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Microbial Decomposition of Cellulose in Soil: Insights Into the Roles of Resource Stoichiometry and Water Content

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ABSTRACT

Decomposition kinetics of carbon (C) substrates in soil vary linearly with changing soil conditions until specific thresholds are reached, where metabolic pathways change completely. These thresholds challenge process-based modeling, e.g., by determining whether nitrogen (N) addition promotes or suppresses microbial respiration. Here, we aimed to identify such thresholds in cellulose decomposition imposed by C, N, and oxygen limitation by manipulating resource stoichiometry and water content in controlled experiments. Agricultural soils were incubated for 35 days under different cellulose amendments, at different water contents with or without nutrient addition. Resource stoichiometry coinciding with microbial biomass C/N ratios imposed a clear threshold behaviour on growth dynamics. Under C limitation (resource C/N <8), cumulative C release scaled with input; whereas under N limitation (C/N $>>8$), this relationship broke down. When N-limited, N shortened the exponential growth phase by determining the onset of growth retardation, while N excess (C/N <8) delayed microbial growth across all stages. In both cases, the onset of growth retardation scaled linearly with resource C/N ratio, but at different rates for C and N limitation. Further, a distinct threshold behaviour was observed for water contents beyond field capacity. In soil with low microbial activity, wetter conditions accelerated growth by reducing resource limitation without changing cumulative C release. The same wetting reduced cumulative C release in soil with higher microbial activity, potentially due to oxygen limitation. These findings underscore the importance of identifying soil condition thresholds, beyond which microbial respiration shifts unpredictably, invalidating linear assumptions in process-based models.

1 | Introduction

Efforts to increase carbon (C) sequestration in soil pose a pressing challenge for mitigating climate change. A crucial aspect of this endeavour is to understand the mechanisms of soil carbon turnover during the decomposition of organic compounds (Cotrufo et al. 2013). Carbon enters the soil mainly through plant residues and rhizodeposition and is released in the form of CO_2 as a result of microbial growth and heterotrophic respiration during the utilization of organic substrates. The rate of plant residue decomposition depends on several biotic and abiotic factors (Neupane et al. 2023; Bao et al. 2023). Although plant residues consist of diverse organic compounds, cellulose is the most abundant polysaccharide in plant cell walls and is thus often considered a model for studying plant residue decomposition (Blagodatskaya et al. 2014; Miao et al. 2021). As a large

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Summary

- Resource stoichiometry matching microbial biomass induces threshold behaviour in microbial growth.
- N excess slows down microbial growth in the course of cellulose degradation in soil.
- N addition does not alter cumulative CO_2 release by the end of the degradation process.
- Delicate balance exists between oxygen supply and demand around field capacity (60%–75% WHC).

biopolymer, cellulose is taken up by soil microorganisms only after it is degraded by enzymes to more labile glucose (Kästner et al. 2021).

Microbial activity and growth depend largely on the amount of substrate-C available relative to the microbial biomass carbon (MBC) (Blagodatskaya and Kuzyakov 2008). In addition, it depends on environmental factors such as soil temperature, moisture (Salazar et al. 2016), nutrient availability, and pH, as well as the inherent characteristics of the microbiome (Roller and Schmidt 2015). The interactions between these environmental factors create a complex and multifaceted impact on microbial activity. Moreover, complexity is further increased by the fact that the impact of changing environmental conditions is often not linear, and may change substantially if thresholds are exceeded. For instance, enzyme kinetics parameters were shown to exhibit non-linear responses to a temperature change of only 5°C (Razavi et al. 2015). Thus, even minor changes in environmental conditions can result in substantial shifts in microbial physiology, affecting microbial activity.

Among the range of soil properties, nutrient availability was shown to have a strong impact on the functional traits of soil microorganisms (Loeppmann et al. 2020; Mou et al. 2023) as well as on all phases of microbial growth—including lag time, exponential growth phase, and growth retardation—when utilizing labile C sources such as glucose (Nordgren 1992; Endress et al. 2024). In this regard, nitrogen (N) plays a particular role in influencing microbial activity and growth in soil, since microbial growth and functional activities need sufficient N in a balanced stoichiometry. However, the N content is relatively low in most structural compounds of plant material. N availability may have contrasting effects on soil heterotrophic respiration. Studies have confirmed that adding nitrogen to soil can increase microbial activity and respiration rates (Zhang et al. 2022), possibly by providing essential nutrients that promote microbial growth and enzyme production (Sinsabaugh et al. 2005). However, other research suggested that adding nitrogen can suppress microbial respiration (Janssens et al. 2010; Ramirez et al. 2010), likely due to exceeding thresholds and consequently causing a shift to more efficient communities that release less CO_2 per growing biomass and demand greater N amounts (Ågren et al. 2001). Additionally, some studies indicate that nitrogen may have little to no impact on heterotrophic respiration (Sun et al. 2014). The question of whether the variability of heterotrophic respiration in response to N addition is erratic or perhaps linked to the amount of N relative to the amount of plant residue carbon (resource C/N ratio) needs to be addressed to explain a discrepancy occurring in the literature. In this regard, the typical microbial biomass C/N ratio determines the minimum N requirement for microbes (Zechmeister-Boltenstern et al. 2015; Yuan et al. 2019). However, the optimum resource stoichiometry may be slightly larger than the microbial biomass C/N ratio, as microbes need C not only for biosynthesis purposes but also as an energy source (USDA-NRCS 2011). Consequently, when the resource C/N ratio falls below or exceeds the microbial biomass C/N ratio, microbes face C and N limitations, respectively. Therefore, it remains unclear how N, relative to C availability, affects microbial respiration, particularly during the intensive respiration of the exponential growth phase when the respiration rates are at the highest level.

Another reason for ambiguous findings on the effect of N addition on heterotrophic respiration could be that soil conditions differ between studies. Water availability is among the most important factors influencing microbial growth and activity, as resource accessibility at low water content frequently serves as a limiting factor for microorganisms in terrestrial ecosystems (Manzoni, Schimel, and Porporato 2012). In soil, the supply of dissolved substrate, nutrients, and oxygen is facilitated by diffusion through the liquid phase, particularly considering that the majority of cells in soils are sessile (Schimel and Schaeffer 2012; Joergensen and Wichern 2018). This is specifically important when the dissolved C or nutrient supply is the limiting factor of microbial growth. In addition, water facilitates the mobility of microorganisms and extracellular enzymes in the soil. Thus, microbial growth increases proportionally with water content. However, at high water contents above a certain threshold, oxygen limitation becomes the greater constraint for heterotrophic respiration (Tecon and Or 2017), which might lead to a 10-fold decrease in mineralization rate as a result of a shift from aerobic to anaerobic metabolism (Keiluweit et al. 2017). The water contents at which anaerobic conditions occur can vary for the same reasons as stated above for N addition (Schlüter et al. 2025). The combination of two environmental factors, like water content and nutrient availability, may even lead to directional changes in soil processes and microbial communities (Rillig et al. 2019). This calls for systematic incubation studies under well-controlled conditions to investigate under which circumstances high N supply has a positive or negative effect on microbial respiration.

The objective of our study was to identify threshold behaviour in cellulose decomposition dynamics imposed by C, N, and oxygen limitation so that a gradual change in environmental conditions would no longer lead to a linear change in the dynamics of CO_2 release as an indication of microbial growth stages. To this end, we conducted incubation experiments for 35 days using long-term unfertilized and fertilized agricultural soils. By providing the same amount of nutrient solution across different amounts of cellulose-C addition, we created a range of resource C/N ratios. Additionally, to investigate the effect of potential oxygen limitation on heterotrophic respiration, we implemented two water regimes, one around field capacity and another wetter treatment. Respiration rates and cumulative CO_2 efflux were determined continuously for these incubations. To assess the

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potentially limiting effect of N on heterotrophic respiration, the mineral N content was determined destructively during and at the end of the incubation.

In light of the context provided, we hypothesized that (i) Resource stoichiometry coinciding with that of microbial biomass imposes a threshold behaviour on cumulative C respired during the exponential growth phase. When resource C/N is less than that of microbial biomass (C limitation), respiration should be proportional to substrate-C input, as C availability drives microbial growth. In the other case (N limitation), respiration should be independent of substrate-C input and constrained by the available nitrogen required for biomass synthesis. (ii) Resource stoichiometry governs the timing of growth retardation. We expect that the onset of growth retardation accelerates with increasing resource C/N ratio, as nitrogen shortage limits growth. Finally, we hypothesized that (iii) the resource stoichiometry effect depends on the soil water content. Under high initial microbial activity, an increase from field capacity to high water content may impose oxygen limitation, reducing microbial respiration. Under lower initial microbial activity, the same water excess should predominantly improve substrate and nutrient accessibility, leading to accelerated microbial respiration.

2 | Material and Methods

2.1 | Soil Origin, Properties, and Pre-Incubation

Haplic Luvisol soil material was collected from the Dikopshof long-term fertilization trial established in 1904 at the University of Bonn, Germany. The trial comprises a five-year crop rotation with sugar beet, winter wheat, winter rye, legume (mainly Persian clover), and potato (oat until 1953). Two treatments of this trial were considered for this study: a farmyard manure treatment (FYM) that had received cattle manure at a mean annual rate of 5–12 t ha^{-1} since 1904 and an unfertilized (UF) treatment. Soil material was collected with a spade from the topsoil (A horizon) to a depth of 20 cm in September 2021. The soil was air-dried and passed through a 2 mm sieve. Afterward, the soil was homogenized and stored at room temperature. MBC content of the soil was determined using preincubated soil by the chloroform fumigation extraction (CFE) method with 0.05 M K_2SO_4 (Vance et al. 1987). A comprehensive characterization of the soil is provided by Lorenz et al. (2024) with selected characteristics in Table 1. For further details on the field experiment, we refer to Holthusen et al. (2012) and Seidel et al. (2021).

Prior to incubation, the soil was pre-incubated in a plastic tray where it was rewetted to 40% of the final water content, which was later adjusted for the subsequent cellulose incubation experiment. The preincubation tray was covered by aluminum foil with a few holes to ensure both minimum dehydration of soil and sufficient oxygen availability. Preincubation lasted for 7 days at a room temperature of around 22°C, to imitate the conditions in fresh soil and to avoid the potential pulse in CO_2 emission directly after rewetting (Birch 1958; Fierer and Schimel 2003). During preincubation, water loss was replenished by frequent water addition, and the seedlings grown were manually removed from the soil.

TABLE 1 | Selected chemical and biological characteristics of the Dikopshof soil in fertilized (FYM) and unfertilized (UF) states.

Parameter	Unit	FYM	UF
TOC[a]	[%]	0.74	0.69
TN[b]	[%]	0.08	0.08
N_{min}	[mg kg^{-1}]	25.8	13.1
C/N		9.5	8.8
pH	(in $CaCl_2$)	6.3	6.1
P	[mg kg^{-1}]	57	30
K	[mg kg^{-1}]	161	26
MBC[c]	[µg C g^{-1} soil]	190.3	79.0
Clay	[%]	15.1	16.2
Silt	[%]	68.9	67.3
Sand	[%]	15.9	16.4
Basal respiration	[µg CO_2 g^{-1} h^{-1}]	0.72	0.66

[a]Total organic carbon.
[b]Total nitrogen.
[c]Microbial biomass carbon.

2.2 | Experimental Treatments

Various treatments were designed to investigate the effect of cellulose amount, nutrient addition, and soil water content on soil heterotrophic respiration, using farmyard manure fertilized (FYM) and unfertilized (UF) soils that varied in terms of microbial activity at the start of the incubation (Table 1). For the sake of comparability, the amount of C added was reported relative to the amount of soil MBC. For the unfertilized soil, a relatively narrow range (0–4 times MBC) was examined, whereas the fertilized soil was subjected to a broader range (0–16 times MBC) as it had a higher nutritional status (Table 1). For the absolute values see Table 2. The cellulose addition of four times MBC was carried out in both soils for a direct comparison. Incubations were performed by 30.46 g of soil (DW) in 280 mL vessels, where cellulose was added uniformly as a powder to the preincubated soil to ensure a homogeneously distributed mixture. The soils were packed to a bulk density of 1.15 g/cm^3, resulting in a soil height of ~1 cm. The soil water content was adjusted via water addition to a final gravimetric water content of 30% or 39% (w/w).

To investigate the effect of nutrients on microbial respiration, treatments with FYM soil were considered with or without the addition of a nutrient solution ($(NH_4)_2SO_4$ 9.5 g/L, KH_2PO_4 14.75 g/L, $MgSO_4(H_2O)_7$ 19 g/L), where 5.46 mL of the solution or deionized water was added to the preincubated soil, respectively. The amount of water or nutrient solution adjusted the soil moisture to the gravimetric soil water content of 30% (w/w).

For examining the response of microbial respiration to different soil water contents, two gravimetric water contents of 30% and 39%, corresponding to 57% and 74% of water holding capacity (WHC) were considered. WHC was determined with the same amount of soil packed to the same bulk density after

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TABLE 2 | Overview on the experimental treatments and the corresponding experimental incubation conditions.

Treatment label	Soil fertilization	Relative amount of substrate-C added (times of MBC)	Absolute amount of cellulose-C added ($\mu g\,C\,g^{-1}$)	Gravimetric soil water content	Nutrient status	Resource C/N ratio
UF_0×MBC	Unfertilized	0	0	30%	Without nutrient	NA
UF_0.1×MBC	Unfertilized	0.1	8	30%	Without nutrient	0.6
UF_1×MBC	Unfertilized	1	80	30%	Without nutrient	6.1
UF_4×MBC	Unfertilized	4	320	30% and 39%	Without nutrient	24.4
FYM_0×MBC	FYM-fertilized	0	0	30%	Without nutrient	NA
FYM_4×MBC	FYM-fertilized	4	800	30% and 39%	Without nutrient	32
					With nutrient	2.1
FYM_8×MBC	FYM-fertilized	8	1600	30%	Without nutrient	62
					With nutrient	4.1
FYM_16×MBC	FYM-fertilized	16	3200	30%	Without nutrient	124
					With nutrient	8.3

Note: Additional information about the soil properties and incubation conditions is given in the text and Table 1.
Abbreviations: FYM, farmyard manure; MBC, microbial biomass carbon; UF, unfertilized.

free drainage on a membrane-supported Büchner funnel. These water contents corresponded to matric potentials of pF = 1.8 and pF < 1.0 as determined in parallel water retention curve experiments with the same soil. At the lower water content, conditions resemble those near field capacity (Ghezzehei et al. 2019; Schlüter et al. 2025) whereas at the higher water content, some soil macropores start to become water-filled. The moisture comparison was performed in FYM soil, with and without nutrient addition, and once in UF soil without nutrient addition (Table 2). Again, this selection was made to provide different initial microbial activity.

2.3 | Respiration Measurement

The CO_2 efflux was measured continuously from the incubation vessels placed inside the water bath of a Respirometer (Respicond V, Sweden) at a constant temperature of 22°C for 35 days. This process involved trapping CO_2 with 10 mL KOH (0.6 M) and simultaneous measurement of the electrical conductivity of the KOH solution (Chapman 1971; Nordgren 1988). The concentration of the KOH solution was chosen such that saturation of the base trap during incubation was avoided. Three to four replicates, depending on device capacity, were measured for each treatment. An empty vessel was also considered as a blind treatment for data processing.

2.4 | Mineral Nitrogen Measurement

In parallel to the main incubations (see Section 2.2), additional incubations were carried out under the same conditions to measure mineral N (N in the form of NH_4^+ and NO_3^-) in the course of incubation. Destructive samples were taken on days 3 and 35 after cellulose addition from all treatments, with an extra sampling day during the first week of incubation for each treatment adjusted by the dynamics of the CO_2 evolution rate.

Mineral nitrogen was determined by extracting 5 g of soil with 20 mL of 1 M KCl. The samples were shaken on a rotary shaker for 1.5 h with a subsequent filtration (Whatman Schleicher and Schuell 595 1/5 Ø 270 mm filter). A flow injection analyzer (FIAstar 5000, Foss GmbH, Rellingen, Germany) was used for the measurement of NH_4^+ and NO_3^-.

2.5 | Ergosterol Measurement

Ergosterol content of the soil was determined from 300 mg of the soil extracted with 1.5 mL methanol (HPLC Isocratic grade, Baker, USA). The samples were shaken on a vortex shaker at 2300 min^{-1} for 30 s and centrifuged at 8200 rpm for 5 min. The supernatant was passed through a filter (Minisart RC 0.45 μm). The ergosterol concentration in the filtered samples was determined by an HPLC utilizing a reverse phase RP18 column (150 × 3 mm, 5 μm) (Sepserv). The mobile phase was 100% methanol (HPLC Isocratic Grade, Baker, USA), with a flow rate of 0.7 mL min^{-1} (~114 bar pressure). The column temperature was maintained at 30°C. Detection was performed using a Diode Array Detector (DAD) at a wavelength of 282 nm, with a bandwidth of 8 nm (Sundberg et al. 1999; Högberg 2006).

2.6 | Data Processing and Statistical Analysis

The raw data of the CO_2 efflux rates were processed to reduce noise and remove long-term drifts in the electrical conductivity signal, which occurred in uncontrollable ways due to

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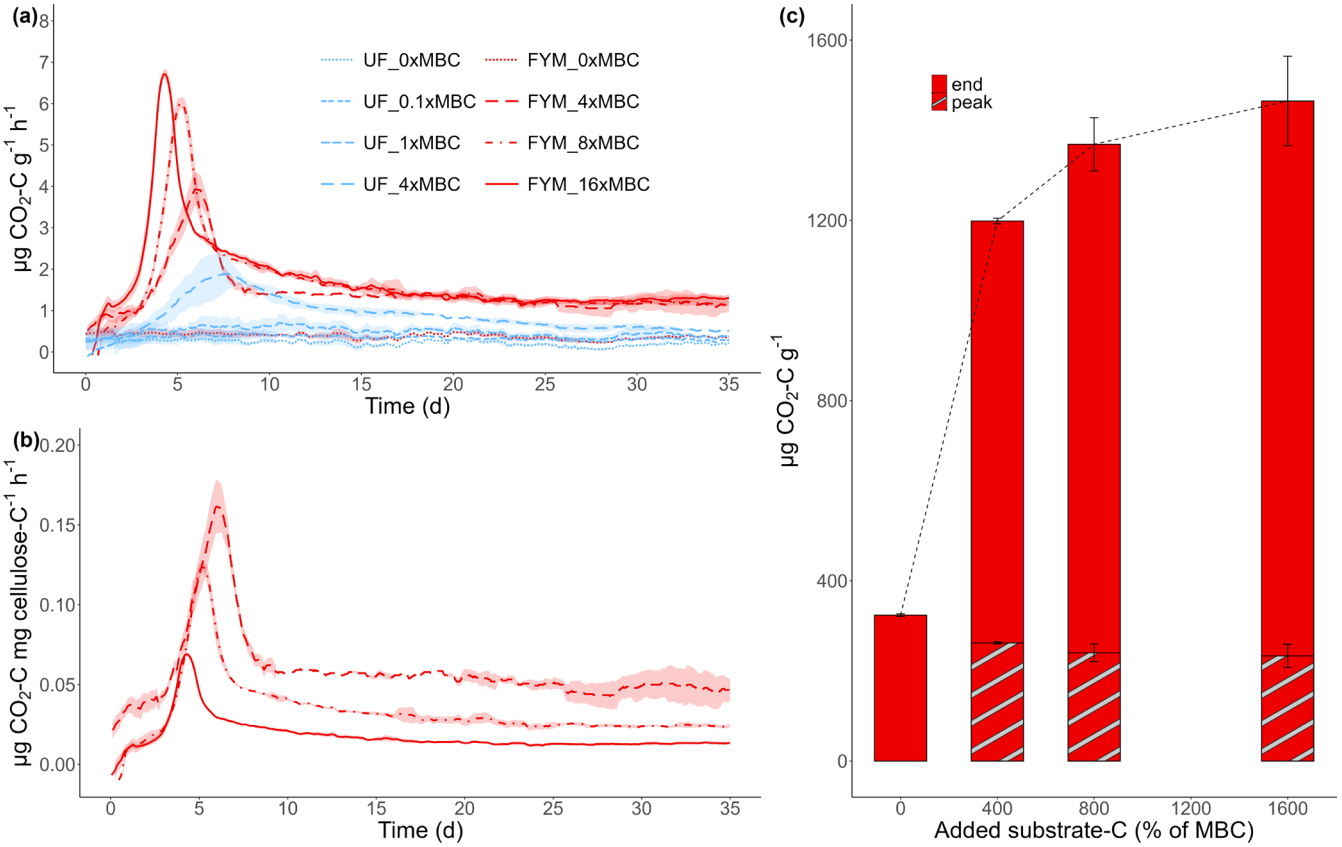

FIGURE 1 | (a) Effect of cellulose quantity on CO_2 evolution rate over 35 days from fertilized (FYM) and unfertilized (UF) soils at a water content of 30% (w/w). (b) Effect of cellulose quantity on CO_2 evolution rate normalized per C added from fertilized soil for those treatments with an explicit growth peak induced by the added substrate (C-cellulose ≥ 4×MBC). (c) Cumulative C efflux from fertilized soil at different amounts of C-cellulose based on MBC up to the end of incubation (as simple bars and dotted line), and at the corresponding times of peak points (patterned bars). Shadows and error bars indicate a 95% confidence interval ($n = 4$).

conditions of the laboratory. The three growth stages were identified based on the pattern of the CO_2 time series: a lag period of several days prior to the peak, exponential growth with a peak in CO_2 efflux after five to ten days, and growth retardation, as indicated by descending slopes in CO_2 emission rate accompanied by the long tailing after the peak. Data processing was achieved by first employing a moving median with a window size of 5 h for outlier removal. Subsequently, the CO_2 efflux rates in the blind treatment (empty vessel) were subtracted to correct for the long-term drift of the electrical conductivity signal. The average time series of all replicates was further processed with a moving average with a window size of 24 h that was extended to 72 h in the case of high-frequency noises in the area prior to the peak, as well as the later tailing. The application of variable smoothing window size was necessary to preserve the smoothness of the long tailing and height of the CO_2 release peak for those treatments in which they occurred.

All statistical analyses were performed to test the effect of treatments using the *t.test* function with p-value = 0.05 in base R (4.4.1). Figures were created in R using the ggplot2 package (Wickham 2016).

3 | Results

3.1 | Effect of Cellulose Amount on Microbial Respiration

Only the addition of cellulose in amounts of 4×MBC and above resulted in CO_2 emission curves with distinct peaks, irrespective of the fertilization status (Figure 1a). By increasing the initial cellulose amount from 4×MBC to 16×MBC, the CO_2 emission peaks (maximum activity) were reached earlier with higher values. More specifically, in FYM soil, CO_2 emission curves peaked on day 4.3, 5.2, and 6.1 after cellulose addition in 16×MBC, 8×MBC, and 4×MBC treatments, respectively. The same trend was present when comparing UF and FYM treatments with 4×MBC cellulose addition. In this case, peaks of CO_2 emission occurred on days 8.2 (UF) and 6.1 (FYM) after cellulose addition, with larger values of CO_2 evolution rate for FYM treatment compared to the UF treatment. All other treatments receiving less than 4×MBC cellulose resulted in near basal respiration values (Figure 1a). Normalization of CO_2 emission curves by the amount of added C revealed an alignment across all FYM treatments in the ascending slope of CO_2 emission curves, differing only in the initiation of the decrease in CO_2 emission rate (peak time) (Figure 1b).

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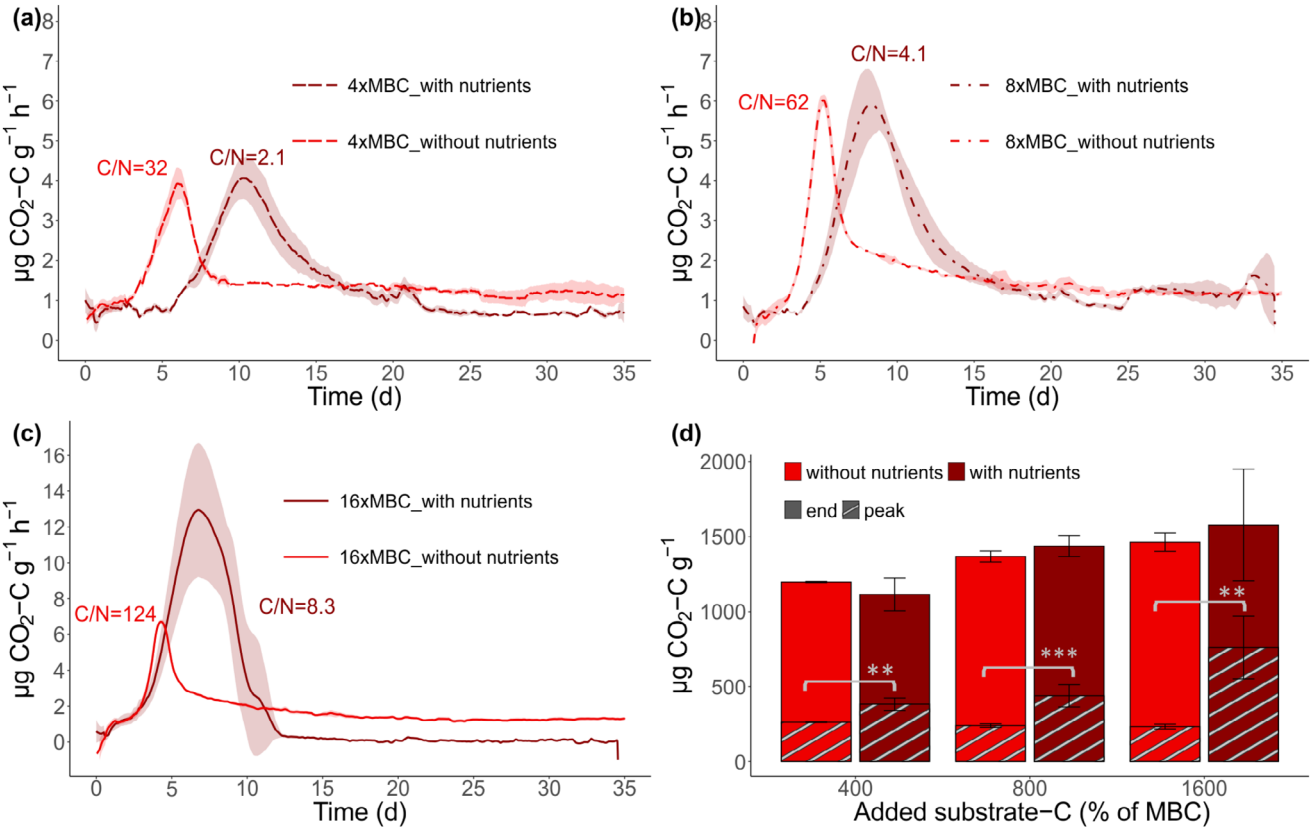

FIGURE 2 | CO_2 evolution rate over 35 days from fertilized soils with and without nutrient addition at a soil water content of 30% (w/w) at the amount of C-addition of (a) 4×MBC (b) 8×MBC and (c) 16×MBC (note that the scale of *y* axis in sub-figure c is different from that of sub-figures a and b). (d) Cumulative C efflux from fertilized soil with and without nutrient addition at different amounts of C-cellulose based on MBC up to the end of incubation (as simple bars), and at the corresponding times of peak points (patterned bars). Shadows and error bars indicate a 95% confidence interval ($n = 3$ and 4). Asterisks denote significant differences in treatments with and without nutrient addition. *** indicates significance at the 0.001 level, ** indicates significance at the 0.01 level.

The total amount of C respired in FYM treatments demonstrated similar values ranging from 233 to 262 µg CO_2-C g^{-1} at the respective peak times, i.e., day 4.3, 5.2, and 6.1 for 16×MBC, 8×MBC, and 4×MBC treatments, respectively (Figure 1c). At the end of the incubation, the amount of C released in these treatments increased disproportionately to the amount of substrate-C added, revealing a saturation pattern (dashed line in Figure 1c).

3.2 | Effect of Nutrients on Microbial Respiration

The addition of nutrients to the 4×MBC-FYM soil treatment slowed down microbial growth (Figure 2a). In the nutrient-amended treatment, the onset of increased CO_2 efflux started at a later time, with CO_2 emission peaks occurring significantly later, on day 10.4 compared to day 6.1 (Figure 2a). This pattern was consistent across all treatments and was attenuated by increasing the initial amount of cellulose, from 4×MBC to 16×MBC (Figure 2a–c).

The cumulative C release at the respective peak times of all treatments amended with nutrients showed a significantly higher amount of C release compared to the treatments without nutrient amendment (patterned bars in Figure 2d). Nevertheless, among all treatments, cumulative C release at the end of the incubation (day 35) in treatments with nutrient amendment was not considerably different from those in treatments without amendment (simple bars in Figure 2d).

3.3 | Dynamics of Mineral Nitrogen and Correlation of Peak Time With the Resource C/N

In the treatments without nutrient addition, the levels of mineral N decreased by 62%, 61%, and 22% in the 4×MBC, 8×MBC, and 16×MBC treatments, respectively, on days 6, 5, and 4 when compared to their respective amounts at day 3 (Figure 3a, separated by NH_4^+ and NO_3^- in Figure S2). Note that the N content is an order of magnitude greater in the nutrient-amended treatment (Figure 3b). By day 35, the 8×MBC and 16×MBC treatments exhibited a mineral N reduction of 73% and 66%. In nutrient-amended treatments, the reduction in the mineral N during the incubation (ΔN_{min}) scaled monotonically but not linearly with cellulose addition (Figure 3c).

Both the treatments with and without nutrient addition demonstrated a linear correlation between the timing of the CO_2 release peak and the resource C/N (ratio of added carbon to mineral nitrogen), yet with different slopes (Figure 3d).

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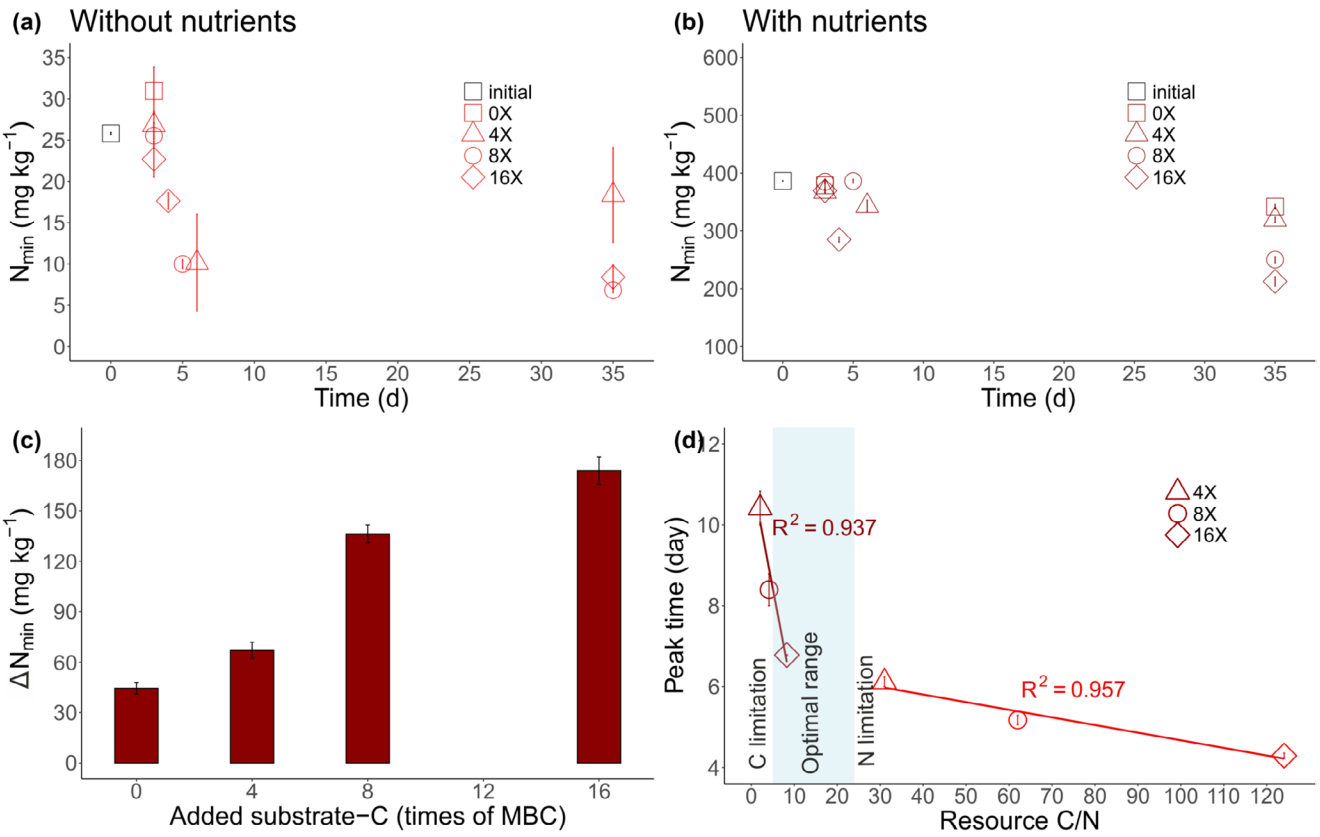

FIGURE 3 | Amounts of mineral nitrogen in treatments without nutrient addition (a) and in treatments with nutrient addition (b) over 35 days from fertilized soils at a soil water content of 30% (w/w) and 39% (w/w) for different amounts of C-addition. (c) Mineral N consumption during 35 days from fertilized soils with nutrient addition at a soil water content of 30%. (d) time of peak (maximum activity) in CO_2 evolution rate for different ratios of added C to mineral N at different amounts of C addition with and without the addition of nutrient solution. The shadowed area represents the optimum C/N for microbes. The error bars indicate a 95% confidence interval ($n = 3$ or 4).

3.4 | Ergosterol Content

The addition of a nutrient solution in 8×MBC and 16×MBC treatments increased the ergosterol content by 37% and 158% at the end of the incubation (day 35). In contrast, the nutrient addition did not have a significant effect on the ergosterol content in 4×MBC and 0×MBC treatments (Figure S1).

3.5 | Effect of Soil Water Content on Microbial Respiration

In UF soil, an increase in water content from 30% to 39% significantly accelerated microbial respiration gauged by a reduction in peak time from day 8.2 to 5.5 (Figure 4a). Despite the different dynamics of CO_2 evolution rate, the total C released in these treatments was similar at both the peak time and the end of the incubation (Figure 4d).

In the FYM soil without nutrient amendment, increasing the water content from 30% to 39% expedited the growth, resulting in a 1.6-day earlier CO_2 peak at day 4.5. In addition to a poorly defined peak at day 4.5, the treatment with 39% water content revealed a secondary peak at day 20.7 (Figure 4b).

In the FYM soil with nutrient addition, an increase in water content did not considerably alter the timing of the CO_2 emission peak. Nevertheless, the increased water content strongly reduced the respiration rates. The treatment with 39% water content displayed a strong variability among replicates throughout the peak area (Figure 4c). For both FYM treatments, with and without nutrient addition, a higher water content resulted in lower cumulative C release until peak time and also until the end of incubation, though clear differences in final amounts of respired C were somewhat overcast by large variation in the wetter treatments (Figure 4d).

4 | Discussion

4.1 | C as the Driving Factor and N as the Limiting Factor of Microbial Exponential Growth

In line with the first hypothesis, the amount of cumulative C release until peak time (end of the exponential growth phase) featured completely different behaviour under high and low resource C/N ratios. Under N limitation (resource C/N > microbial biomass C/N), all treatments released the same C amounts until peak time, despite being amended with different C amounts (patterned bars in Figure 1c). The cumulative CO_2 release at the end of incubation was not proportional to substrate input but had a clear saturation behaviour, further confirming the proposed nutrient limitation (dotted line in Figure 1c). However, when amended with nutrients (resource C/N < microbial biomass

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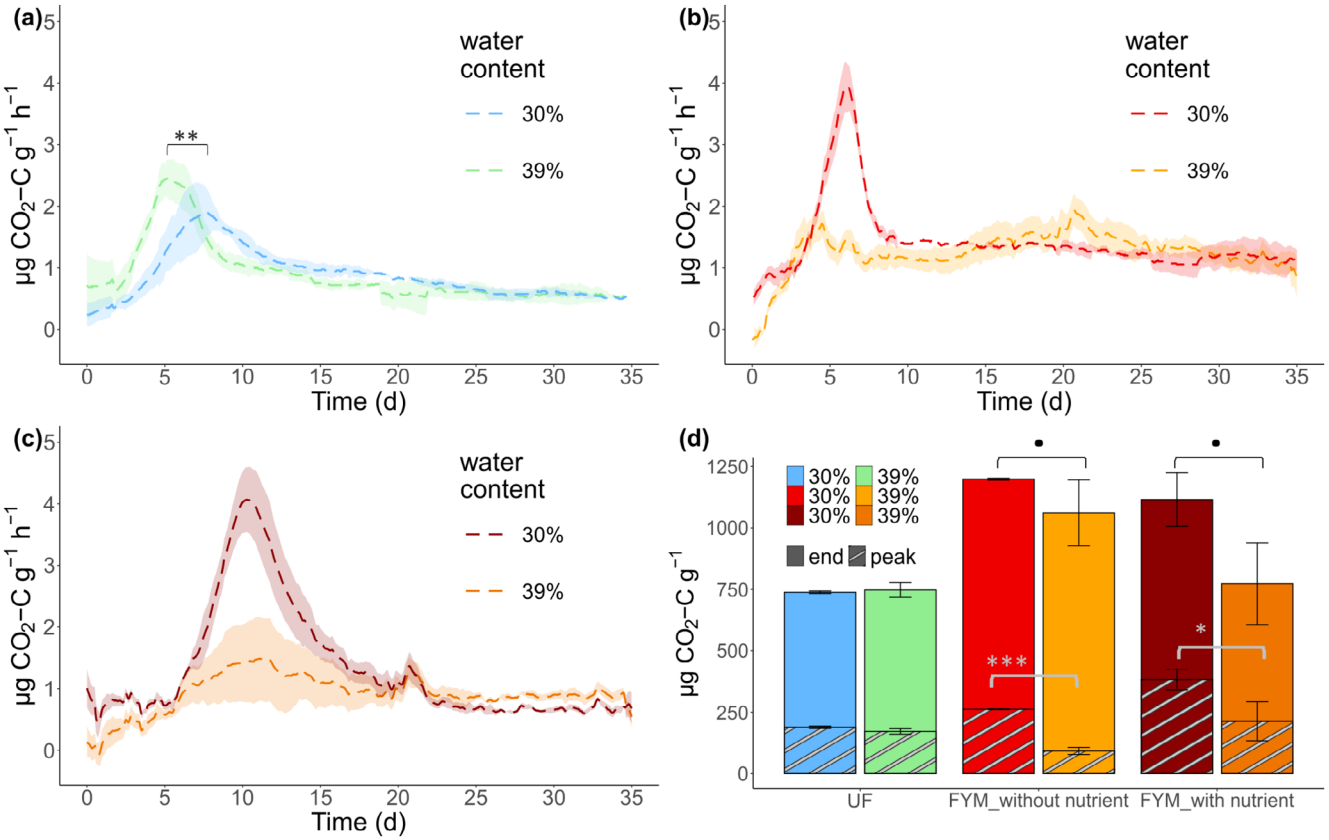

FIGURE 4 | CO_2 evolution rate over 35 days in the amount of C-addition of 4×MBC at soil water contents of 30% and 39% (w/w) from (a) unfertilized soil (UF) without nutrient addition (b) fertilized soil (FYM) without nutrient addition (c) fertilized soil (FYM) with nutrient addition. (d) Cumulative C efflux from UF and FYM soil with and without nutrient addition at 4×MBC C addition at soil water contents of 30% and 39% (w/w) up to the end of incubation (as simple bars), and at the corresponding times of peak points (patterned bars). Shadows and error bars represent a 95% confidence interval ($n = 3$ or 4). Asterisks denote significant differences between the 30% and 39% water content treatments. *** indicates significance at the 0.001 level, ** indicates significance at the 0.01 level, * indicates significance at the 0.05 level, and • indicates significance at the 0.1 level.

C/N), the cumulative C released until peak time scaled linearly with the amount of C added (Figure 2d and Figure S3). The observed similar C release until peak time in treatments without nutrients is in large part because N limitation suppressed exponential growth in those treatments as a result of an unbalanced resource stoichiometry (resource C/N = 32–124). Notably, in treatments without nutrient amendment, by normalizing the CO_2 release curves to the added cellulose-C (Figure 1b), the ascending slopes of the various C amendment treatments in FYM soil collapsed onto each other. The normalization further indicated that higher cellulose addition led to a smaller normalized peak because the decrease in C mineralization rate began earlier. An elevated substrate supply can create hot spots that involve more microorganisms feeding on C, promoting faster N consumption and limitation (Marschner et al. 2012; Kuzyakov and Blagodatskaya 2015). This is evident from the sharp decline in the mineral N level in the 4×MBC and 8×MBC treatments observed at days 6 and 5, coinciding with CO_2 evolution peak times (Figure 3a). In contrast, the rather high N level at day 4 in the 16×MBC treatment might be attributed to sampling occurring before the actual peak time (~8 h earlier). Given the rapid changes in the CO_2 evolution rate around the peak area, N depletion is likely highly sensitive to the precise timing of sampling as it corresponds to the pattern of CO_2 emission. Notably, the levels of mineral N were not fully depleted at the peak time, and even by the end of the incubation (Figure 3a), indicating that either mineral N was still available further away from microbial hotspots or that other nutrients were limiting growth (Kuzyakov and Blagodatskaya 2015; Endress et al. 2024). Considering the first situation, the local N demand must be fulfilled by N mining from more recalcitrant organic compounds (Moorhead and Sinsabaugh 2006) or even from the microbial necromass in the soil (Kästner et al. 2021).

In line with our second hypothesis, the onset of growth retardation was inversely proportional to resource C/N ratios (Figure 3d), resulting in an earlier peak time for higher cellulose additions caused by more vigorous growth. Interestingly, a clear threshold behaviour was evident in that the reduction in this peak time with increasing resource C/N ratio was stronger under C limitation (resource C/N range of 2–8) than under N limitation (resource C/N range of 30–130). Such a clear pattern also suggests that decomposition dynamics are driven by resource stoichiometry rather than by substrate amount. In line with our first hypothesis, this resource C/N threshold occurred around the optimal resource stoichiometry corresponding to the C/N ratio of soil microbial biomass. It is worth mentioning that in this study, we consider the optimal C/N ratio for microbes ranging from 5 to 24, where the upper end of the range accounts for C catabolized for energy gain, in addition to microbial C

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requirements for anabolism (USDA NRCS 2011; Russell 2014). N addition to the 16×MBC treatment shifted the resource C/N ratio from 124 to 8.3, which is within the range of the soil microbial biomass C/N ratio. This strongly increased the maximum CO_2 emission rate and prolonged the exponential growth phase to day 6.8 due to increased microbial activity as a result of the better availability of N (Manzoni, Taylor, et al. 2012), but did not affect the lag time (Figures 2c and 3d). However, this was not the case for 4×MBC and 8×MBC treatments, where a strong excess of N increased the preparation time for growth, but did not increase maximum CO_2 emission rates (Figure 2a,b). This could be attributed to a potential change in microbial community structure in the latter treatments when nutrients were available in excess (Cusack et al. 2011). Specifically, in those treatments, the resource C/N ratio was below the optimal range for soil microorganisms (Figure 3d). This may strongly reduce microbial competition and activate slow-growing microorganisms (Mau et al. 2015). This might also lead to a temporal niche differentiation (Wang and Kuzyakov 2024) where initially fungi become dominant and decompose the more recalcitrant cellulose, and only thereafter bacteria start to grow on the more labile products. When there is no nutrient limitation, changes in community composition and growth strategy might occur even at higher trophic levels (van Bommel et al. 2024). Increased levels of ergosterol, as a biomarker for fungi, after N addition in 8×MBC and 16×MBC treatments might also indicate the intensive development of fungi in those treatments (Figure S1). The observed rise in fungal growth in nutrient-amended treatments could be linked to the alleviation of N limitation caused by high C content (high resource C/N) (Rousk and Bååth 2007). This was plausible, particularly in the case of 8×MBC and 16×MBC treatments, where the resource C/N ratio without N addition was far above the optimal range of C/N for soil microorganisms (Figure 3d).

While our ergosterol measurements indicate increased fungal biomass under N addition, we did not directly quantify bacterial versus fungal contributions to respiration. Fungi generally require less N than bacteria, since fungal cell walls are mostly composed of chitin, whereas bacterial cell walls consist of peptidoglycan (Bartnicki-Garcia 1968; Schleifer and Kandler 1972). Therefore, it is often assumed that bacteria dominate under a low resource C/N ratio, while fungi are more prevalent at higher C/N ratios. However, this might not always hold completely true. This is because fungi possess a broader but not necessarily larger range of required C/N ratios compared to bacteria (Strickland and Rousk 2010). Additionally, other factors beyond C and N, such as the availability of phosphorus, can also determine whether bacteria or fungi are more dominant during litter decomposition in soil (Güsewell and Gessner 2009). Finally, the quality of substrate, i.e., accessibility of C and N, also plays a considerable role in determining which group prevails. For example, Rousk and Bååth (2007) found that bacteria and fungi indicated larger growth rates on substrates with C/N ratios of 15 and 75, respectively. Notably, when mineral N was added to these substrates, bacterial activity decreased while fungal activity increased, emphasizing the role of N form in addition to the amount. Therefore, the relative contribution of bacteria and fungi under different resource C/N ratios might not be straightforward to interpret. Future studies are needed to employ qPCR or PLFA analyses in controlled incubations, taking into account the aforementioned factors to better clarify the contribution of bacteria and fungi in respiration under different resource C/N ratios.

Across all treatments, the addition of N to the soil was found to increase the cumulative C up to the peak time, but this effect was not observed for the cumulative C release until the end of the incubation (Figure 2d). When N addition created an optimal resource C/N (Figure 2c, Figure 3d), there was no change in the lag time, but an increased CO_2 evolution rate was observed. However, when N addition resulted in a resource C/N ratio falling below the optimal range (Figures 2a,b and 3d), it prolonged the lag time for growth but did not alter the maximum CO_2 evolution rate. Thus, N excess can retard the start of exponential growth. This potentially leads to misinterpretations, as some studies, primarily focusing on soil organic matter cycling, suggest that N amendments "inhibit" heterotrophic respiration (Janssens et al. 2010; Ramirez et al. 2010). In reality, respiration may not be inhibited, and the organic matter may be decomposed, but with some delay, which is likely caused by functional changes in the microbial community due to N excess. This is particularly relevant considering that the turnover rate for cellulose applied in our study is in the order of days, while the sequential transformation of soil organic matter can extend from months to decades (Schmidt et al. 2011). Indeed, contrasting situations can occur when the treatments with and without nutrients are compared: (i) at the early stages of decomposition, the respiration rates are lower in the treatments with, versus without nutrients. This can happen due to extended lag time at an excess of nutrients. (ii) Delay in exponential growth at nutrient excess results in greater respiration as compared to the nutrient-deficient treatment already entering the growth retardation stage. (iii) In the subsequent stage, the respiration rate levels off at higher values under nutrient limitation compared to unlimited conditions. In those situations, a higher level of heterotrophic respiration at treatments without nutrients (Figure 2a–c) might be due to overflow respiration (Tempest and Neijssel 1992; Russell and Cook 1995; Manzoni and Porporato 2009) under an unbalanced resource stoichiometry. The studies corresponding to the first and third situations can result in a conclusion that N inhibits respiration, especially when heterotrophic respiration is measured with low frequency. The studies corresponding to the second situation will suggest that N enhances respiration. Although N addition strongly affected microbial respiration dynamics, total C release until the end of the incubation in both treatments with and without N indicated that respiration was unaffected by N addition. Therefore, in general, the impact of N addition on soil heterotrophic respiration depends on the various stages of microbial growth as well as the temporal scale and resolution of the study. This phenomenon may explain some of the contradictory findings regarding the effects of N on soil heterotrophic respiration (Janssens et al. 2010; Zhang et al. 2022; Yang et al. 2022; Liu et al. 2023; Li et al. 2024). Though plant residues and natural organic matter differ strongly from pure cellulose in their composition and stoichiometry, such well-controlled incubations with cellulose and nutrient amendments help to reconcile these contradicting findings. It is worth mentioning that not all CO_2 released might be cellulose-originated due to the priming effect (Kuzyakov et al. 2000; Fontaine et al. 2003). It is well-known that the magnitude and direction of priming

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are shaped not only by substrate quantity (Blagodatskaya and Kuzyakov 2008) but also by nutrient availability, with the strongest positive C-related priming typically occurring around the optimal C/N ratio of ~25 (Zhang et al. 2025). In our study, priming may have contributed to increased CO_2 evolution, as a total primed-C of 10%–15% was observed in a cellulose incubation in the same fertilized soil (Wirsching et al. 2025). However, it is anticipated that the majority of C respired up to the CO_2 peak time, before substrate availability has substantially declined (Wang et al. 2025), originates from cellulose. Any contribution from priming at this stage is expected to be due to apparent priming via pool substitution (Blagodatskaya and Kuzyakov 2008). Therefore, soil priming is not expected to alter the timing of CO_2 release peaks. Consequently, the identification of threshold behaviour in decomposition dynamics remains robust, as priming amplifies CO_2 release but does not necessarily change the peak timing or position of the C/N threshold. In any case, future research utilizing stable isotope probing techniques is needed to partition the CO_2 origin between cellulose and SOM with sufficient frequency during the incubation to clarify the potential role of soil priming on the C/N thresholds.

4.2 | Soil Water Content Might Accelerate or Decelerate Microbial Growth Depending on Soil Nutrient Status

In accordance with our last hypothesis, increasing the soil water content from 30% to 39% in unfertilized soil strongly accelerated the respiration rate during the exponential growth phase (Figure 4a). This acceleration was possibly a result of faster microbial growth, as both diffusive fluxes of dissolved carbon and nutrients were supported simultaneously by increased water availability (Manzoni et al. 2014; Nissan et al. 2023). In addition, elevated water content can also enhance the mobility of microbes and extracellular enzymes (e.g., cellulases) and as a result their accessibility to the C source. While this change in soil moisture expedited the ascending slope of the C mineralization rate, it did not change the cumulative CO_2 emissions at the peak and at the end of the incubation for both treatments (Figure 4d). In contrast to the UF treatment, the acceleration effect was less pronounced or even absent (in the case of nutrient-amended treatment) in FYM treatments (Figure 4b,c). Most likely, increased microbial activity in the presence of more nutrients leads to excessive oxygen consumption in microbial hotspots, shifting the metabolic pathways to anaerobic processes (Keiluweit et al. 2016; Schlüter et al. 2019). It is worth mentioning that the higher variability in nutrient-amended treatment with 39% soil moisture (Figure 4c) indicates the heterogeneous distribution of anaerobic microsites, which is a result of a highly variable flow of oxygen within the soil matrix (Schlüter et al. 2022). Reduced levels of CO_2 released at a water content of 39% both at the peak time and at the end of incubation (Figure 4d) in both FYM treatments were possibly due to the stabilization of added C by anaerobicity (Keiluweit et al. 2017). The balance between oxygen consumption and supply in the O_2-deficient hotspots may shift in time (Schlüter et al. 2025). This might have caused a secondary peak around day 20.7 under the soil water content of 39% in the FYM treatment without the addition of nutrients (Figure 4b). Although reduced respiration levels suggest anaerobicity, considering that mineralization rate under anaerobic respiration can be up to 10 times lower than under aerobic respiration (Keiluweit et al. 2017), future research needs to substantiate anaerobicity via direct measurement of O_2 dynamics.

In summary, the completely different response of UF and FYM at 39% water content (above field capacity) (Figure 4a,b) shows the delicate balance between O_2 supply and demand beyond field capacity, giving rise to a threshold behaviour in oxygen limitation that occurs at different water contents depending on initial nutrient status and microbial activity in soil.

5 | Conclusion

Our results indicated a delicate balance between physical and physiological constraints for microbial respiration during the utilization of cellulose in soil. We confirmed a threshold behaviour in growth dynamics at the transition from C limitation to N limitation for a resource stoichiometry around the C/N ratio of microbial biomass, most probably due to the changes in life strategy and a shift in microbial community structure. We also confirmed a threshold behaviour in growth dynamics for water contents beyond field capacity imposed by oxygen limitation. Further away from these thresholds, many features of microbial growth on cellulose scaled linearly with C and N availability. Pinpointing these thresholds to well-defined physiological and thermodynamic principles or estimating them from basic soil information by empirical relations will help to incorporate them into process-based modeling of soil carbon cycling. Future research should focus on the determination of MBC during cellulose decomposition and its relationship with carbon use efficiency in the context of substrate addition and nutrient dynamics. This study provided novel insights into the role of resource stoichiometry on the dynamics of CO_2 evolution during microbial growth in soil. Our findings further revealed significant implications for decomposition dynamics in soils, in particular in nutrient-rich microhabitats around plant roots (rhizosphere) and litter (detritusphere), where resource stoichiometry and water content can vary widely and change quickly.

Author Contributions

Fatemeh Dehghani: conceptualization, investigation, writing – original draft, methodology, visualization, writing – review and editing, formal analysis, data curation. **Robin Christian Wagner:** investigation, methodology, writing – review and editing, formal analysis, visualization. **Evgenia Blagodatskaya:** conceptualization, funding acquisition, supervision, writing – review and editing. **Steffen Schlüter:** conceptualization, funding acquisition, writing – review and editing, supervision. **Thomas Reitz:** supervision, writing – review and editing, funding acquisition, conceptualization.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** Supporting Information.

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