

Supplementary materials for

Elucidating the links between N₂O dynamics and changes in microbial communities following saltwater intrusions

Rongrong Xie ^{1,2,3*#}, Laichang Lin^{1#}, Chengchun Shi⁴, Peng Zhang⁵, Peiyuan Rao¹, Jiabing Li ^{1,2}, Dandan Izabel-Shen^{6,7**}.

1. College of Environmental and Resource Science, Fujian Normal University, Fuzhou 350007, China

2. Key Laboratory of Pollution Control and Resource Recycling of Fujian Province, Fujian Normal University, Fuzhou 350007, China

3. *Leibniz Institute for Baltic Sea Research, Warnemünde, Rostock 18119, Germany*

4. Fujian Research Academy of Environmental Sciences, Fuzhou 350013, China

5. School of Environmental and Municipal Engineering, North China University of Water Resources and Electric Power, Zhengzhou 450046, China

6. *Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Oldenburg 26129, Germany*

7. Alfred-Wegener-Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven 27570, Germany

The authors contributed equally to this work.

*Corresponding author. Fujian Normal University, Wulongjiang middle Rd. 18, 350117, Fuzhou, China.

***Corresponding author: Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Ammerländer Heerstraße 231, 26129, Oldenburg, Germany.*

21	Contents
22	Supplementary Text
23	Supplementary Table S1
24	Supplementary Table S2
25	Supplementary Table S3
26	Supplementary Table S4
27	Supplementary Table S5
28	Supplementary Table S6
29	Supplementary Table S7
30	Supplementary Table S8
31	Supplementary Table S9
32	Supplementary Table S10
33	Supplementary Figure S1
34	Supplementary Figure S2
35	Supplementary Figure S3

Supplementary Text

Calculation of dissolved N₂O concentration and N₂O saturation

The concentration of dissolved N₂O was calculated based on the Bunsen solubility coefficient for N₂O as a function of temperature and salinity (Li et al. 2022; Weiss and Price 1980). as shown in Eq. (1):

$$C_w = [(C_a - C_0) \times V_a + C_a \times \beta \times R \times T \times V_w] / V_w \quad (1)$$

where C_w is the dissolved N₂O concentration in water (nmol L⁻¹); C_a is the measured N₂O concentration in the headspace gas (nmol L⁻¹); and C_0 is the N₂O concentration in the atmosphere. According to the latest “China Blue Book on Climate Change (2021),” the average N₂O concentration at Waliguan Global Atmospheric Background Station in Qinghai Province (C_0) is 0.33 ppm (CMA Climate Change Centre 2021), which is widely used in N₂O researches (Wan et al. 2021; Marushchak et al. 2021; Rabaey et al. 2022); V_a and V_w are the volumes (in L) of the headspace and water in the bottle, respectively; β is the Bunsen solubility of N₂O (mol L⁻¹ atm⁻¹); R is the ideal gas constant (8.314 m³ Pa mol⁻¹ K⁻¹); and T is the air temperature (in Kelvin). β was calculated according to Eq. (2) (Li et al. 2022; Weiss and Price 1980):

$$\ln \beta = A_1 + A_2(100/T) + A_3 \ln(T/100) + S[B_1 + B_2(T/100) + B_3(T/100)^2] \quad (2)$$

where A_1 , A_2 , A_3 , B_1 , B_2 , and B_3 are constants with values of -62.7062, 97.3066, 24.1406, -0.05842, 0.033193, and -0.0051313, respectively; T is the water temperature; and S is the salinity.

The release and accumulation of N₂O in the water were determined by calculating

57 the N₂O saturation (%) as shown in Eq. (3):

58
$$S_{N_2O} = C_w / C_{eq} \times 100 \quad (3)$$

59 where C_w (nmol L⁻¹) is the measured concentration of dissolved N₂O; C_{eq} (nmol L⁻¹)
60 is the expected concentration at which water is in equilibrium with the atmosphere at the
61 sampling water temperature. C_{eq} was calculated according to Eq. (4) (Weiss and Price
62 1980; Zhou et al. 2022):

63
$$C_{eq} = C_0 \times \exp((A_1 + A_2 \times (100/T) + A_3 \times \ln(T/100) + A_4 \times (T/100)^2)) \quad (4)$$

64 where the values of the constants A₁, A₂, A₃, and A₄ are -165.8806, 222.8743,
65 92.0792, and -1.48425, respectively; and T is the temperature in Kelvin.

66 **Table legends**

67 **Table S1** Hydrological characteristics recorded at each sampling time.

Date	Time	Flow ($\text{m}^3 \text{s}^{-1}$)	Depth (m)
2020.12.20	7:10	3673.23	1.78
	9:00	2250.00	0.92
	11:00	-1725.45	3.20
	13:00	-7127.86	4.38
	15:00	-4466.28	5.33
	17:00	3250.00	4.49
2021.4.17	7:00	3460.00	2.81
	9:00	1440.00	1.84
	10:15	-2314.70	1.83
	11:30	-6787.56	2.71
	12:45	-6281.91	3.86
	14:00	-2458.54	4.36
	15:15	3600.00	4.33
2021.8.29	6:40	4040.00	2.54
	8:40	3320.00	1.42
	10:50	-579.56	1.92
	12:50	-7229.03	4.10
	14:50	-5043.54	5.12
	16:50	2430.00	4.51

68 Note: a negative value indicates that the direction of water flow is from the ocean to the
69 river, and a positive value the opposite

Table S2 Sampling information during field trips and the type of sampling. For microbial community characterization and functional gene quantification, samples were taken only during saltwater intrusions.

Date	Group	Time	Parameters		
			Physical	N speciation	Microbe
2020.12.20	NW / EG	7:10	√	√	×
		9:00	√	√	×
		11:00	√	√	√
		13:00	√	√	√
		15:00	√	√	√
		17:00	√	√	√
2021.4.17	NW / EG	7:00	√	√	×
		9:00	√	√	×
		10:15	√	√	√
		11:30	√	√	√
		12:45	√	√	√
		14:00	√	√	√
		15:15	√	√	√
2021.8.29	NW / EG	6:40	√	√	×
		8:40	√	√	×
		10:50	√	√	√
		12:50	√	√	√
		14:50	√	√	√
		16:50	√	√	√

Note: “√” indicates that samples were taken and evaluated accordingly; “×” indicates that no samples were taken for the analysis.

75 **Table S3** Characterization of physiochemical parameters, N contents, and N₂O dynamics in the NW and EG samples during
76 December 2020 and April and August 2021. The values inside and outside the parentheses represent the average of biological
77 duplicates and the standard deviation, respectively.

Month	group	Time	Salinity	pH	Temperature (°C)	DO (mg L ⁻¹)	Turbidity (NTU)	NH ₄ ⁺ -N (mg L ⁻¹)	NO ₃ -N (mg L ⁻¹)	NO ₂ -N (mg L ⁻¹)	Dissolved N ₂ O (nmol L ⁻¹)	N ₂ O saturation
December	NW	7:10	10.70 (0.16)	8.30 (0.12)	13.00(0.78)	19.80 (0.57)	301.00 (4.25)	0.18 (0.04)	1.26(0.01)	0.0021 (0.0002)	33.28(1.30)	284.49% (4.89%)
			8.08 (0.03)	8.40 (0.18)	13.70(0.07)	20.60 (0.85)	531.00 (8.47)	0.31 (0.00)	1.32(0.02)	0.0030 (0.0005)	37.54(3.96)	328.88% (15.82%)
		11:00	6.25 (0.06)	8.40 (0.16)	13.40(0.14)	18.80 (0.64)	86.80 (2.69)	0.19 (0.01)	1.36(0.02)	0.0047 (0.000)	43.50(1.41)	377.13% (12.37%)
			10.70 (0.11)	8.10 (0.08)	13.40(0.07)	20.00 (0.34)	452.00 (3.71)	0.11 (0.01)	1.25(0.00)	0.0013 (0.000)	30.03(2.52)	260.32% (10.33%)
		15:00	14.70 (0.09)	7.90 (0.12)	12.60(0.07)	15.80 (0.16)	256.00 (4.27)	0.03 (0.01)	1.07(0.01)	0.0013 (0.000)	24.63(2.63)	207.58% (10.07%)
			14.20 (0.11)	8.00 (0.18)	12.30(0.14)	20.90 (0.50)	85.50 (1.41)	0.07 (0.05)	1.04(0.01)	0.0041 (0.002)	28.03(0.11)	233.74% (0.43%)
	EG	7:10	8.00 (0.07)	8.00 (0.16)	12.80(0.25)	19.70 (0.49)	40.20 (1.71)	0.24 (0.02)	1.28(0.05)	0.0163 (0.003)	19.14(2.98)	163.62% (11.23%)
		9:00	8.77 (0.04)	8.10 (0.08)	13.30(0.17)	20.00 (0.67)	42.30 (2.23)	0.36 (0.07)	1.21(0.10)	0.0041 (0.000)	23.72(1.82)	207.84% (23.27%)
			8.13 (0.05)	8.20 (0.13)	13.70(0.07)	19.40 (0.33)	36.27 (1.67)	0.43 (0.04)	1.38(0.05)	0.0069 (0.005)	32.26(1.81)	279.70% (7.21%)

Month	group	Time	Salinity	pH	Temperature (°C)	DO (mg L ⁻¹)	Turbidity (NTU)	NH ₄ ⁺ -N (mg L ⁻¹)	NO ₃ ⁻ -N (mg L ⁻¹)	NO ₂ ⁻ -N (mg L ⁻¹)	Dissolved N ₂ O (nmol L ⁻¹)	N ₂ O saturation
April		13:00	8.95 (0.09)	8.20 (0.12)	13.60(0.07)	19.60 (0.57)	45.00 (3.11)	0.19 (0.03)	1.41(0.01)	0.0036 (0.004)	31.42(2.85)	272.45% (32.13%)
		15:00	10.31 (0.10)	8.00 (0.12)	13.20(0.14)	18.70 (0.47)	44.50 (2.15)	0.19 (0.03)	1.27(0.07)	0.0047 (0.003)	26.65(0.40)	224.62% (1.53%)
		17:00	11.97 (0.13)	8.20 (0.13)	12.80(0.04)	19.30 (0.26)	41.80 (1.54)	0.31 (0.04)	1.16(0.06)	0.0182 (0.002)	25.34(1.19)	211.30% (4.74%)
	NW	7:00	9.51 (0.03)	8.30 (0.07)	18.70(0.00)	9.40 (0.07)	93.90 (3.13)	0.04 (0.00)	0.52(0.02)	0.0086 (0.000)	16.76(1.08)	173.98% (4.90%)
		9:00	6.98 (0.02)	7.90 (0.00)	19.60(0.14)	10.70 (0.14)	167.00 (4.68)	0.11 (0.00)	0.52(0.03)	0.0049 (0.0002)	16.51(0.94)	176.48% (5.05%)
		10:15	6.67 (0.02)	7.30 (0.00)	20.30(0.21)	13.30 (0.00)	171.00 (2.28)	0.10 (0.04)	0.49(0.01)	0.0046 (0.0002)	16.67(1.88)	182.27% (9.38%)
		11:30	10.50 (0.05)	7.20 (0.07)	20.40(0.21)	13.40 (0.14)	61.10 (3.84)	0.08 (0.02)	0.44(0.01)	0.0059 (0.000)	13.49(0.90)	147.96% (4.29%)
		12:45	12.10 (0.05)	7.10 (0.00)	20.10(0.00)	13.40 (0.07)	113.00 (2.40)	0.06 (0.00)	0.35(0.01)	0.0065 (0.0002)	8.99(0.58)	97.65% (2.71%)
		14:00	13.60 (0.04)	7.30 (0.14)	20.30(0.14)	14.20 (0.14)	69.20 (5.25)	0.05 (0.03)	0.26(0.00)	0.0065 (0.000)	8.26(0.32)	90.25% (1.63%)
		15:15	13.20 (0.07)	7.40 (0.07)	19.90(0.00)	14.00 (0.00)	23.70 (1.73)	0.01 (0.01)	0.17(0.01)	0.0065 (0.000)	6.66(0.77)	71.83% (3.57%)
	EG	7:00	2.94 (0.01)	8.40 (0.00)	18.00(0.00)	9.80 (0.00)	90.40 (5.44)	0.19 (0.03)	0.52(0.01)	0.0025 (0.015)	4.41(0.20)	44.74% (3.11%)
		9:00	4.09 (0.01)	7.80 (0.07)	19.00(0.00)	11.00 (0.00)	65.00 (7.50)	0.33 (0.04)	0.37(0.02)	0.0046 (0.047)	9.85(0.96)	103.28% (9.60%)

Month	group	Time	Salinity	pH	Temperature (°C)	DO (mg L ⁻¹)	Turbidity (NTU)	NH ₄ ⁺ -N (mg L ⁻¹)	NO ₃ -N (mg L ⁻¹)	NO ₂ -N (mg L ⁻¹)	Dissolved N ₂ O (nmol L ⁻¹)	N ₂ O saturation
August		10:15	4.60 (0.05)	7.40 (0.07)	21.10(0.14)	13.1 (00.42)	85.80 (1.91)	0.30 (0.13)	0.26(0.03)	0.0010 (0.0096)	3.43(0.11)	38.46% (4.17%)
		11:30	5.40 (0.04)	7.40 (0.00)	20.60(0.00)	12.90 (0.50)	114.00 (1.41)	0.39(0.05)	0.29(0.05)	0.0020 (0.0021)	12.23(0.52)	134.99% (10.78%)
		12:45	6.46 (0.21)	7.30 (0.00)	19.80(0.14)	12.50 (0.00)	35.00 (0.42)	0.36(0.04)	0.13(0.04)	0.0032 (0.0039)	32.42(1.63)	348.76% (6.47%)
		14:00	7.80 (0.02)	7.30 (0.07)	19.20(0.07)	12.60 (0.36)	47.75 (5.69)	0.05(0.02)	0.24(0.01)	0.0017 (0.0006)	12.07(2.11)	127.32% (5.85%)
		15:15	8.93 (0.07)	7.10 (0.14)	19.40(0.07)	13.00 (0.64)	34.15 (4.46)	0.01(0.00)	0.22(0.07)	0.0017 (0.0003)	13.27(3.40)	140.91% (5.18%)
		6:40	3.63 (0.23)	8.50 (0.00)	29.30(0.28)	5.80 (0.28)	79.60 (1.43)	0.11(0.00)	1.19(0.02)	0.0011 (0.000)	0.58(0.001)	8.35% (0.18%)
	NW	8:40	2.01 (0.06)	8.40 (0.07)	29.90(0.07)	5.70 (0.07)	115.00 (1.89)	0.07(0.00)	1.08(0.03)	0.0010 (0.000)	1.22(0.002)	17.99% (0.37%)
		10:50	0.57 (0.01)	8.30 (0.07)	30.60(0.14)	5.80 (0.07)	55.00 (0.84)	0.03(0.00)	1.25(0.01)	0.0010 (0.000)	3.60(0.01)	53.85% (1.72%)
		12:50	2.99 (0.04)	8.60 (0.50)	30.40(0.14)	5.90 (0.28)	94.60 (3.27)	0.29(0.02)	1.16(0.01)	0.0011 (0.000)	2.16(0.01)	32.05% (1.31%)
		14:50	4.75 (0.09)	9.00 (0.28)	29.80(0.00)	6.00 (0.35)	119.00 (2.57)	0.40(0.01)	1.12(0.01)	0.0027 (0.000)	0.22(0.01)	3.19% (1.27%)
		16:50	5.78 (0.13)	9.00 (0.21)	29.70(0.14)	6.10 (0.28)	25.70 (1.05)	0.37(0.01)	1.11(0.01)	0.0068 (0.0002)	0.27(0.001)	3.92% (0.10%)
	EG	6:40	0.93 (0.09)	10.40 (0.00)	29.70(0.28)	6.70 (0.21)	46.40 (3.31)	0.02(0.00)	1.06(0.00)	0.0357 (0.0325)	1.10(0.01)	16.03% (1.99%)

Month	group	Time	Salinity	pH	Temperature (°C)	DO (mg L ⁻¹)	Turbidity (NTU)	NH ₄ ⁺ -N (mg L ⁻¹)	NO ₃ -N (mg L ⁻¹)	NO ₂ -N (mg L ⁻¹)	Dissolved N ₂ O (nmol L ⁻¹)	N ₂ O saturation
		8:40	1.72 (0.08)	9.40 (0.07)	28.70(0.35)	5.90 (0.28)	38.00 (1.43)	0.31(0.17)	0.83(0.01)	0.0396 (0.000)	2.66(0.01)	37.54% (1.38%)
		10:50	1.36 (0.08)	8.30 (0.50)	29.80(0.14)	4.70 (0.07)	35.20 (2.74)	0.15(0.01)	0.93(0.00)	0.0394 (0.0097)	6.64(0.06)	96.91% (1.57%)
		12:50	1.43 (0.02)	7.90 (0.21)	29.90(0.00)	4.80 (0.42)	39.10 (2.35)	0.13(0.02)	1.08(0.02)	0.0232 (0.015)	11.14(0.01)	163.07% (1.71%)
		14:50	2.39 (0.07)	9.00 (0.50)	29.40(0.28)	5.00 (0.21)	27.70 (1.27)	0.21(0.00)	1.03(0.00)	0.030 (0.000)	9.86(0.02)	142.30% (2.66%)
		16:50	3.98 (0.20)	8.70 (0.07)	29.30(0.21)	5.60 (0.21)	35.70 (3.52)	0.32(0.01)	1.02(0.08)	0.012 (0.0137)	9.66(0.02)	138.89% (2.93%)

78 **Table S4** Primer sequences used for the qPCR analysis and amplicon sequencing.

Primer	Target	Oligo-sequence (5' -3')	Reference
16S rDNA V3-V4_F	16S rDNA	ACTCCTACGGGAGGCAGCAG	Munyaka et al.; 2015
	(qPCR,amplicon		
16S rDNA V3-V4_R	sequencing)	GTGGACTACHVGGGTWTCTAAT	Munyaka et al.; 2015
amoA-AOA_F	amoA-AOA	STAATGGTCTGGCTTAGACG	Francis et al.; 2005
amoA-AOA_R	(qPCR)	GCGGCCATCCATCTGTATGT	Francis et al.; 2005
amoA-AOB_F	amoA-AOB	GGGGTTTCTACTGGTGGT	Rotthauwe et al.; 1997
amoA-AOB_R	(qPCR)	CCCCTCKGSAAAGCCTTCTTC	Rotthauwe et al.; 1997
nxrA_F	nxrA	CAGACCGACGTGTGCGAAAG	Attard et al.; 2010
nxrA_R	(qPCR)	TCCACAAGGAACGGAAGGTC	Attard et al.; 2010
nirK_F	nirK	ATCATGGTSCTGCCGCG	Braker et al.; 1998
nirK_R	(qPCR)	GCCTCGATCAGRTTGTGGTT	Braker et al.; 1998
nirS_F	nirS	GTSAACGTSAAGGARACSGG	Throbäck et al.; 2004
nirS_R	(qPCR)	GASTTCGGRTGSGTCTTGA	Throbäck et al.; 2004
nosZ_F	nosZ	CGYTGTTCMTCGACAGCCAG	Throbäck et al.; 2004

Primer	Target	Oligo-sequence (5' -3')	Reference
nosZ_R	(qPCR)	CGSACCTTSTTGCCSTYGCG	Throbäck et al.; 2004
nrfA_F	nrfA	CARTGYCAYGTBGARTA	Welsh et al.;2014
nrfA_R	(qPCR)	TWNGGCATRTGRCARTC	Welsh et al.;2014

79 **Table S5** Exchange efficiency of water to dialysis bag during salinity intrusion

Date	Time	α (h ⁻¹)
2020.12.20	11:00	0.13
	13:00	0.16
	15:00	0.12
	17:00	0.22
	average	0.16
2021.4.17	10:15	0.16
	11:30	0.11
	12:45	0.13
	14:00	0.15
	15:15	0.17
	average	0.14
2021.8.29	10:50	0.16
	12:50	0.02
	14:50	0.15
	16:50	0.24
	average	0.14

80 **Table S6** Results of Wilcoxon tests analyzing N contents and dissolved N₂O
81 concentrations in NW and EG before vs. during the saltwater intrusions.

	December		April		August	
	NW	EG	NW	EG	NW	EG
Salinity	0.058	0.028	0.028	0.028	0.116	0.028
NH ₄ ⁺ -N	0.028	0.028	0.345	0.917	0.028	0.345
NO ₃ ⁻ -N	0.028	0.917	0.028	0.046	0.248	0.046
NO ₂ ⁻ -N	0.028	0.917	0.916	0.917	0.066	0.027
N ₂ O	0.028	0.463	0.028	0.028	0.463	0.028

82 Significance is shown in bold ($P < 0.05$).

83 **Table S7** Results of Wilcoxon tests analyzing the differences in the relative abundances of the bacterial genera involved in N cycling processes
84 between NW and the EG. Abbreviations: Max.Rel, maximal relative abundance of the genus; Min.Rel, minimal relative abundance of the genus.
85 ‘Corresponding sample’ indicates at which sampling time and in which samples the maximal or minimal relative abundance of a particular genus
86 was detected.

N-related bacteria	Genus	December	April	August	Max.Rel	Corresponding sample	Min.Rel	Corresponding sample
Denitrifier	<i>Vogesella</i>	0.109	0.005**	0.012**	20.05%	August-EG-2	0.00%	December-EG-2
	<i>Flavobacterium</i>	0.144	0.005**	0.779	21.86%	April-EG-5	0.12%	April-NW-5
	<i>Pseudomonas</i>	0.068	0.005**	0.012**	8.19%	April-EG-1	0.07%	April-NW-4
	<i>Aeromonas</i>	0.068	0.005**	0.012**	4.36%	August-EG-2	0.05%	December-NW-3
	<i>Dechloromonas</i>	0.715	0.005**	0.012**	5.77%	April-EG-1	0.00%	December-EG-2
	<i>Rheinheimera</i>	0.068	0.005**	0.263	3.49%	August-NW-2	0.03%	April-NW-5
	<i>Rhodobacter</i>	0.273	0.042*	0.068	2.59%	August-EG-3	0.00%	December-EG-2
	<i>Comamonas</i>	0.715	0.043*	0.068	1.38%	August-EG-2	0.02%	April-NW-4
	<i>Pseudohongiella</i>	0.680	0.043*	0.068	1.60%	April-NW-5	0.01%	August-EG-1
	<i>Oceanimonas</i>	0.705	0.225	0.144	3.04%	August-NW-2	0.00%	December-NW-3

N-related bacteria	Genus	December	April	August	Max.Rel	Corresponding sample	Min.Rel	Corresponding sample
	<i>Cloacibacterium</i>	0.465	0.043 *	0.068	0.92%	August-EG-3	0.00%	December-NW-3
	<i>Acidovorax</i>	0.465	0.043 *	0.465	1.01%	April-EG-2	0.01%	December-EG-3
	<i>Altererythrobacter</i>	0.465	0.043 *	0.068	0.74%	August-NW-3	0.02%	December-EG-1
Nitrifier	<i>Nitrospira</i>	0.273	0.043 *	0.068	2.77%	December-NW-2	0.01%	April-EG-5
DNRA bacteria	<i>Bacillus</i>	1.000	0.144	0.017 **	17.02%	December-EG-2	0.00%	August-EG-2

87 Significance codes: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

88 **Table S8** Absolute and relative abundances of functional genes in NW and EG during December 2020 and April and August 2021. The values
89 inside and outside the parentheses represent the average of biological duplicates and the standard deviation, respectively.

Index	Month	Sample	<i>amoA-AOA</i>	<i>amoA-AOB</i>	<i>nxrA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	<i>nrfA</i>	<i>16S rRNA</i> gene
Absolute abundance (copy g ⁻¹)	December	NW-1	8.03E+06	7.27E+06	1.80E+04	5.33E+07	1.66E+07	5.30E+08	1.23E+06	1.12E+10
			(4.25E+05)	(3.42E+05)	(1.24E+03)	(7.19E+05)	(1.08E+06)	(1.53E+07)	(7.97E+03)	(5.73E+08)
		NW-2	8.48E+06	8.54E+06	1.90E+04	1.31E+07	2.64E+07	5.18E+08	6.38E+06	2.62E+10
			(4.44E+05)	(1.07E+05)	(1.63E+03)	(6.26E+04)	(7.46E+05)	(1.01E+07)	(2.63E+05)	(1.63E+09)
		NW-3	4.15E+06	1.83E+07	3.48E+04	1.70E+07	1.88E+07	5.12E+08	1.64E+05	1.29E+10
			(2.07E+05)	(4.38E+05)	(1.16E+03)	(5.45E+05)	(1.04E+06)	(5.62E+06)	(3.33E+03)	(3.02E+08)
		NW-4	7.53E+06	9.57E+06	1.21E+04	8.79E+05	1.65E+07	5.15E+08	2.87E+05	7.23E+09
			(2.84E+05)	(2.21E+04)	(1.17E+03)	(8.30E+03)	(7.02E+05)	(1.89E+06)	(2.80E+03)	(3.35E+08)
		EG-1	7.01E+06	7.30E+06	1.54E+04	5.04E+05	1.31E+07	5.14E+08	1.83E+05	1.09E+10
			(8.65E+04)	(4.54E+05)	(1.03E+02)	(9.75E+03)	(1.91E+05)	(2.41E+07)	(1.11E+04)	(3.69E+08)
		EG-2	4.76E+06	8.49E+06	5.33E+03	2.98E+06	1.84E+07	5.20E+08	2.34E+06	4.55E+09
			(6.22E+04)	(3.87E+04)	(4.69E+02)	(1.89E+05)	(1.03E+06)	(2.28E+07)	(1.24E+05)	(3.51E+07)
		EG-3	4.44E+06	1.03E+07	1.61E+04	1.79E+07	2.35E+07	5.19E+08	6.94E+05	7.15E+09

Index	Month	Sample	<i>amoA-AOA</i>	<i>amoA-AOB</i>	<i>nxrA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	<i>nrfA</i>	<i>16S rRNA gene</i>
			(9.91E+04)	(6.95E+05)	(9.76E+02)	(2.48E+05)	(1.33E+06)	(2.18E+07)	(3.93E+04)	(2.72E+08)
		EG-4	9.80E+06	7.79E+06	3.23E+04	7.66E+05	1.48E+07	5.22E+08	5.19E+06	8.85E+09
			(4.84E+05)	(9.57E+04)	(2.77E+03)	(2.33E+04)	(9.23E+05)	(3.05E+07)	(2.42E+05)	(2.86E+07)
		NW-1	9.28E+05	1.85E+05	2.68E+04	1.36E+06	1.53E+08	1.21E+05	5.77E+05	4.78E+08
			(1.70E+05)	(1.47E+04)	(3.25E+03)	(1.14E+05)	(1.12E+07)	(1.03E+04)	(1.63E+04)	(1.84E+06)
		NW-2	8.98E+05	2.43E+05	3.12E+04	1.67E+06	1.14E+08	2.61E+05	1.42E+06	4.69E+08
			(1.73E+05)	(4.72E+03)	(1.05E+04)	(4.06E+04)	(1.37E+07)	(1.04E+04)	(4.91E+05)	(2.44E+08)
		NW-3	7.42E+05	5.38E+05	1.99E+04	2.30E+06	1.16E+08	7.10E+05	1.09E+06	5.35E+08
			(5.62E+04)	(2.28E+05)	(2.99E+03)	(4.85E+05)	(3.05E+07)	(4.16E+05)	(5.17E+04)	(3.28E+08)
	April	NW-4	7.95E+05	2.62E+05	2.28E+04	1.27E+06	1.17E+08	1.40E+05	1.70E+06	3.04E+08
			(4.91E+05)	(1.24E+05)	(4.86E+03)	(4.38E+05)	(3.86E+07)	(8.26E+04)	(1.27E+06)	(2.66E+07)
		NW-5	7.22E+05	2.05E+05	4.41E+04	1.61E+06	2.01E+08	1.60E+05	6.16E+05	1.05E+09
			(2.71E+05)	(7.35E+04)	(1.14E+04)	(6.88E+04)	(1.73E+07)	(3.32E+04)	(6.00E+04)	(5.61E+08)
		EG-1	7.24E+05	4.96E+05	2.36E+04	2.18E+07	2.07E+08	3.51E+07	2.03E+06	6.62E+09
			(1.63E+05)	(7.66E+04)	(6.82E+03)	(6.42E+06)	(5.45E+07)	(2.24E+07)	(7.38E+05)	(5.89E+08)
		EG-2	2.80E+05	2.07E+05	1.89E+04	5.85E+06	1.66E+08	4.90E+06	8.32E+05	4.57E+09

Index	Month	Sample	<i>amoA-AOA</i>	<i>amoA-AOB</i>	<i>nxrA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	<i>nrfA</i>	<i>16S rRNA</i> gene
			(5.61E+04)	(4.84E+04)	(4.88E+03)	(3.51E+05)	(3.62E+07)	(2.12E+05)	(1.84E+05)	(3.20E+09)
		EG-3	3.31E+05	3.19E+05	1.78E+04	2.98E+06	1.21E+08	2.78E+06	4.69E+05	4.18E+08
			(1.08E+04)	(2.03E+05)	(2.69E+03)	(3.44E+05)	(2.38E+07)	(2.72E+05)	(1.13E+04)	(1.83E+08)
		EG-4	6.61E+05	2.09E+05	3.70E+04	8.62E+06	3.20E+08	1.51E+07	1.86E+06	2.31E+09
			(2.65E+05)	(7.11E+04)	(6.89E+03)	(1.32E+06)	(2.00E+08)	(1.12E+07)	(6.02E+05)	(1.68E+09)
		EG-5	6.50E+05	3.06E+05	2.27E+04	9.93E+06	1.86E+08	3.27E+07	2.84E+06	1.31E+09
			(3.27E+05)	(8.62E+04)	(5.84E+03)	(3.06E+06)	(1.35E+06)	(1.19E+07)	(1.27E+06)	(1.39E+08)
		NW-1	2.39E+05	2.74E+05	1.92E+04	3.68E+06	2.02E+08	8.73E+05	5.84E+05	4.15E+08
			(4.97E+04)	(9.04E+04)	(8.41E+03)	(1.16E+06)	(5.06E+06)	(3.58E+05)	(1.28E+05)	(1.02E+08)
		NW-2	7.61E+05	1.39E+05	2.19E+04	4.64E+06	1.55E+08	8.32E+05	1.05E+06	2.62E+08
			(6.10E+04)	(2.18E+04)	(1.52E+03)	(1.73E+06)	(6.67E+07)	(4.10E+05)	(6.75E+04)	(1.15E+08)
		NW-3	5.01E+05	5.17E+04	1.36E+04	2.61E+05	3.25E+07	3.88E+04	4.04E+05	4.59E+08
			(1.72E+05)	(3.17E+04)	(6.47E+03)	(1.04E+05)	(3.19E+06)	(1.49E+04)	(1.17E+05)	(9.00E+07)
		NW-4	2.48E+06	2.93E+05	9.76E+04	1.92E+06	5.06E+08	2.21E+05	9.18E+05	1.80E+09
			(1.41E+06)	(3.49E+04)	(5.59E+04)	(6.33E+05)	(2.89E+08)	(5.47E+04)	(2.42E+04)	(7.54E+08)
		EG-1	1.35E+06	8.98E+05	1.73E+05	6.64E+08	3.80E+08	1.99E+08	5.05E+06	3.55E+10
	August									

Index	Month	Sample	<i>amoA-AOA</i>	<i>amoA-AOB</i>	<i>nxrA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	<i>nrfA</i>	<i>16S rRNA</i> gene
Relative abundance	December	EG-2	(5.12E+05)	(5.64E+05)	(1.10E+05)	(6.03E+08)	(1.29E+07)	(1.86E+08)	(2.70E+06)	(3.35E+10)
			2.45E+06	1.34E+06	1.70E+05	8.41E+08	3.94E+08	2.83E+08	6.36E+06	9.43E+10
			(1.65E+06)	(1.88E+05)	(1.68E+03)	(3.40E+07)	(7.42E+07)	(7.90E+07)	(8.70E+05)	(1.68E+10)
		EG-3	1.02E+06	2.07E+05	5.77E+04	7.77E+07	2.30E+08	1.34E+07	4.37E+05	9.61E+09
			(4.59E+04)	(1.09E+04)	(3.57E+03)	(2.85E+06)	(1.31E+07)	(2.88E+05)	(2.40E+04)	(5.70E+08)
		EG-4	1.09E+06	1.54E+06	1.35E+05	7.81E+08	1.65E+08	5.13E+08	4.11E+06	1.66E+11
			(2.49E+04)	(1.40E+06)	(1.11E+05)	(7.26E+08)	(4.56E+07)	(5.05E+08)	(3.35E+06)	(1.54E+11)
		NW-1	0.0719%	0.0651%	0.0002%	0.4771%	0.1485%	4.7451%	0.0110%	
			(0.0038%)	(0.0031%)	(0.0000%)	(0.0064%)	(0.0097%)	(0.1367%)	(0.0001%)	
		NW-2	0.0759%	0.0765%	0.0002%	0.1170%	0.2360%	4.6358%	0.0571%	
			(0.0017%)	(0.0004%)	(0.0000%)	(0.0002%)	(0.0029%)	(0.0387%)	(0.0010%)	
		NW-3	0.0371%	0.1634%	0.0003%	0.1519%	0.1687%	4.5862%	0.0015%	
			(0.0016%)	(0.0034%)	(0.0000%)	(0.0042%)	(0.0081%)	(0.0434%)	(0.0000%)	
		NW-4	0.0674%	0.0857%	0.0001%	0.0079%	0.1475%	4.6101%	0.0026%	
			(0.0039%)	(0.0003%)	(0.0000%)	(0.0001%)	(0.0097%)	(0.0261%)	(0.0000%)	
		EG-1	0.0627%	0.0653%	0.0001%	0.0045%	0.1173%	4.6055%	0.0016%	

Index	Month	Sample	<i>amoA-AOA</i>	<i>amoA-AOB</i>	<i>nxrA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	<i>nrfA</i>	<i>16S rRNA</i> gene
			(0.0008%)	(0.0041%)	(0.0000%)	(0.0001%)	(0.0017%)	(0.2202%)	(0.0001%)	
		EG-2	0.0426%	0.0760%	0.0000%	0.0266%	0.1647%	4.6579%	0.0210%	
			(0.0014%)	(0.0008%)	(0.0000%)	(0.0042%)	(0.0226%)	(0.5015%)	(0.0027%)	
		EG-3	0.0398%	0.0925%	0.0001%	0.1600%	0.2104%	4.6468%	0.0062%	
			(0.0014%)	(0.0097%)	(0.0000%)	(0.0035%)	(0.0186%)	(0.3046%)	(0.0005%)	
		EG-4	0.0878%	0.0697%	0.0003%	0.0069%	0.1324%	4.6752%	0.0464%	
			(0.0055%)	(0.0011%)	(0.0000%)	(0.0003%)	(0.0104%)	(0.3450%)	(0.0027%)	
		NW-1	0.1941%	0.0387%	0.0056%	0.2853%	31.9334%	0.0253%	0.1206%	
			(0.0356%)	(0.0031%)	(0.0000%)	(0.0238%)	(2.3470%)	(0.0022%)	(0.0034%)	
		NW-2	0.1914%	0.0518%	0.0066%	0.3549%	24.3800%	0.0557%	0.3026%	
			(0.0369%)	(0.0010%)	(0.0022%)	(0.0086%)	(2.9205%)	(0.0022%)	(0.1047%)	
	April	NW-3	0.1389%	0.1005%	0.0037%	0.4306%	21.6283%	0.1327%	0.2038%	
			(0.0105%)	(0.0427%)	(0.0006%)	(0.0908%)	(5.7082%)	(0.0778%)	(0.0097%)	
		NW-4	0.2614%	0.0861%	0.0075%	0.4172%	38.5091%	0.0462%	0.5598%	
			(0.1616%)	(0.0407%)	(0.0016%)	(0.1442%)	(12.6937%)	(0.0272%)	(0.4193%)	
		NW-5	0.0685%	0.0195%	0.0042%	0.1526%	19.0153%	0.0152%	0.0584%	

Index	Month	Sample	<i>amoA-AOA</i>	<i>amoA-AOB</i>	<i>nxrA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	<i>nrfA</i>	<i>16S rRNA</i> gene
		EG-1	(0.0257%)	(0.0070%)	(0.0011%)	(0.0065%)	(1.6376%)	(0.0032%)	(0.0057%)	
			0.0109%	0.0075%	0.0004%	0.3296%	3.1223%	0.5307%	0.0307%	
			(0.0025%)	(0.0012%)	(0.0001%)	(0.0970%)	(0.8240%)	(0.3386%)	(0.0112%)	
		EG-2	0.0061%	0.0045%	0.0004%	0.1281%	3.6334%	0.1073%	0.0182%	
			(0.0012%)	(0.0011%)	(0.0001%)	(0.0077%)	(0.7927%)	(0.0046%)	(0.0040%)	
		EG-3	0.0792%	0.0763%	0.0043%	0.7131%	29.0414%	0.6661%	0.1121%	
			(0.0026%)	(0.0485%)	(0.0006%)	(0.0823%)	(5.6945%)	(0.0650%)	(0.0027%)	
		EG-4	0.0286%	0.0090%	0.0016%	0.3727%	13.8588%	0.6527%	0.0805%	
			(0.0115%)	(0.0031%)	(0.0003%)	(0.0572%)	(8.6627%)	(0.4829%)	(0.0260%)	
		EG-5	0.0495%	0.0233%	0.0017%	0.7561%	14.1667%	2.4868%	0.2160%	
			(0.0249%)	(0.0066%)	(0.0000%)	(0.2325%)	(0.1026%)	(0.9055%)	(0.0967%)	
August		NW-1	0.0576%	0.0660%	0.0046%	0.8868%	48.6468%	0.2102%	0.1407%	
			(0.0120%)	(0.0218%)	(0.0020%)	(0.2798%)	(1.2179%)	(0.0862%)	(0.0308%)	
		NW-2	0.2910%	0.0533%	0.0084%	1.7725%	59.2234%	0.3181%	0.4013%	
			(0.0233%)	(0.0083%)	(0.0006%)	(0.6627%)	(25.4985%)	(0.1569%)	(0.0258%)	
		NW-3	0.1091%	0.0113%	0.0030%	0.0569%	7.0730%	0.0084%	0.0880%	

Index	Month	Sample	<i>amoA</i> -AOA	<i>amoA</i> -AOB	<i>nxrA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	<i>nrfA</i>	<i>16S rRNA</i> gene
			(0.0376%)	(0.0069%)	(0.0014%)	(0.0226%)	(0.6939%)	(0.0033%)	(0.0255%)	
		NW-4	0.1380%	0.0163%	0.0054%	0.1070%	28.1679%	0.0123%	0.0511%	
			(0.0787%)	(0.0019%)	(0.0031%)	(0.0353%)	(16.0894%)	(0.0030%)	(0.0013%)	
		EG-1	0.0038%	0.0025%	0.0005%	1.8700%	1.0703%	0.5618%	0.0142%	
			(0.0014%)	(0.0016%)	(0.0003%)	(1.6977%)	(0.0363%)	(0.5247%)	(0.0076%)	
		EG-2	0.0026%	0.0014%	0.0002%	0.8919%	0.4178%	0.2997%	0.0067%	
			(0.0010%)	(0.0002%)	(0.0000%)	(0.0361%)	(0.0787%)	(0.0838%)	(0.0009%)	
		EG-3	0.0107%	0.0022%	0.0006%	0.8082%	2.3988%	0.1391%	0.0045%	
			(0.0005%)	(0.0001%)	(0.0000%)	(0.0297%)	(0.1363%)	(0.0030%)	(0.0003%)	
		EG-4	0.0007%	0.0009%	0.0001%	0.4713%	0.0997%	0.3095%	0.0025%	
			(0.0000%)	(0.0008%)	(0.0001%)	(0.4377%)	(0.0275%)	(0.3044%)	(0.0020%)	

90 **Table S9** Results of Wilcoxon tests analyzing the differences in the absolute abundance of functional genes and the 16S rRNA gene between the
 91 NW and the EG samples.

	<i>amoA-AOA</i>	<i>amoA-AOB</i>	<i>nxrA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	<i>nrfA</i>	<i>16S rRNA</i> gene
December	0.715	0.144	0.715	0.273	0.465	0.715	1.000	0.273
April	0.074	0.646	0.386	0.005**	0.074	0.005**	0.386	0.022*
August	0.263	0.036*	0.017*	0.012*	0.208	0.012*	0.050	0.012*

92 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table S10 Results of Kruskal-Wallis tests analyzing the difference in the relative abundance of N functional genes within NW or EG. Paired comparison tests were performed to further analyze the significance of the comparison pairs and the level of significance.

	December		April		August	
	NW	EG	NW	EG	NW	EG
<i>amoA-AOA - amoA-AOB</i>	1.000	1.000	1.000	1.000	1.000	1.000
<i>amoA-AOA-nxrA</i>	1.000	1.000	0.006**	0.838	0.035*	1.000
<i>amoA-AOA-nirK</i>	1.000	1.000	1.000	0.213	1.000	0.011*
<i>amoA-AOA-nirS</i>	1.000	1.000	0.068	0.000***	0.192	0.028*
<i>amoA-AOA-nosZ</i>	0.477	0.664	1.000	0.110	1.000	0.529
<i>amoA-AOA-nrfA</i>	1.000	1.000	1.000	1.000	1.000	1.000
<i>amoA-AOB-nxrA</i>	0.380	0.236	0.827	1.000	1.000	1.000
<i>amoA-AOB-nirK</i>	1.000	1.000	0.173	0.042*	0.620	0.001**
<i>amoA-AOB-nirS</i>	1.000	1.000	0.000***	0.000***	0.001**	0.004**
<i>amoA-AOB-nosZ</i>	1.000	1.000	1.000	0.020*	1.000	0.111
<i>amoA-AOB-nrfA</i>	1.000	1.000	1.000	1.000	1.000	1.000
<i>nxrA-nirK</i>	0.477	1.000	0.000***	0.000***	0.004**	0.000***
<i>nxrA-nirS</i>	0.041*	0.027*	0.000***	0.000***	0.000***	0.000***
<i>nxrA-nosZ</i>	0.001**	0.001**	1.000	0.000***	0.596	0.002**
<i>nxrA-nrfA</i>	1.000	1.000	0.001**	0.087	0.024*	0.529
<i>nirk-nirS</i>	1.000	1.000	1.000	1.000	0.937	1.000
<i>nirk-nosZ</i>	1.000	0.266	0.100	1.000	1.000	1.000
<i>nirk-nrfA</i>	1.000	1.000	1.000	1.000	1.000	0.072
<i>nirS-nosZ</i>	1.000	1.000	0.000***	1.000	0.008**	1.000
<i>nirS-nrfA</i>	0.380	0.477	0.238	0.007**	0.262	0.160
<i>nosZ-nrfA</i>	0.014*	0.031*	0.666	1.000	1.000	1.000

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

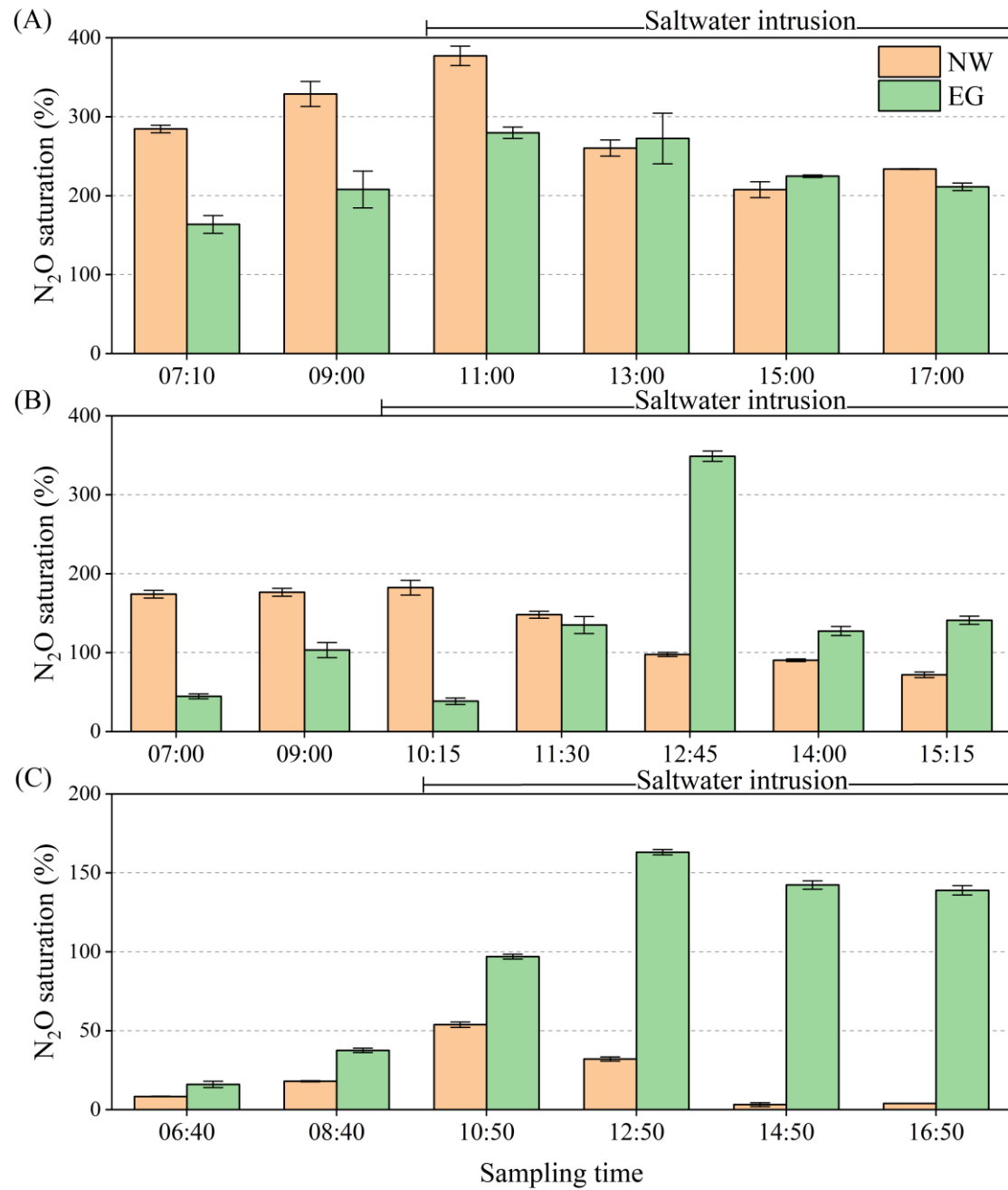


Figure S1 Patterns of N_2O saturation before and during saltwater intrusion at each sampling timepoint in December 2020 (A), April 2021 (B), and August 2021 (C). The results are presented as the average of biological duplicates and the standard deviation.

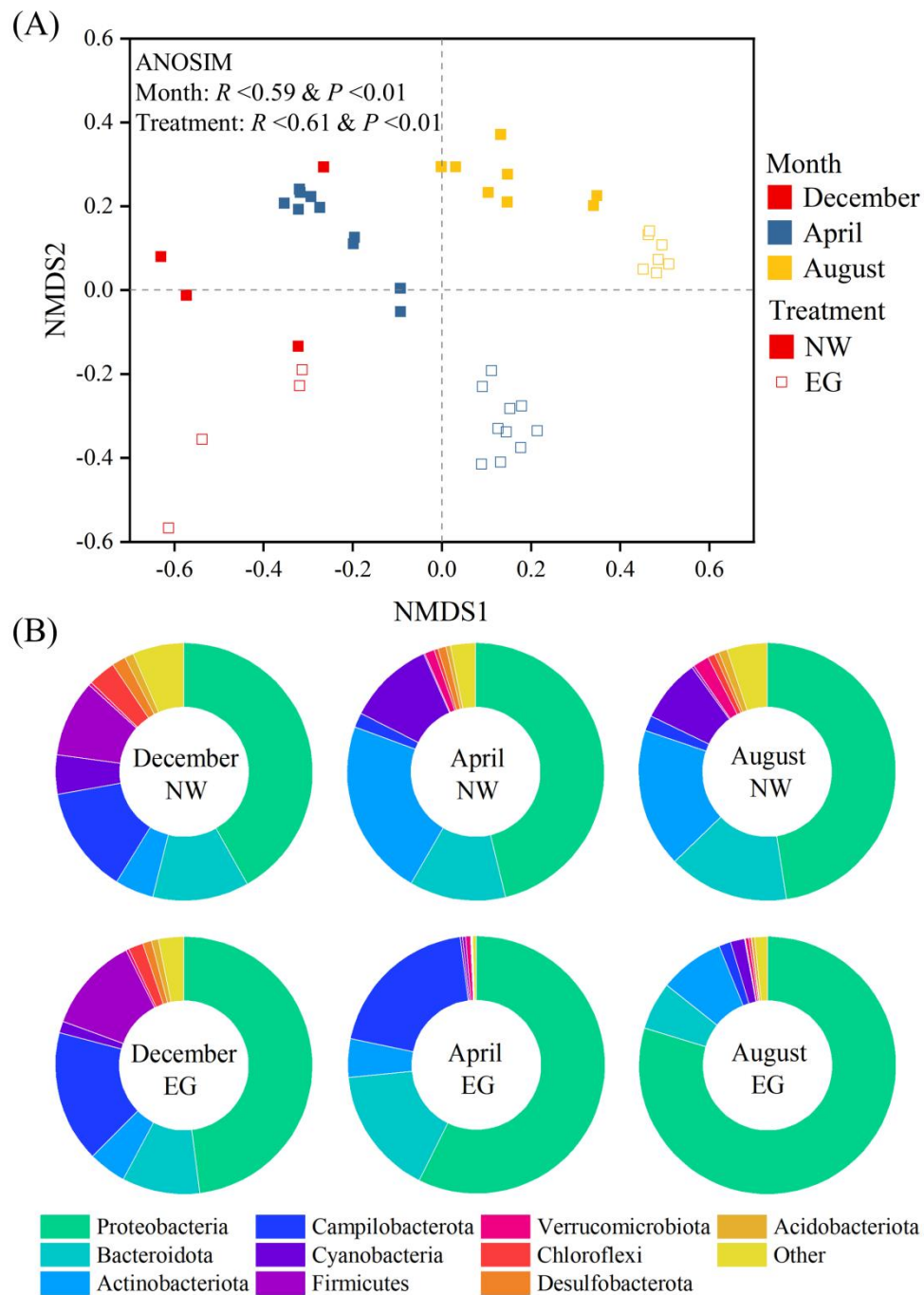


Figure S2 Bacterial beta-diversity, visualized using non-metric multidimensional scaling (NMDS) (A) and the phylum-level distribution in NW and EG (B).

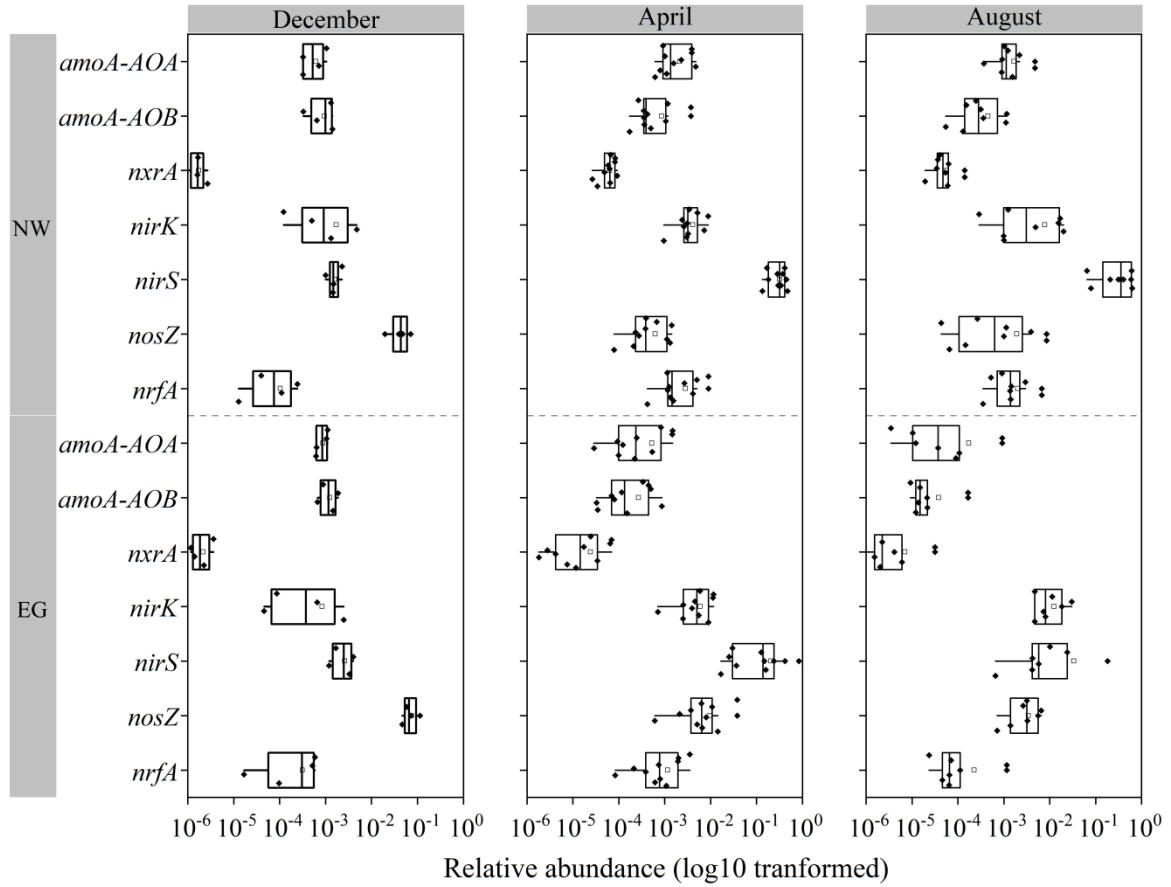


Figure S3 Relative abundances of the functional genes involved in N cycling in NW and EG during December 2020 and April and August 2021. Each point in the boxplot represents the average of biological replicates for each sample.

References

- Li, X.; Qi, M.; Gao, D.; Liu, M.; Sardans, J.; Peñuelas, J.; Hou, L. 2022. Nitrous oxide emissions from subtropical estuaries: Insights for environmental controls and implications. *Water Res.* **212**: 118110.
- Weiss, R.F.; Price, B.A. 1980. Nitrous oxide solubility in water and seawater. *Mar. Chem.* **8**: 347–359.
- CMA Climate Change Centre. Blue Book on Climate Change in China (2021). Science Press, 2021.
- Wan, J.; Tokunaga, T. K.; Brown, W.; Newman, A. W.; Dong, W.; Bill, M.; Beutler, C. A.; Henderson, A. N.; Harvey-Costello, N.; Conrad, M. E.; Bouskill, N. J.; Hubbard, S. S.; Williams, K. H. 2021. Bedrock Weathering Contributes to Subsurface Reactive Nitrogen and Nitrous Oxide Emissions. *Nat. Geosci.* **14**: 217–224.
- Marushchak, M.E.; Kerttula, J.; Di´akov´a, K.; Faguet, A.; Gil, J.; Grosse, G.; Knoblauch, C.; Lashchinskiy, N.; Martikainen, P.J.; Morgenstern, A.; Nykamb, M.; Ronkainen, J.G.; Siljanen, H.M.P.; van Delden, L.; Voigt, C.; Zimov, N.; Zimov, S.; Biasi, C. 2021. Thawing Yedoma permafrost is a neglected nitrous oxide source. *Nat. Commun.* **12**: 7107.
- Rabaey, J.; Cotner, J. 2022. Pond greenhouse gas emissions controlled by duckweed coverage. *Front. Environ. Sci.* **10**: 889289.
- Zhou, Y.; Toyoda, R.; Suenaga, T.; Aoyagi, T.; Hori, T.; Terada, A. 2022. Low nitrous oxide concentration and spatial microbial community transition across an

131 urban river affected by treated sewage. *Water Res.* **216**: 118276.

132 Munyaka, P. M.; Khafipour, A.; Wang, H.; Eissa, N.; Khafipour, E.; Ghia, J. 2015.

133 Mo1774 Prenatal Antibiotic Treatment Increases Offspring's Susceptibility to

134 Experimental Colitis: A Role of the Gut Microbiota. *Plos One*, **148**: S-708.

135 Francis, C.A.; Roberts, K.J.; Beman, J.M.; Santoro, A.E.; Oakley, B.B. 2005.

136 Ubiquity and diversity of ammonia-oxidizing archaea in water columns and

137 sediments of the ocean. *PNSA*, **102**: 14683-14688.

138 Rotthauwe, J.H.; Witzel, K.P.; Liesack, W. 1997. The ammonia monooxygenase

139 structural gene *amoA* as a functional marker: molecular fine-scale analysis of

140 natural ammonia-oxidizing populations. *Applied and Environmental*

141 *Microbiology*, **63**: 4704-4712.

142 Attard, E.; Poly, F.; Commeaux, C.; Laurent, F.; Terada, A.; Smets, B.F.; Recous, S.;

143 Roux, X. L. 2010. Shifts between *Nitrospira* and *Nitrobacter*-like nitrite oxidizers

144 underlie the response of soil potential nitrite oxidation to changes in tillage

145 practices. *Environmental Microbiology*, **12**: 315-326.

146 Braker, G.; Fesefeldt, A.; Witzel, K. P. 1998. Development of PCR primer systems

147 for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying

148 bacteria in environmental samples. *Applied and Environmental Microbiology*, **64**:

149 3769-3775.

150 Throbäck, I. N.; Enwall, K.; Jarvis, Å.; Hallin, S. 2004. Reassessing PCR primers

151 targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying

152 bacteria with DGGE. *FEMS Microbiology Ecology*, **49**: 401-417.

153 Welsh, A.; Chee-Sanford, J. C.; Connor, L. M.; Loffler, F. E.; Sanford, R. A. 2014.
154 Refined nrfA phylogeny improves PCR-based nrfA gene detection. Applied and
155 Environmental Microbiology, **80**: 2110-2119.