Supporting Materials

Extraction and purification of total genomic DNA

The OMEG Soil Genome Extraction Kit (D5625-01Soil DNA Kit) was used for genomic DNA extraction and purification. (1) Weigh 0.15–0.25 g of pre-treated samples into a 10 mL centrifuge tube, add 500 mg of glass beads, then add 1 mL of Buffur SLX Mlus, and mix with vigorous shaking on a vortex shaker for 3–5 min. (2) Add 100 μL Buffer DS and mix well on a vortex shaker. (3) Incubate the sample at 70 ℃ for 10 min, during which the sample is gently shaken once to make the mixture homogeneous. (4) Centrifuge at 3000 rpm for 3 min at room temperature, transfer 800 μL of supernatant into a new 1.5 ml tube, add 270 μL of Buffer SP2, and vortex to mix well. (5) Ice-bath for 5 min and then put into a micro benchtop centrifuge 13000 g centrifugation at 4 ℃ for 3 min. (6) Transfer the supernatant into a new sterilised 1.5 mL centrifuge tube, add 0.7 times the volume of isopropanol, and mix via inversion 20–30 times to obtain a well-mixed solution. Precipitate the DNA for about 1 h at -20°C. (7) Centrifuge the DNA at 13000 g at 4°C for 10 min for precipitation to occur. (8) Carefully remove the supernatant and invert the centrifuge tube to dry on absorbent paper for 1 min. (9) Add 200 μL of the Elution Buffer and vortex for 10 s. Incubate at 65 ℃ for 15 min to fully dissolve the DNA. (10) Add 75 μL of the HTR Reagent and vortex well for 10 s. (11) Allow it to stand at room temperature for 2 minutes, and centrifuge at 13000g for 2 min at 4 ℃. (12) Transfer the supernatant into a new sterilised 1.5 mL centrifuge tube and repeat steps 10 to 12 once to ensure that the humus and other impurities in the sample can be completely removed so as not to affect the subsequent reaction. (13) Add an

equal volume of XP2 Buffer and vortex well to mix. (14) Add the mixed solution obtained in the previous step into the HiBind adsorption column (the adsorption column was put into a 2 mL centrifugal collection tube), centrifuge at 10000 g for 1 min at room temperature, and pour out the waste liquid in the collection tube. (15) Add 300 μL XP2 Buffer, centrifuge at 12000 rpm for 30 seconds, centrifuge at 10000 g for 1 min at room temperature, and pour out the waste liquid in the collection tube. (16) Remove the adsorption column and put it into a new 2 mL centrifugal collection tube. Add 400 μL of a rinse solution SP2 Wash Buffer, centrifuge at 10000 rpm for 1 min, and discard the waste solution. (17) Repeat step 16 twice. (18) Place the adsorption column back into the empty collection tube and centrifuge at 13,000 for 3 min to remove as much of the rinse solution as possible to prevent the inhibition of downstream reactions by residual ethanol in the rinse solution. (19) Put the adsorption column into a newly sterilised 1.5 mL centrifuge tube, add 60 μL of the Elution Buffer, and put it into a constant temperature water bath at 65°C for 12 min to incubate, so that the DNA on the adsorption column can be fully dissolved. (20) Centrifuge at 13000 for 1 min to elute DNA. (21) Repeat steps 19–20 to elute the DNA on the adsorption column again, and store the extracted genomic DNA in a refrigerator at -20 ℃. The extracted genomic DNA should be stored in the refrigerator at -20 ℃ and detected via 1% agarose gel electrophoresis.

Polymerase chain reaction (PCR)

Nine target fragments of the total bacteria, archaea and seven functional genes of the denitrification functional group were quantitatively analysed. These included the 16S

rDNA fragments of the total bacteria, 16S rDNA fragments of archaea, and fragments of the archaeal ammonia monooxygenase gene *AOA-amoA*, bacterial *AOB-amoA*, nitrite oxidoreductase nor, and the membrane-bound nitrate reductase gene. The fragments of bacterial *AOB-amoA*, nitrite oxidoreductase nor, membrane-bound nitrate reductase, *narG*, Cu-type nitrite reductase, *nirK*, cytochrome cd1-containing nitrite reductase, *nirS*, and nitrous oxide reductase, *nosZ*, were subjected to PCR (polymerase chain reaction). A 25 μL amplification system was used: 2.5 μL of 10×buffer (containing Mg^{2+}), 2 μL of dNTP at 2.5 mmol L-1, 1 μL of the sample DNA solution, 0.25 μL of Taq enzyme at 2.5 U/μL, 10 mmol L-1 of forward and reverse primers (primer sequences are shown in Table 2.4), and the addition of sterile double-distilled water (ddH_2O) to a total volume of 25 μL. The reaction procedure is shown in Table S1.

Fluorescence quantitative PCR

The ABI PRISM 7300 system was used to quantify the functional bacteria for denitrification, as well as the functional genes of the samples. The total volume of the amplification reaction was 20 μL, containing 10 μL of SYBR Green I mix (Applied Biosystems) and 1 μL of the DNA template (samples or cloning products used as standard curves), the amount of each addition of the 10 μmol/L primer is shown in Table 2.4 (converted to a 20 μL system), and the reaction procedure is shown in Table 2.4. After the reaction, the temperature in the reaction tube slowly increased from 60℃ to 95℃, the double chain of the amplification product was gradually denatured and unchained to a single chain with the increase in temperature, SYBR GreenI was released, and the fluorescence signal was decayed, and the instrument detected the change in the

fluorescence signal in the reaction tube point by point and then plotted the melting curve of the fluorescence signal of the amplification product with the change in temperature (Dissociation curve). The fragment genes have different lengths, GC contents and base compositions, as well as Tm values, and the shapes and peaks on the melting curve are also different. Accordingly, the specificity of amplification can be analysed by the melting curve. The amplification products were stored at -20℃. Sterile water was used as the negative control.

High-throughput sequencing

PCR amplification products were purified and then subjected to high-throughput sequencing using the Illumina MiSeq PE300 platform. This was performed by Shanghai Meiji Biomedical Technology Co. For the raw downstream double-end sequencing data obtained from amplicon sequencing, FastQC software was used to assess the data quality, and Trimmomatic software was used to perform quality control on the raw data, filtering the low-quality bases at both ends of the READ. After passing the quality control, the data were spliced into a single sequence based on the overlap relationship between PE reads using the FLASH splicing software, and a valid sequence was obtained. Based on the 97% sequence similarity level, OUT (operational taxonomic units) clustering was performed using UPARSE software (version 7.1 http://drive5.com/uparse/), and single sequences and chimeras without duplicates were removed during the clustering process. Species classification was annotated for each sequence using the RDP classifier (http://rdp.cme.msu.edu/), and the OUT abundance table was obtained by comparing to the Silva database (SSU132) and setting the

comparison threshold at 70%. Based on the results of OTU clustering analysis, multiple diversity indices were analysed for OTUs, as well as the detection of the sequencing depth; based on the taxonomic information, statistical analyses of community structure were performed at each taxonomic level.

Table S1. Primers used in the PCR experiments.

Functional gene	Primer name	Primer Sequence (5'–3')
Bacterial 16S rRNA	338F	ACTCCTACGGGAGGCAGCAG
	806R	GGACTACHVGGGTWTCTAAT
nirS	cd3AF	GTSAACGTSAAGGARACSGG
	R3cd	GASTTCGGRTGSGTCTTGA
nirK	nirK1F	GCCTCGATCAGRTTRTGG
	nirK5R	TCGCCSATYCCGGCSATGTC
nosZ	NosZ2F	CGCRACGGCAASAAGGTSMSSGT
	NosZ2R	CAKRTGCAKSGCRTGGCAGAA
narG	narG-f	TCGCCSATYCCGGCSATGTC
	narG-R	GAGTTGTACCAGTCRGCSGAYTCSG
nxrA	F1norA	CAGACCGACGTGTGCGAAAG
	R1norA	TCYACAAGGAACGGAAGGTC
AOA	Arch-amoAF	STAATGGTCTGGCTTAGACG
	Arch-amoAR	GCGGCCATCCATCTGTATGT
AOB	bamoA-1F	GGGGTTTCTACTGGTGGT
	bamoA-2R	CCCCTCKGSAAAGCCTTCTTC