

## Supplementary File 1: Supplementary methods to this study.

### Method 1.1 Sediment physicochemical properties analysis

Soil water content was measured using the oven-drying method <sup>[1]</sup>. Mixing sediment with deionized water at a 1:2.5 ratio was used to measure salinity and pH with a YSI-30 portable salinity meter and pH meter <sup>[2]</sup>. The sulfide concentration was measured using Orion sure-flow® combined silver sulfide electrodes (Thermo Scientific Orion)<sup>[3]</sup>. Total extractable Fe was extracted from 0.5 g of fresh sediment with 30 mL of 0.5 M anoxic HCl for 1 hour. The total extracted Fe was then measured via a 1,10-phenanthroline and 1% (w/v) hydroxylamine hydrochloride method, followed by Fe(II) via a 1,10-phenanthroline assay. Fe(III) was calculated by the difference between the total Fe and Fe(II) <sup>[1,3]</sup>. Exchangeable NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup> were measured using a continuous-flow analyzer after 2 M KCl extraction <sup>[4]</sup>. The total organic carbon (TOC) and total carbon (TC) content in sediments were measured using an automatic TOC analyzer with a SSM-5000A detector (Shimadzu, Japan) <sup>[1]</sup>.

### Method 1.2 Measurement of potential denitrification rates

The concentrations of <sup>29</sup>N<sub>2</sub> and <sup>30</sup>N<sub>2</sub> produced during the incubation were determined by membrane inlet mass spectrometry (MIMS), and the PDNF rates were calculated from these concentrations <sup>[5]</sup>. The respective contributions of denitrification to <sup>29</sup>N<sub>2</sub> production were quantified as follows:

$$P_{29} = D_{29} + A_{29} \quad (1)$$

where  $P_{29}$  (nmol g<sup>-1</sup> h<sup>-1</sup>) denotes the total <sup>29</sup>N<sub>2</sub> production rates during the soil-slurry experiments, and  $D_{29}$  and  $A_{29}$  (nmol g<sup>-1</sup> h<sup>-1</sup>) are the production rates of <sup>29</sup>N<sub>2</sub> from denitrification process. The <sup>14</sup>N and <sup>15</sup>N produced from <sup>14</sup>NO<sub>3</sub><sup>-</sup> or <sup>15</sup>NO<sub>3</sub><sup>-</sup> follow random isotope pairing, and  $D_{29}$  can be expressed as follows:

$$D_t = D_{29} + 2 \times D_{30} \quad (2)$$

$$A_{29} = P_{29} - D_{29} \quad (3)$$

where  $D_t$  (nmol g<sup>-1</sup> h<sup>-1</sup>) denotes the potential rates of denitrification.

### Method 1.3 Measurement of N<sub>2</sub>O emission rates

The production rates of N<sub>2</sub>O during the incubation were calculated using the following equation <sup>[5]</sup>:

$$R = \frac{(N_f - N_i) \times V}{T} \quad (4)$$

where,  $R$  (nmol <sup>15</sup>N g<sup>-1</sup> h<sup>-1</sup>) denotes the N<sub>2</sub>O production rate,  $N_i$  and  $N_f$  (nmol mL<sup>-1</sup>) indicate the total contents of N<sub>2</sub>O dissolved in the initial and final slurry samples, respectively, which were calculated as described below.  $V$  (mL) is the volume of the incubation vials, and  $T$  (h) represents the incubation time.

The headspace equilibrium technique and gas chromatography (Shimadzu GC-14B, Shimadzu Co., Kyoto, Japan) with a detection limit of 0.001  $\mu\text{mol L}^{-1}$  were used to detect the dissolved  $\text{N}_2\text{O}$  concentration.  $N_f$  and  $N_i$  indicate the total amount of  $\text{N}_2\text{O}$  dissolved in slurry samples at the end and the beginning of incubation, respectively, which were calculated using the following equation [5,6]:

$$N_{i(f)} = \frac{N_{i(f) - \text{headspace}} \times R \times T \times 10^{-3}}{V(\text{headspace}) \times kb(\text{N}_2\text{O})} + \frac{N_{i(f) - \text{headspace}}}{V(\text{headspace})} \quad (5)$$

where,  $kb(\text{N}_2\text{O})$  ( $\text{Pa L mol}^{-1}$ ) is Henry coefficient;  $R$  is the gas constant  $8.314$  ( $\text{Pa mol}^{-1} \text{K}^{-1}$ );  $N_{i(f) - \text{headspace}}$  indicates the amount of  $\text{N}_2\text{O}$  in the headspace at the beginning ( $N_{i - \text{headspace}}$ ) and end ( $N_{f - \text{headspace}}$ ) of incubation (nmol);  $T$  is the incubation temperature (K);  $V$  represents the volume of water sample (L);  $V(\text{headspace})$  is the volume of headspace (L).

#### Method 1.4 16S rRNA sequence analyses

Sequence splicing was conducted using Mothur software ([https://www.mothur.org/wiki/Download\\_mothur](https://www.mothur.org/wiki/Download_mothur)), and QIIME (<http://qiime.org/install/index.html>) was used to analyze the 16S rRNA gene sequences further. After noise reduction, the complete sequences of the V3–V4 hypervariable region of the 16S rRNA gene were finally obtained. Sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity cutoff using UCLUST software (<http://www.drive5.com/uclust>). According to the UPARSE algorithm (<http://www.drive5.com/uparse>), the sequence with the highest frequency in each OTU was selected as the representative sequence, which was then annotated using the RDP database (<http://rdp.cme.msu.edu>). Based on a Bayesian algorithm, the RDP classifier was used to classify each representative OTU sequence at the phylum, class, order, family, and genus levels. Finally, the relative abundance of sequences classified at the different levels was calculated. Relative abundance refers to the ratio of the number of sequences annotated at different taxonomic levels to the total number of all sequences in each sample. Alpha diversity of the samples was analyzed based on OTUs.

## References

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