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Arbuscular mycorrhizal fungi reduce N_2O emissions from degraded residue patches

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Nitrous oxide (N_2O) is a potent greenhouse gas, and agricultural soils represent a major anthropogenic source. Crop residues provide nutrients for plants but also act as hotspots of N_2O production. The hyphae of arbuscular mycorrhizal fungi (AMF) could proliferate in organic patches, utilize released N from the organic patches, and potentially mitigate N_2O emissions. However, the effect of AMF on N_2O emissions in degraded residue patches and the possible microbial mechanism remain uncertain. Here, a mesocosm experiment was conducted to investigate the impact of AMF (*Funneliformis mosseae*) inoculation on N_2O emissions, availabilities of carbon and nitrogen, extracellular enzyme activities, and the abundance of key N-cycling genes in degraded residue patches. Our results showed that AMF hyphae significantly reduced N_2O emissions from degraded residue patches. Quantitative PCR analysis of key functional genes involved in N_2O production (*amoA*, *nirK*, *nirS*) and consumption (*nosZ*) showed that AMF significantly reduced the abundance of the bacterial *amoA* and *nirS* genes. NH_4^+, NO_3^-, total dissolved nitrogen (TDN), total nitrogen (TN), and dissolved organic carbon (DOC) contents decreased drastically in the presence of AMF. In addition, the activities of all tested extracellular enzymes were significantly decreased by AMF and positively correlated with DOC content. Multiple stepwise regression analysis demonstrated that the abundance of the *nirS* gene primarily influenced N_2O emissions and was positively correlated with DOC content in degraded residue patches. Our findings indicate that AMF suppressed N_2O producers, particularly *nirS*-type denitrifiers, by slowing down the release of C and N from degraded residues, thereby leading to a cascade effect on the decrease of N_2O emissions. This study provides a promising approach to mitigate N_2O emissions by enhancing AMF in the agroecosystems.

KEYWORDS

nitrous oxide, mycorrhizal fungi, crop residue, denitrification, extracellular enzyme activities

1 Introduction

Nitrous oxide (N_2O) is a potent, long-lived greenhouse gas, with 273 times more warming potential than CO_2 per molecule, and accelerates ozone depletion (Ravishankara et al., 2009; Arias et al., 2021). Agricultural soils are the major source of N_2O, accounting for 50% of anthropogenic N_2O emissions (Tian et al., 2020). Microbial processes, such as nitrification and denitrification, are the major pathways for N_2O production in agricultural soil (Butterbach-Bahl et al., 2013). The oxidation of ammonia into nitrite via hydroxylamine was the first step of nitrification which was primarily carried out by two types of microbes: ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). N_2O is produced as a byproduct during the decomposition of hydroxylamine (NH_2OH) (Prosser and Nicol, 2012). Nitrification predominates under aerobic conditions (Dobbie et al., 1999). Denitrification is the stepwise reduction of NO_3^- to N_2 via NO_2^-, NO, and N_2O and is performed by a diverse range of facultatively or obligate anaerobic organisms when O_2 becomes scarce. N_2O is an obligate intermediate of denitrification, and its net emissions depend on the balance between N_2O-producing and N_2O-consuming rates. The *nirK/nirS* genes encoding nitrite reductases are key genes involved in the reduction of NO_2^- to NO, and their abundance is often used to predict N_2O-producing rates. The *nosZ* gene, encoding N_2O reductase, is the only known biotic sink for N_2O, and its abundance is an indicator of N_2O-consuming rates (Chapuis-Lardy et al., 2007; Hallin et al., 2018). In reality, N_2O emissions from agricultural soils exhibit fluctuations and spatiotemporal variability, and both nitrification and denitrification processes likely occur simultaneously (Kravchenko et al., 2017).

The majority of N_2O production in agricultural soils occurs within small soil volumes (<1 cm^3) associated with crop residues (Kravchenko et al., 2017). For example, more than 80% of total N_2O emissions were traced to an 80-mg plant remnant inside the soil core (Parkin, 1987). A large amount of crop residues is generated during agricultural production and annual production reaching approximately 990 million tons in China and 5 billion tons globally (Dai et al., 2018; Roberto et al., 2018). Returning crop residues to agricultural soils can alleviate soil erosion, promote nutrient cycling, and increase soil carbon sequestration. However, the decomposition of crop residues can also result in higher N_2O emissions. During residue decomposition, released NH_4^+, NO_2^-, and NO_3^- become available to nitrifiers and denitrifiers, and dissolved organic carbon (DOC) serves as a carbon source and electron donor for denitrifiers (Korom, 1992). Moreover, water absorption from surrounding soils by crop residues can create an anaerobic microenvironment favoring denitrification (Kravchenko et al., 2017). The annual N_2O emissions derived from crop residues amount to 0.4 Tg N, partially offsetting the benefits of increased soil carbon sequestration from residues (Mosier et al., 1998).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in soils and form symbiotic associations with more than two-thirds of terrestrial plants (Smith and Read, 2008), playing a crucial role in N cycling (Veresoglou et al., 2012). Recent studies have found that AMF can reduce N_2O emissions by altering nitrogen substrate availability, water content, or the community of nitrifying and denitrifying microorganisms (Bender et al., 2014; Lazcano et al., 2014; Bender et al., 2015; Zhang et al., 2015; Storer et al., 2017; Gui et al., 2021; Shen and Zhu, 2021). The extraradical hyphae of AMF can extend into organic patches away from the roots to acquire nutrients (Leigh et al., 2009; Hodge and Fitter, 2010). Although AMF lack the lytic exo-enzymatic repertoire to exploit organic patches alone (Joner and Johansen, 2000; Tisserant et al., 2012; Tisserant et al., 2013), they can influence the decomposition of plant residues by interacting with saprophytic microorganisms (Nuccio et al., 2013) or impacting the soil structure (Rillig and Mummey, 2006). Accumulating evidence suggests that AMF promote the decomposition and N release of residues and acquire the released N for their own growth or transfer some to the host plant (Hodge et al., 2001; Hodge and Fitter, 2010). However, results regarding the release of carbon in crop residues affected by AMF are inconsistent (Hodge et al., 2001; Hodge, 2014; Verbruggen et al., 2016), mainly due to the differences in the C/N and/or the labile-to-recalcitrant fraction of carbon (Cornwell et al., 2008; Hattenschwiler et al., 2011). Generally, it is acknowledged that AMF stimulate the decomposition of fresh residues, whereas for older or degraded residues, AMF tend to inhibit decomposition and slow down the release of DOC by promoting soil aggregates (Hodge et al., 2001; Leifheit et al., 2015; Verbruggen et al., 2016). Taken together, the changes in C and N substrate availability during residue decomposition affected by AMF can trigger cascading effects on soil nitrification and denitrification. However, it remains unclear 1) whether AMF-mediated N_2O emission is related to the decomposition of plant residues and 2) the microbial mechanisms of AMF regulating N_2O emission during the residue decomposition. Here, we conducted a mesocosm experiment to assess the impact of AMF on N_2O emissions from the organic patches after approximately 30 days of crop residue degradation. Soil mineral nitrogen and the abundance of key genes involved in nitrification and denitrification were also analyzed. Given that AMF promote N absorption and slow down the release of DOC from older or degraded residues, we hypothesized that i) AMF reduce the availability of nitrogen substrates in organic patches and slow down the release of DOC from degraded residues and ii) AMF inhibit nitrification and denitrification due to the low availability of C and N, thereby reducing N_2O emissions in the organic patches.

2 Materials and methods

2.1 Growth media

The soil was collected from a long-term experimental field at the Quzhou Experimental Station (36.87°N, 115.02°E) in Quzhou County, Hebei Province, North China. The soil is classified as a Cambisol with a silt loam texture. The background soil properties were as follows: pH 7.24 (H_2O), 7.95 g kg^{-1} organic carbon (SOC), 0.90 g kg^{-1} total N (TN), 12.01 mg kg^{-1} Olsen-P (AP), and 176.2 mg kg^{-1} available K (AK) (Zhang et al., 2016). The collected soil was

sieved through a 2-mm sieve, air-dried, and mixed with sand to a soil-to-sand ratio of 1:1 (m/m) as the cultivated medium. The medium was gamma-irradiated with a maximum dose of 32 kGy to eliminate indigenous AMF. To ensure nutrient supply, 100 mg kg^{-1} of N (calcium nitrate tetrahydrate), 20 mg kg^{-1} of P (monopotassium phosphate), and 100 mg kg^{-1} of K (monopotassium phosphate) were added to the medium prior to the experiment.

2.2 Experimental design and process

The experiment followed a full factorial design with two factors: 1) patch types: organic patch and control patch and 2) inoculation treatments: inoculated with AMF (+AMF) and without AMF (−AMF).

Mesocosm units were constructed by two joining compartments (one host compartment, 3 × 10 × 15 cm^3; one hyphal compartment, 10 × 10 × 15 cm^3) via a 30-μm mesh that allowed hyphae, but not plant roots, access to the hyphal compartment (Supplementary Figure 1). The host compartment contained 450 g of the sterilized medium and 50 g of inoculum of *Funneliformis mosseae* in the +AMF treatment or 50 g of the sterilized inoculum with microbial filtrate from the unsterilized inoculum in the −AMF treatment. *Funneliformis mosseae* is a common AMF in Chinese agricultural soils. The inoculum consisted of spores, mycelium, and fine root segments and was propagated using the same cultivated medium and planted with *Zea mays* L. for 4 months in a greenhouse. The hyphal compartment contained 1,500 g of the sterilized medium. A sterile PVC pipe (3.3 cm wide, 11 cm high) was inserted into the hyphal compartment 3 cm from the host compartment and 4 cm from the base of the hyphal compartment. Two opposing aperture windows (2.5 cm wide, 4 cm high) were opened 1 cm from the base of the PVC pipe. These windows and the base of the pipe were covered with a 30-μm mesh (Supplementary Figure 1).

Two pregerminated maize seeds were grown in each host compartment and thinned to one plant after emergence. The patch was added to the PVC pipe 29 days after maize planting. The patch materials contained 14 g of the sterilized medium mixed with 7 g of milled dried roots of maize plants (total carbon 40.62%, total N 1.43%, C:N ratio 28.41) as the organic patch or 50 g of the sterilized medium as the control patch (to ensure equal volume with the organic patch). Then, 5 mL of microbial filtrate was pipetted into each patch to equalize the initial microbial communities. The microbial filtrate was produced from the same fresh field soil according to the previously described method (Koide and Li, 1989; van der Heijden et al., 2006). The mesocosm experiment was conducted in a greenhouse at China Agricultural University, Beijing. Soil moisture was maintained at approximately 50%–60% of water-filled pore space (WFPS) with deionized water.

2.3 Gas sampling and analyses

Thirty days after patch addition, the PVC pipe was sealed at the top with a rubber plug that included a syringe needle and a three-way valve to form a gas sampling port (5 cm high). The total internal volume of the gas port was approximately 35 cm^3 (Supplementary Figure 1). Two days after sealing the pipe, the gas within the pipe was sampled with a syringe through the three-way valve. A 10-cm^3 gas sample was slowly removed from the pipe, waiting for an additional 5 s to allow the sample to mix inside the syringes before removing the syringes. The N_2O concentrations of the gas samples were determined using a model 7890A gas chromatograph (Agilent, Santa Clara, CA, USA).

2.4 Harvest and physiochemical analyses

Mesocosms were destructively sampled after gas sampling. Samples of patches in the hyphal compartment were collected and divided into three portions: one for the determination of soil gravimetric water content, mineral nitrogen, and DOC and total dissolved nitrogen (TDN) contents was stored at 4°C; one for the analysis of total carbon (TC) and TN contents and mycorrhizal hyphal length density (HLD) was stored at room temperature; and the remaining soil for molecular analysis was immediately frozen with liquid nitrogen and stored at −80°C.

In the host compartment, the shoots of the maize plants were removed from the soil surface. The roots of the plants were extracted from the medium, washed, and divided into three portions: a fraction for the estimation of AMF root colonization was soaked in 50% ethanol, and the rest of the roots and shoots were dried (65°C; 48 h) for the determination of N contents and dry weights.

The gravimetric water content of the patches was measured using the oven-dried method. The ammonium (NH_4^+-N) and nitrate (NO_3^--N) contents of the patches were measured using a continuous flow analysis (TRAACS 2000, Bran and Luebbe, Norderstedt, Germany) after the fresh patch samples were extracted with 0.01 mol L^{-1} of $CaCl_2$ solution (Peng et al., 2013). DOC and TDN in the extracts were measured by infrared spectrometry after combustion at 850°C. The total N and C contents of the plant shoots, roots, and patch samples were determined using a FLASH Elemental Analyzer 1112 (Thermo Finnigan, Waltham, MA, USA) after the samples were ground in a ball mill.

2.5 AMF root colonization rate and hyphal length density in patches

The subsamples of roots were cleared with 10% KOH, acidified with 1% HCl, and stained with 0.05% trypan blue (Phillips and Hayman, 1970). Thirty pieces of roots from each sample were randomly selected, arranged on slides, and then mounted under a light microscope (Axioplan, Zeiss, Germany). The AMF root colonization rate was determined using the magnified intersection method (Mcgonigle et al., 1990).

The HLD of AMF in patches was determined by a modified aqueous extraction and membrane filter technique using air-dried samples of patches in triplicate (Jakobsen et al., 1992). Hyphal

length density was calculated according to the modified Newman formula for calculating root length (Tennant, 1975).

2.6 Enzyme assays of the patch samples

The potential extracellular enzyme activities of the patch samples were measured using fluorogenically labeled substrates (Bell et al., 2013). Five fluorogenic enzyme substrates based on 4-methylumbelliferone (MUF) were used to assess enzyme activities: MUB-α-D-glucopyranoside for α-1,4-glucosidase, MUF-β-D-glucopyranoside for β-1,4-glucosidase, MUF-β-D-cellobioside for β-cellobiosidase, MUB-β-D-xylopyranoside for β-1,4-xylosidase, and MUB-*N*-acetyl-β-D-glucosaminide dehydrate for β-1,4-*N*-acetylglucosaminidase. L-Leucine-7-amido-4-methylcoumarin was used to analyze the activity of leucine amino peptidase. Briefly, fresh patch samples (equivalent to 1 g dry weight) were suspended in 100 mL of 50 mM sodium acetate buffer (pH 6.0) by shaking for 30 min, and then 200 μL of the suspensions and 50 μL of a 200-μM substrate (labeled with the MUB) were pipetted into a 96-well microplate and incubated in the dark at 25°C for 4 h. All solutions and suspensions were prepared with sterile water. A negative control was arranged with blanks (without soil suspension). After a 4-h incubation period, the microplates were measured fluorometrically with an automated luminescence spectrophotometer (FLx800 microplate, Bio-Tek Instruments, Winooski, VT, USA; emission at 450 nm, extinction at 345 nm). After correcting for the negative control and quenching effects, enzyme activities were expressed in units of nmol h^{-1} g^{-1} dry sample as MUB release.

2.7 Quantification of key N-cycling genes in patches

The genomic DNA was extracted from fresh patch samples using the Fast DNA® SPIN Kit for soil (MP Biomedicals, Cleveland, OH, USA) according to the manufacturer's instructions. The quantity and quality of DNA were checked using a Nanodrop spectrophotometer (Thermo Fisher, Waltham, MA, USA). Real-time quantitative polymerase chain reactions (qPCRs) of the bacterial and archaeal *amoA* genes involved in nitrification and *nirK*, *nirS*, and *nosZ* genes involved in denitrification were conducted using a CFX96 Optical Real-Time detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Each reaction system was performed in a 20-μL solution containing 10 μL of SYBR Premix Ex Taq (Tli RNaseH Plus, 2×, Takara Bio, Shiga, Japan), 1 μL of DNA template, 0.5 μL of each primer (10 mM), and 8 μL of ddH_2O. The qPCR primer pairs and thermal conditions are shown in Supplementary Table 1. Standard curves were obtained using a 10-fold dilution series (10^2–10^8 copies) of plasmid DNA containing target genes with known copy numbers. Each sample was performed in technical triplicate. The amplification efficiencies were 90%–105%, and the R^2 value of the standard curves was 0.99–1.00 for all genes.

2.8 Statistical analyses

All data were first tested for normality and homogeneity of variances. To fulfill normality and homogeneity of variances, the copy numbers of N-cycling genes were $\log_{10}(x)$-transformed, and AM fungal variables (AMF root colonization rate and HLD) were transformed by square roots. The effects of patch types, inoculation treatments and their interactions on plant and AMF variables, patch N_2O concentration, and gene copies were examined by two-way analysis of variance (ANOVA). The effects of patch types, inoculation treatments, and their interactions on soil physicochemical properties and activities of extracellular enzymes in patches which clearly did not fit the homogeneity of variances were examined by the Scheirer–Ray–Hare test. The effects of inoculation treatments on the variables within each patch type were determined by the two-tailed unpaired *t*-test. Multiple stepwise regression was employed to identify the most influential variables affecting N_2O concentration. Independent variables include NH_4^+ and NO_3^- contents, as well as the copy numbers of N-cycling genes. To assess the overall extracellular enzyme activities, we calculate the standardized scores (*Z* scores) of six extracellular enzyme activities and sum them up to obtain a single index (*Z* value). Pearson correlation analysis was conducted to test the relationships between the copy numbers of N-cycling genes, HLD, physiochemical properties, and the *Z* value of extracellular enzyme activities. The above Scheirer–Ray–Hare test and Pearson correlation analysis were conducted using R version 4.0.4. The rest of the statistical analysis was performed using the SPSS statistical software package version 22 (SPSS, Inc., Chicago, IL, USA).

3 Results

3.1 AMF root colonization of maize plants and hyphal length density in patches

The roots of the maize plants were successfully colonized by AMF. The AMF root colonization rates were greatly increased in the +AMF treatment but not significantly affected by patch types (Figure 1A and Supplementary Table 2).

The HLD in the +AMF treatment was 7.41 m g^{-1} on average, which was significantly higher than that in the –AMF treatment, where a small number of extraradical hyphae were detected. The HLD values in the organic patches were higher than those in the control patches (Figure 1B and Supplementary Table 2).

3.2 Patch water, nitrogen, and carbon contents

The water, NH_4^+, DOC, TDN, TC, and TN contents in the organic patches were much higher than those in the control patches (Figures 2, 3 and Supplementary Table 3). AMF significantly reduced the NH_4^+, NO_3^-, DOC, TDN, and TN contents in the organic patches. In the control patches, only the NO_3^- and TDN

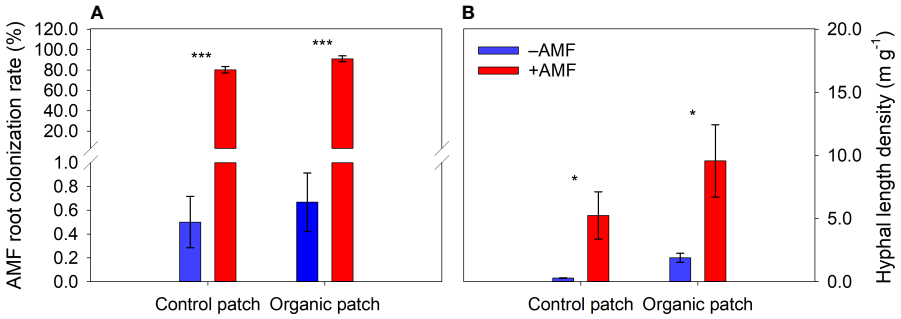

FIGURE 1
Arbuscular mycorrhizal fungi (AMF) root colonization rate of maize plants **(A)** and hyphal length density in patches **(B)** between +AMF and –AMF treatments under different patch types. Values represent means ± standard error, n = 4. +AMF and –AMF indicate the inoculation with and without *Funneliformis mosseae* in the host compartment, respectively. Asterisks indicate significant differences between +AMF and –AMF treatments within each patch type according to the two-tailed unpaired t-test (*, $P < 0.05$; ***, $P < 0.001$).

contents were reduced by AMF. The patch water and TC contents were not affected by AMF, regardless of patch type (Figures 2, 3 and Supplementary Table 3).

3.3 The activities of extracellular enzymes in patches

The activities of the six extracellular enzymes in the organic patches (α-1,4-glucosidase, β-1,4-glucosidase, β-cellobiosidase, β-1,4-xylosidase, β-1,4-*N*-acetyl-glucosamidase, and leucine amino peptidase) were approximately 30–230 times higher than those in the control patches. AMF significantly reduced the activities of the six extracellular enzymes in the organic patches by 38% to 70%. In the control patches, the activities of α-1,4-glucosidase, β-1,4-glucosidase, and leucine amino peptidase were significantly reduced, while the activity of β-1,4-*N*-acetylglucosaminidase was increased by AMF. The activities of the other two extracellular enzymes (β-cellobiosidase and β-1,4-xylosidase) were not significantly affected by AMF in the control patches (Figure 4 and Supplementary Table 3).

3.4 N_2O concentration and the abundance of key N-cycling genes in patches

AMF significantly reduced the N_2O concentration from the organic patches and did not significantly affect the N_2O concentration from the control patches (Figure 5A and Supplementary Table 2). The

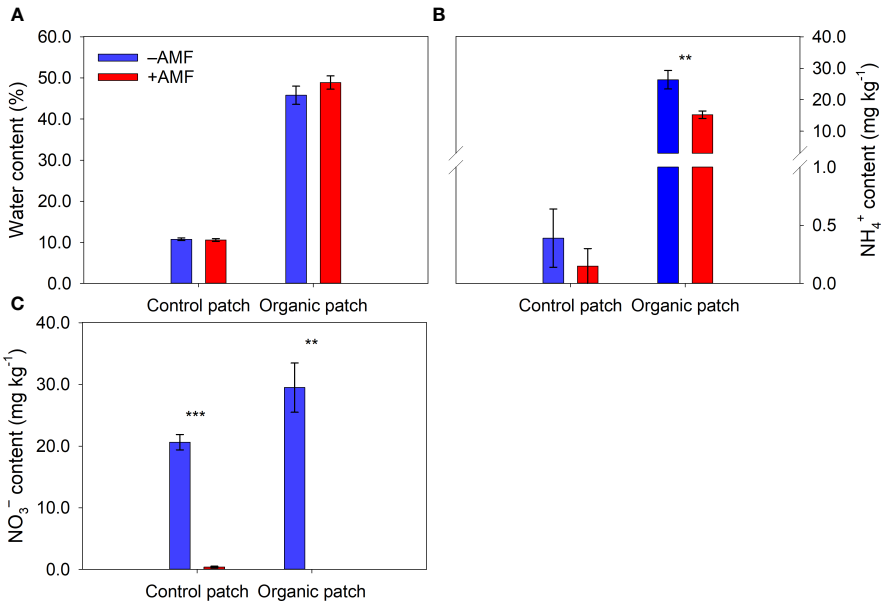

FIGURE 2
Water **(A)**, NH_4^+-N **(B)**, and NO_3^--N **(C)** contents in patches of different types between +AMF and –AMF treatments. Values represent means ± standard error, n = 4. +AMF and –AMF indicate the inoculation with and without *Funneliformis mosseae* in the host compartment, respectively. Asterisks indicate significant differences between +AMF and –AMF treatments within each patch type according to the two-tailed unpaired t-test (**, $P < 0.01$; ***, $P < 0.001$).

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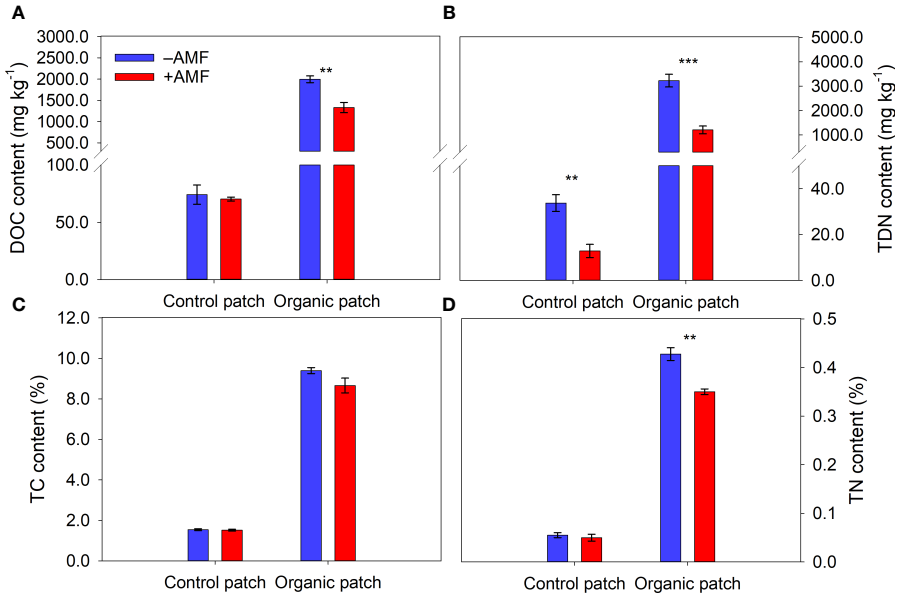

FIGURE 3
Dissolved organic carbon (DOC) **(A)**, total dissolved nitrogen (TDN) **(B)**, total carbon (TC) **(C)**, and total nitrogen (TN) **(D)** in patches of different types between +AMF and –AMF treatments. Values represent means ± standard error, n = 4. +AMF and –AMF indicate the inoculation with and without *Funneliformis mosseae* in the host compartment, respectively. Asterisks indicate significant difference between +AMF and –AMF treatments within each patch type according to the two-tailed unpaired t-test (**, $P < 0.01$; ***, $P < 0.001$).

abundance of the bacterial *amoA* gene ranged from 8.60×10^5 to 3.56×10^6 copies g^{-1} dry soil. The values were several times lower than those of the archaeal *amoA* gene (2.56×10^6 to 1.35×10^7 copies g^{-1} dry soil). In the organic patches, AMF significantly reduced the abundance of the bacterial *amoA* gene and did not affect the abundance of the archaeal *amoA* gene, while in the control patches, AMF significantly increased the abundance of the bacterial and archaeal *amoA* genes (Figures 5B, C and Supplementary Table 2).

The abundance of the *nirK* gene ranged from 2.82×10^7 to 7.88×10^9 copies g^{-1} soil, which was approximately an order of magnitude higher than that of the *nirS* gene in patches (ranged from 7.58×10^6 to 5.50×10^8 copies g^{-1} soil). The abundance of the *nirK*, *nirS*, and *nosZ* genes in the organic patches was higher than that in the control patches. In the organic patches, AMF significantly reduced the *nirS* gene abundance by 67% on average and did not significantly affect the abundance of the *nirK* and *nosZ* genes. In the control patches, AMF significantly increased the *nirK* gene abundance and did not significantly affect the abundance of the *nirS* and *nosZ* genes (Figures 5D–F and Supplementary Table 2).

3.5 Regression and correlation analysis

Multiple stepwise regression analysis showed that the abundance of the *nirS* gene alone was the main factor controlling the N_2O concentration in the organic patches, explaining 68.2% of the variation in N_2O concentration. In the control patches, no relevant independent variables were entered into the model (Supplementary Table 4). The abundance of the bacterial *amoA* and *nirS* genes was negatively correlated with HLD in the organic patches. In the control patches, HLD was positively correlated with the abundance of the bacterial *amoA* gene and showed no significant linear correlation with the *nirS* gene abundance (Figures 6A, C). The abundance of the bacterial *amoA* gene was positively correlated with NH_4^+ content (r = 0.72, P = 0.043), and the abundance of the *nirS* gene was positively correlated with DOC content (r = 0.76, P = 0.028) in the organic patches but not in the control patches (Figures 6B, D). The Z value of extracellular enzyme activities was positively correlated with DOC content in the organic patches (r = 0.75, P = 0.031; Figure 6E).

3.6 Biomass, nitrogen content, and uptake of maize plants

The shoot and root biomass and nitrogen uptake of maize plants were significantly affected by the inoculation treatment but not by patch type (Supplementary Table 5). AMF significantly increased the shoot and root biomass of host plants by 1.79 and 1.65 times, respectively. The shoot and root N contents of maize plants were not significantly affected by AMF except for a slight but significant decrease in shoot N content in the control patches. AMF also significantly enhanced the shoot and root N uptake of maize plants by 1.58 and 1.73 times, respectively (Supplementary Table 6).

4 Discussion

4.1 AMF reduced N_2O emissions from degraded residue patches

AMF hyphae can penetrate into crop residue patches to search for patchily distributed nutrients and alter carbon and

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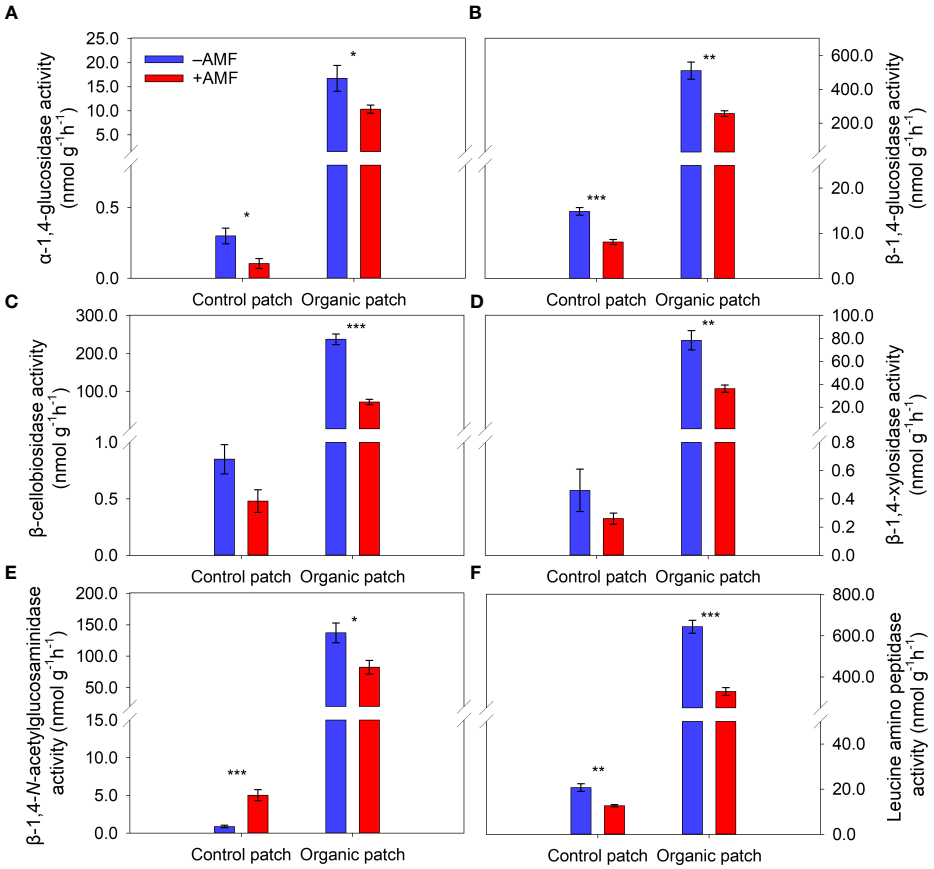

FIGURE 4
Activities of extracellular enzymes α-1,4-glucosidase **(A)**, β-1,4-glucosidase **(B)**, β-cellobiosidase **(C)**, β-1,4-xylosidase **(D)**, β-1,4-*N*-acetylglucosaminidase **(E)**, and leucine amino peptidase **(F)** in patches of different types between +AMF and –AMF treatments. Values represent means ± standard error, $n = 4$. +AMF and –AMF indicate the inoculation with and without *Funneliformis mosseae* in the host compartment, respectively. Asterisks indicate significant differences between +AMF and –AMF treatments within each patch type according to the two-tailed unpaired *t*-test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

nitrogen cycling during the decomposition of crop residues (Hodge et al., 2001). Growing evidence shows that AMF reduce soil N_2O emissions in the mycorrhizosphere or hyphosphere (Bender et al., 2014; Zhang et al., 2015; Storer et al., 2017; Gui et al., 2021; Zhao et al., 2021). However, the reasons for the decrease were variable and environmentally dependent. The successful colonization of extraradical hyphae in patches is a prerequisite for the effect of AMF on patch N_2O emissions. The hyphal growth rate of *Funneliformis mosseae* after initial infection with host plants is only about 3 mm d^{-1} (Smith and Read, 2008), and time is needed for substantial hyphae to proliferate in patches. In the present study, the average HLD in the +AMF treatment after approximately 30 days of patch addition was 7.41 m g^{-1}, much higher compared to the –AMF treatment (Figure 1B), which is consistent with previous reports (Hodge and Fitter, 2010; Gui et al., 2021). In the present study, AMF reduced N_2O concentration from the organic patches after approximately 30 days of patch addition (Figure 5A), when crop residues in the organic patches had undergone a period of rapid degradation (Wu et al., 2019). This reduction in N_2O emissions from degraded residue patches by AMF could potentially be attributed to the decreased abundance of *nirS*-type denitrifiers and ammonia-oxidizing bacteria, as well as the decrease in N-substrate availability, which will be discussed in detail below.

4.2 AMF suppressed *nirS*-type denitrifiers in degraded residue patches

AMF significantly reduced the abundance of the *nirS* gene in organic patches which negatively correlated with HLD (Figures 5E, 6C). Multiple stepwise regression analysis suggested that the abundance of the *nirS* gene best explained the decrease of N_2O concentration from organic patches (Supplementary Table 4). Denitrification is carried out by microbes as an alternate respiration to conserve energy under O_2 constraints (Zumft, 1997), with either organic carbon (for heterotrophic denitrification) or reduced inorganic compounds (for autotrophic denitrification) as electron donors (Korom, 1992). In the present study, the DOC content in the organic patches was significantly decreased by AMF and positively correlated with the abundance of the *nirS* gene (Figures 3A, 6D). This result indicates that the growth of the *nirS*-type denitrifiers may be limited by the availability of organic carbon, which is a result of AMF interference in residue

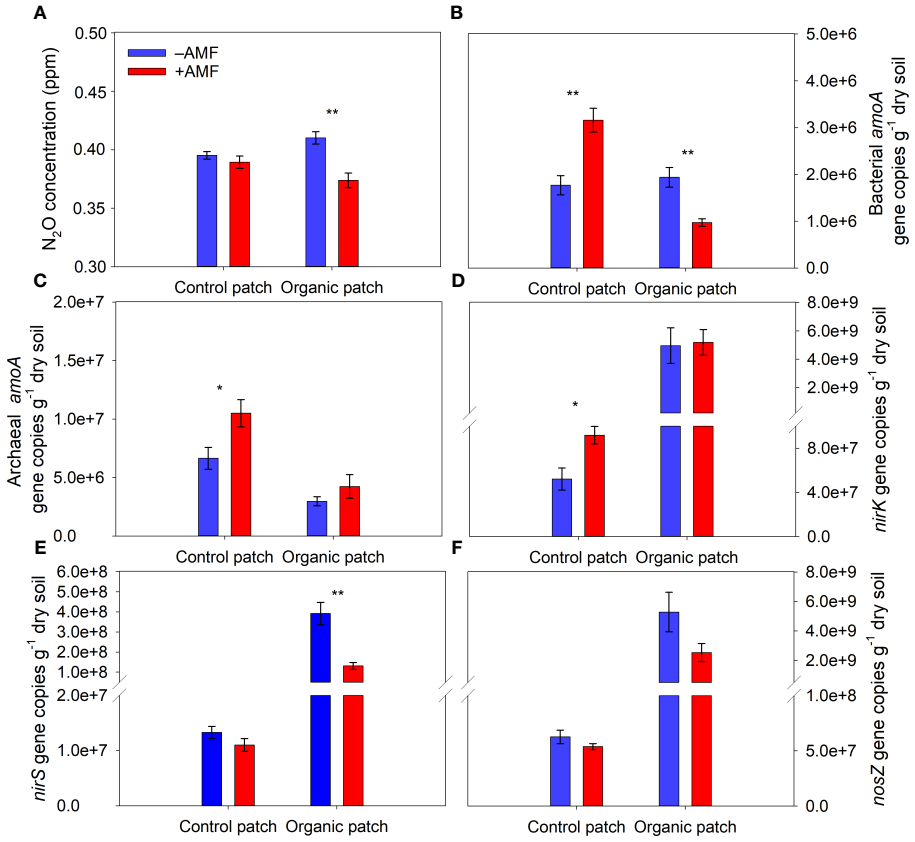

FIGURE 5
N_2O concentration **(A)** and copies of the bacterial *amoA* **(B)**, archaeal *amoA* **(C)**, *nirK* **(D)**, *nirS* **(E)**, and *nosZ* **(F)** genes in patches of different types between +AMF and –AMF treatments. Values represent means ± standard error, n = 4. +AMF and –AMF indicate the inoculation with and without *Funneliformis mosseae* in the host compartment, respectively. Asterisks indicate significant differences between +AMF and –AMF treatments within each patch type according to the two-tailed unpaired t-test (*, $P < 0.05$; **, $P < 0.01$).

degradation. This finding is inconsistent with a previous study that found that AMF increased the content of DOC in patches, possibly due to hyphal exudates (Li et al., 2023). The possible explanation for this inconsistency is that the ratio of crop residues in the present study was 33%, which is much higher than in the previous study (1%), and the contribution of carbon released from degraded residues outweighs the carbon exuded by AMF hyphae in the patch DOC pool.

In the present study, inoculation with AMF significantly reduced all tested extracellular enzyme activities in the organic patches (Figure 4). This demonstrates that the presence of AMF inhibits the activity of decomposers in degraded residue after approximately 30 days of incubation. This finding is consistent with previous studies in which AMF suppressed the decomposition of woody plant litters and wheat stubbles after incubation for 3 months in the field (Leifheit et al., 2015; Verbruggen et al., 2016). In contrast, AMF have also been reported to accelerate litter decomposition (Hodge et al., 2001). The discrepancy may be attributed to the quality, quantity, composition, and decomposing time of the residues. AMF could initially stimulate decomposers by providing hyphal exudates in the early stages of residue decomposition (Herman et al., 2012; Kaiser et al., 2015). Over time, AMF hyphae efficiently scavenge mineral nutrients, such as N released from the organic patches, and potentially suppress these decomposers by competing substrates (Leifheit et al., 2015). This is consistent with lower N availability including NH_4^+, NO_3^-, TDN, and TN in the organic patches due to AMF in the present study (Figures 2, 3). The low decomposition rate in the presence of AMF may mitigate the release of DOC in plant residues and offer great potential to occlude C within soil aggregates (Leifheit et al., 2015; Verbruggen et al., 2016). The decrease in DOC content by AMF in the organic patches, which is positively correlated with the Z value of extracellular enzyme activities, supports this speculation (Figures 3A, 6E). However, AMF only significantly decreased the abundance of the *nirS* gene, but not the *nirK* gene, in the organic patches (Figures 5D, E). Compared with *nirK*-type denitrifiers, *nirS*-type denitrifiers are more sensitive to DOC, and their populations increase with soil DOC content within a certain range. Meanwhile, *nirK*-type denitrifiers have a broader profile of carbon substrate utilization and do not significantly respond to alterations in soil DOC content (Hou et al., 2018; Langarica-Fuentes et al., 2018). In addition, one limitation of the study is that it did not adequately reflect the impact of AMF on the early residue degradation phase. Future studies should focus on improving the experimental design and the dynamic monitoring

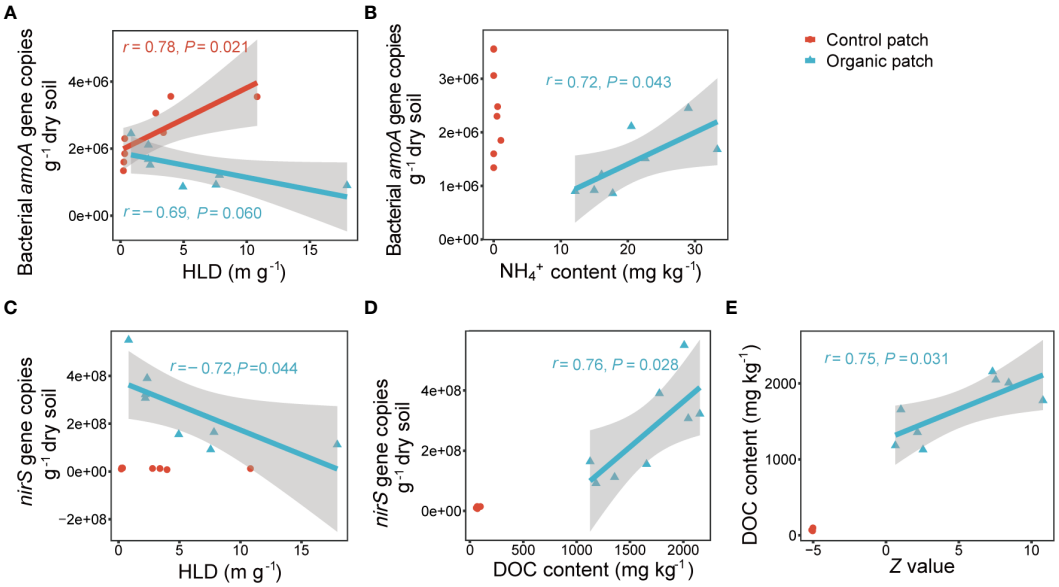

FIGURE 6
Correlations between the bacterial *amoA* gene copies with HLD **(A)** and NH_4^+ content **(B)**, between *nirS* gene copies with HLD **(C)** and DOC content **(D)**, and between DOC content with the *Z* value of extracellular enzyme activities **(E)**. HLD indicates hyphal length density, DOC indicates dissolved organic carbon. Correlation analysis based on Pearson correlation coefficient. Gray shades visualize the 95% confidence interval and correlations with $P < 0.1$ are listed.

throughout the entire cultivation period to obtain a more comprehensive understanding of the impact of AMF on residue degradation.

4.3 AMF hyphae reduced the availability of N substrate and suppressed ammonia-oxidizing bacteria in degraded residue patches

In the present study, AMF significantly decreased NH_4^+ and NO_3^- contents in the organic patches resulting in low N-substrate availability to nitrifying and denitrifying microbes (Figure 2). AMF can detect, proliferate, and acquire N from decomposing organic matter such as clover, cellulose, albumin, DNA, and chitin (Bukovska et al., 2018) and, consequently, utilize this N primarily for their own growth and transfer some to the host plant. This is supported by the significantly improved N uptake of maize plants in the presence of AMF (Supplementary Table 6). It is estimated that nearly a third of the total N detected in the AMF hyphae is derived from the organic patches (Hodge and Fitter, 2010).

In the present study, AMF hyphae significantly reduced the abundance of the bacterial *amoA* gene in the organic patches (Figure 5B), which is consistent with previous studies that showed AMF suppressed AOB and soil potential nitrification rates (Veresoglou et al., 2011; Bukovska et al., 2018). AMF can absorb NH_4^+, NO_3^-, and some simple organic N from the soil, with a preference for NH_4^+ (Govindarajulu et al., 2005; Tanaka and Yano, 2005). AOB are a group of slow-growing microbes and generally thought to be weak competitors for NH_4^+ compared with AMF (Bollmann et al., 2002; Storer et al., 2017). In the present study, AMF hyphae proliferated extensively in the organic patches and increased the N uptake of maize plants (Figure 1B and Supplementary Table 6). The NH_4^+ content in the organic patches decreased nearly by 50% in the presence of AMF, which was also positively correlated with the abundance of the bacterial *amoA* gene (Figures 2B, 6B). This demonstrates that AMF outclassed AOB in acquiring NH_4^+ in degraded residue patches and subsequently suppressed the growth of AOB. In contrast to the organic patches, AOB and AOA abundance was promoted by AMF in the control patches (Figures 5B, C), which is consistent with increased AOB abundance after urea application by native AMF in a pot experiment (Teutscherova et al., 2019). A possible explanation is that AMF promoted soil aeration by improving soil aggregation (Rillig and Mummey, 2006; Morris et al., 2019).

5 Conclusion

Crop residues serve as hotspots of N_2O emissions in agriculture. Here, we showed that AMF inhibited all the tested extracellular enzyme activities in degraded residue patches. AMF hyphae significantly reduced N_2O emissions, the abundance of the bacterial *amoA* and *nirS* genes (functional genes involved in N_2O production), and N-substrate availability in degraded residue patches after approximately 30 days of incubation. Additionally, the decrease of the *nirS* gene abundance by AMF had substantial consequences for reduced N_2O emissions in degraded residue patches and was positively correlated with DOC contents. However, the reduction of AMF abundance and diversity in intensive agricultural practices might have cascading effects on residue decomposition and N cycling. Agricultural management

strategies aimed at strengthening hyphal networks for sustainable agriculture will reinforce the interaction between AMF and the N-cycling microbiome, leading to increased N uptake, carbon sequestration, and mitigation of N_2O emissions.

Author contributions

XL and RZ conceived the research and drafted the initial version of the manuscript. XL and GH conducted the experiments. SB and GY performed the data analysis. All authors contributed to the discussion and writing of the manuscript. All author(s) read and approved the final manuscript.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1224849/full#supplementary-material

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