Supplementary Information

Sample site

The soil used for the experiments was collected from the Luancheng Agro-Ecosystem Experimental Station (37°90′ N, 114°70′ E) in the North China Plain, a typical grain production region characterized by high nitrogen fertilizer application (400 kg N ha^{-1} yr^{-1}), significant nitrogen loss, and high N_2O emissions. The cropping system follows a summer maize-winter wheat rotation. Detailed descriptions of the soil, climate, and management practices were provided in previously published study [1]. Fresh soil samples (0-20 cm depth) were collected, passed through a 2-mm sieve, and used immediately.

Reference

[1] Wang Y, Hu C, Ming H, Zhang Y, Li X, et al. 2013. Concentration profiles of CH_4, CO_2 and N_2O in soils of a wheat–maize rotation ecosystem in North China Plain, measured weekly over a whole year. *Agriculture, Ecosystems Environment* 164:260-272 https://doi.org/10.1016/j.agee.2012.10.004

Metagenome and Virome Analysis

Soil DNA was extracted from 0.5 g soil samples using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), following the instructions of manufacturer strictly. These DNA samples were then sent to Magigene Company for metagenomic sequencing and analysis. Metagenomic sequencing was performed on the Illumina NovaSeq 6000 platform with 2×150 bp paired-end reads. Quality-controlled reads were assembled using MEGAHIT v1.2.9 with default parameters.

Viral sequences were identified and classified from the assembled contigs using CheckV and Virsorter2, retaining only DNA viruses for further analysis. Clean reads were mapped to the viral contigs using BWA to determine viral abundance, and Prokka was employed for gene prediction on the viral contigs, excluding short genes to ensure accurate annotations. Viral contigs were further analyzed to assess their potential impact on nitrogen cycling by examining the abundance and distribution of key viral taxa. This streamlined virome analysis facilitates the accurate identification and quantification of viral populations, elucidating their roles in regulating denitrifying microbial communities and influencing greenhouse gas emissions in soil ecosystems.