

Distinct Denitrifying Phenotypes of Redominant Bacteria Modulate Nitrous Oxide Metabolism in Two Typical Grassland Soils

Qiaoyu Wu¹ · Mengmeng Ji¹ · Siyu Yu¹ · Jili¹ · Xiangang Wu¹ · Xiaotang Ji² · Birbin Liu³ · Xiaojun Zhang¹ 

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Abstract

Denitrification is a major pathway for N₂O release from soil. However, the denitrifying phenotypes of redominant bacteria in soil and their effects on N₂O metabolism are still unclear. In this study, we investigated the denitrifying phenotypes of redominant bacteria in two typical grassland soils (BBF and PFP) using a combination of metagenomic and metatranscriptomic approaches. The results showed that the denitrifying phenotypes of redominant bacteria in BBF and PFP soils were distinct. The redominant bacteria in BBF soil were *Rhodanobacter* and *Castellanella*, while the redominant bacteria in PFP soil were *Rhodanobacter* and *Castellanella*. The denitrifying phenotypes of redominant bacteria in BBF soil were characterized by the presence of *niS*, *niK*, and *nosZ* genes, while the redominant bacteria in PFP soil were characterized by the presence of *niS*, *niK*, and *nosZ* genes. The results suggest that the denitrifying phenotypes of redominant bacteria in soil play a key role in modulating N₂O metabolism.

Keywords

Introduction

The denitrification process is a major pathway for N₂O release from soil. The denitrifying phenotypes of redominant bacteria in soil and their effects on N₂O metabolism are still unclear. In this study, we investigated the denitrifying phenotypes of redominant bacteria in two typical grassland soils (BBF and PFP) using a combination of metagenomic and metatranscriptomic approaches.

The results showed that the denitrifying phenotypes of redominant bacteria in BBF and PFP soils were distinct. The redominant bacteria in BBF soil were *Rhodanobacter* and *Castellanella*, while the redominant bacteria in PFP soil were *Rhodanobacter* and *Castellanella*. The denitrifying phenotypes of redominant bacteria in BBF soil were characterized by the presence of *niS*, *niK*, and *nosZ* genes, while the redominant bacteria in PFP soil were characterized by the presence of *niS*, *niK*, and *nosZ* genes. The results suggest that the denitrifying phenotypes of redominant bacteria in soil play a key role in modulating N₂O metabolism.

...
N₂O ... A ...
...
... T ...
... N₂O ...
niS ... **niK** ... **niG** ...
... N₂O ...
... H ...

Gas Kinetics During Anaerobic Incubation

The N_2O concentration (nmol g⁻¹ soil dry weight) was measured during anaerobic incubation (0–96 h) for CK, N250, N250+G, BF, and FF treatments. The concentration of N_2O increased over time in all treatments, with BF and FF showing the highest concentrations (Fig. 1a). The concentration of N_2O was significantly higher in BF and FF compared to CK, N250, and N250+G (ANOVA, $P < 0.05$). The concentration of N_2O was also significantly higher in BF and FF compared to CK (SEM-DF, $P < 0.05$). The concentration of N_2O was significantly higher in BF and FF compared to N250 (ANOVA, $P < 0.05$). The concentration of N_2O was significantly higher in BF and FF compared to N250+G (ANOVA, $P < 0.05$). The concentration of N_2O was significantly higher in BF and FF compared to CK (SEM-DF, $P < 0.05$). The concentration of N_2O was significantly higher in BF and FF compared to N250 (ANOVA, $P < 0.05$). The concentration of N_2O was significantly higher in BF and FF compared to N250+G (ANOVA, $P < 0.05$).

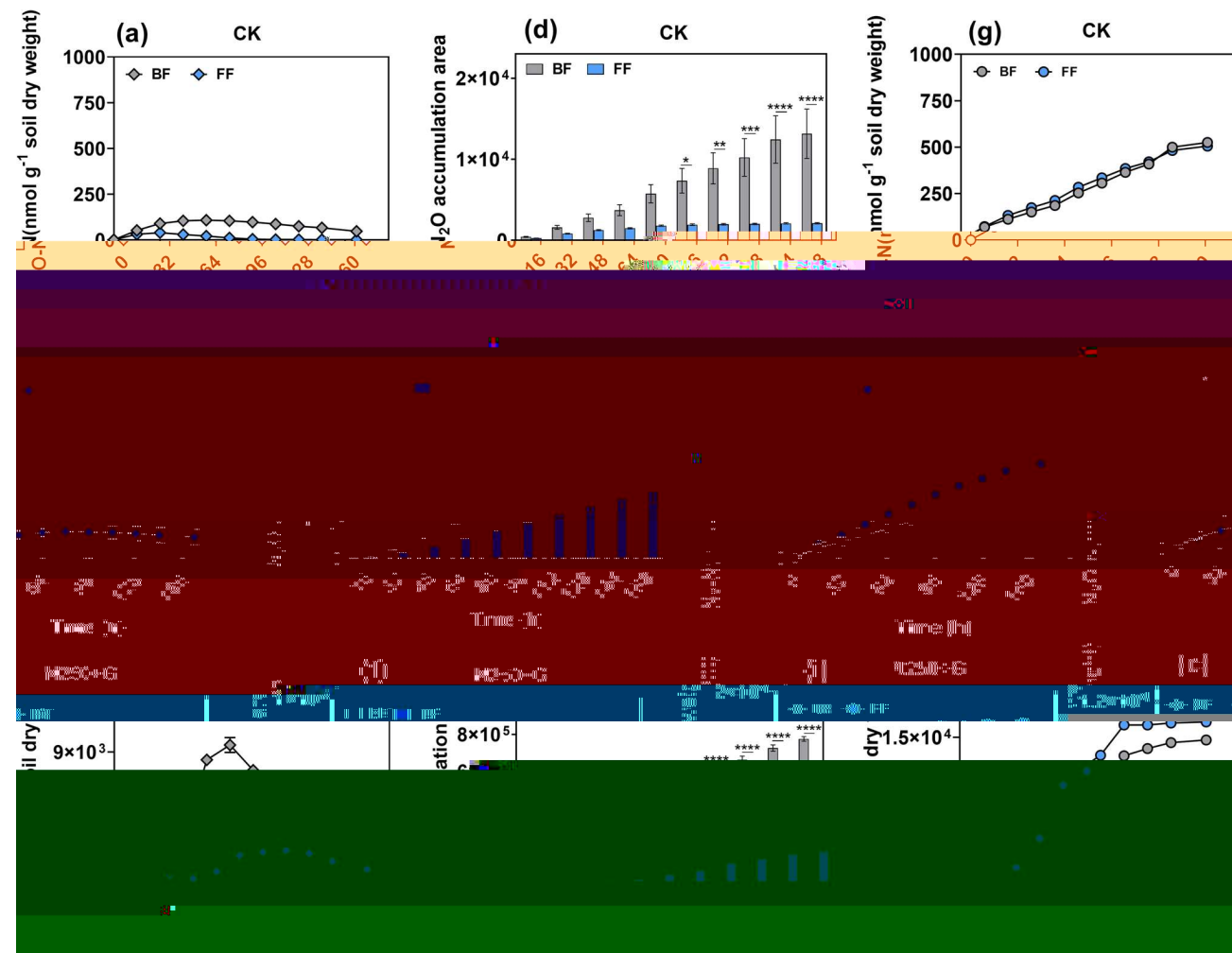


Fig 1 N_2O concentration (nmol g⁻¹ soil dry weight) and H_2O accumulation area (a–d) and N_2O concentration (nmol g⁻¹ soil dry weight) (e–g) during anaerobic incubation (0–96 h) for CK, N250, N250+G, BF, and FF treatments. The concentration of N_2O was significantly higher in BF and FF compared to CK, N250, and N250+G (ANOVA, $P < 0.05$). The concentration of N_2O was significantly higher in BF and FF compared to CK (SEM-DF, $P < 0.05$). The concentration of N_2O was significantly higher in BF and FF compared to N250 (ANOVA, $P < 0.05$). The concentration of N_2O was significantly higher in BF and FF compared to N250+G (ANOVA, $P < 0.05$).

Variations in Microbial Community Structures

(F = 1.513).

The effect of N₂O flux on soil microbial community structure was also examined. The effect of N₂O flux on soil microbial community structure was also examined. The effect of N₂O flux on soil microbial community structure was also examined.

Quantity of Denitrifying and 16S rRNA Genes

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Fig 2 Quantification of denitrifying and 16S rRNA genes in soil. (a) *narG*, (b) *niK*, (c) *nosZ*, (d) *narGnosZ*, (e) *niKnosZ*, (f) *niSnosZ*. Error bars represent SEM. ANOVA: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

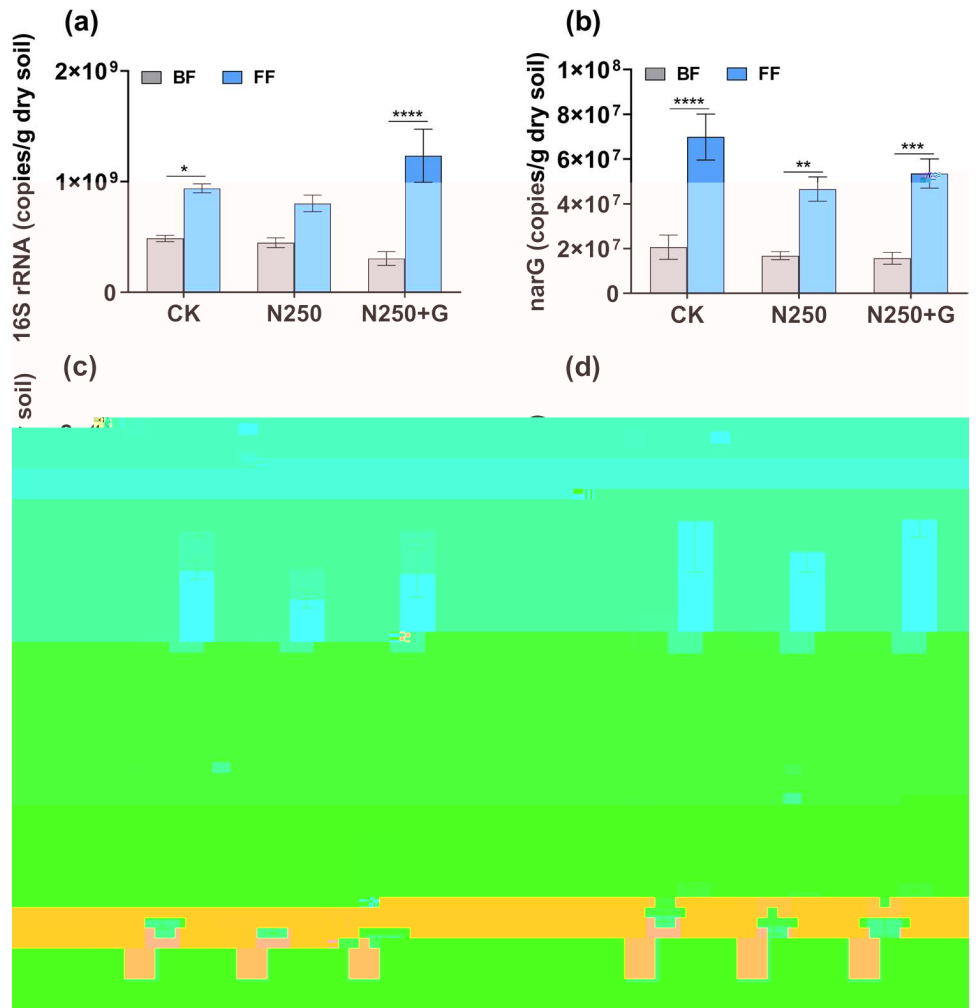
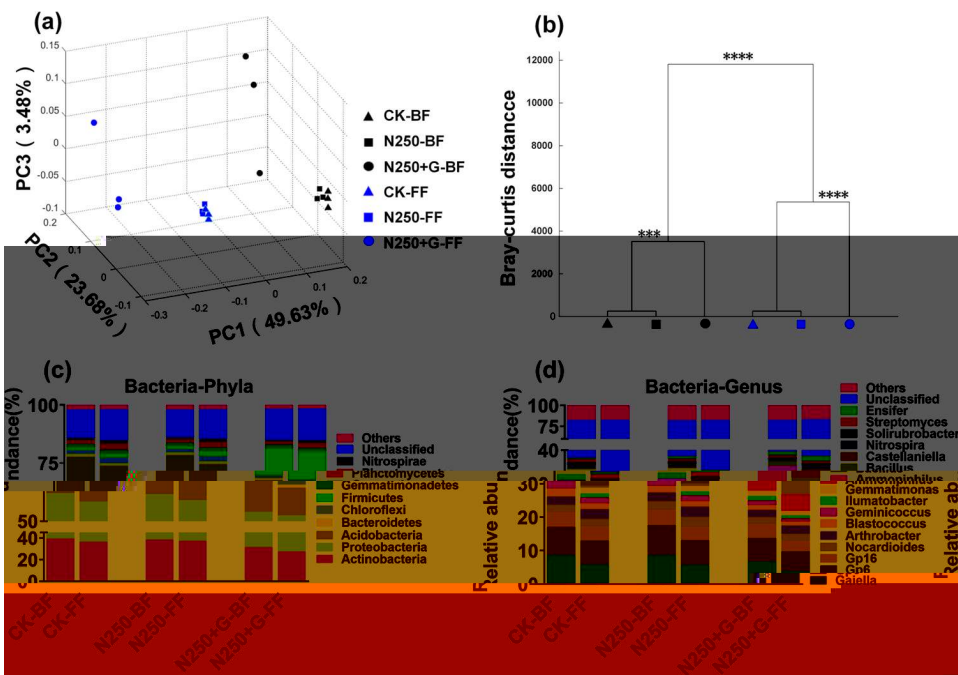


Fig 3

MANOVA (p < 0.0001).
p < 0.0001
p < 0.0001
p < 0.0001
p < 0.0001



MANOVA (p < 0.0001).
p < 0.0001
p < 0.0001
p < 0.0001
p < 0.0001

Gaiella Gp6 Gp16 Nocardioides
Arthrobacter Blastococcus Gemnicoccus Ilumatobacter
Gemmatimonas Ammoriphilus Bacillus Castellariella
Nitrospira Sclerotobacter Streptomyces Ensifer

Ammoriphilus
riella Bacillus

Prediction of Functional Denitrifiers

OTU 6536
norB nosZ
norB

nosZ

norB nosZ

norB nosZ

MANOVA (p < 0.0001).
p < 0.0001
p < 0.0001

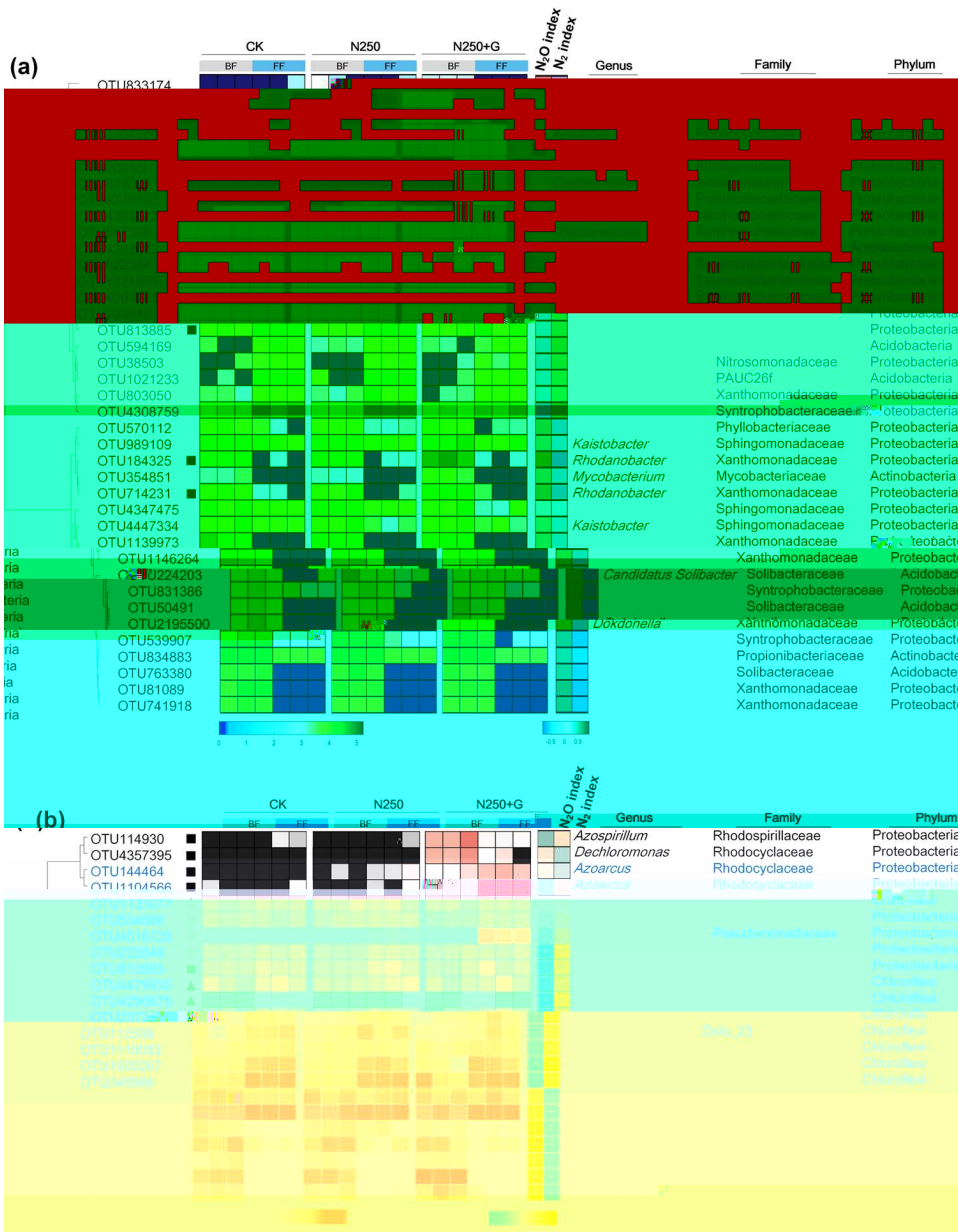
norB-Containing Bacteria in Two Types of Soils

norB
norB
nosZ
Candidatus Solibacter Dkd
donella Kaistobacter Lysobacter Phyllobacterium
Themononas Castellariella
norB nosZ
Azospinillum Dethiobacter Devosia
Rhodrobacter
Azospinillum
Kaisto
bacter Rhodrobacter

Denitrification Functions of Representative Bacterial Isolates

Fig 4

nosZ a
nosZ b
nosB
nosZ
nosZ *nosB*
nosZ
nosB
nosZ
R



nosZ-Containing Bacteria in Two Types of Soils

nosZ
nosZ
nosZ *nosB*
nosZ *nosB*
Rhodanbacter
Hyphonicidium *Chlorflexi*
Rhodanbacter

Denitrification Functions of Representative Bacterial Isolates

Rhodanbacter
Rhodanbacter
Rhodanbacter

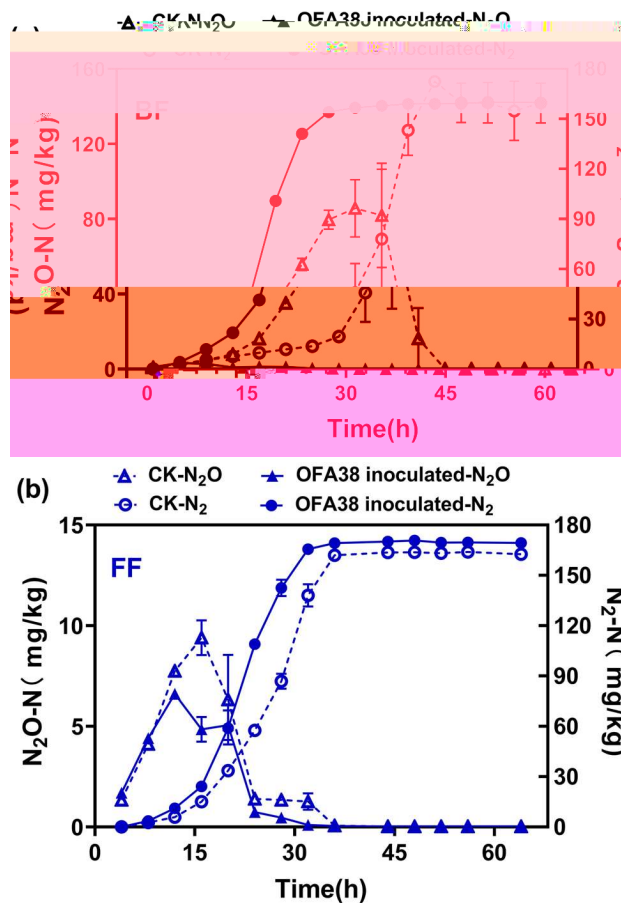
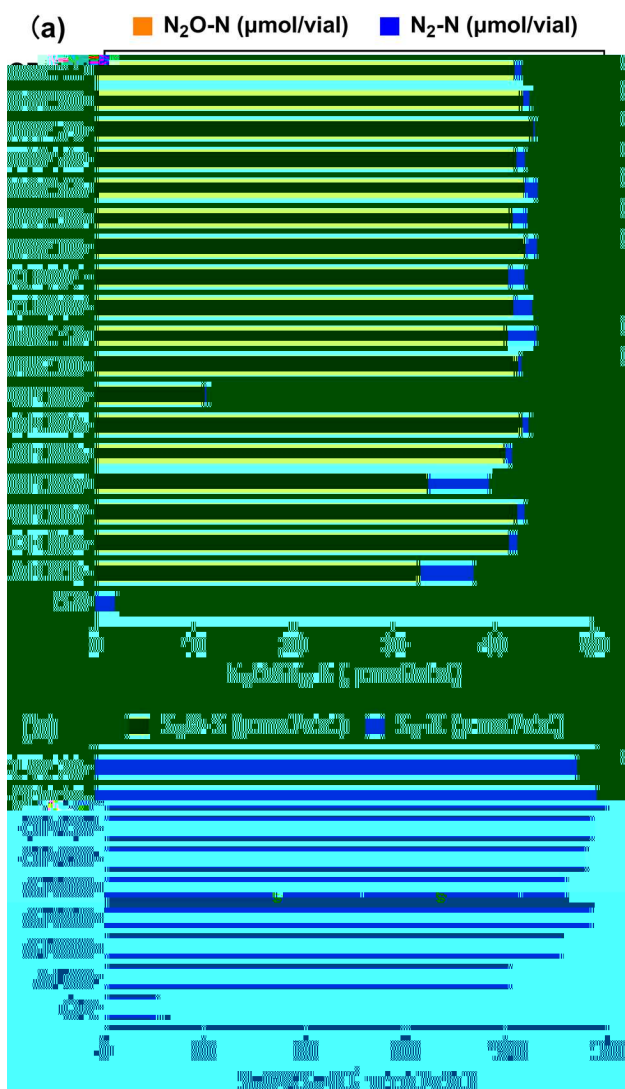
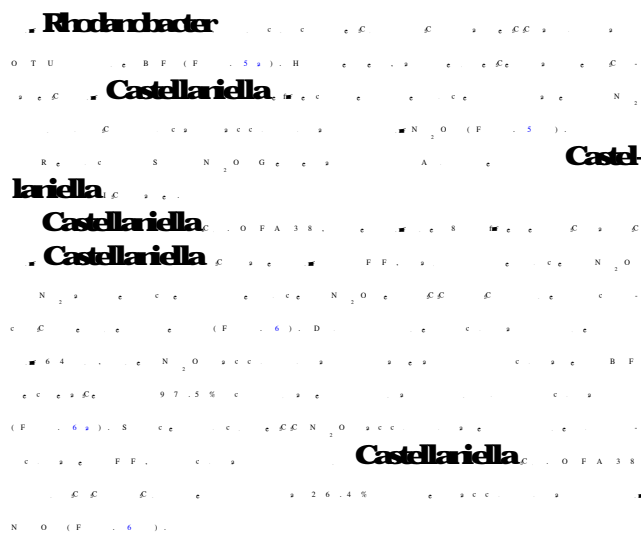
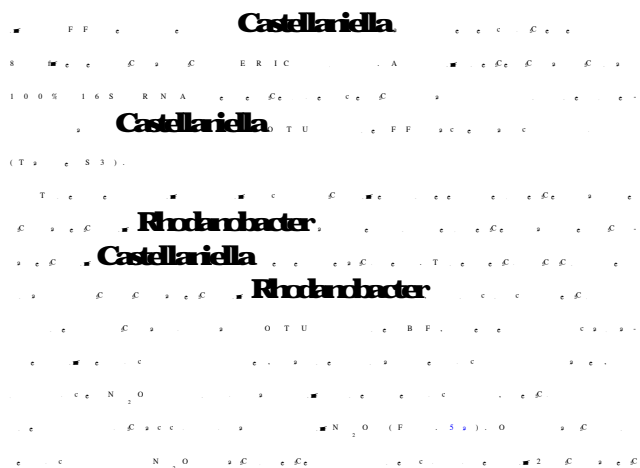


Fig 5 Phylogenetic tree of *Castellariella* and *Rhodanobacter* species. The tree shows the evolutionary relationships between the two genera, with *Castellariella* as the outgroup and *Rhodanobacter* as the ingroup. Bootstrap values are provided for the nodes.

Fig 6 Time-course of N₂O-N and N₂-N concentrations in soil for CK and OFA38 inoculated treatments. The graph shows the concentration of N₂O-N (mg/kg) and N₂-N (mg/kg) over time (h) for CK and OFA38 inoculated treatments. Error bars represent standard deviation.

Discussion

Effect of Nitrate and Glucose on Bacterial Communities

The effect of nitrate and glucose on bacterial communities was studied in a laboratory experiment. The results showed that the addition of nitrate and glucose significantly altered the composition and diversity of the bacterial community. The most prominent changes were observed in the phyla *Amoriphilus*, *Bacillus*, and *Castellaniella*. The presence of nitrate and glucose led to a decrease in the relative abundance of *Amoriphilus* and *Castellaniella*, while the relative abundance of *Bacillus* increased. These changes are likely due to the fact that *Bacillus* is a well-known nitrifier and is capable of utilizing nitrate as a nitrogen source. In contrast, *Amoriphilus* and *Castellaniella* are known to be involved in the denitrification process, which converts nitrate back into atmospheric nitrogen. The addition of glucose, which is a readily available carbon source, may have favored the growth of *Bacillus* over the other two genera. The overall effect of nitrate and glucose on the bacterial community was to shift the balance of the system towards nitrification and away from denitrification.

Effect of Soil Nitrogen and Carbon on N_2O Accumulation

The effect of soil nitrogen and carbon on N_2O accumulation was studied in a laboratory experiment. The results showed that the addition of soil nitrogen and carbon significantly increased the accumulation of N_2O in the soil. The most significant increases were observed in the treatments with the highest levels of soil nitrogen and carbon. These increases are likely due to the fact that soil nitrogen and carbon are essential for the growth and activity of soil microorganisms, which are the primary producers of N_2O . The addition of soil nitrogen and carbon also led to an increase in the relative abundance of *Bacillus*, which is a well-known nitrifier and is capable of producing N_2O as a byproduct of its metabolic activity. The overall effect of soil nitrogen and carbon on N_2O accumulation was to increase the amount of N_2O produced and retained in the soil. This increase in N_2O accumulation is a concern because N_2O is a potent greenhouse gas and contributes to global warming. The results of this study suggest that the addition of soil nitrogen and carbon to agricultural systems may lead to an increase in N_2O emissions, which could have negative consequences for the environment.

Declarations

Conflict of Interest

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