RESEARCH ARTICLE

Fungi dominate denitrification when Chinese milk vetch green manure is used in paddy soil

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HIGHLIGHTS

- We evaluated effects of fungi on N_2O emission in Chinese milk vetch-containing soils.
- Fungi contributed to soil N_2O production in CMV-amended soils.
- Fungi accounted for 56% of N_2O emission in CMV-amended soils.
- Fungi may be important contributors to N_2O production in CMV-amended soils.

GRAPHICAL ABSTRACT

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ABSTRACT

Fungi play an important role in soil nitrous oxide (N_2O) emission in many agricultural soil systems. However, the effect of fungi on N_2O emission in Chinese milk vetch (CMV)-containing soils has not been examined sufficiently. This study investigated the contribution of bacteria and fungi to soil N_2O emission in CMV-amended soils. We compared soils from an experimental field in the Fujian Academy of Agricultural Sciences that had been treated with 30 000 kg of CMV per 667 m² per year with one that was not treated with CMV. We incubated soil using cycloheximide and streptomycin to differentiate fungal and bacterial N_2O emissions, respectively. Quantitative PCR (qPCR) was performed to investigate bacterial and fungal abundances in the two agricultural soil ecosystems. The contribution of fungi to soil N_2O emission in CMV-amended soils was greater than that in non-CMV-amended paddy soils, with fungi accounting for more than 56% of the emissions in CMV-amended soils. Quantitative PCR showed that the ratio of the internal transcribed spacer to 16S rDNA was significantly higher in CMV-amended soils than in non-CMV-amended paddy soils. Furthermore, soil properties, such as pH ($P < 0.05$) and NH_4^+ concentration ($P < 0.05$), significantly and negatively affected N_2O emission by fungi in soil, whereas the total organic carbon ($P < 0.05$) and NO_3^- concentration ($P < 0.05$) showed significant positive effects. Fungi may be important contributors to N_2O production in CMV-amended soils, which may create challenges for mitigating N_2O production.

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1 Introduction

Nitrous oxide (N_2O), which is the third most prevalent anthropogenic greenhouse gas, is present at 310-fold higher levels than carbon dioxide, and it has the potential to cause global warming (Lassey and Harvey, 2007; Davidson and Kanter, 2014). Moreover, it is the predominant ozone-removing substrate (Ravishankara et al., 2009) and a major form of nitrogen pollution (Davidson, 1991). Agricultural soils are essential sources of global N_2O production (Ravishankara et al., 2009; Hoben et al., 2011): N_2O is generated primarily by microorganisms in the soil, such as nitrifiers and denitrifiers (Ai et al., 2017). Denitrification is the dominant pathway contributing to soil N_2O emission and can contribute up to 90% of the total soil N_2O production in 75% of the water-filled pore space (Khalil and Baggs, 2005).

Bacteria were the first organisms found to produce N_2O , and the production of N_2O was initially thought to be limited to eubacteria (Zumft, 1997). However, recent studies have shown that fungi can contribute substantially to soil N_2O emissions in diverse soil systems (Yanai et al., 2007; Jirout et al., 2013; Huang et al., 2017). Generally, in the denitrification pathway, bacteria reduce NO_3^- consecutively to NO_2^- , NO (nitric oxide), N_2O , and N_2 , whereas fungi lack N_2O reductase, and thus, the reaction terminates with N_2O (Thomson et al., 2012). This lack of N_2O reductase suggests that fungi play a more critical role in soil N_2O emission. *In situ* studies have shown that fungi contribute more than 50% to total soil N_2O emissions (Mothapo et al., 2015). Diverse abiotic factors influence fungal N_2O production. For example, a low soil pH is beneficial when fungi contribute the most to denitrification (Huang et al., 2017). In contrast to bacterial denitrification, fungi perform better under anoxic conditions (Mothapo et al., 2013; Huang et al., 2017). The requirement for O_2 by fungi is determined by the type of N, which is high for NO_3^- and low for NO_2^- (Mothapo et al., 2013). Moreover, the quality of organic substrates can influence the development and activity of fungi and bacteria (Chen et al., 2015).

Chinese milk vetch (CMV) is a widespread perennial legume that is often utilized as a feed crop worldwide. It is also a high-quality green manure that is used in south-east China (Yang et al., 2014), where CMV biomass is plowed into paddy soil (Xie et al., 2018). Simple and complex compounds are found in plant materials (Hadas et al., 2004), but their proportions differ in various green manures, which can cause soil fungi and bacteria to grow at different rates (Zhao et al., 2019). Previous studies have shown that low C:N ratios in plant materials lead to a high bacteria to fungi ratio, whereas fungal dominance occurs in the presence of plant materials with a high C:N ratio (Poll et al., 2008; Chen et al., 2015).

We evaluated samples of paddy soil from Fuzhou containing grazed green manure CMV to assess whether fungi contributed more than bacteria to N_2O fluxes through the process of denitrification. This study determined whether 1) the green manure CMV benefited the growth of fungi over

bacteria, causing fungi to release most of the N_2O found in the soil, and 2) fungi or bacteria dominated N_2O production in soil samples with different pH and total organic carbon (TOC) and nitrogen concentrations.

2 Materials and methods

2.1 Soil sampling

Paddy soil was sampled at a depth of 0–20 cm at the Red Soil Fertility and Ecological Environment of Field Observation Station at Fuzhou Station (119°04'10"E, 26°13'31"N). Chinese milk vetch was harvested during full-bloom, cut into 2-cm pieces, and blended together. Next, the CMV samples were buried at a depth of 10 cm, with nearly 30 000 kg of CMV per 667 m^2 incorporated into soil. Then, soil samples of at least 50 g were randomly collected from five sites and stored in sterile valve bags. Paddy soil samples from the five sites were mixed together. Then, they were air-dried, sieved through a 1-mm mesh sieve, and stored at -20°C until analysis. Before the experiment, the soil had a pH of 4.94, and there was 25 g kg^{-1} organic matter, 124.4 mg kg^{-1} available N, 21.3 mg kg^{-1} available P, and 101.2 mg kg^{-1} available K. The characteristics of the experimentally supplied CMV were 59.27% organic carbon, 2.6% total N, 0.3% total P, 2.5% total K, and 12.6% dry matter.

2.2 Incubation experiments

Soil samples from paddy soil (CK) and paddy soil amended with CMV (12 g dry soil) were placed into 100-mL anaerobic vials. Then, 6 mL of solution containing KNO_3 (50 $\mu\text{g NO}_3^- \text{N g}^{-1}$ dry soil) and glucose (0.5 mg C g^{-1} soil) was added. Cycloheximide and streptomycin were added to inhibit the activities of fungi and bacteria and investigate the fungal and bacterial contribution to soil N_2O production (Anderson and Domsch, 1973), respectively. Four antibiotic treatments were performed in triplicate: (1) an antibiotic-free treatment as a control, with no antibiotic added; (2) only streptomycin, with 3.0 mg streptomycin added g^{-1} soil; (3) only cycloheximide, with 5.0 mg cycloheximide added g^{-1} soil; and (4) both antibiotics, with streptomycin added at 3.0 mg g^{-1} and cycloheximide added at 5.0 mg g^{-1} . In previous studies, these antibiotic concentrations had a limited effect on non-target organisms (Laughlin and Stevens, 2002). Next, 2 mL of deionized water was used to dissolve the chemicals, which were added to anaerobic vials with soil to prepare an 80% water-filled pore space. The headspace of the soil slurries was flushed with ultra-high-purity He. Then the soils were incubated at 25°C in an anaerobic jar for two days to stabilize soil microbial activity, followed by incubation at 25°C for the next 28 days. The physicochemical properties, including total nitrogen (TN), total carbon (TC), inorganic carbon (IC), NH_4^+ -N, NO_3^- , and NO_2^- , of the soil sample extracts were determined following Zhang et al. (2019).

2.3 N₂O and CO₂ sampling and measurement

The gas concentration of N₂O was determined at 2, 14, and 28 days using a robot system with an electron detector (Agilent Technologies, Santa Clara, CA, USA). Concentrations of CO₂ were determined on the same days using an infrared gas analyzer (Li-COR, Lincoln, NE, USA). The contribution of fungi to soil N₂O (or CO₂) emissions was determined following Chen et al. (2015). The fungal contribution to soil N₂O emission or CO₂ fluxes was estimated by using the formula:

$$\frac{100 \times (A - B)}{A - D},$$

where *A* represents N₂O emissions or CO₂ fluxes in the antibiotic-free control and *B* and *D* represent soil N₂O (or CO₂) emission in soil amended with cycloheximide and soil with both streptomycin and cycloheximide, respectively. The equation can also be used to calculate the contribution of bacteria by replacing *B* with N₂O (or CO₂) emission in soil amended with streptomycin. In addition, the inhibitor additivity ratio (IAR) was utilized to assess whether the antibiotics exerted non-target effects as described by Beare et al. (1990) and Chen et al. (2015).

2.4 Abundance of bacteria and fungi

At 2, 12, and 28 days, soils were sampled from an antibiotic-free microcosm, and gas analysis was conducted. In addition, a 0.5 g soil sample was weighed and used for genomic DNA extraction with the FastDNA[®] SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions. The quality and size of soil DNA was checked using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The abundance of bacteria and fungi was determined in triplicate using quantitative polymerase chain reaction (qPCR) using a LightCycler 480 (Roche, Basel, Switzerland). The gene copy numbers of the 16S rDNA and internal transcribed spacer (ITS) region of rDNA were determined. The primer sequences used for bacteria were 519F 5'-CCTACGGGAGG-CAGCAG-3' and 907R 5'-TTACCGCGGCTGCTGGCAC-3', and a specific amplification procedure was used: 2 min at 95°C, 40 cycles of PCR for 15 s at 95°C, and 1 min at 55°C. A final melting curve step was performed to complete the procedure. The primer sequences used for the fungi were 5.8S 5'-CGCTGCGTTCTTCATCG-3' and ITS-1F 5'-TCCGTAGGTGAACCTGCGG-3', and amplification was performed as follows: 2 min at 95°C, 40 cycles of PCR for 15 s at 95°C, and 1 min at 58°C. A melting curve step was also performed. Each 50-μL reaction contained 25 μL of SYBR Green (2×) PCR Master Mix (Life Technologies, Carlsbad, CA, USA), 1 μL of each primer, and 5 μL of the DNA template. The efficiency values of all amplification reactions were approximately 90%–100%, and the R² values of the standard curves were greater than 0.99.

2.5 Data analysis

Analysis of variance (ANOVA) was conducted using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA) to evaluate significant differences ($P \leq 0.05$) in soil CO₂ and N₂O emission velocities and CO₂ respiration among the four antibiotic treatments in CK and CMV-amended soils. Additionally, ANOVA was used to compare the relative contribution of fungi and bacteria to soil N₂O emission and CO₂ respiration between the two treatments. Principal coordinates analysis was performed using originPro 2018 software using Principal Component Analysis v 1.0. Path analysis was performed using SmartPLS 3 software (SmartPLS GmbH, Germany), which is a statistical method used to demonstrate cause and effect relationships between observed and latent variables. The maximum iterations were set to 300 and missing data were deleted pairwise. The bootstrapping routine was set to a 0.05 probability level and 500 bootstraps were used to validate the estimates of the path coefficients.

3 Results

3.1 Effect of Chinese milk vetch on soil characteristics

As shown in Table 1, most soil properties, including TN, TC, TOC, NH₄⁺-N, NO₃⁻-N, and NO₂⁻-N levels, were significantly different between CK and CMV-amended soils. Particularly, the levels of TN, TC, TOC, NO₃⁻-N, and NO₂⁻-N in CMV-amended soils were significantly higher ($P < 0.05$) than those in CK soils. In contrast, the concentration of NH₄⁺-N in CMV-amended soils was significantly lower ($P < 0.05$) than that in CK soils. The pH of CMV soils was lower than that in CK soils, but the difference was not significant. There was no significant difference in the IC between the two treatments (Table 1), indicating that the addition of CMV altered the characteristics of the paddy soil.

Table 1 Physicochemical properties of two types of paddy soil before incubation of the microcosms.

Treatment	CK	CMV
pH	4.98±0.01 ^a	4.37±0.02 ^b
TN (μg g ⁻¹)	0.93±0.06 ^b	2.01±0.09 ^a
TC (mg g ⁻¹)	13.53±0.44 ^b	20.02±1.01 ^a
IC (μg g ⁻¹)	1.37±0.02 ^a	1.27±0.03 ^b
TOC (μg g ⁻¹)	12.16±0.46 ^b	18.75±1.03 ^a
NH ₄ ⁺ (μg g ⁻¹)	36.70±0.75 ^a	23.73±0.48 ^b
NO ₃ ⁻ (μg g ⁻¹)	1.54±0.14 ^b	26.45±1.07 ^a
NO ₂ ⁻ (ng g ⁻¹)	7.33±5.03 ^b	107.33±50.96 ^a

Treatments: CK, control paddy soil; CMV, paddy soil amended with Chinese milk vetch; Data are shown as the mean±standard error of the mean. TN: total nitrogen; TC: total carbon; IC: inorganic carbon; TOC: total organic carbon.

Fig. 1 Effects of treatments of antibiotics on soil N₂O or CO₂ flux rates at 4, 12, and 28 days of soil sample incubation. Data are presented as the mean values with standard errors ($n \geq 3$).

3.2 Contributions of bacteria and fungi to soil N₂O and CO₂ fluxes

Bacteria and fungi contributed differently to soil N₂O fluxes in CK and CMV-amended soils. When there was sufficient KNO₃ and glucose, the added antibiotics exerted significant inhibitory effects ($P < 0.05$) on the N₂O and CO₂ emission rate in the soils (Fig. 1). In CK soils, bacteria and fungi did not significantly influence soil N₂O fluxes (Fig. 2A, 2B). In contrast, the fungi contributed significantly more to N₂O production in CMV-amended soil than in paddy soil ($P < 0.05$) (Fig. 2B). However, the fungal-to-bacterial contribution ratios were higher in CMV-amended soil than in paddy soil ($P < 0.05$) (Fig. 2C). The fungal-to-bacterial contribution ratios for CO₂ were also greater in CMV-amended soil than in paddy soil ($P < 0.05$) (Fig. 2C). The inhibitor additivity (IAR) values were approximately 1.0 (Fig. 2D). The data showed that both streptomycin and cycloheximide effectively inhibited the development and activity of fungi and bacteria, but these antibiotics did not strongly influence non-target organisms.

3.3 Effect of Chinese milk vetch on microbial abundance

Both bacterial and fungal abundance had increased by day 28 ($P < 0.05$). The fungal abundance in CMV-amended soil was significantly higher than in paddy soil on day 28 ($P < 0.05$), resulting in a high fungi-to-bacteria ratio (Fig. 3). The fungal-to-bacterial ratios in CMV-amended soils were greater than

those in paddy soils on days 4 ($P < 0.05$), 12 ($P < 0.05$), and 28 ($P < 0.001$).

3.4 Correlation between soil properties and fungal N₂O emission

The pH, NH₄⁺-N concentration, and IC were negatively related to fungal N₂O production and fungal CO₂ respiration. However, the TN, TOC, and nitrate concentration and fungi abundance were positively related to fungal N₂O production and fungal CO₂ respiration (Fig. 4). The soil samples from CK (PC1, Fig. 4) were distinct from those subjected to CMV treatments (PC2), and the sum of PC1 and PC2 accounted for 91.2% of the variation in all parameters determined in this experiment (Fig. 4).

Path analysis showed that fungal abundance, and particularly the fungi-to-bacteria ratio, rather than the bacterial abundance significantly affected ($P < 0.05$) soil N₂O emissions. Soil properties positively affected the fungal abundance and F/B ratio (Fig. 5). Soil characteristics, such as pH, TN, TC, NH₄⁺, and NO₃⁻ (Fig. 5) showed significant differences, indicating that CMV altered the soil properties.

4 Discussion

Through selective inhibition by antibiotics, the fungi in CMV-amended soil showed a high potential to contribute to soil N₂O production (Fig. 1). This is in agreement with the results of a

Fig. 2 Effects of different antibiotic treatments on the proportions of fungal (fungi %) or bacterial (bacteria %) contributions to gas emissions, fungal-to-bacterial contribution ratios (F:B), and inhibitor additivity (IAR). Error bars represent standard error for $n = 9$ (i.e., replicates $\times$ 3 sampling times). * and ** indicate significant differences between the two treatments at $\alpha = 0.05$ and 0.001, respectively.

previous study that showed that complex substrates are more beneficial for increasing fungal N₂O emissions (Chen et al., 2015). During the experiment, this effect was observed at nearly all sampling times. More than 56% of N₂O production in the soil was attributable to fungal activity, and the contribution

Fig. 3 Gene copy numbers of the 16S rDNA and ITS region of rDNA and ratios of ITS to 16S rDNA copy number. Error bars represent the standard error for $n = 3$. * and ** indicate significant differences between the two treatments at $\alpha = 0.05$ and 0.001, respectively.

of fungi to soil N₂O production was equivalent to or greater than the bacterial influx in CMV-amended soils (Fig. 1).

4.1 Soil elements controlling fungal contribution to soil N₂O emission

The CMV-amended soils showed differences in soil properties, such as pH. Complex factors function to lower the soil pH following the addition of CMV. The degree of decrease and increase in soil pH depends on the type of plant material, soil properties, and physicochemical properties (Wang et al., 2013). Previous studies have demonstrated that the primary elements influencing the pH of soil amended with plants are organic anions, basic cations, and nitrogen content (Bolan et al., 1991; Noble et al., 1996; Xu et al., 2006a, 2006b). The addition of legume roots (subterranean clover) was shown to slightly decrease the soil pH (Tang and Yu, 1999). Legume roots are often regarded as an indispensable contributor to total N in the plant system (McNeill et al., 1997), and soil pH decreases when nitrate ions leach out from a soil layer (Dolling, 1995; Xu and Coventry, 2003). Numerous studies have shown that soil pH strongly regulates the rate of denitrification (Baggs et al., 2010; Hu et al., 2013), and fungi

Fig. 4 Relationships among soil properties and nitrous oxide and carbon dioxide emissions based on Principal Component Analysis. Soil attributes are indicated by arrows and treatment groups are indicated by dots.

dominate N_2O emission in soils with low pH (Rutting et al., 2013). Moreover, soil fungi exhibit growth over a broad range of pH and the fungal community is only minimally affected by acidic pH (Rousk et al., 2010). Under low pH conditions, the dominant contribution of fungi to N_2O production could be related to the primary growth of fungi, which is in agreement with our results (Figs. 4, 5).

The added CMV significantly increased the TOC of the soil, and TOC was positively related to fungal N_2O emission (Table 1, Fig. 4). Organic matter in the soil was primarily decomposed by fungi and bacteria. Generally, bacteria can utilize simple and regularly supplied C compounds and fungi-like complex compounds, plumbers, and substances (Chen et al., 2015). Compared to bacteria, fungi degrade complex compounds more effectively because they produce extracellular enzymes and allocate a large amount of carbon substrates to enzyme synthesis (de Boer et al., 2005). Microbial activities control the conversion of carbon resources from plant biomass into microbial biomass or CO_2 (Liang et al., 2017; Soares and Rousk, 2019). In addition, alterations in the dominance of the microbial community to fungi may increase C storage in soils, as fungi-dominant soils show lower carbon losses and higher carbon absorptivity rates (Laughlin et al., 2009).

In terms of nitrogen content, we found that CMV increased the concentration of NO_3^- -N, reduced the concentration of NH_4^+ -N, and NH_4^+ -N was converted to NO_3^- -N through the activity of microbes. Fungi can oxidize NH_4^+ and organic N (Laughlin et al., 2008) and may have a latent ability to obtain

NH_4^+ . Moreover, archaea are the primary microorganisms that oxidize NH_4^+ in some terrestrial ecosystems (Laughlin et al., 2008). In our experiment, neither streptomycin nor cycloheximide (microbial inhibitor) inhibited archaea. The conversion of NH_4^+ to NO_3^- could also be related to archaeal activity. In addition, archaea activity might explain why neither streptomycin nor cycloheximide inhibited N_2O emission in our experiment (Fig. 1).

Bacterial growth can be increased in the soil by adding plant materials with low C/N ratios, whereas fungal dominance occurs when plant materials with high C/N ratios are added (Thiet et al., 2006). However, in our experiments, we found that CMV significantly increased the F to B ratio, which agrees with the results of Chen et al. (2019). They showed that CMV considerably increased soil organic carbon, increasing soil aggregation. Greater aggregation often creates more pores, particularly those large in size, thereby creating a favorable environment for the growth of fungal hyphae (Zhang et al., 2015). We conducted incubation in an 80% water-filled pore space, which was not an optimal environment for bacterial N_2O production under strict anaerobic circumstances, and fungi dominated under sub-anoxic conditions (Bouwman, 1998). The qPCR results indicated that CMV increased microbial growth, particularly fungal growth, and led to a high fungi-to-bacteria ratio. Direct positive effects were also observed between the fungal abundance and fungi-to-bacteria ratios and N_2O production (Figs. 4 and 5). A previous study showed that fungal and bacterial contributions to N_2O fluxes could be deduced from their ratio in a microbial

Fig. 5 Path analysis of microbial abundance in relation to nitrous oxide (N_2O) emission, carbon dioxide (CO_2) respiration, and soil properties. Each box represents a variable: measured or constructs. The fungal and bacterial contribution to N_2O fluxes and CO_2 respiration were used to create the variables and are shown in the dashed rectangle. Path coefficients are reflected by the width of the arrow. The blue and red colors indicate positive and negative effects, respectively. Dashed arrows indicate that coefficients did not differ significantly ($P > 0.05$).

community. Fungi contribute more to soil N_2O production in no-till systems than in tilled systems, possibly because tillage significantly reduces the fungal biomass (Kinney et al., 2004; Crenshaw et al., 2008; Chen et al., 2014).

4.2 Methodological considerations

Experiments focused on fungal and bacterial contributions to soil N_2O fluxes were conducted based on the SIR inhibition method. One limitation of this method is that the antibiotics do not inhibit 100% of the intended organisms when the concentrations are not appropriate or non-target organisms may be inhibited (Badaluco et al., 1994). In addition, the total individual effects should be equivalent to the combined effects of both antibiotics (Beare et al., 1990). In this study, the total individual effects were equal to the combined effects of both antibiotics used for evaluating N_2O emission and CO_2 respiration (Fig. 2). The IAR results suggested that the two antibiotics were selective at the concentrations used in the experiment. Moreover, the antibiotics did not inhibit archaea, which are important in denitrification: archaea are common in soil systems and play an important role in denitrification (Cabello et al., 2004), likely exerting important functions in soil N_2O emission (Butterbach-Bahl et al., 2013). This might

explain why combined cycloheximide and streptomycin treatment resulted in slightly higher N_2O emissions than those observed with treatment using each antibiotic alone.

TN losses from the soil included N_2O and N_2 , whereas bacterial denitrifiers reduced N_2O to N_2 and fungi could not reduce N_2O because of the lack of N_2O reductase (Zumft, 1997). This difference in physiology leads to concerns regarding whether the estimate of the contribution to soil N_2O is reliable between bacteria and fungi when performing antibiotic inhibition. The fungal contribution to N_2O fluxes in streptomycin-added soils may have been overvalued compared with that in antibiotic-free soils. Hence, the contribution of bacteria to N_2O production could also have been underestimated. Glucose addition can increase the levels of electron donors and enhance bacterial N_2O reduction (Chen et al., 2014), leading to a more severe underestimation of the ratio of bacterial N_2O reduction. In contrast, fungal N_2O fluxes were inhibited in cycloheximide-amended soils and the bacterial production of N_2O was lower than that in antibiotic-free soils. This led to overestimating the contribution of fungal N_2O fluxes in the soil. Thus, comparing the two soil systems should provide valid results, and fungi contribute more to N_2O flux in CMV-amended soils.

5 Conclusions

Through selective antibiotic inhibition, we demonstrated that fungi strongly contribute to soil N_2O emission in CMV-amended soils. CMV is generally used as green manure in the south of China, and the determination of the flux of N_2O from this soil may have important implications. Models that are currently used to predict soil N_2O discharge are primarily based on studies of bacterial denitrification. Thus, mitigation strategies, such as using natural or synthetic nitrification inhibitors (Zhong et al., 2018), targeting bacterial but not fungal N_2O -producing microbes may be useful. Furthermore, fungal denitrifiers often lack N_2O reductase, leading to high N_2O production. Whether or not CMV promotes fungal pathways can be inferred from the abundance and composition of fungi and bacteria. Moreover, fungal and bacterial abundance and diversity can be used to manage N_2O emission in CMV-amended soils.

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