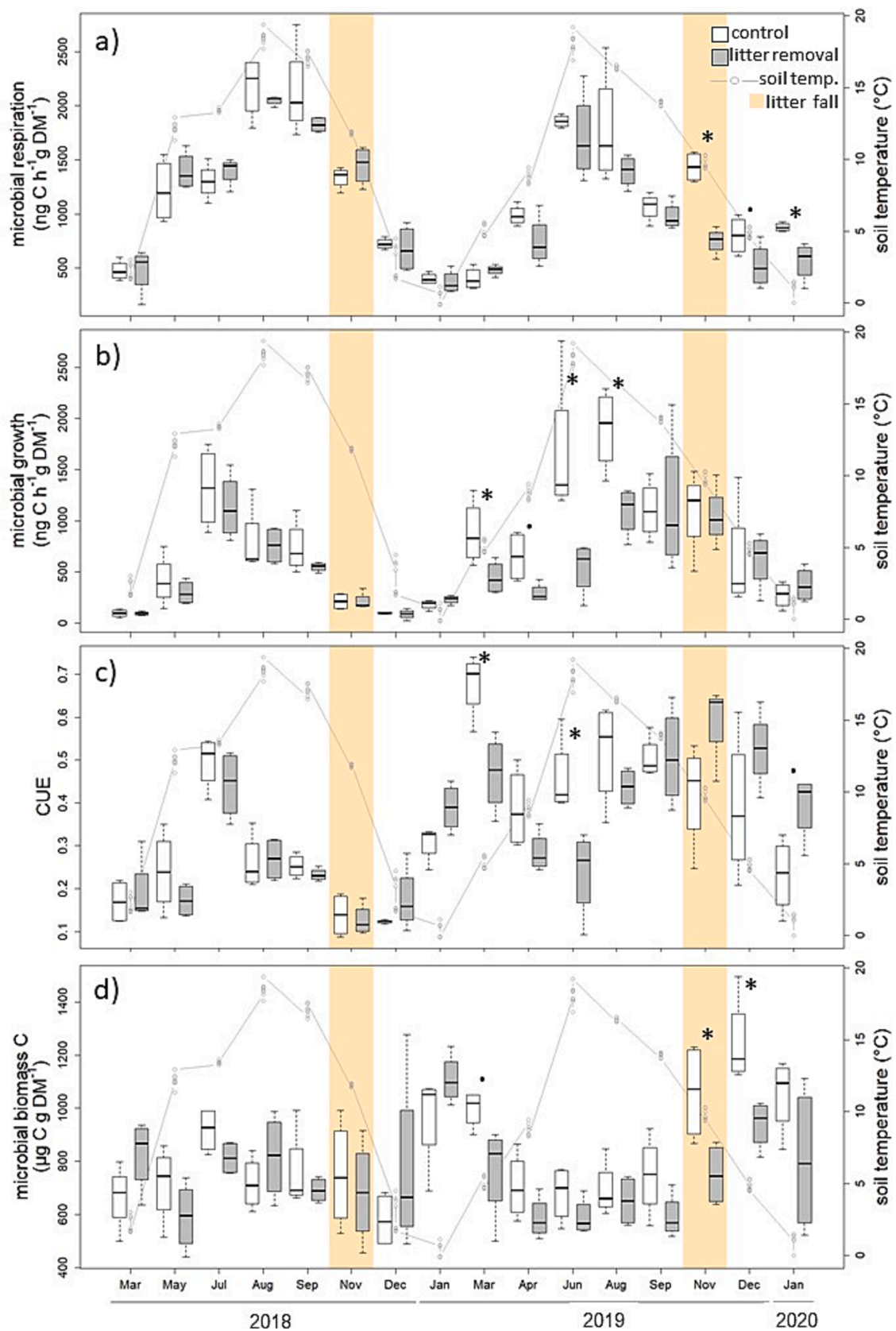
Fig. 3. Seasonal dynamics of microbial respiration a), microbial gross growth b), CUE c) and MBC d) at the forest site. Grey lines and symbols in the background show the seasonality of soil temperature. Brown background indicates litterfall. White boxes are mean values for the control plots that received leaf litter. Grey boxes are mean values for the plots where leaf litter was removed. Dots and asterisks above the boxes indicate differences between the field treatments at the respective harvest with the following levels of significance:. p < 0.1, * p < 0.05, ** p < 0.01. Detailed statistical results can be found in Table 2 and Table S4.

Table 2
Results from mixed-effects models to test differences of the measured parameters between sampling timepoints, between treatments and their interaction. Mean values are depicted in Fig. 2 and Fig. 3. Statistically significant effects are in bold.

|		sampling F-value	p-value	treatment F-value	p-value	sampling:treatment F-value	p-value	R code
agricultural	microbial respiration	**205.7**	**<0.0001**	0.270	0.6035	**6.300**	**<0.0001**	**gls(log(resp) ~ harvest*treatment,weights = varIdent(form = ~1|harvest))**
	microbial gross growth	**98.76**	**<0.0001**	**58.88**	**<0.0001**	**8.930**	**<0.0001**	**gls(log(growth) ~ harvest*treatment,weights = varIdent(form = ~1|harvest))**
	CUE	**46.20**	**<0.0001**	**34.90**	**<0.0001**	**5.722**	**<0.0001**	**gls(log(CUE) ~ harvest*treatment,weights = varIdent(form = ~1|harvest))**
	microbial biomass C	**15.20**	**<0.0001**	**35.27**	**<0.0001**	**5.260**	**<0.0001**	**gls(log(MBC) ~ harvest*treatment,weights = varIdent(form = ~1|harvest))**
forest	microbial respiration	**86.14**	**<0.0001**	**14.07**	**0.0003**	**4.329**	**<0.0001**	**gls(resp ~ harvest*treatment,weights = varIdent(form = ~1|harvest))**
	microbial gross growth	**51.79**	**<0.0001**	**11.87**	**0.0009**	**2.220**	**0.0105**	**gls(log(growth) ~ harvest*treatment,weights = varIdent(form = ~1|harvest))**
	CUE	**24.10**	**<0.0001**	0.550	0.46	**3.979**	**<0.0001**	**gls(CUE ~ harvest*treatment, data.f.models)**
	microbial biomass C	**11.07**	**<0.0001**	**17.77**	**0.0001**	**2**	**0.0230**	**gls(log(MBC) ~ harvest*treatment,weights = varIdent(form = ~1|harvest))**
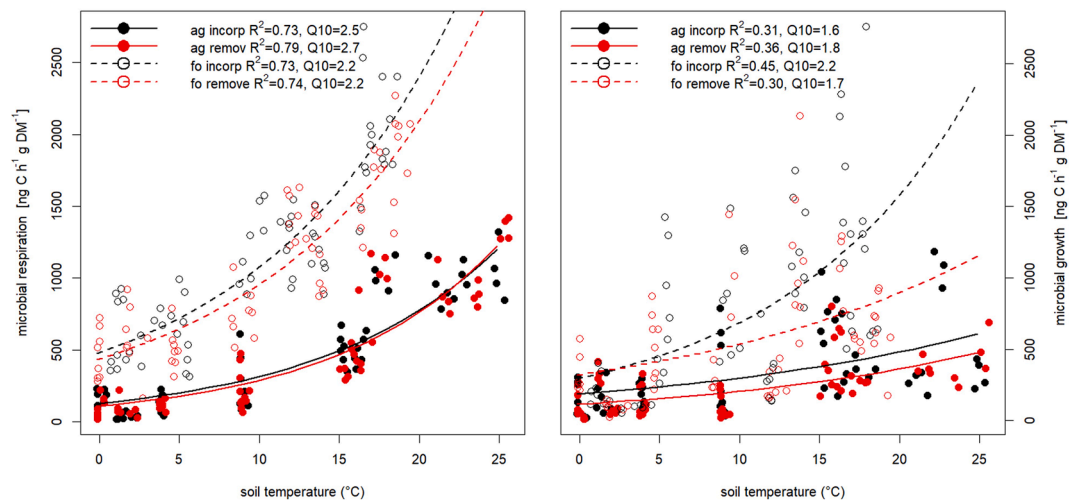
Fig. 4. Exponential regressions between soil temperature and microbial respiration a) and soil temperature and microbial gross growth b). Open symbols represent samples from the forest site, full symbols represent samples from the agricultural field site. R^2 as indicators for goodness of fit were calculated form the log-linearized exponential regressions. Q10 values were calculated from the respective model formulas.

agricultural soils could thus be interpreted as C not being as limiting at the investigated forest site.

4.2. Seasonality of microbial carbon dynamics

Despite the differences in magnitude and range of the measured parameters at the two sites, seasonal dynamics of microbial respiration and growth followed similar patterns and indicate common drivers of microbial C dynamics in soil. Microbial respiration increased with temperature (Fig. 4), peaked in summer and declined to low values in winter. This pattern was observed in both years and at both field sites. The effects of temperature on respiration are well known and have been studied for years in numerous ecosystems (Lloyd and Taylor, 1994; Fierer et al., 2006). Furthermore, seasonality of soil microbial respiration is sometimes also associated with plant productivity and increases with increasing aboveground net primary productivity and C inputs in the form of e.g. litterfall (Raich and Tufekcioglu, 2000). Our results show that litterfall only affected microbial respiration at the forest site in the second year (Fig. 3a). This is in accordance with findings that litter removal effects only set in some time after their establishment (Fekete et al., 2014; Lajtha et al., 2014) and the here investigated litter removal treatments, which had been established only one year before the start of

the measurements, might still have been too young. At the agricultural field site, harvest residue removal had little and inconsistent effects on microbial respiration (Fig. 2a) and we did not find differences in SOC content between treatments in this study. Spiegel et al. (2018) however reported small but significant increases in SOC following crop residue incorporation for the same study site. The discrepancy between the two studies might have been caused by the greater sampling depth in Spiegel et al. (2018). The missing effect of crop residue removal at the agricultural site in the present study might further be connected to the agricultural practices, especially tillage, at the site which has strong negative effects on soil C content (Conant et al., 2007) and microbial parameters (Kandeler et al., 1999). The missing or small influence of harvest residue on SOC and respiration rates at this particular site might further be explainable by the importance of belowground plant inputs as main source for the soil C cycle and the formation of soil organic matter (Austin et al., 2017).

Similar to microbial respiration microbial growth was correlated to soil temperature but temperature explained less variability in measured growth rates at both sites (Fig. 4). The temperature dependency of microbial growth has already been shown in a number of studies and concepts (Manzoni et al., 2012; Simon et al., 2020; Walker et al., 2018). Q10 values, a proxy for temperature sensitivity, were lower for growth

Table 3

Effects of different variables on microbial respiration and gross growth in addition to soil temperature and the interactive effects of soil temperature and the other factors. Statistically significant effects are in bold.

		agricultural control		agricultural removal		forest control		forest removal	
		F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Microbial respiration	Soil temperature	**362.5**	**<0.0001**	**267.6**	**<0.0001**	**341.0**	**<0.0001**	**383.5**	**<0.0001**
	plants/foliage	0.424	0.518	0.024	0.877	0.787	0.379	2.853	0.098
	water content	1.702	0.199	0.463	0.500	**7.203**	**0.010**	**3.879**	**0.055**
	EOC	0.676	0.415	1.873	0.178	**4.249**	**0.045**	**9.016**	**0.004**
	TEN	0.137	0.713	0.210	0.649	**5.255**	**0.026**	**17.70**	**0.0001**
	MBC	**16.00**	**0.0002**	0.302	0.585	**15.91**	**0.0002**	**9.092**	**0.004**
	plants/foliage × temperature	1.102	0.299	0.975	0.329	**17.30**	**0.0001**	**10.42**	**0.002**
	WC × temperature	**25.39**	**<0.0001**	**23.81**	**<0.0001**	0.316	0.577	0.376	0.543
	EOC × temperature	0.110	0.742	1.500	0.227	3.987	0.052	**0.0004**	**0.984**
	TEN × temperature	0.275	0.603	**7.277**	**0.001**	2.122	0.152	1.025	0.316
	MBC × temperature	0.062	0.804	0.520	0.475	**14.51**	**0.0004**	2.094	0.154
microbial gross growth	Soil temperature	**81.62**	**<0.0001**	**65.91**	**<0.0001**	**52.60**	**<0.0001**	**35.64**	**<0.0001**
	plants/foliage	**30.99**	**<0.0001**	**7.503**	**0.009**	1.609	0.211	0.328	0.569
	water content	**26.26**	**<0.0001**	**6.822**	**0.012**	**29.62**	**<0.0001**	**20.71**	**<0.0001**
	EOC	**26.85**	**<0.0001**	0.002	0.961	0.176	0.677	**7.315**	**0.009**
	TEN	0.182	0.672	**11.90**	**0.001**	0.325	0.571	**10.27**	**0.002**
	MBC	**9.756**	**0.003**	2.489	0.122	3.257	0.077	**4.895**	**0.032**
	plants/foliage × temperature	**54.82**	**<0.0001**	0.250	0.620	0.795	0.377	0.942	0.337
	WC × temperature	**6.213**	**0.017**	0.839	0.365	0.483	0.491	**13.58**	**0.001**
	EOC × temperature	2.945	0.093	**24.25**	**<0.0001**	1.311	0.258	0.007	0.936
	TEN × temperature	3.830	0.057	**6.230**	**0.017**	0.103	0.750	0.655	0.422
	MBC × temperature	**9.648**	**0.003**	0.270	0.606	0.226	0.637	**5.152**	**0.028**

(1.6 to 2.2) than for respiration (2.2 to 2.7). This is in line with other recent studies that concluded that growth is less sensitive to temperature shifts than respiration (Pietikäinen et al., 2005; Cruz-Paredes et al., 2021). A consequence of the differences in temperature sensitivity of growth and respiration could be an explanation for the high CUE values, that are calculated from growth and respiration, found during winter in our study. This is in line with findings by Frey et al. (2013) who found in a short-term laboratory experiment using a different method, a decrease in efficiency with increasing temperature. Besides temperature, plant presence at the agricultural site affected microbial growth (Table 3). This indicates that the availability of plant-derived, easily available C sources, which can be assumed to be higher when plants are present is higher than during times when no plants are present, might have stimulated microbial growth. This is corroborated by the higher growth values during the first winter at the agricultural site during which winter wheat was present compared to the second winter where the agricultural field was bare (Fig. 2). Contrastingly, in removal plots the positive effect of plant presence in winter on microbial growth was not found. In the investigated agricultural field, even the low photosynthetic activity of the present plants in winter seems to have stimulated microbial gross growth, but only when harvest residues were present. Together with the higher microbial growth rates in control plots at the forest site and agricultural control plots during the second summer this suggests that the positive effect of active plants on microbial growth is only established when enough C is available from other sources. Interestingly, litter and residue removal effects were only visible in the second year of our investigation. This discrepancy between the years may further be explained by the difference in soil water content, which was especially low during summer and fall 2018. Low water availability in summer might have hampered microbial access to easily available C sources (Schimel, 2018) such as root exudates but also leachates from leaf litter. The effect of litter removal might thus have been masked since also in control plots no C could have been leached into the soil due to the lack of precipitation. Drought in summer and fall of 2018 could also have affected microbial biomass and could have been the reason for a delayed increase in MBC compared to the following winter. Microbial biomass at the forest site increased only in January 2019 (Fig. 1, Table S3) while during the second winter microbial growth and biomass already increased in November 2019 where soil water content was higher than the year before. At this point it has to be mentioned that we might have

captured an artificial increase in respiration and growth in the first summer and fall by amending field-fresh soil which was low in water content with ^{18}O labelled water. Strong increases in soil respiration have been found during rewetting of dry soils already decades ago (Birch, 1958). A recent study has revealed that similar to respiration, growth is strongly affected by rewetting (Canarini et al., 2020). Drought itself or an artificial Birch effect, caused by rewetting in the laboratory, might also be the reason for the correlations we found between respiration and soil water content (Table 3). Soils were however only excessively dry (<30 % of WHC; Table S2 and S3) during some of the summer samplings and the 2018 fall sampling. Furthermore, temperature explained more than 70 % (Fig. 4) of the seasonal variability in respiration at both sites (Table 3). Because soils were only excessively dry at some sampling dates, we think that the general dynamics presented here are still valid. Nevertheless, we cannot exclude the possibility that an artificial Birch effect might have masked potential field treatment effects during the first year of our experiments. It might however also have been the soil drought itself that led to reduced substrate availability; a strong enough disturbance to mask potential treatment effects (Schimel, 2018).

4.3. Effects of agricultural management practices on microbial C dynamics

In addition to seasonal dynamics in microbial growth, respiration, and microbial biomass, we also found effects of agricultural management practices i.e. tillage and crop harvest. It should be noted that the here presented study was not explicitly focused on agricultural practices and the sampling timepoints were chosen to be at least two weeks after harvest or tillage. Tillage led to a significant reduction in microbial respiration in both field treatments and years, and to a significant reduction in microbial growth (Table S3) during the first year. And while there was also a decrease in soil temperature from September to November 2018, especially the strong decrease in respiration to levels as low or below during the following winter, cannot be a pure temperature effect. Tillage has been shown to affect microbial C cycling in soils especially since it destroys soil aggregates (Grandy and Robertson, 2007) and disrupts fungal hyphae (Rosner et al., 2018) which leads to reductions in microbial biomass (Zuber and Villamil, 2016). We did not find any immediate effects of tillage on microbial biomass, potentially because tillage effects on microbial biomass or fungi are usually only

found after years, or when tillage and non-till systems are compared (Zuber and Villamil, 2016).

Crop harvest strongly reduced microbial growth from July 2018 to August 2018 in both treatments (64 % in control and 43 % in removal plots), and from June 2019 to August 2019 in control treatments (69 %). In 2019, when no plants were present on the plots, we also found a 40 % reduction of the microbial biomass that had built up over the winter in control treatments. Microbial gross growth and biomass abruptly decreased following crop harvest (Fig. 3, Table S4). This together with the effects of plant presence that led to the difference in microbial biomass dynamics in 2018 and 2019 highlights the role of fresh belowground plant inputs as the main source for microbial growth, biomass build up and potentially soil organic matter formation (Kätterer et al., 2011; Austin et al., 2017).

4.4. Microbial C dynamics in winter

In contrast to spring, summer and fall where we observed clear seasonal dynamics of microbial respiration, growth and biomass that were explainable by temperature and substrate dependencies, our findings for winter were surprising and not as clear. In particular, the strong increase of MBC during the winter of the first year in both systems and during the second year in forest soils that seemed to be decoupled from microbial growth was unexpected. Increases in MBC and microbial abundance in winter have been observed in other temperate and boreal sites (Schmidt and Lipson, 2004; Zhang et al., 2014; Isobe et al., 2018). The reasons for this winter peak are however not fully understood. In our results, the increase in MBC at the agricultural site was only observed when plants were present. In control plots at the agricultural site, microbial biomass C peaked in January 2019 and remained high while MBC gradually decreased in the removal plots from January to June 2019. In the second winter when no winter wheat was grown, MBC did not increase, irrespective of field treatment, which might be explained by the lack of plant cover and its C inputs. The positive effect of plant cover might have been caused by an insulation effect and thus higher soil temperatures. At the investigated site here, wheat plants were only up to 10 cm high making an insolation effect unlikely. It is rather the lack of C input by active plants, even if it is small, that might have hampered an increase in MBC in the second winter. MBC at the forest site was only elevated from January 2019 on when soil water content must have been sufficiently high to leach easily available C from the fresh leaf litter. In the second year the increase in MBC during winter already started during litter fall and was higher in control plots than in removal plots.

MBC winter peaks at both sites were not associated with higher growth rates or increases in absolute DNA contents and were only correlated with MBN at the forest site. This indicates that microorganisms took up C and N and did not divide. Instead, microorganisms might have produced either storage compounds or cryoprotectants (Tribelli and López, 2018), cold-shock proteins (Weber et al., 2002) to protect themselves against freezing and the associated osmotic stress. Increases in storage compounds at cold temperatures as well as other adaptations to low temperatures have been shown in other studies (Mason-Jones et al., 2022; Schnecker et al., 2023) and could have contributed to the observed peaks in MBC in winter in this study. High MBC values in winter might have also been caused by methodological issues. Dead or inactive plant roots which are more abundant in winter, could have further contributed to chloroform extracted C (Friedel et al., 2002). The differences between the treatments, that should not have affected root abundance, at the forest site in the second year however rule out that additional C extracted from plant roots was the cause for the observed increase in MBC in winter. Following winter, the accumulated MBC declined during spring and summer.

4.5. CUE as indicator for C dynamics and C sequestration

Microbial CUE is frequently used to represent microbial physiology in microbial soil C models and concepts (Cotrufo et al., 2013; Poeplau et al., 2019; Pold et al., 2019; Sokol et al., 2019) and has become a widely considered parameter for microbial physiology and C availability, especially since substrate-independent measurements have been introduced (Spohn et al., 2016a; Geyer et al., 2019). While doing so, it is often neglected that microbial CUE is not a directly measured parameter, but is calculated as the fraction of microbial growth divided by microbial C uptake (Manzoni et al., 2012), which is calculated as the sum of microbial growth and respiration.

Our data show: Microbial respiration and growth have different temperature relationships and can exhibit a wide range of values (Figs. 2 and 3). Our data indicate that respiration is strongly constrained by temperature while microbial growth was not only affected by temperature but additionally by plant presence and C availability in the agricultural field. As shown in our data, high CUE, as in winter, is not necessarily connected to an increase in microbial growth. In contrast during summer when microbial growth was high, even higher respiration rates led to low CUE. Changes in CUE are thus a result of microbial adaptations in respiration and growth and CUE is not controlled or affected directly. Our data for CUE further ask for caution to use CUE alone as a proxy for soil organic matter storage or formation (Tao et al., 2023). In a study of 16 forests along a 4000 km transect in eastern China, Wang et al. (2021) found CUE to range from 0.1 to 0.6 and that CUE was clearly and significantly related to SOC. CUE at both of our sites ranged from 0.1 to 0.7 (Table S3 and S4) within the course of a single year with no change in SOC content. Our findings thus support the conclusions drawn from a field experiment (Simon et al., 2020) and from a modeling exercise (Hagerty et al., 2018): Examination of CUE alone is inconclusive in terms of soil C cycling. It is necessary to consider underlying processes such as microbial respiration and microbial growth explicitly in order to correctly interpret microbial physiological status as well as potential soil C sequestration.

4.6. Conclusions

Our data show that microbial respiration, microbial gross growth, and MBC display high seasonal variability in two contrasting temperate soil systems, i.e. in an agricultural soil and a forest soil. Seasonal dynamics of microbial respiration and growth showed similar patterns, even though the magnitude and range of individual parameters differed between the agricultural soil and the forest soil. While microbial respiration was tightly controlled by temperature, growth also depended on the availability of C and soil water content. In contrast, microbial CUE did not clearly follow seasonal temperature fluctuations and exhibited peaks during winter. As the high CUE values were not accompanied by higher growth rates or increases in DNA, CUE alone should only be used with great caution to describe soil microbial C cycling. Over the course of a year CUE at both sites ranged from around 0.1 to 0.7, which is in the same range as CUE values found in a 4000 km long transect across 16 forest sites with SOC contents from 1.6 % to 13 % and thus questions a mechanistic connection of CUE and soil C stocks or C sequestration. We further observed surprising microbial C dynamics during winter. Our data suggest that soil microorganisms might increase internal storage of C and N instead of dividing. The increase in MBC during winter was dependent on the presence of plants at the agricultural site and declined again in the following spring. While the concrete mechanisms still have to be elucidated, our findings indicate that winter with its bloom and following decline in microbial biomass could constitute the main season for microbial C cycling in temperate soil systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Author Contributions

JS conceived the study. AR, TS, HS and SZ-B contributed to the conceptual planning of the study and the experimental setup. JS, LB, PG, MP, ES, FS and CU sampled soil at the field sites and processed and analyzed the samples in the laboratory. JS analyzed the data and wrote the manuscript. All authors contributed to data interpretation and provided feedback on earlier versions of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116693.

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