Taxonomic and denitrifying bacteria communities associated with the interception of nitrate leaching by carbon amendment in the subsoil

CURRENT STATUS: POSTED

Chen Chen
China Agricultural University

Hui Han
China Agricultural University

Ya Meng
China Agricultural University

Hangyu Wu
China Agricultural University

Rui Jia
China Agricultural University

Ting Xu
China Agricultural University

guo-chun Ding
China agriculture university

gc_ding@cau.edu.cn *Corresponding Author*
ORCID: <https://orcid.org/0000-0002-5468-5979>

Ji Li
China Agricultural University

DOI:

10.21203/rs.2.20232/v1

SUBJECT AREAS

General Microbiology

KEYWORDS

Carbon amendment, subsoil zone, nitrate leaching, denitrifying bacteria, bacterial diversity

Abstract

Nitrate leaching is severe in greenhouse agriculture where excessive nitrogen is often applied to maintain high crop productivities. Carbon amendment in the subsoil, where denitrification is limited by the availability of carbon, might mitigate nitrate leaching. In this study, we investigated the effects of carbon amendment in the subsoil on nitrate leaching and the emission of greenhouse gases (CH₄ and N₂O) emissions using a soil column experiment. Diversity and abundance of total and nirS-, nirK-, and nosZ-type denitrifying bacteria were investigated by high throughput sequencing of PCR amplicons and quantitative real-time PCR. The amounts of nitrate leaching were >39% less in the treatments with carbon amendment than in the non-amended control without fertilization or in treatments fertilized by two doses (1600 or 3200 kg N ha⁻¹) of ammonia or nitrate. No effects of carbon amendment on the emissions of CH₄ or N₂O were observed. The total N content in the subsoil zone with carbon amendment increased from 20.74% to 70.54%. Strikingly, the abundance of nirS, nosZ and 16S rRNA was higher in the treatment than the corresponding controls while no significant effects were detected for nirK. Carbon amendment rather than fertilization was the primary factor that influenced the community composition of the three denitrifying bacterial communities and explained 14%, 10%, and 4% of the variation in the community of nosZ, nirS, and nirK, respectively. Decreased alpha-diversity and increased variability in beta-diversity were observed for the carbon amended treatment for total and denitrifying bacteria. On average, genera such as Anaerovorax, Pseudobacteroides, Magnetospirillum, Prolixibacter, Sporobacter, Ignavibacterium, Syntrophobacter, Oxobacter, Hydrogenispora, Desulfosporomusa, Mangrovibacterium, and Sporomusa were enriched more than seven times in soil amended with carbon. In summary, carbon amendment in the subsoil mitigated nitrate leaching and increased the nitrogen pool by possible activation of denitrifying and anaerobic bacterial populations.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

Figure 1

Schematic diagram of the experimental design of this study.

Figure 2

The effects of carbon amendment on nitrate leaching accumulation (a), soil total nitrogen in the subsoil zone (b), soil CH₄ concentration(c), and soil N₂O concentration (d) (n=3).

Significant difference ($p < 0.05$, t-test) between carbon amendment and non-amended control was indicated by different letters. Carbon: carbon amendment; CK: non-amendment.

Non-fertilizer: no fertilizer; NH₄⁺Low: with ammonia fertilization at 1600 kg N ha⁻¹; NH₄⁺High: with ammonia fertilization at 3200 kg N ha⁻¹; NO₃⁻Low: with nitrate fertilization at 1600 kg N ha⁻¹; NO₃⁻High: with nitrate fertilization at 3200 kg N ha⁻¹.

Figure 3

Log10 transformed gene copy numbers of 16S rRNA (a), nirK (b), nirS (c), and nosZ (d) (n=3). Significant difference (p<0.05, t-test) between carbon amendment and non-amended control was indicated by different letters. Carbon: carbon amendment; CK: non-amendment.

Non-fertilizer: no fertilizer; NH₄⁺Low: with ammonia fertilization at 1600 kg N ha⁻¹; NH₄⁺High: with ammonia fertilization at 3200 kg N ha⁻¹; NO₃⁻Low: with nitrate fertilization at 1600 kg N ha⁻¹; NO₃⁻High: with nitrate fertilization at 3200 kg N ha⁻¹.

Figure 4

Effect of carbon amendment on alpha-and beta-diversity of nirS-, nirK- and nosZ-type denitrifying bacterial communities in the subsoil zone soils. (a) The amount of variation in denitrifying communities explained by carbon amendment. (b) The heterogeneity in community composition. (c) Pielous evenness indices. Forward: the forward dataset; Reverse: the reverse dataset.

Figure 5

Heatmap analysis of the most abundant five nirS OTUs in each sample. Red cross (+) indicate the phylotype with significantly higher relative abundance in the treatment with the

carbon amendment soil. Relative abundance in permille was indicated by color. ND: not detected. The percentage behind OTU indicated the similarity between the representative sequence of the OTU to the reference sequences. Accession number was in the bracket.

Carbon: carbon amendment; CK: non-amendment. Non-fertilizer: no fertilizer; NH₄+Low: with ammonia fertilization at 1600 kg N ha⁻¹; NH₄+High: with ammonia fertilization at 3200 kg N ha⁻¹; NO₃-Low: with nitrate fertilization at 1600 kg N ha⁻¹; NO₃-High: with nitrate fertilization at 3200 kg N ha⁻¹.

Figure 6

Effect of carbon amendment on bacterial diversity in the subsoil. (a) Relative abundance of dominant phylum. (b) UPGMA cluster analysis. (c) The heterogeneity in community composition. (d) Heatmap analysis of enriched genera by carbon-amended and the magnitude of enrichment (e) Shannon, Pielou's evenness. Carbon: carbon amendment; CK: non-amendment. Non-fertilizer: no fertilizer; NH₄+Low: with ammonia fertilization at 1600 kg N ha⁻¹; NH₄+High: with ammonia fertilization at 3200 kg N ha⁻¹; NO₃-Low: with nitrate fertilization at 1600 kg N ha⁻¹; NO₃-High: with nitrate fertilization at 3200 kg N ha⁻¹.

Figure 7

A mechanical model of carbon amendment on the mitigation of nitrate leaching in the subsoil zone.

This is a list of supplementary files associated with this preprint. Click to download.

[FigS3.tif](#)

[FigS1.tif](#)

[FigS4.tif](#)

[Table S1.docx](#)

[Table S2.docx](#)

[Table S3-6.docx](#)

[FigS2.tiff](#)