

SUPPLEMENTARY INFORMATION for

Denitrification rates in lake sediments of mountains affected by high atmospheric nitrogen deposition

Carlos Palacin-Lizarbe¹, Lluís Camarero², Sara Hallin³, Christopher M Jones³, and Jordi Catalan^{1,4}

¹CREAF, Campus UAB, Cerdanyola del Vallès, Spain, ²Center for Advanced Studies of Blanes, (CEAB–CSIC), Girona, Spain,

³Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Uppsala, Sweden, ⁴CSIC, Cerdanyola del Vallès, Spain

Current file content:

- **Table S1.** Sediment and water descriptors by habitat.
- **Table S2.** Actual (r_a , no nitrate added) and potential (r_p , 28 μ M nitrate added) denitrification rates in mountain lake sediments.
- **Table S3.** Multiple linear regression models relating the actual and potential denitrification rates to several types of explanatory variables.

Table S1. Sediment and water descriptors by habitat.

			Sediment habitat ^a					
			All	R	C	I	D	E
Temperature ^b	° C	mean	9.1	8.4	9.8	16.5	7.4	16.4
		SD	5.3	4.3	6.5	0.8	4.5	0.4
		min	0.8	0.8	0.8	15.0	3.6	16.2
		max	17.5	12.9	16.2	17.5	16.1	16.9
[NO ₃ ⁻] ^b	μM	mean	5	9	6	0	5	0
		SD	6	7	6	0	6	0
		min	0	2	0	0	0	0
		max	17	16	13	0	17	0
[NO ₂ ⁻] ^b	μM	mean	0.10	0.15	0.09	0.06	0.10	0.04
		SD	0.06	0.07	0.04	0.04	0.06	0.02
		min	0.02	0.07	0.04	0.03	0.03	0.02
		max	0.27	0.21	0.15	0.14	0.27	0.06
[NH ₄ ⁺] ^b	μM	mean	5.6	5.6	8.8	1.0	6.3	1.0
		SD	9.4	9.5	9.9	0.1	10.2	0.2
		min	0.8	1.0	1.2	0.8	1.0	0.8
		max	51.3	25.0	25.0	1.2	51.3	1.2
[SO ₄ ²⁻] ^b	μM	mean	14	18	24	10	14	11
		SD	7	10	15	1	5	0
		min	6	6	7	9	6	10
		max	40	32	40	13	27	11
DOC ^b	mg * L ⁻¹	mean	66	52	119	94	58	57
		SD	53	32	108	33	48	5
		min	2	8	2	60	6	52
		max	301	86	233	155	301	62
z (water column depth) ^{b, c}	m	mean	17.4	0.5	0.5	0.5	25.3	0.5
		SD	22.8	0.0	0.0	0.0	23.9	0.0
		min	0.5	0.5	0.5	0.5	4.0	0.5
		max	72.0	0.5	0.5	0.5	72.0	0.5
Organic matter ^d	%	mean	32	6	29	50	32	49
		SD	14	5	18	12	9	6
		min	1	1	6	30	17	42
		max	66	14	48	66	48	54
Nitrogen (N) ^d	%	mean	1.39	0.20	1.10	1.82	1.46	1.99
		SD	0.59	0.21	0.81	0.41	0.42	0.30
		min	0.02	0.02	0.08	1.21	0.76	1.75
		max	2.44	0.57	1.93	2.44	2.34	2.32
δ ¹⁵ N ^d	‰	mean	-0.92	1.37	-0.32	0.02	-1.46	-0.68
		SD	1.73	1.75	0.71	0.89	1.65	0.81
		min	-4.36	-1.10	-1.09	-1.14	-4.36	-1.52
		max	3.52	3.52	0.45	1.42	1.93	0.09
Carbon (C) ^d	%	mean	14.24	2.73	13.54	21.83	14.09	20.51
		SD	6.48	2.54	8.99	5.45	4.42	2.26
		min	0.20	0.20	2.01	14.05	7.47	18.43
		max	29.35	6.02	22.96	29.35	24.18	22.91
δ ¹³ C ^d	‰	mean	-24.45	-23.52	-23.44	-20.49	-25.70	-19.02
		SD	3.53	2.59	3.82	2.68	2.96	1.07
		min	-31.73	-27.54	-26.61	-24.36	-31.73	-20.10
		max	-17.47	-20.11	-18.62	-17.47	-21.26	-17.96
C/N ^d	a/a	mean	12.49	16.06	17.53	14.08	11.25	12.09
		SD	3.10	4.32	6.75	2.30	0.99	0.50
		min	9.38	12.35	13.52	11.64	9.38	11.52
		max	29.52	22.82	29.52	18.17	15.21	12.45
Dry weight / wet weight ^d		mean	0.13	0.56	0.23	0.08	0.08	0.08
		SD	0.17	0.29	0.24	0.04	0.03	0.01
		min	0.02	0.25	0.09	0.05	0.02	0.08
		max	0.88	0.88	0.65	0.18	0.14	0.09
Sediment density ^d	g * cm ⁻³	mean	1.55	2.21	1.72	0.83	1.60	0.99
		SD	0.47	0.45	0.48	0.37	0.29	0.24
		min	0.45	1.59	1.29	0.45	1.10	0.84
		max	2.64	2.64	2.48	1.41	2.49	1.27
Sediment grain size ^d	μm	mean	328	438	282	361	308	394
		SD	90	130	135	107	63	58
		min	130	288	130	182	197	328
		max	627	627	433	511	440	435

			Sediment habitat ^a					
			All	R	C	I	D	E
[DNA] ^e	ng * m ⁻²	mean	1.6E+08	2.5E+08	1.3E+08	9.5E+07	1.6E+08	1.2E+08
		SD	9.8E+07	2.3E+08	5.3E+07	5.2E+07	7.6E+07	1.8E+07
		min	9.9E+05	4.3E+07	6.8E+07	5.1E+07	9.9E+05	1.0E+08
		max	7.0E+08	7.0E+08	2.0E+08	1.8E+08	3.3E+08	1.4E+08
[16S] ^e	copies * m ⁻²	mean	4.5E+13	5.9E+13	4.2E+13	2.2E+13	4.9E+13	3.0E+13
		SD	3.5E+13	3.8E+13	1.2E+13	1.1E+13	3.9E+13	2.8E+12
		min	3.2E+11	1.3E+13	2.6E+13	1.0E+13	3.2E+11	2.6E+13
		max	2.1E+14	1.1E+14	5.3E+13	3.8E+13	2.1E+14	3.1E+13
[nirS] ^e	copies * m ⁻²	mean	3.3E+12	1.0E+12	1.7E+12	3.1E+12	3.8E+12	4.5E+12
		SD	3.2E+12	6.4E+11	1.7E+12	3.5E+12	3.5E+12	6.0E+11
		min	1.6E+10	2.7E+11	4.4E+11	6.8E+11	1.6E+10	4.0E+12
		max	1.4E+13	1.8E+12	4.7E+12	1.1E+13	1.4E+13	5.1E+12
[nirK] ^e	copies * m ⁻²	mean	1.4E+11	3.7E+11	1.4E+11	6.4E+10	1.2E+11	9.1E+10
		SD	1.8E+11	4.2E+11	7.3E+10	3.9E+10	1.4E+11	1.4E+10
		min	7.8E+08	5.9E+10	5.1E+10	3.2E+10	7.8E+08	7.7E+10
		max	1.2E+12	1.2E+12	2.5E+11	1.5E+11	8.8E+11	1.1E+11
[nosZ1] ^e	copies * m ⁻²	mean	1.2E+11	4.2E+11	6.6E+10	5.7E+10	9.5E+10	5.2E+10
		SD	1.9E+11	5.6E+11	3.9E+10	4.9E+10	8.8E+10	1.5E+10
		min	4.4E+08	4.6E+10	3.1E+10	1.3E+10	4.4E+08	4.2E+10
		max	1.5E+12	1.5E+12	1.3E+11	1.4E+11	4.2E+11	6.9E+10
[nosZ2] ^e	copies * m ⁻²	mean	4.5E+10	1.1E+11	1.8E+10	3.0E+10	4.3E+10	3.1E+10
		SD	6.5E+10	1.8E+11	1.6E+10	2.8E+10	4.1E+10	1.3E+11
		min	1.8E+08	4.8E+09	1.0E+10	3.6E+09	1.8E+08	1.8E+10
		max	4.8E+11	4.8E+11	4.6E+10	7.7E+10	2.0E+11	4.4E+10

^a Habitats: littoral sediments from rocky areas (R), helophyte (*Carex rostrata*) belts (C), beds of isoetids (I) and elodeid (E) macrophytes, and non-vegetated deep (D) sediments.

^b Water (overlying the sediment core) and sediment (^d abiotic or ^e molecular) descriptors used for modelling denitrification rates. Sediment molecular data used in this study is a subset of data previously published in Palacin-Lizarbe, et al. (2019) *Front Microbiol*. DNA and gene abundances expressed in ng DNA m⁻² or gen copies m⁻².

^c Water column depth coded as 0.5 m for littoral sediments.

^f Dry weight (DW).

Table S2. Actual (r_a , no nitrate added) and potential (r_p , 28 μM nitrate added) denitrification rates in mountain lake sediments.

		Sediment habitat ^a					
		All	R	C	I	D	E
Actual denitrification rates (r_a) ($\mu\text{mol N}_2\text{O m}^{-2} \text{ h}^{-1}$)	mean	1.5	3.0	2.6	2.4	1.0	0.7
	SD	1.6	2.3	2.1	2.6	1.0	1.0
	min	0.0	0.0	0.0	0.0	0.0	0.0
	max	9.1	9.1	6.4	8.0	4.1	1.9
Potential denitrification rates (r_p) ($\mu\text{mol N}_2\text{O m}^{-2} \text{ h}^{-1}$)	mean	14.6	17.9	22.2	22.8	12.3	22.1
	SD	10.2	12.9	7.5	7.9	9.6	8.8
	min	1.4	3.2	11.6	14.1	1.4	12.3
	max	45.8	37.6	28.0	38.8	45.8	33.1

^a Studied sediment habitats sorted by the mean r_a : littoral sediments from rocky areas (R), helophyte (*Carex rostrata*) belts (C), beds of isoetids (I) and elodeid (E) macrophytes, and non-vegetated deep (D) sediments.

Table S3. Multiple linear regression models relating the actual (r_a , models 1-5) and potential (r_p , 28 μ M nitrate added, models 6-12) denitrification rates to several types of explanatory variables.

Model	Explanatory var	Formula: Fixed part	Random part	AICc (gls)	Fixed R ²	Global R ²
Actual denitrification rates (n = 69)						
lake		$\sqrt{r_a} = 0.05$	$\sim 1 \text{lake}$	202	0	0.24
habitat		$\sqrt{r_a} = 0.31$	$\sim 1 \text{habitat}$	195	0	0.22
1a	Sediment (molecular)	$\sqrt{r_a} = 0.62 \times \log_{10}(\text{nosZII}) - 0.48 \times \sqrt{\text{nirS}} - 0.32 \times \sqrt{\text{DNA}}$	—	192 (201)	0.18	0.18
1b	Sediment (molecular)	$\sqrt{r_a} = 0.49 \times \log_{10}(\text{nosZI}) - 0.32 \times \sqrt{\text{DNA}} - 0.29 \times \sqrt{\text{nirS}}$	—	192 (202)	0.17	0.17
2a	Sediment (abiotic)	$\sqrt{r_a} = -1.16 \times \text{N} + 0.85 \times \text{C}$	—	188 (193)	0.20	0.20
2b	Sediment (abiotic)	$\sqrt{r_a} = 0.25 - 0.35 \times \text{N}$	$\sim 1 \text{habitat}$	193	0.11	0.28
2c	Sediment (abiotic)	$\sqrt{r_a} = 0.32 - 0.33 \times \text{OM}$	$\sim 1 \text{habitat}$	194	0.10	0.28
2d	Sediment (abiotic)	$\sqrt{r_a} = 0.31 - 0.30 \times \text{C}$	$\sim 1 \text{habitat}$	195	0.08	0.27
3a	Water (abiotic)	$\sqrt{r_a} = 0.61 \times \text{NO}_3^- + 0.46 \times \text{Temperature}$	—	185 (192)	0.24	0.24
3b	Water (abiotic)	$\sqrt{r_a} = 0.06 + 0.71 \times \text{NO}_3^- + 0.57 \times \text{Temperature}$	$\sim 1 \text{lake}$	193	0.28	0.40
3c	Water (abiotic)	$\sqrt{r_a} = 0.09 + 0.90 \times \text{NO}_3^- + 0.59 \times \text{Temperature}$	$\sim 1 + \text{NO}_3^- \text{lake}$	193	0.38	0.48
3d	Water (abiotic)	$\sqrt{r_a} = 0.34 + 0.37 \times \text{NO}_3^-$	$\sim 1 \text{habitat}$	189	0.12	0.33
3e	Water (abiotic)	$\sqrt{r_a} = 0.23 + 0.53 \times \text{NO}_3^- + 0.29 \times \text{Temperature} \star$	$\sim 1 \text{habitat}$	190	0.16	0.35
4	Landscape	$\sqrt{r_a} = 0.30 \times \log_{10}(\text{Catchment})$	—	195	0.08	0.08
5a	All	$\sqrt{r_a} = 0.58 \times \text{Temperature} + 0.37 \times \text{NO}_3^- - 0.29 \times \text{N} + 0.26 \times \log_{10}(\text{Catchment}) - 0.24 \times \sqrt{\text{DNA}}$	—	177 (192)	0.38	0.38
5b	All	$\sqrt{r_a} = 0.51 \times \text{Temperature} + 0.47 \times \text{NO}_3^- - 0.31 \times \text{N} - 0.27 \times \sqrt{\text{DNA}}$	—	179 (192)	0.35	0.35
5c	All	$\sqrt{r_a} = 0.10 + 0.93 \times \text{NO}_3^- + 0.71 \times \text{Temperature} - 0.27 \times \sqrt{\text{nirS}}$	$\sim 1 + \text{NO}_3^- \text{lake}$	193	0.44	0.54
5d	All	$\sqrt{r_a} = 0.09 + 0.96 \times \text{NO}_3^- + 0.63 \times \text{Temperature} - 0.25 \times \sqrt{\text{DNA}}$	$\sim 1 + \text{NO}_3^- \text{lake}$	193	0.43	0.53
5e	All (+ habitats)	$\sqrt{r_a} = -0.12 + 0.80 \times \text{Carex} + 0.78 \times \text{Rocky} + 0.56 \times \text{NO}_3^- + 0.43 \times \text{Temperature} - 0.27 \times \sqrt{\text{DNA}}$	—	179	0.37	0.37

Model	Explanatory var	Formula: Fixed part	Random part	AICc (gls)	Fixed R ²	Global R ²
		Potential denitrification rates (n = 52)				
lake		$\sqrt{r_p} = -0.13$	~1 lake	110	0	0.77
habitat		$\sqrt{r_p} = 0.24$	~1 habitat	152	0	0.20
6a	Sediment (molecular)	$\sqrt{r_p} = -0.52 \times \log_{10}(nosZI) + 0.50 \times \sqrt{nirS}$	—	139 (146)	0.26	0.26
6b	Sediment (molecular)	$\sqrt{r_p} = -0.06 + 0.30 \times \sqrt{nirS} - 0.27 \times \log_{10}(nosZI)$	~1 lake	111	0.08	0.79
6c	Sediment (molecular)	$\sqrt{r_p} = 0.42 + 0.68 \times \sqrt{nirS} - 0.65 \times \log_{10}(nosZI)$	~1 habitat	136	0.35	0.55
7a	Sediment (abiotic)	$\sqrt{r_p} = 0.49 \times C + 0.43 \times \delta^{15}N$	—	124 (132)	0.46	0.46
7b	Sediment (abiotic)	$\sqrt{r_p} = 0.48 \times OM + 0.43 \times \delta^{15}N$	—	125	0.45	0.45
7c	Sediment (abiotic)	$\sqrt{r_p} = 0.49 \times \delta^{15}N + 0.48 \times N$	—	125	0.44	0.44
8	Water (abiotic)	$\sqrt{r_p} = 0.48 \times \text{Temperature} + 0.48 \times \log_{10}(SO_4^{2-}) - 0.43 \times NO_3^-$	—	115	0.53	0.53
9a	Landscape	$\sqrt{r_p} = -0.75 \times \text{Altitude} - 0.65 \times \log_{10}(\text{Catchment})$	—	117 (125)	0.52	0.52
9b	Landscape	$\sqrt{r_p} = -0.03 - 0.81 \times \text{Altitude} - 0.63 \times \log_{10}(\text{Catchment}) \star$	~1 lake	110	0.44	0.77
10a	All	$\sqrt{r_p} = -0.77 \times \log_{10}(nosZI) + 0.69 \times \text{Temperature} + 0.64 \times \sqrt{nirS} - 0.47 \times N + 0.34 \times \log_{10}(SO_4^{2-}) - 0.33 \times \text{Altitude}$	—	90 (112)	0.77	0.77
10b	All (+ habitats)	$\sqrt{r_p} = -0.14 - 0.77 \times \log_{10}(nosZI) + 0.77 \times \text{Carex} + 0.68 \times \text{Isoetes} + 0.68 \times \text{Temperature} + 0.66 \times \sqrt{nirS} - 0.54 \times OM + 0.36 \times \log_{10}(SO_4^{2-}) - 0.30 \times \text{Altitude}$	—	90 (114)	0.79	0.79
10c	All	$\sqrt{r_p} = 0.08 + 0.52 \times \text{Temperature} - 0.50 \times \text{Altitude} - 0.46 \times \log_{10}(nosZI) + 0.41 \times \sqrt{nirS}$	~1 lake	106	0.61	0.88
11	All (but molecular)	$\sqrt{r_p} = -0.99 \times \log_{10}(\text{Catchment}) - 0.77 \times \text{Altitude} + 0.51 \times \log_{10}(SO_4^{2-})$	—	100	0.65	0.65
12	All (but landscape)	$\sqrt{r_p} = -0.73 \times \log_{10}(nosZI) + 0.72 \times \text{Temperature} + 0.57 \times \sqrt{nirS} + 0.45 \times \log_{10}(SO_4^{2-}) - 0.33 \times N$	—	103	0.69	0.69

Note: Models with all components significant and with a ΔAICc value ≤ 2 for each set of explanatory variables are shown. Models with lake/habitat effect in the random part are shown when are similar or improve the fixed models. The intercept of all models was always not significant (p>0.05); they were not shown when having negligible values (< 1 × 10⁻¹⁵). In bold are indicated the best models, i.e. trade-off between being simple and explicative (AICc and ANOVA test). The star “★” indicate model components with p-value higher than 0.05, occurring in models 3e (p=0.058) and 9b (p=0.051).