

## ORIGINAL ARTICLE

## Fundamental Soil Science

# Linking changes in soil microbial biomass size and nitrogen availability to increased N<sub>2</sub>O production

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## Abstract

Microbial biomass (MB) plays a critical role in the soil nitrogen (N) cycle. However, its effect on nitrous oxide (N<sub>2</sub>O) production, and how this is influenced by N availability and exogenous carbon (C) inputs, remains unknown. In this study, grassland soil (GS) and cropland soil (CS) originating from the same parent material but differing in soil matrix were selected. MB size was manipulated by preincubating soils with glucose. The soils with altered MB sizes were then amended with exogenous C sources, either ryegrass (*Lolium perenne* L.) residue or water-extracted ryegrass, which differed in C:N ratios (low and high, respectively), with or without N addition, to examine their effects on N<sub>2</sub>O production. In the absence of exogenous C and N inputs, the increased MB led to a 105% and 18% increase in N<sub>2</sub>O production in CS and GS, respectively. Among the GS treatments, combining low C:N ryegrass residues and N addition resulted in the greatest N<sub>2</sub>O production, while in CS, high C:N water-extracted ryegrass and N addition induced the highest N<sub>2</sub>O production. Furthermore, the CS with increased MB responded primarily to exogenous C inputs, while GS showed a greater sensitivity to N addition, particularly through shifts in microbial biomass N and inorganic N, which are key regulators of N<sub>2</sub>O emissions. These findings highlight the importance of MB size in shaping N<sub>2</sub>O emissions in response to external C or N inputs, and demonstrate how the stoichiometric traits of exogenous C sources interact with microbial and soil properties to drive N<sub>2</sub>O production.

**Abbreviations:** +MB, MB increased; CS, cropland soil; DI, deionized; DOC, dissolved organic carbon; EF, emission factor; GS, grassland soil; IN, inorganic nitrogen; MB, microbial biomass; MBN, microbial biomass nitrogen; R + N, ryegrass residue plus inorganic nitrogen; SOC, soil organic carbon; WHC, water holding capacity; WR, water-extracted ryegrass residue; WR + N, water-extracted ryegrass plus inorganic nitrogen.

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### Plain Language Summary

N<sub>2</sub>O production was accelerated by increased microbial biomass size. Increased soil microbial biomass and N input controlled N<sub>2</sub>O production. Residues and N addition had contrasted effect on N<sub>2</sub>O production for cropland and grassland soils.

## 1 | INTRODUCTION

Nitrous oxide (N<sub>2</sub>O) is a potent greenhouse gas that contributes significantly to climate change and the depletion of the ozone layer (Oertel et al., 2016; Ravishankara et al., 2009). It is estimated that approximately 60% of anthropogenic N<sub>2</sub>O emissions arise from agricultural soils, where microbial processes such as nitrification and denitrification serve as the primary pathways. These processes are directly influenced by soil microbial activities, soil inorganic nitrogen (IN) content, and labile carbon (C) availability (Weiler et al., 2018). Microbes are generally considered as the driving force behind organic matter decomposition processes (Domeignoz-Horta et al., 2020). They act as both a source and a sink for available nutrients, driving processes such as organic matter turnover, nitrogen (N) cycling, and nutrient mobilization/immobilization, (Butterbach-Bahl et al., 2013). The size of the microbial biomass (MB) is a key factor influencing these processes, serving as an indicator of changes in total soil organic matter (Powlson et al., 1987; Zhang et al., 2017), and its role in regulating soil C cycling has been well documented (Li et al., 2018; Li et al., 2019a). Given the strong interconnection between C and N cycles (Guenet et al., 2020), the size of MB likely plays a role in regulating N cycling as well.

In general, a large portion of soil mineralizable N is derived from microbial biomass N (MBN) (Bonde et al., 1988), and greater MB may promote microbial reactivation or reproduction, potentially stimulating soil N<sub>2</sub>O emissions (Li et al., 2019b). Several studies have shown that MB size is positively correlated with N<sub>2</sub>O production in soils (Zhang et al., 2017). This relationship is primarily attributed to the availability of C and N, which support microbial processes that convert N compounds into N<sub>2</sub>O (Chen et al., 2013). Nevertheless, a global meta-analysis showed that N enrichment increased MBN in cropland but significantly decreased both MBN and microbial biomass C (MBC) in grassland and forest ecosystems (Jia et al., 2020), suggesting that the relationship between MB and soil N<sub>2</sub>O production likely varies with land use. Therefore, understanding how MB size influences N<sub>2</sub>O emissions under different C and N conditions is important, as shifts in microbial activity can significantly alter the fate of N in the system.

Beyond MB, crop residues are another major source of C and N inputs to soils and can affect N<sub>2</sub>O emissions both directly and indirectly (Olesen et al., 2023). Crop residues

vary in chemical composition and C:N ratio, which together define their quality and biodegradability, which strongly affect soil N cycling, particularly N<sub>2</sub>O emissions. Readily decomposable compounds, such as water-extractable organic C, provide energy for denitrifying bacteria, promote electron flow, and increase denitrification rates (Guo et al., 2020). In general, the addition of crop residues with low C:N ratio (relatively rich in readily decomposable C and N) stimulates the growth of heterotrophic decomposers and alters their oxygen and nutrient demands, as well as ammonification and microbial N assimilation processes, all of which influence the availability of mineral N in soils (Li et al., 2013). In contrast, crop residues with high C:N ratios (relatively low in readily decomposable C and N), the decomposition rate slows, and microbial growth may be limited due to higher energy demand (Shi & Marschner, 2014). As a result, the addition of crop residues with low C:N ratios generally increase soil N<sub>2</sub>O emissions compared to soils without residue additions (Shan & Yan, 2013; Weiler et al., 2018), while the addition of crop residues with high C:N ratios tend to promote N immobilization, reducing N<sub>2</sub>O production as microbes compete for available soil N (Booth et al., 2005; Cheng et al., 2017). Moreover, the C:N ratio of crop residues also influences soil N<sub>2</sub>O emissions indirectly by regulating MB through changes in C and N availability. This, in turn, affects N immobilization and N<sub>2</sub>O production through complex microbial transformations (Pugesgaard et al., 2017; Schmatz et al., 2020). Understanding how crop residue inputs and N addition influence N mineralization and immobilization is essential for developing strategies to mitigate N<sub>2</sub>O emissions.

The addition of N fertilizer significantly impacts N<sub>2</sub>O emissions, particularly when combined with crop residue inputs (Li et al., 2023). While N inputs are necessary to sustain crop yields, they can also lead to an increase in N<sub>2</sub>O production. Fertilizer N addition may alleviate N limitation for some microbes such as nitrifiers and denitrifiers, which rely on IN substrates (Daly & Hernandez-Ramirez, 2020). Nitrification and denitrification are the two primary microbial pathways for N<sub>2</sub>O production, with nitrification being an autotrophic process and denitrification a heterotrophic one (Goldberg & Gebauer, 2009; Kraus et al., 2015; Tian et al., 2020). Both pathways are sensitive to substrate availability and soil environmental conditions, including soil water content, temperature, pH, labile C availability, and oxygen concentration (Butterbach-Bahl et al., 2013; Song et al., 2019). However,

the effect of N addition on MB varies across land use types. Studies have shown that N inputs tend to decrease soil MB in unmanaged ecosystems but increase MB under long-term fertilization cropping systems (Geisseler & Scow, 2014; Jia et al., 2020; Zhou et al., 2017). These contrasting responses are likely driven by underlying differences in soil properties shaped by land use history. For example, soils under unmanaged systems such as grassland, are typically characterized by high levels of labile C and greater microbial diversity compared to croplands (Conant et al., 2017). Globally, grasslands and croplands contribute approximately 6%–9% and 22%–27% of total N<sub>2</sub>O emissions (Tian et al., 2013, 2016; Zhuang et al., 2012), respectively, and N availability is widely recognized as a key limiting factor for N<sub>2</sub>O emissions in these systems (Guo et al., 2022). Given that N<sub>2</sub>O production is closely linked to MB as well as C and N availability, it is important to investigate the mechanisms by which soil N<sub>2</sub>O emissions respond to exogenous C and N inputs in these land use systems.

To investigate the impact of MB on N<sub>2</sub>O emissions and assess how interactions between crop residue and N addition modifies this impact, we preincubated two soils (grassland soil [GS] and cropland soil [CS]) with contrasting soil organic carbon (SOC) content with and without glucose to change soil MB size (Li et al., 2018). We hypothesized that the treatment of increased MB size would significantly enhance N<sub>2</sub>O emissions in GS, while the combined addition of crop residues and N would amplify N<sub>2</sub>O emissions in CS. We further hypothesized that the influence of MB on N<sub>2</sub>O emissions would vary depending on SOC content and land management history, reflecting the complex interactions between C and N cycling. By identifying the drivers of N<sub>2</sub>O emissions, particularly the role of MB and its interactions with exogenous C and N inputs, this study aims to provide insights into potential strategies for mitigating N<sub>2</sub>O emissions from agricultural soils, a critical step toward addressing climate change.

### Core Ideas

- N<sub>2</sub>O production was accelerated by increased microbial biomass size.
- Increased soil microbial biomass and N input controlled N<sub>2</sub>O production.
- Residues and N addition had contrasted effect on N<sub>2</sub>O production for cropland and grassland soils.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample preparation

Soils were sampled from the Russell Ranch Sustainable Agricultural Facility of the University of California- Davis. The climate is characterized by a Mediterranean climate with an average annual temperature of 17°C and an average annual rainfall of 460 mm (Moreno-Cornejo et al., 2015). The soils are classified as Mollic Haploxeralf with a silty clay loam texture (Soil Survey Staff, 2006). Samples were collected from a conventionally CS under wheat (*Triticum aestivum* L.)–tomato (*Solanum lycopersicum* L.) rotation for 23 years and an adjacent unmanaged GS. In spring 2015, five soil cores were randomly collected from each replicate plot (three replicates per soil system) to the plow layer (0–20 cm). All the visible plant residues and roots were removed. The soil samples were sieved (<2 mm) and stored at 4°C until used. Selected soil properties are listed in Table 1.

Mature ryegrass was used as an exogenous C source in the incubation (Bird et al., 2003). To acquire ryegrass residues with different quality (different C:N ratios), a portion of the residues was cut into small pieces (< 1 mm) and extracted with deionized (DI) water (0.5:100, w/v) at 75°C for 6 h followed by shaking 15 min (Toosi et al., 2012). All residues were oven dried at 50°C and ball milled before application.

**TABLE 1** The properties of soils and organic substrates used in the incubation experiment.

|                                          | Cropland soil |       | Grassland soil |       | Ryegrass | Water-extracted ryegrass |
|------------------------------------------|---------------|-------|----------------|-------|----------|--------------------------|
| SOC (g kg <sup>-1</sup> )                | 9.61          |       | 19.39          |       | 420      | 440                      |
| TN (g kg <sup>-1</sup> )                 | 0.79          |       | 2.58           |       | 10.10    | 8.10                     |
| C:N                                      | 12.2          |       | 7.5            |       | 41.6     | 54.3                     |
|                                          | Unchanged MB  | +MB   | Unchanged MB   | +MB   |          |                          |
| DOC (μg g <sup>-1</sup> )                | 26.6          | 44.0  | 83.7           | 93.8  | NA       | NA                       |
| MBC (μg g <sup>-1</sup> )                | 190.0         | 365.1 | 565.4          | 805.9 | NA       | NA                       |
| MBN (μg g <sup>-1</sup> )                | 10.6          | 23.9  | 113.7          | 169.4 | NA       | NA                       |
| NO <sub>3</sub> -N (μg g <sup>-1</sup> ) | 14.5          | 38.0  | 42.5           | 91.8  | NA       | NA                       |
| NH <sub>4</sub> -N (μg g <sup>-1</sup> ) | 0.4           | 1.2   | 0.7            | 0.5   | NA       | NA                       |

Abbreviations: +MB, microbial biomass increased; DOC, dissolved organic C; MB, microbial biomass; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; NA, not available; SOC, soil organic carbon.

Table 1 shows the characteristics of ryegrass residue with a low C:N ratio and water-extracted ryegrass residue with a high C:N ratio.

## 2.2 | Increasing soil MB size

To increase soil MB size, bulk soils were preincubated with glucose and N at 50% of water holding capacity (WHC) and 22°C for 7 days. Glucose at 0.24 and 1.6 mg C g<sup>-1</sup> soil was determined as the optimal concentration to induce MB growth without significantly changing microbial community structure and composition for CS and GS, respectively (Li et al., 2018). Nitrogen was added simultaneously as ammonium sulfate to reach the input C:N ratio of 10 to eliminate N limitation for microbial growth (Moreno-Cornejo et al., 2015). Control treatments (MB unchanged [MB]) received DI water instead of glucose and N were incubated under the same conditions. Soil properties after 7 days of preincubation, with and without glucose amendment, are reported in Li et al. (2018) and summarized in Table S1. The detailed methodology for this preliminary study is described in Li et al. (2018).

## 2.3 | Incubation experiment

For each type of preincubated soil (MB or MB increased [+MB]), six treatments were applied, each with three replicates: (1) no amendment (control), (2) IN (N), (3) ryegrass residue (R), (4) ryegrass residue plus inorganic N (R + N), (5) water-extracted ryegrass residue (WR), and (6) water-extracted ryegrass plus inorganic N (WR + N). This resulted in a total of 24 treatment combinations. A summary of the treatments is provided in Table S1. The preparation methods for ryegrass residues of differing quality are described in Section 2.1. Ryegrass residue was applied at 0.5% of dry soil mass, corresponding to 2.1 mg C g<sup>-1</sup> dry soil. IN was added as ammonium sulfate at a rate of 0.09 mg N g<sup>-1</sup> dry soil. Preincubated soil samples (20 g dry weight) were thoroughly mixed with the amended substrates using a spatula and pipette (for N addition), adjusted to 60% of WHC using DI water. The soil samples were placed in specimen cups (120 mL) and set inside mason jars (500 mL) containing 2 mL water at the bottom to maintain soil moisture throughout the incubation. Three jars without soil and residue (blank) were used to monitor background N<sub>2</sub>O concentration. The jars were incubated in the dark at 22°C for 42 days. The detailed description of the experimental design was referred to Li et al. (2018).

## 2.4 | N<sub>2</sub>O analysis

Gas samples were collected from the mason jars on days 0, 1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 18, 21, 28, 35, and 42. After each gas sampling collection, the jars were ventilated with CO<sub>2</sub>-free

air for 10 min and then closed and returned to the incubation conditions described above. Gas samples were analyzed for N<sub>2</sub>O concentration using a gas chromatograph system (GC-2014, Shimadzu) equipped with an electron capture detector.

The emission rate of N<sub>2</sub>O was determined by calculating the increase in N<sub>2</sub>O concentrations in the headspace of each jar:

$$F = \frac{P \times C \times V \times 28}{R \times m \times (273 + T)}$$

where  $F$  is the emission rate of N<sub>2</sub>O (μg N·g<sup>-1</sup>·d<sup>-1</sup>),  $P$  is standard atmospheric pressure (101.3 Pa),  $C$  is the difference in gas concentration between treatment and blank after 24-h incubation (in ppbv),  $V$  is the volume of head space (in L),  $R$  is the universal gas constant (8.314 J mol<sup>-1</sup> K<sup>-1</sup>),  $m$  is the weight of dry soil (g), and  $T$  is the incubation temperature (°C).

Nitrous oxide emission factor (EF, %) of added N with or without residue additions was calculated as the percentage of added N emitted as N<sub>2</sub>O using the following equation:

$$EF\% = (N_2O_{R+N} \text{ or } N_2O_N - N_2O_R \text{ or } N_2O_{\text{control}}) / N \times 100$$

where N<sub>2</sub>O<sub>R+N</sub> or N<sub>2</sub>O<sub>N</sub> represents the cumulative N<sub>2</sub>O production from the soils treated with N and residues addition or N only, N<sub>2</sub>O<sub>R</sub> or N<sub>2</sub>O<sub>control</sub> is the cumulative N<sub>2</sub>O production from soils treated with residues addition only or neither residue nor N, and  $N$  is the added N in the form of ammonium sulfate at the beginning of incubation.

## 2.5 | Soil analysis

Soil samples were collected on days 0, 14, 28, and 42 for analysis. Total soil organic C and N contents of soils and ryegrass residues were determined by elemental analyzer (EAS 4010, Costech). MBC and MBN were measured using the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). The MBC was calculated as the difference in dissolved organic C (DOC) levels between fumigated and unfumigated samples with an efficiency factor of 0.45 (Wu et al., 1990), and the MBN was calculated as the difference in total extractable N contents between fumigated and unfumigated soils with an efficiency factor of 0.54 (Joergensen & Mueller, 1996). ΔMBC (ΔMBC = MBC<sub>+MB</sub> - MBC<sub>MB</sub>) represents the change of MBC between unchanged and increased MB treatments. DOC content was measured by UV-persulfate oxidation (Teledyne-Tekmar Phoenix 8000).

To determine IN, soils were extracted with 0.5 mol L<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub> (1:5 ratio, w: v) for 1 h. The extracts were filtered through a Whatman 57 filter paper, and the supernatant was stored at 4°C. Within 48 h after extraction, samples were analyzed for IN (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) using colorimetric

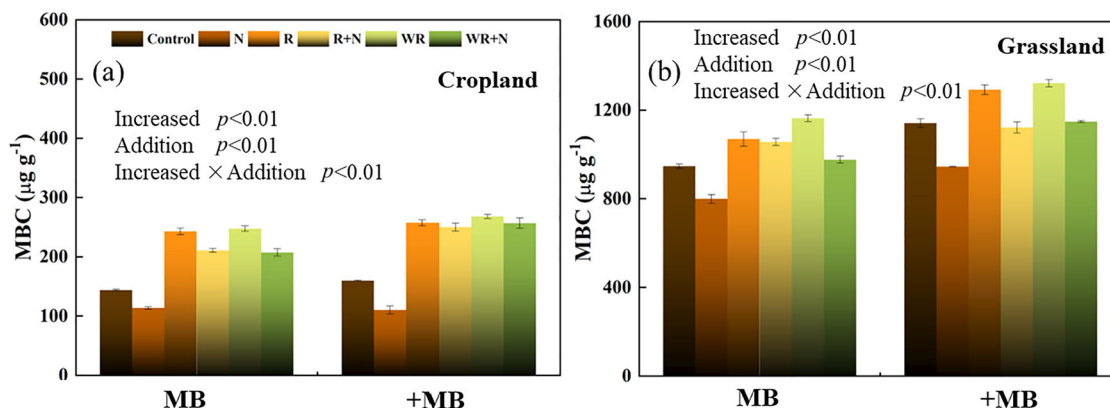


**FIGURE 1** Concentration of microbial biomass carbon (MBC) at the end of 42-day incubation. Treatments are as follows: nitrogen (N), ryegrass (R), ryegrass + N (R + N), water-extracted ryegrass (WR), and water-extracted ryegrass + N (WR + N). Error bars represent standard error of the means ( $n = 3$ ).

methods (Doane & Horwath, 2003; Verdouw et al., 1978).  $\Delta\text{IN}$  ( $\Delta\text{IN} = \text{IN}_{+\text{MB}} - \text{IN}_{\text{MB}}$ ) represents the change of IN content between unchanged and increased MB treatments.

## 2.6 | Statistical analyses

The data are presented as means of three replicates, with standard errors. Duncan's test was used to identify differences in  $\text{N}_2\text{O}$  production rate and EF among treatments at a significant level of  $p < 0.05$  (Webster & Lark, 2018). The Student's  $t$ -test was used to test for differences in these variables between MB size treatments. Three-way analysis of variance was used to determine the interaction of MB size, residue, and N addition on  $\text{N}_2\text{O}$  production. Linear regressions were used to examine the Pearson correlation between the cumulative  $\text{N}_2\text{O}$  and soil MBC, MBN, DOC, and IN concentrations. Statistical analyses were conducted using SPSS version 18.0.

## 3 | RESULTS

### 3.1 | Changes of MB

After preincubation, +MB increased by 72.6 and 368.3  $\mu\text{g C g}^{-1}$  with glucose addition compared to the no-glucose control in CS and GS, respectively (Table 1). After 42 days of incubation, compared to the no-amendment treatment (hereafter control), the addition of ryegrass residue (R treatment) increased MBC in CS soil by 69% and 61% under the MB and +MB treatments, respectively. Similarly, water-extracted ryegrass (WR treatment) increased MBC by 72% and 68% under the MB and +MB treatments, respectively (Figure 1). In GS soil, MBC increased by 13% under the R treatment regardless of MB treatment type, while under the WR treatment, MBC increased by 23% in the MB treatment and 16% in the

+MB treatment, compared to the control. The difference in MBC between MB and +MB treatments ( $\Delta\text{MBC}$ ) generally decline over time, with the largest change observed in GS soil under the R + N treatment (Figure S1). A similar declining trend was observed for  $\Delta\text{MBN}$  in both CS and GS soils over the 42-day incubation period (Figure S1).

### 3.2 | $\text{N}_2\text{O}$ production

Increasing MB size stimulated  $\text{N}_2\text{O}$  production by 105% in CS soil and 18% in GS soil under control (no amendment) conditions (Figure 2). In CS soil, compared to the control, the addition of ryegrass residue (R) and water-extracted ryegrass (WR) increased  $\text{N}_2\text{O}$  production by 320% and 278%, respectively, under the MB treatments. Compared to the MB treatment, the +MB treatment further enhanced  $\text{N}_2\text{O}$  production by 34% under R and 70% under WR. Similarly, in GS soil, the addition of R and WR increased  $\text{N}_2\text{O}$  production by 26% and 33%, respectively, relative to the control under the MB treatment. Under these same conditions, the +MB treatment further increased  $\text{N}_2\text{O}$  production by 2% under R, but decreased it by 14% under WR compared to the MB treatment.

During the first 14 days of incubation, the treatments with N addition (i.e., N, R + N, and WR + N) showed significantly higher  $\text{N}_2\text{O}$  production rates than those without N addition (Figure S2). However, these differences diminished and even disappeared as the incubation progressed. Specifically, in CS soil, +MB treatment stimulated  $\text{N}_2\text{O}$  production by 35% under N addition, while in GS soil, the increase was 23% (Figure 2).

In both soils, the addition of ryegrass, regardless of type, enhanced the effect of N on  $\text{N}_2\text{O}$  production. Among all the treatments, R + N or WR + N treatments produced the highest  $\text{N}_2\text{O}$  emissions in both CS and GS soils (Figure 2). However, no significant interactions were observed among MB size,

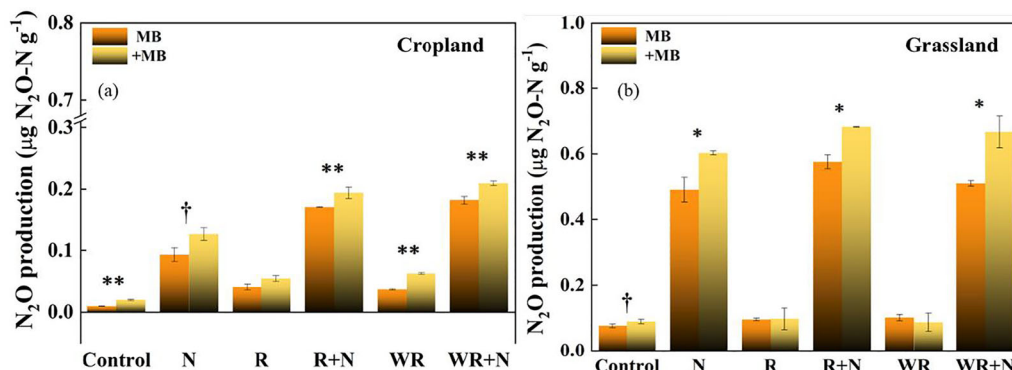


**FIGURE 2** N<sub>2</sub>O production as affected by initial microbial biomass under different residue and nitrogen additions. Treatments are as follows: control, nitrogen (N), ryegrass (R), ryegrass + N (R + N), water-extracted ryegrass (WR), and water-extracted ryegrass + N (WR + N). Error bars represent standard error of the means ( $n = 3$ ). Significant differences in cumulative N<sub>2</sub>O emissions between soils with unchanged and increased microbial biomass are indicated by † $p < 0.1$ , \* $p < 0.05$ , and \*\* $p < 0.01$ .

**TABLE 2** Three-way analysis of variances (ANOVAs) on the effect of microbial biomass size (MB), residue input (R), chemical nitrogen (N), and their interactions on cumulative N<sub>2</sub>O production from cropland soil (CS) and grassland soil (GS) during incubation.

| Source     | CS       |          | GS       |          |
|------------|----------|----------|----------|----------|
|            | <i>F</i> | <i>p</i> | <i>F</i> | <i>p</i> |
| MB         | 54.382   | <0.001   | 32.427   | <0.001   |
| R          | 165.265  | <0.001   | 5.780    | 0.01     |
| N          | 1488.971 | <0.001   | 1551.930 | <0.001   |
| MB × R     | 1.088    | 0.35     | 0.211    | 0.81     |
| MB × N     | 0.735    | 0.40     | 25.412   | <0.001   |
| R × N      | 8.029    | <0.001   | 3.048    | 0.07     |
| MB × R × N | 0.206    | 0.82     | 0.869    | 0.43     |

ryegrass type, and N input on N<sub>2</sub>O production for both soils (Table 2).

### 3.3 | Inorganic N and N<sub>2</sub>O emission factor

After 42 days of incubation, compared to the control, IN levels in CS soil increased by 275%, 250%, and 244% under the MB treatment, and by 243%, 218%, and 206% under the +MB treatments for the N, R + N and WR + N treatments, respectively (Figure 3). In GS soil, IN increased by 67%, 58%, and 57% in the MB treatment, and 61%, 45%, and 45% in the +MB treatment under the same respective treatments. Compared to CS, GS soil exhibited a consistently greater magnitude of ΔIN over the incubation period (Figure S1). The addition of both N and residue (R + N and WR + N) reduced the ΔIN relative to residue-only treatments (R and WR).

Regardless of MB size or residue type, the N<sub>2</sub>O EF was significantly higher in GS soil than in CS soil (Table 2; Figure 4). In GS soil, EF was significantly higher under the +MB treat-

ments, compared to the MB treatment ( $p < 0.05$ ). In contrast, no significant effect of MB size on EF was observed across all treatments in CS soil.

## 4 | DISCUSSION

### 4.1 | The effect of MB size and residue addition on soil N<sub>2</sub>O production

Nitrogen transformation processes are closely mediated by soil microorganisms, and MB plays a critical role in regulating these processes (Li et al., 2019b). In our study, we observed that increasing the soil MB size generally enhanced N<sub>2</sub>O production across all treatments, except under the R treatment in both CS and GS soils and the WR treatment in GS soil (Figure 2). The increase in N<sub>2</sub>O emissions under the +MB treatment was more pronounced in GS soil than in CS soil. This is likely due to the GS soil's lower C:N ratio and initially higher levels of DOC, MB, and microbial activity, which together contribute to a greater potential for N<sub>2</sub>O production (Liu et al., 2021) (Table 1). In soils with initially large MB and low C:N ratio, microbial turnover rates and nutrient availability are generally higher (Li et al., 2019a). While rapid microbial turnover can temporarily immobilize exogenous IN by converting it into microbial necromass and metabolites (Li et al., 2013), it can also accelerate the mineralization of organic N, increasing the availability of IN for microbial processes such as denitrification and subsequent N<sub>2</sub>O production (Figure 3). However, in our study, the differential responses of N<sub>2</sub>O emissions to increased MB size in CS and GS soils cannot be solely attributed to differences in IN availability, as both soils had ample IN throughout the incubation (Table 1; Figure 3) (Firestone & Davidson, 1989). Instead, the enhanced N<sub>2</sub>O emissions in GS soil may be attributed to increased denitrification activity driven by high MB and DOC (Table 1)

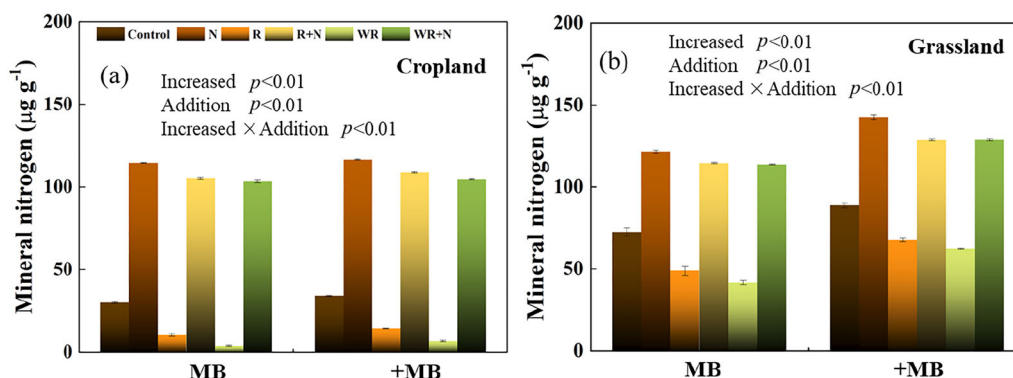


**FIGURE 3** Concentration of inorganic nitrogen at the end of 42-day incubation. Treatments are as follows: nitrogen (N), ryegrass (R), ryegrass + N (R + N), water-extracted ryegrass (WR), and water-extracted ryegrass + N (WR + N). Error bars represent standard error of the means ( $n = 3$ ).

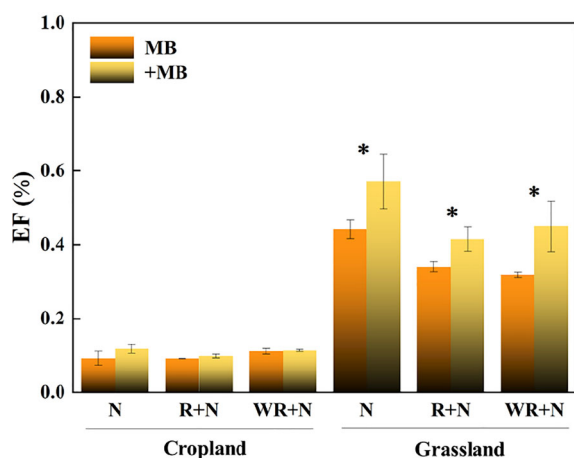


**FIGURE 4** Emission factors (EF) of N<sub>2</sub>O production in response to nitrogen with or without residues additions for cropland and grassland soils with different microbial biomass sizes. Treatments are as follows: nitrogen (N), ryegrass + N (R + N), and water-extracted ryegrass + N (WR + N). Error bars represent standard error of the means ( $n = 3$ ). Asterisks above the bars indicate significant differences between the unchanged and increased biomass soils within each treatment: \* $p < 0.05$ .

(Firestone & Davidson, 1989). Increased MB size in soils with high C availability can enhance oxygen consumption, creating anoxic microsites that facilitate denitrification, a major pathway of N<sub>2</sub>O production under anaerobic conditions (Zhu et al., 2013). Thus, the pronounced N<sub>2</sub>O emissions in GS soil under the +MB treatment likely reflects enhanced denitrification due to increased microbial activity and high C availability.

The response of soil N<sub>2</sub>O production to the addition of exogenous C sources, such as plant residues, is strongly influenced by MB. In CS soil, the addition of WR led to a significant increase in N<sub>2</sub>O emissions under the +MB treatment compared to the MB treatment (Figure 2). Incorporating plant residue into soil increases the availability of C and N to microorganisms, stimulating microbially mediated miner-

alization of native soil organic N (Chèneby et al., 2010) and promoting denitrifier activity, which can lead to enhanced N<sub>2</sub>O production (Hicks et al., 2021; Pimentel et al., 2015). This effect is often modulated by the C:N ratio of the amendment, with higher C:N inputs favoring immobilization over mineralization (Mooshammer et al., 2014). In CS soil, the interaction between +MB and high C:N plant residue stimulated greater N<sub>2</sub>O emissions compared to the high C:N plant residue under the MB treatment (Figure 2), although accounting for mineralization derived from soil or plant residue is challenging. This is likely due to higher MB promoted N mineralization and organic matter decomposition, potentially creating microsites favorable for denitrification, where N<sub>2</sub>O is produced as an intermediate product (Myrold 2014; Miller et al., 2008). This mechanism is supported by the observed positive correlation between N<sub>2</sub>O production and MBC (Figure 5). These findings imply that in CS soil, which has relatively low C availability (Table 1), C limitation plays a key role in regulating N<sub>2</sub>O production through MB. In GS soil, however, the R treatment produced more N<sub>2</sub>O than the WR treatment under the +MB treatment. This suggests that in C-rich soils like GS, microbial activity may not be limited by C availability.

## 4.2 | The effect of N application on soil N<sub>2</sub>O emissions

The application of N increased soil IN concentrations, resulting in increased N<sub>2</sub>O emissions (Figure 3). This suggests that the increased N<sub>2</sub>O emissions were mainly driven by the added N, especially when its input exceeded the microbial immobilization capacity (Peng et al., 2011). Similar findings were reported by Daly and Hernandez-Ramirez (2020), who reported that the majority of N<sub>2</sub>O emissions were derived from N fertilization, rather than native soil N. Under conditions of limited labile C, N addition may also prime the mineralization of soil organic N, a process

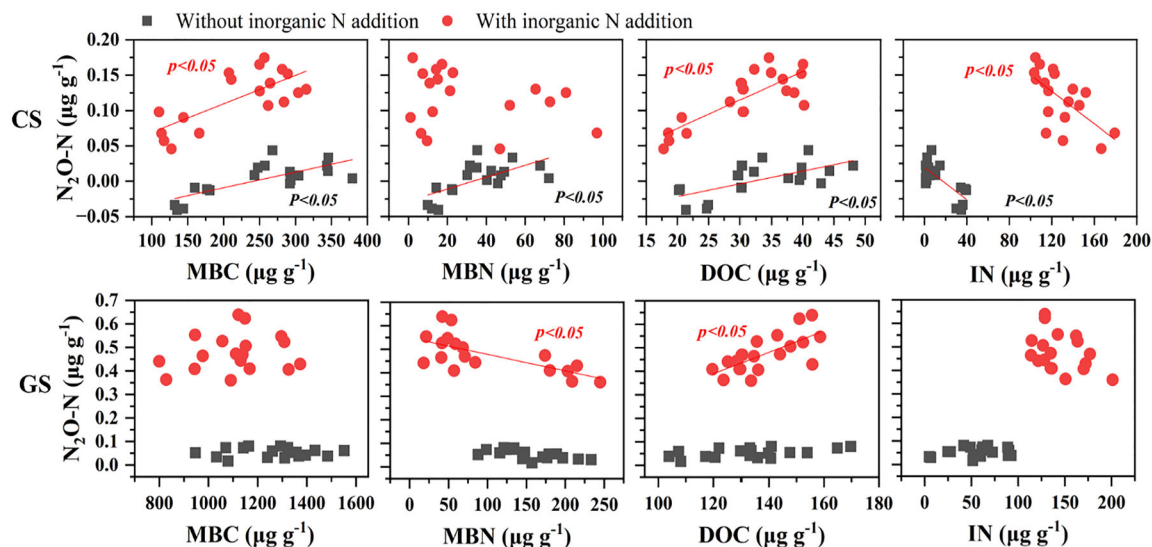


**FIGURE 5** Pearson correlation between  $\text{N}_2\text{O}$  and MBC (microbial biomass carbon), MBN (microbial biomass nitrogen), DOC (dissolved organic carbon), and IN (inorganic nitrogen) over 14, 28, and 42 days in cropland soil (CS) and grassland soil (GS).

by which microbes decompose organic N to meet energy demands, releasing excess N as  $\text{NH}_4^+$  (Farrell et al., 2014), a substrate for  $\text{N}_2\text{O}$  production. While microbial assimilation of  $\text{NO}_3^-$  into MB can reduce  $\text{NO}_3^-$  accumulation in soils and environmental losses (Cheng et al., 2017), this process may be suppressed when N inputs exceed microbial demand.

Compared to treatments receiving only IN input or no N input, co-application of plant residues and IN resulted in higher  $\text{N}_2\text{O}$  emissions. Plant residue amendments influence soil N dynamics and fertilizer requirements depending on their composition, particularly C:N ratio (Jia et al., 2020; Olesen et al., 2023; Pullens et al., 2021). The decomposition of immature, N-rich residues may release more IN than microbes require, further enhancing  $\text{N}_2\text{O}$  emissions (Pullens et al., 2021). This effect was particularly evident in CS soil with plant residue (Table 2), where the R and WR treatments resulted in similar  $\text{N}_2\text{O}$  emissions when combined with N, despite differences in residue quality. In this soil,  $\text{N}_2\text{O}$  emissions correlated significantly with MBC and DOC (Figure 5), indicating the importance of microbial activity and C availability in modulating  $\text{N}_2\text{O}$  emissions. In GS soil, however, the application of high C:N plant residue with IN led to lower  $\text{N}_2\text{O}$  emissions than the low C:N plant residue, consistent with the general negative correlation between residue C:N ratio and  $\text{N}_2\text{O}$  emissions (Huang et al., 2004). When microbes decompose low C:N substrates, the excess N not required for biomass synthesis may be released as  $\text{NH}_4^+$  or other N compounds, contributing to  $\text{N}_2\text{O}$  production. Nitrogen addition can also increase the abundance of  $\text{NH}_4^+$  oxidizing microbes and alter microbial-mediated N cycling toward processes that produce  $\text{N}_2\text{O}$  (Frey et al., 2023).

In our study, the changes in IN concentration during the incubation were largely due to  $\text{NH}_4^+$  depletion in treatments with both plant residue and IN addition (Li et al., 2019a). Conditions in our incubation were likely not sufficiently anaerobic for complete denitrification, which could explain the accumulation of  $\text{N}_2\text{O}$ , as  $\text{N}_2\text{O}$  reduction to  $\text{N}_2$  is more oxygen-sensitive than  $\text{NO}_3^-$  reduction to  $\text{N}_2\text{O}$  (Morley & Baggs, 2010). Interestingly, in GS soil, the addition of plant residue together with N reduced N effect compared to the addition of N alone. One possible explanation is that the plant residue promoted N immobilized into MBN and thereby reducing the effects of N availability on  $\text{N}_2\text{O}$  emission (Figure S1). This is consistent with the observed negative correlation of cumulative  $\text{N}_2\text{O}$  emissions and MBN (Figure 5), as well as the lower EF of applied N in the MB treatment compared to the +MB treatment (Figure 4), where MB is less likely further immobilized added N. Overall, our results emphasize that the interaction of C and N content within MB plays a critical role in regulating  $\text{N}_2\text{O}$  production, highlighting the importance of incorporating MB size into N management strategies tailored to different land use systems (Figure 6).

## 5 | CONCLUSION

This study demonstrated that the initial MB size significantly influenced soil  $\text{N}_2\text{O}$  emissions, with increased MB size enhancing emissions in both conventionally cropland and GSs. In both systems, N addition, rather than the soil itself, was the primary source of  $\text{N}_2\text{O}$  emissions. In CS, the incorporation of high C:N plant residue led to greater  $\text{N}_2\text{O}$  emissions than low C:N residue, whereas in GS, low C:N residue yielded

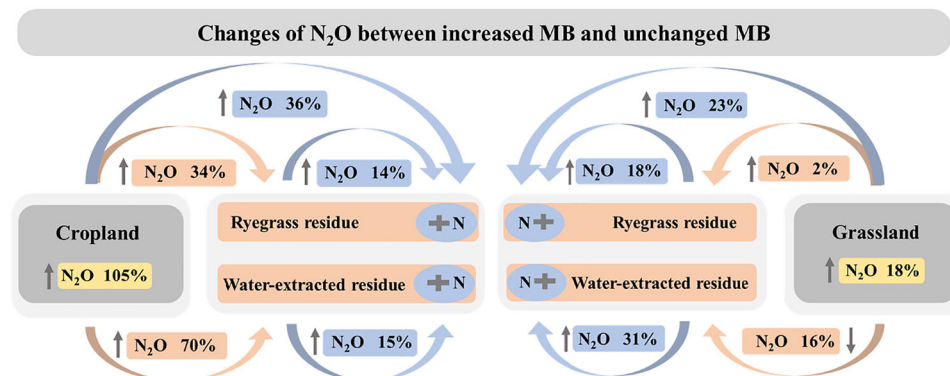


**FIGURE 6** Changes of  $\text{N}_2\text{O}$  production between increased microbial biomass size and unchanged microbial biomass size via residue input and N addition under cropland and grassland soils. The blue color represents treatments that include the addition of inorganic nitrogen; the orange color represents treatments that include the addition of residue.

the highest emissions. A higher EF induced by N application was observed in GS, indicating that the interaction between increased MB size and added N played a key role in  $\text{N}_2\text{O}$  emission. Our results indicate that changes in MBC were the primary factor governing  $\text{N}_2\text{O}$  emission in CS with low organic C content, while changes in MBN and IN were more relevant in the C-rich GS. Overall, this study highlights MB size as a key factor influencing  $\text{N}_2\text{O}$  emissions, modulated by soil C availability and nutrient dynamics.

## AUTHOR CONTRIBUTIONS

**Mengyang You:** Formal analysis; writing—review and editing. **Lu-Jun Li:** Funding acquisition; supervision; writing—review and editing. **William R. Horwath:** Conceptualization; supervision; writing—review and editing. **Xia Zhu-Barker:** Conceptualization; supervision; writing—review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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