Supplemental Material

for

Flow regulates biological NO_3^- and N_2O production in a turbid sub-tropical stream

by

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submitted to

Geochimica et Cosmochimica Acta

1. Gas transfer velocity

We calculated the gas transfer velocity of N_2O (k_{600}, in m d^{-1}) between the surface water and the air using three empirical parameterisations from (Raymond et al., 2012):

(1) $k_{600} = (v \times S)^{0.89} \times h^{0.54} \times 5037$

(2) $k_{600} = 929 \times (v \times S)^{0.75} \times Q^{0.011}$

(3) $k_{600} = 4725 \times (v \times S)^{0.86} \times Q^{-0.14} \times h^{0.66}$

where v is the current velocity (m s^{-1}), h is the water depth (m), S is the streambed slope (unitless), and Q is discharge (m^3 s^{-1}). These equations were used to calculate the net flux of N_2O between the air and the water column in .

Table S1 Stream gas transfer velocity (k_{600}) and N_2O flux over five 24 hr periods. Fluxes were calculated as both the arithmetic mean and the sum of values measured every 1 min^{-1} for each 24 hr period, based on the mean of the three k_{600} calculations (outlined above) and the measured dissolved N_2O concentrations relative to air.

Sampling date	k_{600} *m d^{-1}*	**mean N_2O flux** *mg N m^{-2} d^{1}*	**total N_2O flux** *mg N m^{-2} d^{1}*
D1 (11/04/2017)	1.0 ± 0.8	0.58 ± 0.8	0.21
D2 (12/04/2017)	0.66 ± 0.3	0.77 ± 0.5	0.67
D4 (14/04/2017)	1.3 ± 0.3	1.9 ± 0.5	1.6
D6 (16/04/2017)	0.82 ± 0.1	1.6 ± 0.5	1.3
D8 (18/04/2017)	0.73 ± 0.3	1.8 0.9	1.6

2. Stream metabolism calculations

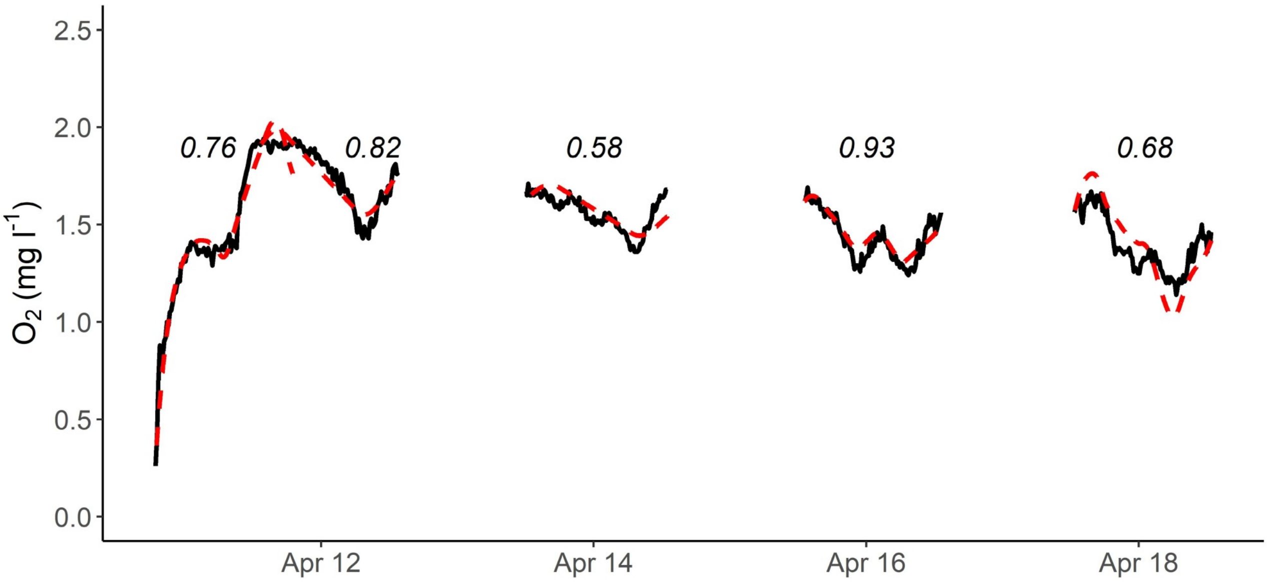

Fig. S1 The measured (black lines) and predicted (red dashed lines) dissolved O_2 concentrations in the surface water at the centre of the sampled reach. Predicted values are based on the best-fit mle solutions in the streamMetabolizer model (Appling et al., 2018). Agreement between measured and modelled values are shown for each 24 h period, based on Kendall correlation analysis.

Table S2 Stream gross primary productivity (GPP) and ecosystem respiration (ER) estimated using the streamMetabolizer R package, using a MLE model for a light saturation effect for GPP and constant ER. Positive fluxes indicate O_2 production, negative fluxes O_2 consumption. The ratio between GPP and ER (P/R) fluxes is also shown.

Sampling date	**GPP** *g O_2 m^{-2} d^{-1}*	**ER** *g O_2 m^{-2} d^{-1}*	**P/R**
D1 (11/04/2017)	2.8	-60 (-65 - -55)	0.05
D2 (12/04/2017)	0.65	-3.3 (-3.3 - -3.3)	0.2
D4 (14/04/2017)	0.32	-2.7 (-2.7 - -2.6)	0.1
D6 (16/04/2017)	0.18	-39 (-38 - -39)	0.005
D8 (18/04/2017)	3.0	-110	0.03

3. Groundwater – surface water mixing calculations

Table S3 The relative contribution of surface water at 20 cm below the streambed surface (f_{sw}) was calculated for each sampled day (D1 – D8) based on the measured conductivity (µS cm^{-1}) in the surface water (*SW*) and hyporheic zone (*HZ*) and range of previously reported local groundwater conductivity (1000 – 5000 µS cm^{-1}).

	D1	D2	D4	D6	D8
Conductivity SW	250	270	300	320	350
Conductivity HZ	680 (50)	610 (100)	650 (80)	990 (100)	1200 (300)
f_{sw} (mean)	0.83	0.86	0.86	0.72	0.62
f_{sw} (high)	0.87	0.92	0.91	0.82	0.82
f_{sw} (low)	0.73	0.75	0.75	0.51	0.24

Table S4 Inputs and outputs from the ^{222}Rn mass balance used to estimate the flux (Q_{GW}) and fraction (f_{GW}) of groundwater in the reach surface water. Losses from radioactive decay (F_{decay}) and water-air evasion (F_{air}) were calculated as dpm $reach^{-1}$, based on h (m) and the stream reach surface area (m^2), using equations from Khadka et al. (2017). Values are reported as mean (min – max) based the minimum and maximum local groundwater ^{222}Rn concentrations for the range of parameters observed over 200 min around daily high (Q_{high}, Rn_{high}) and low (Q_{low}, Rn_{low}) tides.

	D1	D2	D4	D6	D8
F_{decay}	1.5	1.5	1.3	1.4	1.3
F_{air}	57,000	51,000	92,000	76,000	66,000
Q_{low}	0.05 (-0.2 – 0.51)	2.2 (1.9 – 2.6)	5.5 (5.3 – 5.6)	2.0 (1.6 – 2.5)	0.96 (-0.034 – 1.6)
Rn_{low}	4.7 (4.6 – 4.8)	4.1 (4.0 – 4.2)	4.0 (3.9 – 4.0)	4.5 (4.4 – 4.8)	4.1 (4.1 – 4.3)
Q_{high}	3.3 (0.29 – 6.5)	3.3 (3.1 – 3.4)	3.7 (2.5 – 4.7)	2.8 (2.7 – 2.9)	2.3 (1.6 – 3.1)
Rn_{high}	2.9 (1.9 – 3.3)	2.7 (2.1 – 3.2)	3.5 (3.2 – 3.7)	3.8 (3.7 – 3.8)	3.6 (3.6 – 3.7)
Q_{GW}	1.1 (0.25 – 3.0)	1.0 (0.21 – 2.0)	1.9 (0.36 – 3.5)	1.6 (0.33 – 3.2)	1.4 (0.3 – 2.9)
f_{GW}	0.35 ± 0.3	0.32 ± 0.3	0.54 ± 0.4	0.58 ± 0.5	0.61 ± 0.5

4. Additional water chemistry figures and tables

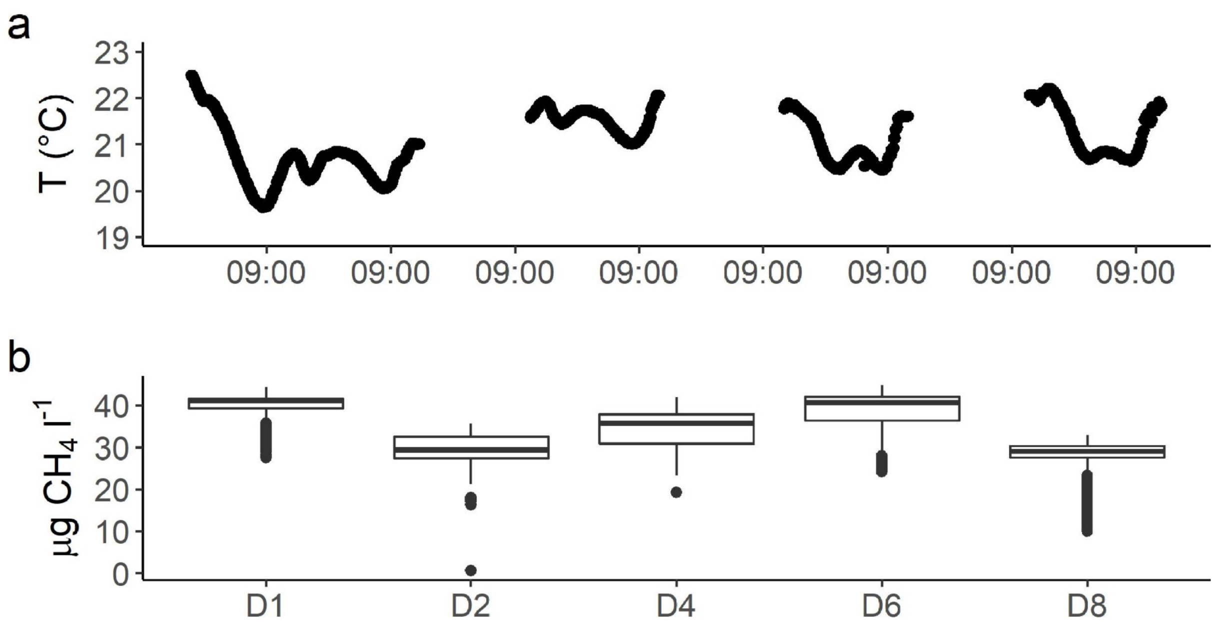

Fig. S2 Changes in surface water temperature (a) and CH_4 (b) over eight days of falling water levels.

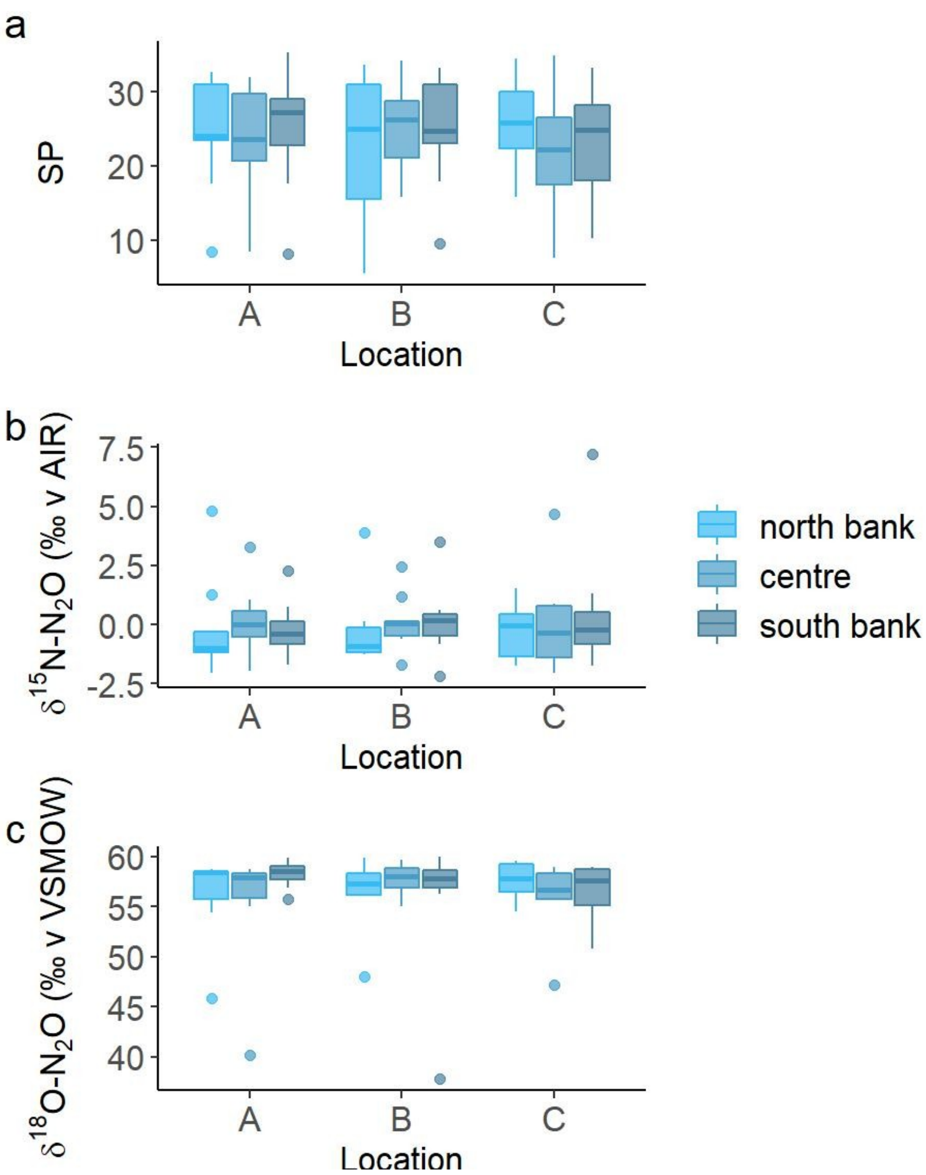

Fig. S3 Differences in the site preference (SP, a), $\delta^{15}N$ (b), and $\delta^{18}O$ (c) composition of surface water N_2O. Boxplots show the mean range of values measured for 10 sampling times over five days. Samples were collected across the width (north bank, centre, and south bank) at three locations along the length of a 50 m stream reach (A, B, C). Values are not corrected for atmospheric mixing.

Table S5 Surface water chemistry, measured at nine locations over the length (A, B, C) and width (north, centre, south) of a stream reach (*SW*). Data shown is the mean (standard deviation) for each sampling location measured 10 times over an eight day period. See Table S8 for statistical tests for the NO_3^- and NH_4^+ data.

Location		NH_4^+ *mg N l⁻¹*	NO_3^- *mg N l⁻¹*	DOC *mg C l⁻¹*	PO_4^{3-} *mg P l⁻¹*
A	North	0.98 (0.8)	1.7 (0.6)	9.4 (2)	0.19 (0.2)
	Centre	0.66 (0.2)	1.7 (0.6)	9.5 (2)	0.18 (0.3)
	South	0.72 (0.03)	1.9 (0.4)	9.5 (1)	0.12 (0.09)
B	North	0.67 (0.07)	1.7 (0.5)	11 (2)	0.10 (0.04)
	Centre	0.69 (0.06)	1.8 (0.5)	9.9 (1)	0.095 (0.01)
	South	0.71 (0.04)	1.9 (0.4)	9.9 (1)	0.091 (0.01)
C	North	0.86 (0.6)	1.8 (0.5)	10 (2)	0.13 (0.1)
	Centre	0.69 (0.05)	1.9 (0.4)	10 (3)	0.094 (0.01)
	South	0.70 (0.04)	1.9 (0.4)	9.9 (1)	0.096 (0.02)

Table S6 Subsurface water chemistry, measured using DET probes inserted to 5 cm below the streambed surface (*S*). Data shown is the mean (standard deviation) for each sampling location measured 10 times over eight days. Note neither DOC nor PO_4^{3-} could be measured in the DET probes.

Location		NH_4^+ *mg N l⁻¹*	NO_3^- *mg N l⁻¹*
A	North	110 (70)	0.32 (0.2)
	Centre	130 (80)	0.23 (0.08)
	South	53 (30)	0.38 (0.2)
B	North	51 (40)	0.42 (0.5)
	Centre	120 (100)	0.51 (0.5)
	South	61 (70)	0.53 (0.3)
C	North	42 (20)	0.31 (0.2)
	Centre	110 (50)	0.38 (0.2)
	South	79 (30)	0.82 (0.5)

Table S7 Subsurface water chemistry, measured in peizometers at 20 cm below the streambed surface (*HZ*). Data shown is the mean (standard deviation) for each sampling location measured 10 times over an eight day period; see Table S8 for statistical analyses of spatial and temporal patterns.

Location		NH_4^+ *mg N l⁻¹*	NO_3^- *mg N l⁻¹*	DOC *mg C l⁻¹*	PO_4^{3-} *mg P l⁻¹*
A	North	4.7 (0.9)	0.047 (0.03)	92 (60)	0.066 (0.05)
	Centre	1.1 (0.8)	0.27 (0.2)	53 (20)	0.073 (0.04)
	South	1.0 (0.3)	1.1 (0.4)	51 (20)	0.21 (0.1)
B	North	1.5 (0.7)	0.17 (0.2)	60 (30)	0.37 (0.2)
	Centre	0.78 (0.08)	1.6 (0.4)	27 (5)	0.099 (0.03)
	South	0.73 (0.05)	1.8 (0.4)	20 (4)	0.097 (0.03)
C	North	0.74 (0.4)	0.14 (0.2)	70 (30)	0.23 (0.2)
	Centre	0.75 (0.07)	1.7 (0.5)	23 (9)	0.17 (0.2)
	South	1.3 (0.6)	1.5 (0.4)	27 (20)	0.26 (0.05)

Table S8 Mixed model results (as Type II Wald F tests and 95% confidence intervals) for changes in NO_3^- concentrations, $\delta^{15}N\text{-}NO_3^-$, and $\delta^{18}O\text{-}NO_3^-$ over time. Sampling locations are treated as repeated measures within each (*SW*, *S*, *HZ*), which were evaluated separately. Specific sample locations are treated as random effects, and time (continuous) and locations, either width-wise (north, centre, south) and length-wise (A, B, C), as fixed factors. **Bold** text indicates significant differences (* = p<0.05, ** = p<0.01, *** = p<0.001).

Depth	Parameter	Time		Width		Time*Width		Length		Time*Length	
SW	NO_3^-	**410*** **	**(7 – 9)**	4.6	(-80 – 400)	1.3	(-3 – 1)	0.60	(-70 – 300)	0.40	(-3 – 1)
	NH_4^+	3.3	(-5 - -1)	1.7	(-700 - -90)	**3.5***	**(0.3 – 6)**	0.54	(-500 – 300)	0.86	(-3 – 5)
	$\delta^{18}O\text{-}NO_3^-$	**24*** **	**(-0.05 - -0.1)**	0.18	(-3 – 2)	0.39	(-0.01 – 0.03)	0.48	(-2 – 3)	0.77	(-0.03 – 0.02)
	$\delta^{15}N\text{-}NO_3^-$	**93*** **	**(0.008, 0.02)**	0.75	(-0.08 – 0.9)	0.79	(-0.006 – 0.004)	1.9	(-1 – 0.3)	0.62	(-0.005 – 0.005)
S	NO_3^-	1.9	(-2 – 2)	2.2	(-200 – 700)	0.83	(-5 – 2)	1.2	(-10 – 600)	0.78	(-5 – 3)
	NH_4^+	3.3	(-5 - -1)	1.7	(-700 - -90)	**3.5***	**(0.3 – 7)**	0.54	(-500 – 300)	0.86	(-3 – 5)
	$\delta^{18}O\text{-}NO_3^-$	0.43	(-0.02 – 0.03)	0.91	(-6 – 6)	**2.8***	**(-0.08 – -0.004)**	0.32	(-8 – 3)	0.82	(-0.02 – 0.06)
	$\delta^{15}N\text{-}NO_3^-$	**9.6** **	**(-0.02 – 0.04)**	1.1	(-8 – 6)	2.3	(-0.03 – 0.1)	0.47	(-4 – 8)	0.71	(-0.08 – 0.02)
HZ	NO_3^-	**17*** **	**(-3 – 0.2)**	**5.8***	**(200 – 1000)**	**12*** **	**(3 – 8)**	0.78	(-800 – 2000)	**4.98** **	**(1 – 6)**
	NH_4^+	**24*** **	**(4 – 10)**	1.3	(-3000 – 800)	2.6	(-8 – 0.1)	1.0	(-3000 – 800)	1.8	(-8 – 0.7)
	$\delta^{18}O\text{-}NO_3^-$	1.1	(-0.005 – 0.06)	**5.4***	**(-8 – 3)**	1.6	(-0.08 – 0.03)	0.31	(-7 – 5)	0.11	(-0.05 – 0.04)
	$\delta^{15}N\text{-}NO_3^-$	0.33	(-0.07 – -0.02)	4.5	(-11 – -3)	**5.0** **	**(0.02 – 0.1)**	1.0	(-10 – 0.8)	1.9	(-0.02 – 0.08)

References

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