Submission to *Water*

Supplementary Materials

Effect of mangrove on nitrogen removal in the intertidal zone of Shenzhen's Deep Bay: From ^{15}N isotope tracing to microbial analysis

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The following are included as additional supplementary materials for this paper:

Section S1. Calculation methods of potential rates of denitrification, anammox, and DNRA

Both anammox and denitrification generated $^{29}N_2$; thus, the respective contributions of each process to the total $^{29}N_2$ production were quantified by Eq. (S1):

$$P_{29} = A_{29} + D_{29} \quad (S1)$$

where, P_{29}, A_{29}, and D_{29} (μmol/(kg·h)) represent the total $^{29}N_2$ production rate, the $^{29}N_2$ production rate from anammox, and the $^{29}N_2$ production rate from denitrification, respectively. Because the $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ generated from denitrification follow random isotope pairing, D_{29} can also be estimated by Eq. (S1):

$$D_{29} = 2 \times P_{30} \times (1 - F_n) \times F_n^{-1} \quad (S2)$$

where P_{30} (μmol/(kg·h)) is the total $^{30}N_2$ production rate, and F_n is the mole fraction of $^{15}N_2$ in the nitrate pool, which can be calculated by the measured concentrations of nitrate before and after the addition of $^{15}NO_3^-$. Consequently, the potential rates of anammox and denitrification were estimated by Eqs. (S3) and (S4):

$$D_{total} = D_{29} + 2 \times D_{30} \quad (S3)$$

$$A_{29} = P_{29} - D_{29} \quad (S4)$$

where D_{total} and A_{29} (μmol/(kg·h)) rate the potential rates of denitrification and anammox, respectively.

The potential rate of DNRA was calculated by Eq. (S5):

$$R_{DNRA} = ([^{15}NH_4^+]_{Final} - [^{15}NH_4^+]_{Initial}) \times V \times W^{-1} \times T^{-1} \quad (S5)$$

Where R_{DNRA} (μmol/(kg·h)) is the measured DNRA rate, $[^{15}NH_4^+]_{Final}$ and $[^{15}NH_4^+]_{Initial}$ (μmol N/L) are the measured concentrations of $^{15}NH_4^+$ in the final and initial samples of the soil slurries, respectively, V (L) is the volume of the vial, W (g) is the dry weight of the soil, and T (h) is the incubation time.

Section S2. Analysis of bacterial community by 454 high-throughput 16S rRNA gene pyrosequencing

Bacterial 16S rRNA genes for pyrosequencing were amplified by PCR using a 10-nucleotide barcoded forward primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 533R (5'-TTACCGCGGCTGCTGGCAC-3'), which targeted the V1–V3 region. The 20 μL PCR mixture contained 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.4 μL of each primer (5μM), 0.5 μL of DNA, and 0.4 μL FastPfu Polymerase (TransStart FastPfu DNA Polymerase, TransGen, China). The thermocycling steps were as follows: 95 °C for 2 min, followed by 25 °C cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 5 min. After purification using the UNIQ-10 PCR Purification Kit (Sangon, Shanghai, China) and quantification using a TBS-380 (Turner BioSystems, Inc., USA), a mixture of amplicons was used for pyrosequencing on a Roche massively parallel 454 GS-FLX Titanium sequencer (Roche 454 Life Sciences, Bran-ford, CT, USA) according to standard protocols. To minimize the effects of random sequencing errors, low-quality sequences were removed by eliminating those without an exact match to the forward primer, without a recognizable reverse primer, of length shorter than 150 nucleotides and containing any ambiguous base calls (Ns). The barcodes and primers were trimmed from the resulting sequences. Finally, a total of 39445 high-quality sequence tags were produced, with an average length of 469 bp per sequence. The operational taxonomic units (OTUs) were clustered with 97% similarity using the MOTHUR program. Rarefaction curves, species richness estimator (Chao1), Shannon diversity index, and Good's coverage were generated in MOTHUR for each sample.

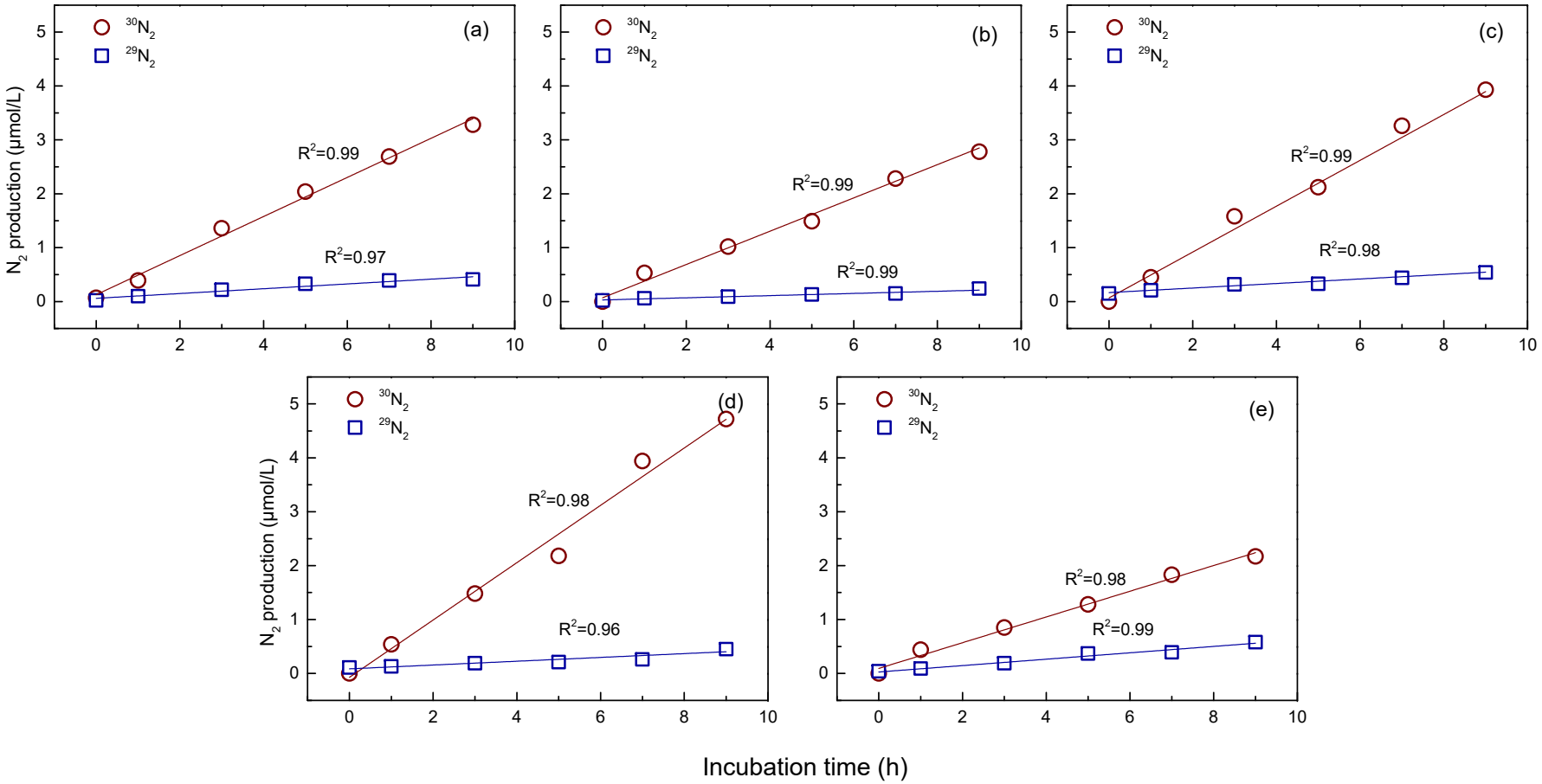

Figure S1 Accumulation of $^{29}N_2$ and $^{30}N_2$ in soil slurries spiked with 100μM $^{15}NO_3^-$ at Sites KC (a), BG (b), SA (c), AC(d), and MF (e). KC, BG, SA, AC, and MF are site abbreviations for *Kandelia candel*, *Bruguiear gymnorrhiza*, *Sonneratia apetala*, *Aegiceras corniculatum*, and mud flats, respectively.