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ORIGINAL ARTICLE

# Arbuscular Mycorrhizal Fungi Shift Soil Bacterial Community Composition and Reduce Soil Ammonia Volatilization and Nitrous Oxide Emissions

**Tangqing He[1,2] · Xuelin Zhang[1] · Jiaqi Du[1] · Frank S. Gilliam[3] · Shuo Yang[1] · Minghui Tian[1] · Chenxi Zhang[1] · Yanan Zhou[1]**

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**Abstract**
Arbuscular mycorrhizal fungi (AMF) establish mutualistic relationships with the majority of terrestrial plants, increasing plant uptake of soil nitrogen (N) in exchange for photosynthates. And may influence soil ammonia ($NH_3$) volatilization and nitrous oxide ($N_2O$) emissions directly by improving plant N uptake, and/or indirectly by modifying soil bacterial community composition for the soil C availability increasing. However, the effects of AMF on soil $NH_3$ volatilization and $N_2O$ emissions and their underlying mechanisms remain unclear. We carried out two independent experiments using contrasting methods, one with a compartmental box device (in 2016) and the other with growth pot experiment (in 2020) to examine functional relationships between AMF and soil $NH_3$ volatilization and $N_2O$ emissions under varying N input. The presence of AMF significantly reduced soil $NH_3$ volatilization and $N_2O$ emissions while enhancing plant biomass and plant N acquisition, and reducing soil $NH_4^+$ and $NO_3^-$, even with high N input. The presence of AMF also significantly reduced the relative abundance within the bacterial orders *Sphingomonadales* and *Rhizobiales. Sphingomonadales* correlated significantly and positively with soil $NH_3$ volatilization in 2016 and $N_2O$ emissions, whereas *Rhizobiales* correlated positively with soil $N_2O$ emissions. High N input significantly increased soil $NH_3$ volatilization and $N_2O$ emissions with increasing relative abundance of *Sphingomonadales* and *Rhizobiales*. These findings demonstrate the contribution of AMF in regulating $NH_3$ and $N_2O$ emission by improving plant N uptake and altering soil bacterial communities. They also suggest that altering the rhizosphere microbiome might offer additional potential for restoration of N-enriched agroecosystems.

**Keywords** Nitrogen management · Arbuscular mycorrhizal fungi · Ammonia volatilization · Nitrous oxide · Bacterial composition

## Introduction

Atmospheric ammonia ($NH_3$) is a major pollutant, leading to eutrophication [1, 2]. Soil nitrous oxide ($N_2O$) is a major greenhouse gas (GHG) with a global warming potential 300 times that of carbon dioxide ($CO_2$) over a 100-year period, contributing about 5–6% of heat trapping by all greenhouse gases [3, 4]. Agricultural fields have become a major anthropogenic source for atmospheric $NH_3$ and $N_2O$, ultimately arising from N fertilization application [5, 6]. Typically, volatilization of $NH_3$ is closely associated with the formation of $NH_4^+$ in the soil [7–9], whereas agricultural $N_2O$ emissions mainly comes from nitrification and denitrification processes in soil [10–12]. Mitigation of $NH_3$ volatilization and $N_2O$ emissions is necessary for environmental protection and sustainable development of agricultural management.

Arbuscular mycorrhizal fungi (AMF) comprise an integral component of the soil microbial community [13]. These fungi are globally distributed, establishing mutualistic relationships with most species of terrestrial plants [14]. It is well documented that AMF may influence soil $N_2O$

✉ Xuelin Zhang
xuelinzhang1998@163.com

[1] College of Agronomy, Co-construction State Key Laboratory of Wheat and Maize Crop Science, Collaborative Innovation Center of Henan Grain Crops in Henan Province, Henan Agricultural University, Zhengzhou 450046, China

[2] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China

[3] Department of Biology, University of West Florida, Pensacola, FL 32514, USA

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emissions [15, 16]. Previous studies have shown that the presence of AMF significantly reduced soil $N_2O$ emissions [15, 17, 18]. In contrast, some studies found that AMF can promote $N_2O$ emissions through increasing labile C for denitrifiers [19], while others have reported minimal effects of AMF potential on $N_2O$ emissions [20, 21]. These conflicting results highlight the need to explicitly quantify the impacts of mycorrhizal hyphae on $N_2O$ emissions [22] and the underlying mechanisms of AMF-induced $N_2O$ mitigation.

Previous studies have shown that the AMF effectively promote the absorption of nutrients such as N for their host plants through hyphal networks embedded in the soil [23–25]. AMF hyphae can proliferate in organic patches and senescent nodules [26], where they acquire substantial amounts of N, transferring much of it to their host plants [16, 27, 28]. An isotopic labelling experiment revealed that the inorganic N absorbed by the fungi outside the root was incorporated into amino acids and transferred from the outer to the inner mycelia in the form of arginine [29], suggesting a possible mechanism by which AMF may reduce soil $N_2O$ emissions. Indirectly, AMF hyphae can impact soil N dynamics by altering soil bacterial communities [22, 30], including denitrifiers [15], thus mediating $N_2O$ production [31]. For example, AMF can inhibit ammonia oxidizers [18, 32] or regulate denitrification as shown by the positive correlation between AMF abundance and *nos*Z-type denitrifiers [15].

Soil microorganisms, especially bacteria, are responsible for regulating biogeochemical cycles in terrestrial ecosystems, promoting plant growth and maintaining ecosystem stability [3, 4]. Mandal et al. [33] found that biochar can reduce $NH_3$ volatilization by altering the composition of bacterial communities, particularly the family *Nitrosomonodaceae*. Zhou et al. [34] found that the abundance of bacterial gene *nor*B was associated with soil $N_2O$ emissions. At present, there are relatively few studies on the response of soil bacteria to AMF, and the mechanism of the influence of bacterial community composition on $NH_3$ volatilization and $N_2O$ emissions is unclear.

Previous studies on the effects of AMF on soil $N_2O$ emissions primarily utilized compartmented microcosm units [18, 19], growth pot experiments [35], or mutant plant strains [15, 20] in the glasshouse. Storer et al. (2018) carried out an experiment to study the effects of AMF on soil $N_2O$ emissions using microcosm units [18], whereas Liang et al. (2019) examined the interactive effects of biochar and AMF on greenhouse gas emissions using pot methods [35]. A few studies, however, have considered the effects of AM fungi on nutrient leaching losses by using different methods under field conditions [22]. Still a few experiments have been conducted to examine the mechanisms and general nature of AMF effects on soil $N_2O$ emission using different experimental approaches, including soil types, crop varieties, and methodologies. Thus, to date, it is unclear whether AMF effects and their regulating mechanisms reported in the literature are a general characteristic of mycorrhizal symbioses under a wide range of conditions.

To address this, we established two independent experiments under ambient conditions of field temperature and precipitation to mimic the field growth environment of the host. One used Plexiglass mesocosms to separate HOST chambers from TEST chambers that were further separated by nylon mesh of different pore sizes to test the effect of AMF with the host maize variety Weike 702 on soil $NH_3$ volatilization and $N_2O$ emission. The other used growth pots with the host maize variety Zhengdan 958 to study the effects of N fertilizer rates, AMF, and their interactions on soil $N_2O$ emissions. We hypothesized that (1) the presence of AMF would reduce soil $NH_3$ volatilization and $N_2O$ emissions and correspond to the variations of soil bacterial community composition even under the contrasting conditions of the two different experimental approaches and different host varieties and (2) the response of soil bacterial community composition to AMF would show similar patterns between two different experimental methods.

## Materials and Methods

### Site Description and Experimental Materials

To quantify the effects of AMF hyphae on soil $NH_3$ volatilization and $N_2O$ emissions, two independent experiments of contrasting methodology were established under the ambient conditions of field temperature and precipitation to simulate the field growth environment, one using compartmented mesocosms (Experiment 1) and the other using growth pots (Experiment 2) (Fig. S1). Experiment 1 was designed to determine impacts of AMF hyphae on both soil $NH_3$ volatilization and $N_2O$ emissions simultaneously, whereas Experiment 2 only on $N_2O$ production.

Experiment 1 was carried out in 2016 in Xiping County, Henan Province, China (114° 02′ E, 33° 20′ N). Experiment 2 was conducted in 2020 at Henan Agricultural University (113° 40′ E, 34° 46′ N). Meteorological conditions were notably closely similar between sites/years during maize growth periods from June 10 to October 8 in 2016 and 2020 (Fig. 1). The same soil type (Shajiang Black Soil) was used in both experiments. Soil samples were collected in June 2016 and 2020 and taken from 0 to 20 cm (tillage layer) of farmland soil under annual wheat and maize rotations in Xiping County, Henan Province. All soil was air dried and screened to pass a 2-mm sieve, with general soil properties of 2016 and 2020 measured. Because these properties did not vary significantly between 2016 and 2020, only soil attributes in 2016 are presented: total N 2.76 g $kg^{-1}$, organic

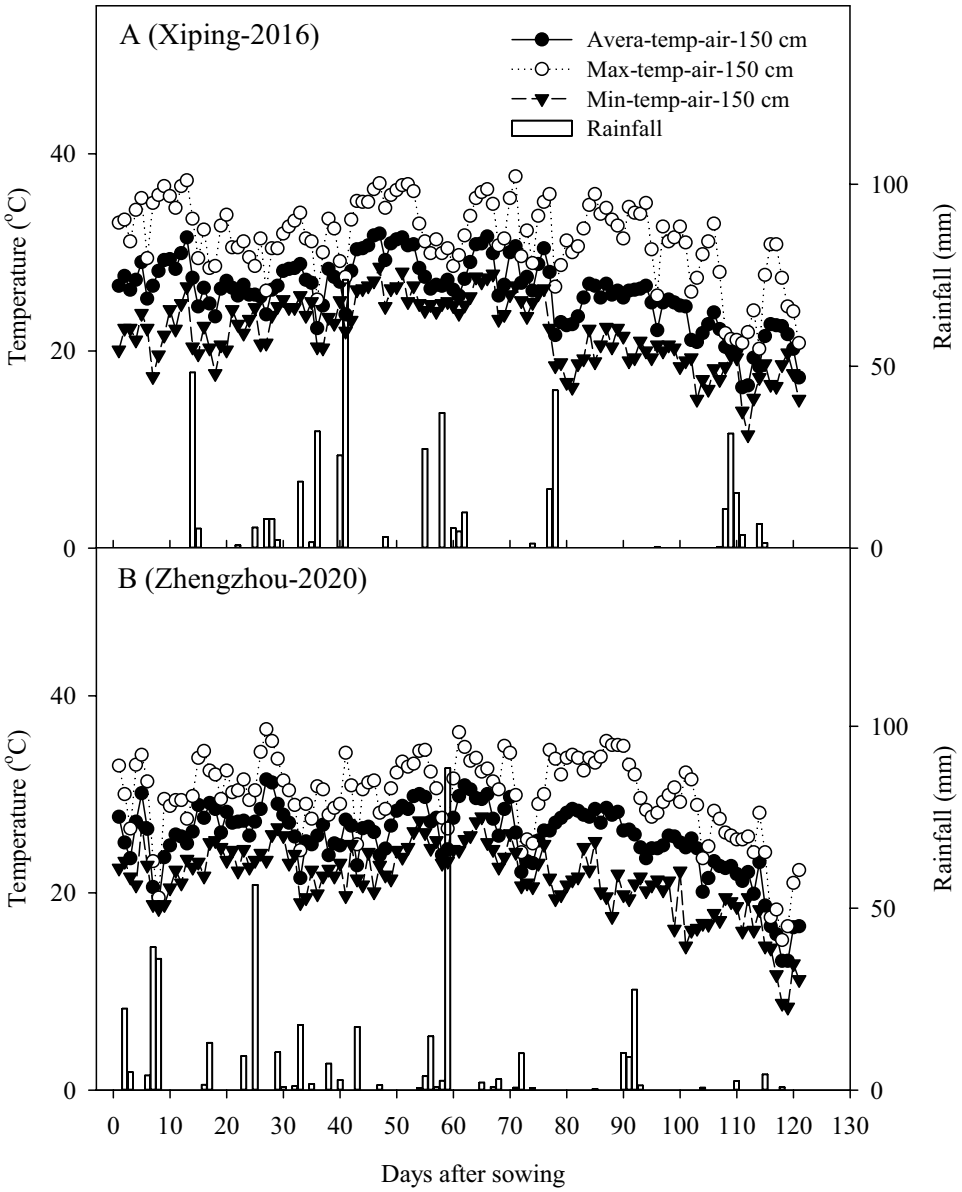


**Fig. 1** Average (Avera-temp-air-150 cm), maximum (max-temp-air-150 cm), and minimum (min-temp-air-150 cm) daily temperature measured 150 cm above the soil surface and daily rainfall (rainfall) during maize growing periods from June 10 to October 8 in Xiping (**A**) in 2016 and Zhengzhou (**B**) in 2020

matter 18.17 g $kg^{-1}$, available N 0.11 g $kg^{-1}$, available phosphorus 0.02 g $kg^{-1}$, available potassium 0.33 g $kg^{-1}$, and pH 6.81. Soil texture was a clay loam 39.1% sand, 21.3% silt, and 39.6% clay. The AMF *Funneliformis mosseae* used in both experiments were obtained from Bank of *Glomeromycota* in China of the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, China, and was propagated with *Zea mays* L. in sterilized sand in a growth chamber for 3 months until sporulation in the greenhouse of Henan Agricultural University. Sand substrates with chopped roots, extraradical mycelium, and spores were stored in plastic bags at room temperature until used. The density of spores in the inocula of each AMF species was estimated by microscopic examination (Nikon SMZ800) after wet-sieving and centrifugation [36, 37]. Spore numbers in the 50 g inoculum of species were 584 in 2016 and 512 in 2020. The medium for storing AMF was sand.

## Experiment Design

### Experiment 1

This experiment examined the effects of both N fertilizer rates and mycorrhizae in a factorial design. Based on our previous studies in a long-term field experiment for N fertilizer rates in Xiping County, Henan Province, China, we found that the lower, optimal, and excessive N input rate is 180 kg N $hm^{-2}$, 270 kg N $hm^{-2}$, and 360 kg N $hm^{-2}$ for maize growth; the lower (180 kg N $hm^{-2}$) and excessive

(360 kg N $hm^{-2}$) rates were used in this experiment and urea as the form of N fertilizer. In order to distinguish the direct effects of AMF on soil $NH_3$ volatilization and $N_2O$ emissions, a modified compartment cultivation system was used [38]. The compartment box (length × width × height = 60 cm × 20 cm × 20 cm) was divided into a HOST chamber (40 cm × 20 cm × 20 cm) and a TEST chamber (20 cm × 20 cm × 20 cm) with a perforated baffle in the middle (Fig. S1). 24 kg air-dried soil was added to each box and mixed with 100 g AMF (*Funneliformis mosseae*) inoculum, which consisted of culture media with spores, hyphae, and colonized root pieces; maize was sown in the HOST chamber. The TEST chamber was used to measure and verify the function of mycorrhizae on soil $NH_3$ volatilization and $N_2O$ emissions. According to the membrane permeability of the intermediate baffle, mycorrhizal impacts included three levels. The control (M0) was a meshwork of 0.45-μm film that isolated the HOST and TEST chambers, such that neither maize roots nor AMF in the HOST chamber could penetrate into the TEST chamber, but permitted diffusion of solutes via mass flow between the two chambers [14, 27]. The AMF treatment (M1) involved separating the HOST and TEST chambers by a 20-μm mesh membrane, wherein only AMF hyphae were allowed to enter the TEST chamber through the baffle. Finally, the ROOT treatment (M2) separated the HOST and TEST chambers by a 0.46-cm nylon mesh that allowed both AMF and roots to enter the TEST chamber through the baffle. Each treatment replicated four times.

Uniformly sized seeds of maize variety Weike 702, characteristically high in disease resistance and grain yield, were disinfected with 10% $H_2O_2$, rinsed with distilled water, and sown on 10 June 2016, constituting days after sowing 0 (DAS 0). Four seeds were sown in the HOST chamber with two seedlings remaining at the 3rd leaf stage. The base fertilizer addition comprised phosphorus ($P_2O_5$) and potassium ($K_2O$) fertilizers applied at 90 kg $hm^{-2}$ and 120 kg $hm^{-2}$, respectively, and mixed with soil before sowing. Half of the N fertilizer was dissolved in $H_2O$ and applied evenly to both HOST and TEST chambers at each of DAS 29 and 59. During maize growth periods, soil in both HOST and TEST chambers was watered with deionized $H_2O$, with moisture periodically determined by the Campbell Scientific HS2 Hydrosense II probe (Campbell Scientific, Logan, UT, USA) (Fig. S2). Experiment 1 continued for 4 months after which the maize was harvested at maturity stage (5 October 2016).

### Experiment 2

The growth pot experiment was also established under open conditions to test the interactive effects of N additions and AMF on $N_2O$ emissions and soil bacterial communities. Urea (dissolved in water) was also used as N fertilizer form, and the N treatment comprised addition at rates of 180 kg N $hm^{-2}$ (N1) and 270 kg N $hm^{-2}$ (N2). The AMF treatments were without (M-) and with (M +) the presence of AMF in the pots. Each pot was 33 cm in diameter and 22 cm in height and filled with 10 kg of soil. The M- pot had 100 g of sand in place of adding AMF, whereas the M + pot was treated with 100 g AMF inoculum, which consisted of culture media containing spores, hyphae, and colonized root pieces. Maize variety Zhengdan 958, widely used in agricultural production, was selected for this experiment and sown on 10 June 2020. All additions of phosphate (at 90 kg $P_2O_5$ $hm^{-2}$) and potassium (at 120 kg $K_2O$ $hm^{-2}$) fertilizers were applied along with half of the N fertilizer made before seed sowing, whereas the remaining half of the N fertilizer rate was dissolved in $H_2O$ and applied to the soil 30 days after sowing. Soil moisture in the pot was periodically determined during maize growth periods (Fig. S2). Experiment 2 continued for 4 months after which the maize was harvested at maturity, on 8 October 2020.

## Soil $NH_3$ volatilization and $N_2O$ Emission Measurements

Soil $NH_3$ volatilization was determined by ventilation method [39] repeatedly during Experiment 1. Soil $N_2O$ emissions were collected during the maize growth period using the static chamber and gas chromatography method [40] repeatedly in both experiments. At DAS 30 and 60 for Experiment 1 and DAS 1 and 30 for Experiment 2, $N_2O$ emissions were sampled for 5 consecutive days after fertilizer-N addition. Before each gas sample was taken, all glass vials were evacuated for 1 min by a vacuum pump. The base was closed for 1 h before 50 ml of headspace gas was drawn. Gas analysis was performed via gas chromatography (Shimadzu GC-2010, Japan).

## Soil and Plant Sampling and Analysis

Soil samples in both the TEST compartments and pots were taken after maize harvesting, with soils mixed evenly and sampled randomly. Sampled soil was placed on ice in coolers and immediately brought to the laboratory. A subsample of each sample was immediately frozen at − 80 °C for microbial determination, and the remaining sample was kept at 4 °C prior to chemical analyses.

Harvests (including grain, aboveground biomass, and roots) were carried out at the maturity stage. Aboveground biomass in 2016 was divided into stem, leaf, leaf sheath, and other plant parts; in 2020, this was stem, leaf, and other plant parts separately. All plant material was oven-dried at 75 ℃ for 72 h and weighed to determine biomass. Oven-dried material was separately and finely ground to powder for determination of N concentration (Tables S1 and S2). All N measurements, including soil inorganic N ($NH_4^+$ and

$NO_3^-$) at maize harvest time, were determined via continuous flow analysis (AA3, SEAL-Analytical, Germany). N accumulation for all plant components was calculated by multiplying biomass with respective N concentrations separately. Aboveground plant N accumulation in 2016 was the sum of N accumulation for stem, leaf, leaf sheath, and other plant parts; for 2020, this included the sum of stem, leaf, and other plant parts. Root and shoot ratios were calculated by dividing root biomass by aboveground biomass. Microbial biomass carbon (MBC) at maize harvest time for Experiment 2 was also determined using with fumigation extraction, and microbial biomass nitrogen (MBN) was measured following alkaline persulfate oxidation of $K_2SO_4$ extracts [41, 42]. Mycorrhizae colonization of plant roots was microscopically determined after roots were stained with acidic glycerol-trypan blue solution at 90 °C for 30 min [43] and scored using gridline intersection [44].

### Bacterial Community Analyses

Soil DNA was extracted from 0.5 g of each soil sample using the Fast DNA® SPIN kit for soil (MP Biomedicals, USA) as per the manufacturer's instructions. DNA quality and concentration were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Extracted DNA was diluted approximately tenfold with nuclease-free water for real-time polymerase chain reaction (qPCR) amplification. Using the diluted genomic DNA as template, the barcoded primers 515F and 806R (GTGCCA GCMGCCGCGG and GGACTACNNGGGTATCTAAT) were used to amplify the V4 regions of the soil bacterial community. All amplification reactions had efficiency values of 95–100%, and $R^2$ values of the standard curves were consistently > 0.99. The amplification specificity of each gene was confirmed by melting curve analysis. The amplification products were sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA).

According to the barcode sequence and PCR amplification primer sequence, the sample data were separated from the offline data, and the effective sequences (effective tags) were obtained through removal of impurity, mosaic, filtering, and removal of chimeras. MiSeq sequencing of bacteria resulted in 1,196,616 and 514,656 reads in 2016 and 2020, respectively, after quality filtering. Uparse software (Uparse v.7.0) was used to cluster all the effective tags, and OTUs (operational taxonomic units) were divided according to 97% similarity. The sequence with the highest frequency of OTUs was selected as the representative sequence of OTUs. Species annotation analysis was carried out based on the SSUrRNA database of Mothur (v.1.36.1) and Silva (http://www.arb-silva.de/) (thresholds were set at 0.8 ~ 1) to obtain the community composition of each sample at different classification levels. MUSCLE (v.3.8.31) software was used for rapid multiple sequence alignment to obtain all OTUs on behalf of the sequence of the system. Data for each sample were homogenized, and the sample with the least number of data was taken as the standard for homogenization. Subsequent alpha diversity analysis was based on data after homogenization. QiIME software (v.1.7.0) was used to calculate alpha diversity index.

### Data Analyses

Variation with sample time and effects of N fertilizer and AMF treatments on soil $NH_3$ volatilization and $N_2O$ emission rate were tested using repeated measure analysis of variance (ANOVA) in a general linear model, with sampling time, N fertilizer, and AMF treatments used as main effects. Two-way ANOVA was used to analyze the effects of N fertilizer rates and mycorrhizae treatments on maize plant biomass, plant N accumulation, root AMF colonization, soil $NH_4^+$-N, $NO_3^-$-N, MBC, and MBN. Differences among treatment means were tested with Duncan's multiple-range tests. Two-way ANOVA was used to compare the alpha diversity parameters of soil bacteria among different treatments, including observed species, estimated indices (Chao1, Shannon, and Simpson), and goods coverage. We used principal coordinate analyses (PCoA) based on Bray–Curtis distances to visualize the effects of treatments on the beta diversity of bacteria using package vegan from the R version 3.6.3. To test effects of N fertilizer and AMF on the beta diversity of bacterial communities, we performed two-way permutational multivariate analyses of variance (PERMANOVA) using the function adonis from the R version 4.1.1 (R Core Team, 2020). Statistical analyses were performed using the software program SPSS version 10.0 (SPSS Inc., Chicago, IL, USA) and plotted using Origin 2022 (Learning Version).

## Results

### Grain Yield, Aboveground Plant Biomass, Root Biomass, N Accumulation, AMF Colonization, Soil N, and Microbial C and N

In Experiment 1, both N fertilizer and mycorrhizae significantly influenced grain yield, aboveground plant biomass, and N accumulation (Fig. 2; Table 1). Compared with M0, M1 and M2 treatments increased grain yield, plant biomass, root biomass, grain yield N accumulation, plant N accumulation, root N accumulation, and AMF colonization at both N1 and N3 input (Fig. 2; Tables 1 and S1), while reducing soil $NH_4^+$-N and $NO_3^-$-N (Table 1). In Experiment 2, the M + treatment increased grain yield, aboveground plant biomass, aboveground plant N accumulation,

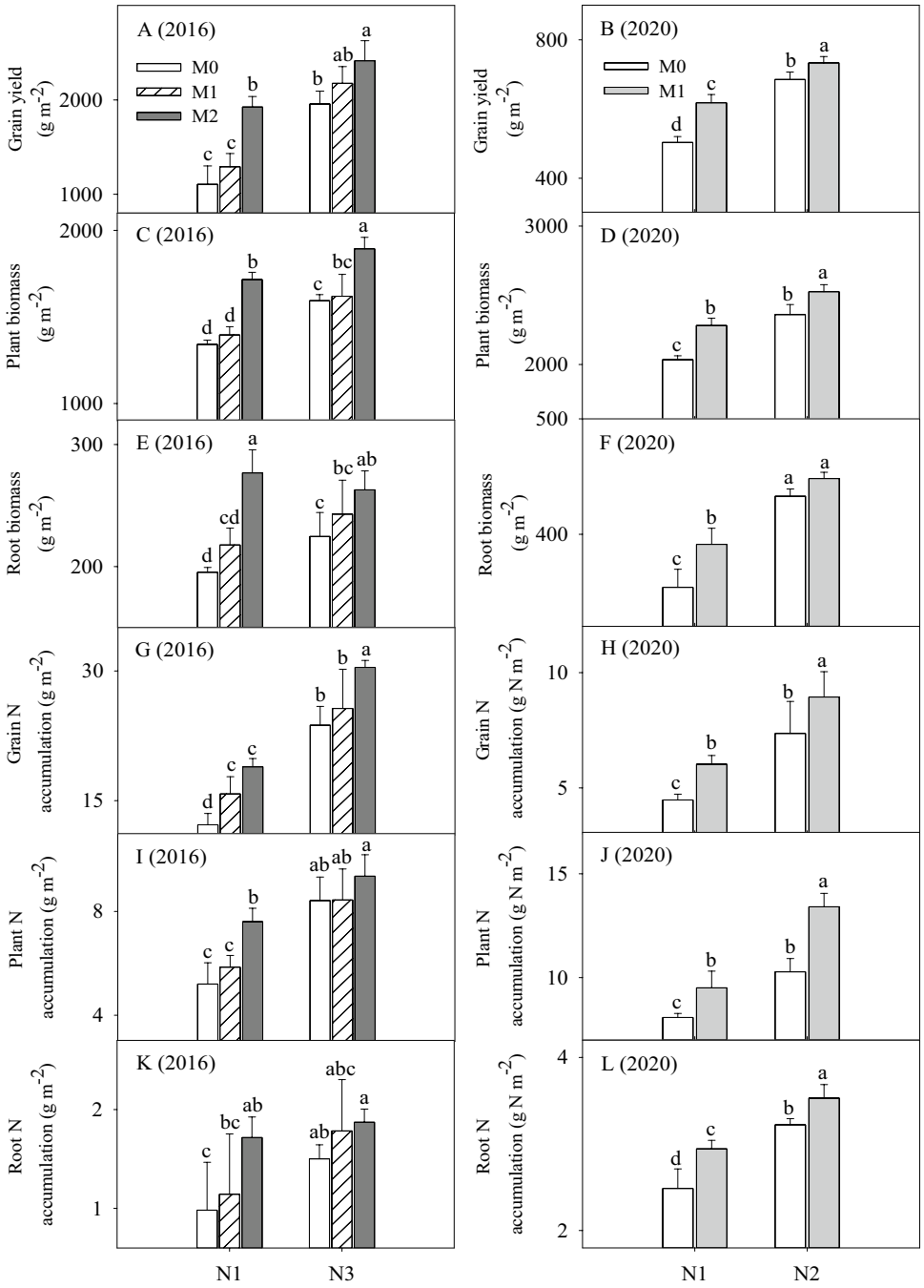


**Fig. 2** Comparison of maize grain yield and their N accumulation, plant biomass and their N accumulation, and root biomass and their N accumulation among the treatments (values are means ± SE, $n = 4$). N1, N2, and N3 represent 180 kg N $hm^{-2}$, N270 kg N $hm^{-2}$, and 360 kg N $hm^{-2}$, respectively. Plant biomass represents aboveground plant biomass; plant N accumulation represents aboveground plant N accumulation. M0 represents neither AM hyphae nor maize roots grow into the TEST compartments; M1 represents only AM hyphae grow into the TEST compartments; M2 represents both AM hyphae and maize roots grow into the TEST compartments, respectively. M- represents without the presence of AMF in the pots, and M + represents with the presence of AMF in the pots. Different letters indicate significant differences among the treatments by one-way ANOVA at $P = 0.05$, and Duncan's multiple-range tests

root biomass, root N accumulation, AMF colonization, and MBC relative to M- at N inputs (Fig. 2; Tables 2 and S2), while reducing soil $NH_4^+$-N and $NO_3^-$-N contents and MBN (Table 2).

## Soil $NH_3$ Volatilization and $N_2O$ Emissions

Both soil $NH_3$ volatilization and $N_2O$ emissions varied temporally during maize growth (Fig. S3, Table S3). In Experiment 1, the N3 treatment increased soil $NH_3$ volatilization and $N_2O$ emissions by 65% and 24%, respectively, relative to N1 (Fig. 3). Compared to the M0 treatment, M1 and M2 treatments reduced soil $NH_3$ volatilization rate by 14% and 18%, respectively, and reduced $N_2O$ emissions by 28% and 52%, respectively, for N1, respectively. At N3, these reductions were 8% and 22%, respectively, for $NH_3$ volatilization rates, and 21% and 56%, respectively, for soil $N_2O$ emissions. In Experiment 2, the M + treatment reduced soil $N_2O$ emissions by 8% compared with M- at N1, and by 13% at N2 (Fig. 3).

**Table 1** The effects of nitrogen (N) fertilizer and mycorrhizae (M) on maize plant biomass, biomass N accumulation, root/shoot ratio, AMF colonization, and soil $NH_4^+$-N, $NO_3^-$-N content (values are means ± SE, $n = 4$) by the two-way ANOVA in 2016

| | Stem | | Leaf | | Other parts | | Root/shoot ratio | AMF colonization (%) | Soil (mg $kg^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Biomass (g $m^{-2}$) | N accumulation (g N $m^2$) | Biomass (g $m^{-2}$) | N accumulation (g N $m^2$) | Biomass (g $m^{-2}$) | N accumulation (g N $m^2$) | | | $NH_4^+$-N | $NO_3^-$-N |
| N1M0 | 463 ± 36d | 1.09 ± 0.47c | 474 ± 34c | 2.52 ± 0.41c | 404 ± 7c | 1.59 ± 0.09b | 0.15 ± 0.001ab | 7.3 ± 0.3d | 6.8 ± 0.7ab | 16.4 ± 0.5c |
| N1M1 | 490 ± 14 cd | 1.44 ± 0.22bc | 480 ± 25c | 2.65 ± 0.45c | 426 ± 18c | 1.77 ± 0.07b | 0.16 ± 0.001ab | 17.0 ± 0.6b | 6.5 ± 0.5b | 15.6 ± 1.4c |
| N1M2 | 593 ± 22ab | 1.65 ± 0.13ab | 534 ± 27b | 3.45 ± 0.51b | 589 ± 28a | 2.50 ± 0.28a | 0.16 ± 0.01a | 19.7 ± 0.9a | 5.7 ± 0.5b | 14.9 ± 1.1c |
| N3M0 | 538 ± 32bc | 2.02 ± 0.32a | 534 ± 31b | 3.44 ± 0.32b | 523 ± 16b | 2.95 ± 0.50a | 0.15 ± 0.02ab | 4.7 ± 0.3e | 8.3 ± 0.6a | 48.4 ± 1.3a |
| N3M1 | 577 ± 74ab | 2.07 ± 0.53a | 538 ± 24b | 3.64 ± 0.54b | 504 ± 60b | 2.73 ± 0.78a | 0.14 ± 0.02b | 12.3 ± 0.3c | 6.8 ± 0.4ab | 30.3 ± 2.9b |
| N3M2 | 640 ± 45a | 1.91 ± 0.30ab | 640 ± 13a | 4.42 ± 0.36a | 613 ± 39a | 3.03 ± 0.49a | 0.14 ± 0.01b | 15.7 ± 0.3b | 6.7 ± .2b | 28.8 ± 2.0b |
| N | 16.8*** | 47.7*** | 29.9*** | 17.4*** | 28.9*** | 27.6*** | 5.5* | 82.6*** | 4.8* | 213.0*** |
| M | 16.3*** | 24.8*** | 46.1*** | 0.9 | 10.8*** | 3.4* | 0.1 | 283.1*** | 4.0* | 23.4*** |
| N × M | 0.5 | 2.1 | 4.2* | 1.8 | 0.01 | 1.8 | 3.6* | 2.0 | 0.8 | 18.0*** |

N, M, and N × M represent the N fertilization effect, mycorrhizae effect, and their interaction effect, respectively. N1 and N3 represent the N fertilization rates of 180 and 360 kg N $hm^{-2}$. M0 represents neither AM hyphae nor maize roots grow into the TEST compartments; M1 represents only AM hyphae grow into the TEST compartments; and M2 represents both AM hyphae and maize roots grow into the TEST compartments, respectively. *, **, and *** indicate the significant difference at the 0.05, 0.01, and 0.001 levels, respectively. Different letters after the data in the same column indicate significant difference in $P < 0.05$ level among treatments

**Table 2** The effects of nitrogen (N) fertilizer and mycorrhizae (M) on maize plant biomass, biomass N accumulation, root/shoot ratio, AMF colonization, $NH_4^+$-N, $NO_3^-$-N content, MBC, and MBN (values are means ± SE, $n = 4$) by the two-way ANOVA in 2020

| | Stem | | Leaf | | Other parts | | Root/shoot ratio | AMF colonization (%) | Soil (mg $kg^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Biomass (g $m^{-2}$) | N accumulation (g N $m^2$) | Biomass (g $m^{-2}$) | N accumulation (g N $m^2$) | Biomass (g $m^{-2}$) | N accumulation (g N $m^2$) | | | $NH_4^+$-N | $NO_3^-$-N | MBC | MBN |
| N1M0 | 943 ± 25c | 2.76 ± 0.06c | 459 ± 9b | 2.36 ± 0.25c | 632 ± 10d | 2.96 ± 0.16c | 0.17 ± 0.007ab | 5.1 ± 2.5c | 4.9 ± 0.2a | 13.2 ± 0.1bc | 333 ± 16 | 14.3 ± 0.6 |
| N1M1 | 1097 ± 7a | 3.49 ± 0.49b | 469 ± 14b | 2.65 ± 0.36bc | 716 ± 44c | 3.37 ± 0.16bc | 0.17 ± 0.008b | 95.3 ± 2.1a | 4.3 ± 0.1bc | 12.7 ± 0.3c | 310 ± 11 | 15.2 ± 0.9 |
| N2M0 | 1062 ± 31b | 3.74 ± 0.19b | 511 ± 18a | 2.94 ± 0.22ab | 788 ± 49b | 3.59 ± 0.44b | 0.18 ± 0.008a | 14.3 ± 2.3b | 4.7 ± 0.1ab | 14.1 ± 0.3a | 308 ± 21 | 14.0 ± 0.8 |
| N2M1 | 1102 ± 15a | 5.30 ± 0.53a | 505 ± 38a | 3.22 ± 0.31a | 918 ± 23a | 4.89 ± 0.32a | 0.18 ± 0.004ab | 91.0 ± 2.8a | 4.1 ± 0.1c | 13.6 ± 0.3ab | 305 ± 20 | 14.4 ± 1.2 |
| N | 32.3*** | 55.5*** | 14.8*** | 15.9** | 102.5*** | 52.3*** | 4.9* | 1.0 | 2.2 | 12.41** | 0.8 | 0.4 |
| M | 80.4*** | 37.4*** | 0.02 | 3.9 | 36.8*** | 32.7*** | 1.4 | 1199.0*** | 13.6*** | 4.65 | 0.6 | 0.5 |
| N × M | 27.4*** | 4.9* | 0.4 | 0.004 | 1.7 | 8.9* | 0.3 | 7.8* | 0.001 | 0.03 | 0.3 | 0.1 |

N, M, and N × M represent the N fertilization effect, mycorrhizae effect, and their interaction effect, respectively. N1 and N2 represent the nitrogen fertilization rates of 180 and 270 kg N $hm^{-2}$. M- represents without the presence of AMF in the pots, and M + represents with the presence of AMF in the pots. *, **, and *** indicate the significant difference at the 0.05, 0.01, and 0.001 levels, respectively. Different letters after the data in the same column indicate significant difference in $P < 0.05$ level among treatments

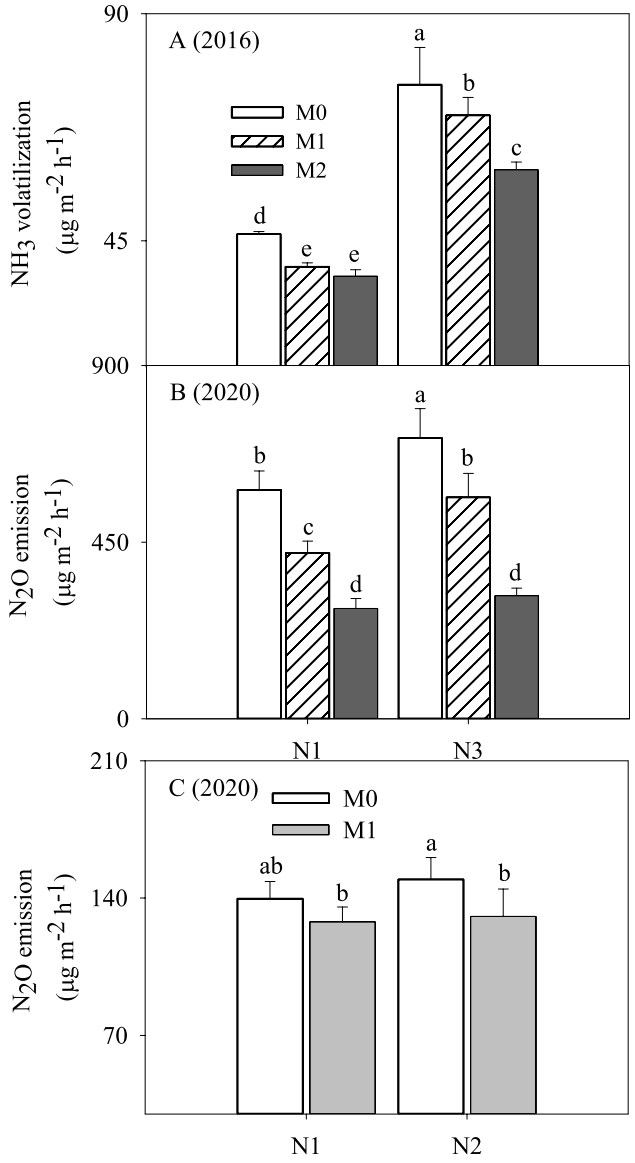


**Fig. 3** Comparison of soil $NH_3$ volatilization and $N_2O$ emission fluxes among the treatments (values are means ± SE, $n = 4$). N1, N2, and N3 represent 180 kg N $hm^{-2}$, N270 kg N $hm^{-2}$, and 360 kg N $hm^{-2}$, respectively. M0 represents neither AM hyphae nor maize roots grow into the TEST compartments; M1 represents only AM hyphae grow into the TEST compartments; M2 represents both AM hyphae and maize roots grow into the TEST compartments, respectively. M- represents without the presence of AMF in the pots, and M + represents with the presence of AMF in the pots. Different letters indicate significant differences among the treatments by one-way ANOVA at $P = 0.05$, and Duncan's multiple-range tests

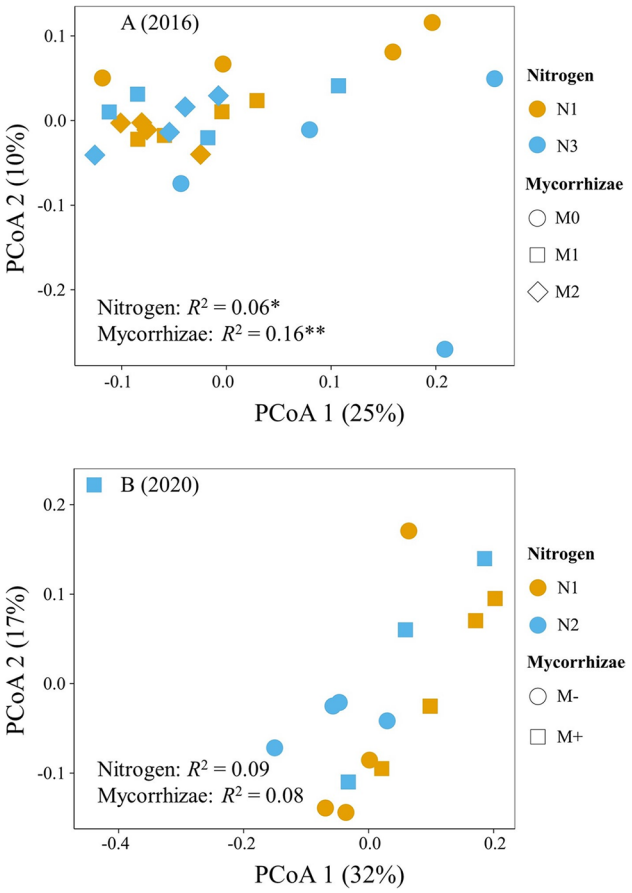


**Fig. 4** Beta diversity was assessed by PCoA at the OTU level based on Bray–Curtis similarity distance. N1, N2, and N3 represent 180 kg N $hm^{-2}$, N270 kg N $hm^{-2}$, and 360 kg N $hm^{-2}$, respectively. M0 represents neither AM hyphae nor maize roots grow into the TEST compartments; M1 represents only AM hyphae grow into the TEST compartments; M2 represents both AM hyphae and maize roots grow into the TEST compartments, respectively. M- represents without the presence of AMF in the pots, and M + represents with the presence of AMF in the pots

## Soil Bacterial Community Composition

All sequences from each soil sample were clustered into operational taxonomic units (OTUs) with more than 97% identity. Observed species displayed significant differences in both experiments (Figs. S4 and S5). In Experiment 1, compared with M0, M1 and M2 treatments increased the Chao1 index over M0 at N treatments of N1 and N3. In Experiment 2, compared with M-, the M + treatment increased the Chao1 index over M- by 13% at N1 and by 5% at N2. Changes in the bacteria communities were revealed in the results of PCoA based on Bray–Curtis distances (Fig. 4).

In Experiment 1, the major bacterial phyla were *Proteobacteria* (43 – 45%), *Acidobacteria* (16 – 22%), and *Actinobacteria* (11 – 15%) (Fig. S6A). The major class was *Alphaproteobacteria* (22 – 25%) (Fig. S6C), and the major orders were *Sphingomonadales* and *Rhizobiales* (Fig. 5A), both of which increased in relative abundance in the N3 treatment by 13% and 4%, respectively, compared to N1 (Fig. S7). At N1, the M1 and M2 treatments both reduced *Sphingomonadales* by 13% and *Rhizobiales* by 5% and 8%, respectively. At N3, these reductions were 21% and 11%,

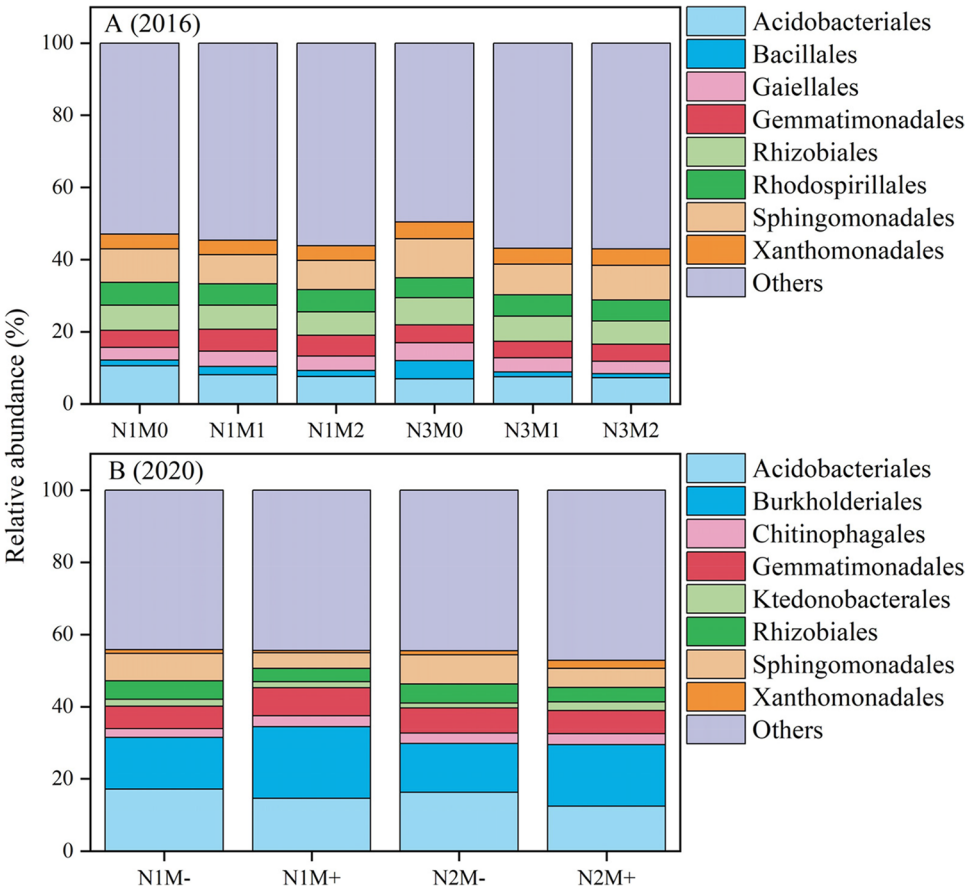


**Fig. 5** Composition of basic bacterial communities with different treatments. The relative abundance of the dominant bacterial taxonomic groups at order level. N1, N2, and N3 represent 180 kg N $hm^{-2}$, N270 kg N $hm^{-2}$, and 360 kg N $hm^{-2}$, respectively. M0 represents neither AM hyphae nor maize roots grow into the TEST compartments; M1 represents only AM hyphae grow into the TEST compartments; M2 represents both AM hyphae and maize roots grow into the TEST compartments, respectively. M- represents without the presence of AMF in the pots, and M + represents with the presence of AMF in the pots

respectively, for *Sphingomonadales* and by 8% and 15%, respectively, for *Rhizobiales*.

In Experiment 2, the major bacterial phyla were *Proteobacteria* (35 − 37%), *Acidobacteriota* (21 − 26%), and *Actinobacteriota* (5 − 7%) (Fig. S6B). Major classes were *Acidobacteriae* (17 − 22%), *Alphaproteobacteria* (11 − 18%), and *Gammaproteobacteria* (17 − 23%) (Fig. S6D), with *Sphingomonadales* and *Rhizobiales* for major orders (Fig. 5B). The N2 treatment increased relative abundance of *Sphingomonadales* and *Rhizobiales* by 11% and 6%, respectively, compared to N1 (Fig. S7). At N1, the M + treatment reduced *Sphingomonadales* and *Rhizobiales* by 43% and 28%, respectively, relative to M-; at N2, reductions were 35% and 24%, respectively (Fig. S7).

## Relationships Between Soil $NH_3$ and $N_2O$ Flux with Plant and Soil Properties

Pearson product-moment correlation showed that both soil $NH_3$ volatilization rates and $N_2O$ emissions rates were significantly and negatively correlated with AMF colonization, whereas they were positively related with soil inorganic N (Table 3). Soil $NH_3$ volatilization rates were positively correlated with the abundance of *Sphingomonadales* in

**Table 3** The relationship between soil $NH_3$ volatilization and $N_2O$ emission rate with maize plant or soil properties in 2016 ($n = 24$) and 2020 ($n = 16$)

| | 2016 | | 2020 |
|---|---|---|---|
| | $NH_3$ rate ($\mu g\ m^{-2}\ h^{-1}$) | $N_2O$ rate ($\mu g\ m^{-2}\ h^{-1}$) | $N_2O$ rate ($\mu g\ m^{-2}\ h^{-1}$) |
| Grain yield ($g\ m^{-2}$) | 0.54** | −0.22 | 0.02 |
| Plant biomass ($g\ m^{-2}$) | 0.29 | −0.45* | −0.14 |
| Root biomass ($g\ m^{-2}$) | 0.01 | −0.54** | 0.02 |
| Grain N accumulation ($mg\ N\ m^{-2}$) | 0.63** | −0.14 | −0.09 |
| Plant N accumulation ($mg\ N\ m^{-2}$) | 0.58** | −0.08 | −0.14 |
| Root N accumulation ($mg\ N\ m^{-2}$) | 0.32 | −0.17 | −0.07 |
| AMF colonization (%) | −0.62** | −0.90** | −0.60* |
| $NH_4^+$-N ($mg\ kg^{-1}$) | 0.57** | 0.547** | 0.60* |
| $NO_3^-$-N ($mg\ kg^{-1}$) | 0.90** | 0.63** | 0.68** |
| MBN ($mg\ kg^{-1}$) | - | - | -0.07 |
| MBC ($mg\ kg^{-1}$) | - | - | −0.14 |
| MBC/MBN | - | - | −0.10 |
| pH | −0.38 | −0.24 | - |

* and ** indicate the significant relationships at the 0.05 and 0.01 levels

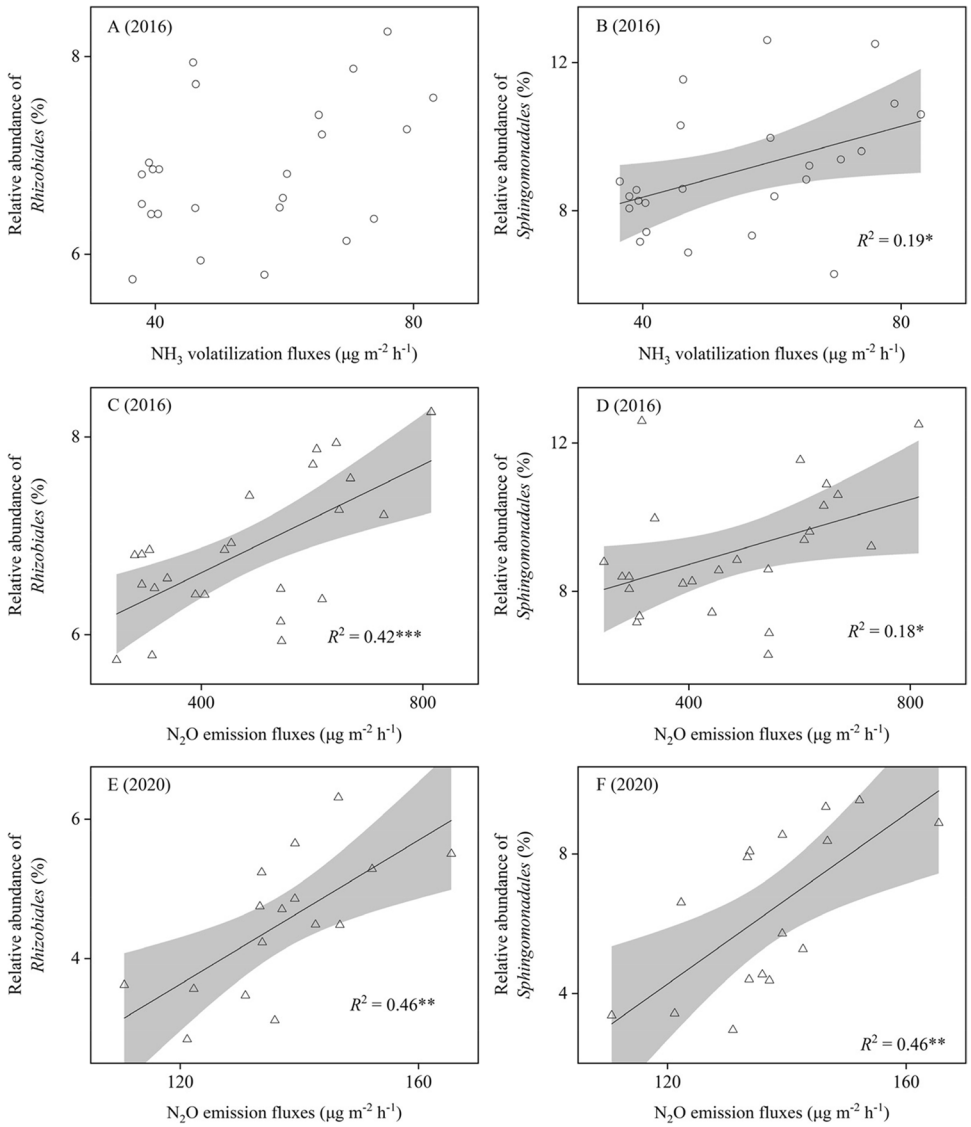


**Fig. 6** Relationships between soil $NH_3$ volatilization, $N_2O$ emission fluxes and *Sphingomonadales* (**A**, **C**, **E**) and *Rhizobiales* (**B**, **D**, **F**) in soil. The significance levels are labelled as $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, respectively

Experiment 1, and soil $N_2O$ emissions were positively correlated with the abundance of *Sphingomonadales* and *Rhizobiales* in both experiments (Fig. 6).

## Discussion

Our results showed that the presence of AMF significantly reduced soil $NH_3$ volatilization and $N_2O$ emissions (Figs. 3 and 7), along with increases in plant biomass and N uptake and changes in soil bacterial communities under different experimental methods and by using different maize varieties as host plant (Figs. 2, 4, and 5). Previous studies found that plant roots and their AM symbionts enhance the ability of plants to obtain inorganic N from soil, increasing the biomass of host plants and reducing soil $NH_4^+$ and $NO_3^-$ content [16, 25]. This indirectly reduces soil $NH_3$ volatilization and $N_2O$ emissions. AMF can form an expansive ectodermal hyphae network with the roots of the host plant and diffuse throughout the soil, increasing N absorption in the rhizosphere [20, 45]. Uptake of $NH_4^+$ by both plant root and AMF limits nitrification, whereas uptake of $NO_3^-$ limits denitrification, reducing the soil $NH_3$ volatilization and $N_2O$ emissions [16, 20]. Wang et al. [46] reported that AMF can absorb $NO_3^-$-N by strongly inducing expression of the putative nitrate transporter gene in roots. Bender et al. [15] also found that AMF increased N immobilization in plant biomass and reduced $N_2O$ emissions from the soil of tomato crops by 33–42%.

Both $NH_3$ volatilization and $N_2O$ emissions are also affected by soil microbial activities [47, 48]. AMF can

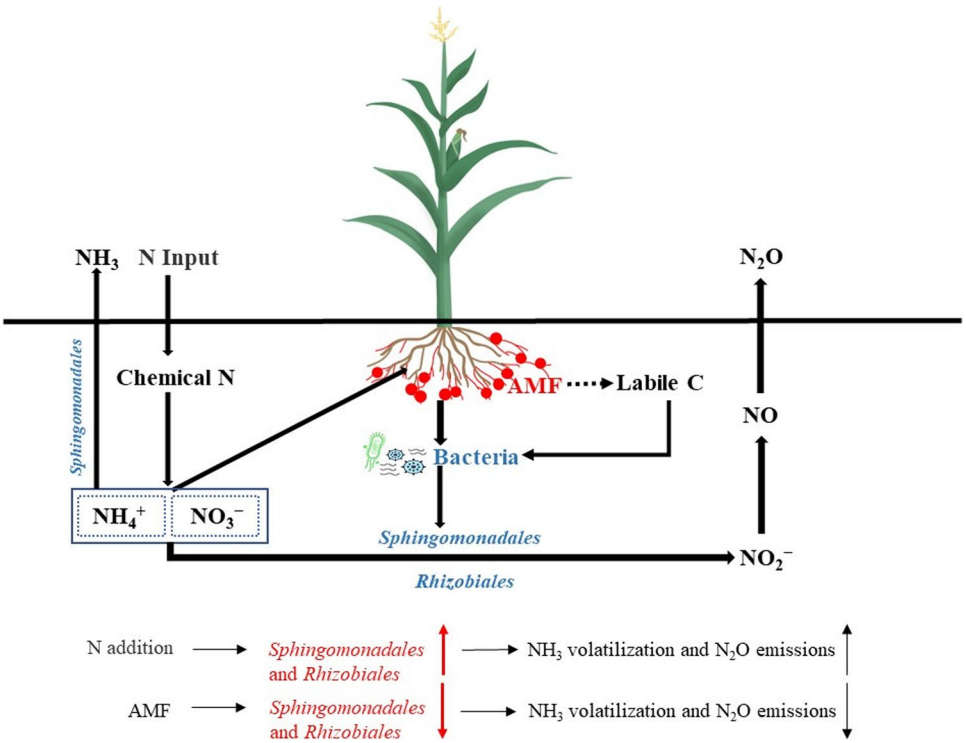


**Fig. 7** Conceptual frameworks that summarize the effects of AMF on soil $NH_3$ volatilization and $N_2O$ emissions with N input. N input increased the relative abundance of *Sphingomonadales* and *Rhizobiales*, indicating by the arrow up, while AMF reduced *Sphingomonadales* and *Rhizobiales* at order level, indicating by the arrow down

affect $N_2O$ emissions by regulating microbial communities involved in nitrification and denitrification [15, 18]. Compared with ammonia oxidizers, AMF utilizes more soil ammonium, eventually reducing the abundance of ammonia oxidizing bacteria and archaea in the soil, decreasing nitrification rate, $NH_3$ volatilization, and $N_2O$ [49, 50]. AMF can also affect $N_2O$ emissions by regulating microbial community composition [51]. Bender et al. [15] found that the abundance of key genes responsible for $N_2O$ production (*nir*K) was negatively correlated with AMF abundance, whereas genes responsible for $N_2O$ consumption (*nos*Z) were positively correlated to AMF abundance. Qiu et al. [52] found that plant roots with AMF reduced the abundance of the *nir*K gene and the ratio of (*nir*K + *nir*S)/*nos*Z under the elevated atmospheric $CO_2$.

In this study, the presence of AMF significantly reduced the abundance of the orders *Sphingomonadales* and *Rhizobiales*, which were positively correlated with $NH_3$ volatilization in 2016 and $N_2O$ emissions (Fig. 6). Previous studies have reported that the relative abundance of *Sphingomodacaeae* contained species that produce $N_2O$ [51, 53]. *Rhizobiales* is associated with most denitrifying bacteria containing *nir*K, suggesting that the *nir*K gene carried by *Rhizobiales* may promote soil denitrification [54, 55]. In all, these suggest that the presence of AMF reduced soil $NH_3$ volatilization and $N_2O$ emissions by altering composition of the soil bacterial community.

Previous studies reported that high N input often suppresses AMF colonization [56–59], and the effect of AMF on soil $N_2O$ emissions might be less significant with high N input. In our two field-based experiments of contrasting methodology, however, we found that AMF reduced soil $NH_3$ and $N_2O$ emissions in agricultural soils with high N input. These results suggest that some AMF species may be resistant to external N input. In addition, N-induced decreases in AMF infection alone may not be indicative for the decline of hyphal biomass and associated uptake of nutrients [52, 60]. Thus, inoculating with AMF could be a useful strategy for mitigating GHG emissions and climate change. It might also play a more important role in mediating farmland restoration even with excessive N fertilizer application in N-rich agroecosystem that has been previously appreciated [16].

Although agronomists working in intensive cropping systems are interested in enhancing crop growth via N fertilization, excess use of fertilizer N has caused farmland degradation and serious environmental problems [61–64]. To restore these farmlands and maximize crop yield without environment damage, a selective approach for soil N management should be developed and employed in intensive cropping systems. As a microbial-based biotechnology for environmental health in agricultural restoration, AMF has numerous applications in sustainable agriculture adaptable to farmland restoration [65, 66]. Li et al. (2013) found that inoculated AMF promoted nutrient absorption by maize in mining areas under drought stress, and using AMF reclamation could increase agricultural production and improve ecological restoration in coal mining regions of Shendong

[67]. Oyewole et al. (2017) found that combinations of AM fungi and biochar are effective in water conservation in sustainable production of sunflowers cultivated in semi-arid environments [68]. Because our results showed that AMF improves N uptake even at high N inputs, they could be applied to numerous scenarios toward maintaining high crop production while mitigating effects of excess N on the environment. Certainly, altering the rhizosphere microbiome, as seen here in increasing relative abundance of *Sphingomonadales* and *Rhizobiales*, highlights its potential for restoration of N-enriched agroecosystems.

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**Author Contribution** X. Z designed this study. Y. Z. and M. T conducted the experiments, and T. H. and X. Z. wrote the first draft. T. H. improved the figures and tables. T. H., J. D., S. Y., M. T., C. Z., F. S G., Q. Y, and C. L edited the draft and provided editorial advice. All authors contributed to the writing.

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**Data Availability** The data underlying this article are available in the article and in its online Supplementary Material.

## Declarations

**Consent to Participate** Informed consent was obtained from all individual participants included in this study.

**Competing Interests** The authors declare no competing interests.

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