Isotopic evidence for alteration of nitrous oxide emissions and producing pathways contribution under nitrifying conditions

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Supplementary material description

Six pages of supplementary material containing one text, two tables and two figures.

Text S1

The experiments that tested the influence of ammonium concentrations on ammonium oxidation and nitrous oxide emissions also supported the nitrifying activity of the reactor. During these experiments, decreases in [NH_4^+], increases in [NO_2^-] and [NO_3^-] were observed, while pH remaining below 8 prevented any relevant loss of ammonium by volatilization. For example, [NH_4^+] decreased from 6.2 to 1.1, from 28.6 to 17 and from 62.1 to 49.1 mg N L-1 by flowing through the nitrifying biomass. At the same time, [NO_2^-] and [NO_3^-] increased from 0 to 0.2-0.3 mg N L-1 and from 1.4-1.8 to 5-10 mg N L-1, respectively.

23 **Table S1. Detailed conditions of oxygenation, temperature and concentration tests.**

inflow [NH_4^+]	**inflow gas rate**	**O_2 in gas mix**	**temperature**
mg N L^{-1}	*L min^{-1}*	*%*	*°C*
	oxygenation tests		
28.6 ±0.5	0.5	21	19.6 ±0.1
20.2 ±0.5	0.5	21	19.5 ±0.1
37.3 ±0.6	0.5	10.5	20.6 ±0.1
37.3 ±0.6	0.5	16.8	20.6 ±0.1
37.3 ±0.6	0.5	4.2	20.5 ±0.1
25.1 ±0.5	0.57	21	19.6 ±0.5
25.1 ±0.5	0.4	0	19.2 ±0.1
25.1 ±0.5	0.53	4.2	19.2 ±0.1
25.1 ±0.5	0.51	10.5	19.2 ±0.1
25.1 ±0.5	0.5	16.8	19.3 ±0.1
23.8 ±0.6	0.53	4.2	19.9 ±0.1
23.8 ±0.6	0.51	10.5	20.2 ±0.1
23.8 ±0.6	0.5	16.8	20.1 ±0.1
	temperature tests		
21.1 ±n.a.	0.5	21	20.3 ±0.1
21.1 ±n.a.	0.5	21	16.2 ±0.1
21.1 ±n.a.	0.5	21	15.5 ±0.1
20.3 ±0.3	0.5	21	22.3 ±0.1
20.3 ±0.3	0.5	21	18.2 ±0.1
20.3 ±0.3	0.5	21	13.5 ±0.2
	NH_4^+ concentration tests		
6.2 ±0.1	0.5	21	19.6 ±0.0
28.6 ±0.5	0.5	21	19.6 ±0.1
62.1 ±0.4	0.5	21	19.8 ±0.0
56.1 ±0.3	0.5	21	19.0 ±0.1
42.9	0.5	21	19.0 ±0.0
42.7 ±1.0	0.5	21	19.3 ±0.0
20.2 ±0.5	0.5	21	19.5 ±0.1

n.a. data not available

24

Table S2. Inflow and outflow ammonium, nitrite and nitrate concentrations measured during the ammonium concentration tests.

$[NH_4^+]$ *(mg N L^{-1})*		**$[NO_2^-]$** *(mg N L^{-1})*		**$[NO_3^-]$** *(mg N L^{-1})*	
inflow	outflow	inflow	outflow	inflow	outflow
6.2 ±0.1	1.1 ±0.2	0	0.2 ±0.1	1.8	5.1 ±0.3
28.6 ±0.5	17 ±0.8	0	0.3 ±0.1	1.4	9.3 ±0.7
62.1 ±0.4	49.1 ±5.9	0	0.3 ±0.3	1.4	10 ±0.2
56.1 ±0.3	37.8 ±2	0.1 ±0.1	0.3 ±0	4.5 ±0.1	17.9 ±1.4
42.9	30.4	0.1	0.3	1.4	13.5
42.7 ±1.0	27.1 ±1.6	1.3	0.2 ±0.1	4.9 ±1.6	14 ±0
20.2 ±0.5	11.5 ±0.2	0.9 ±0.2	0.6 ±0.1	3.1 ±0.4	9.6 ±0.1

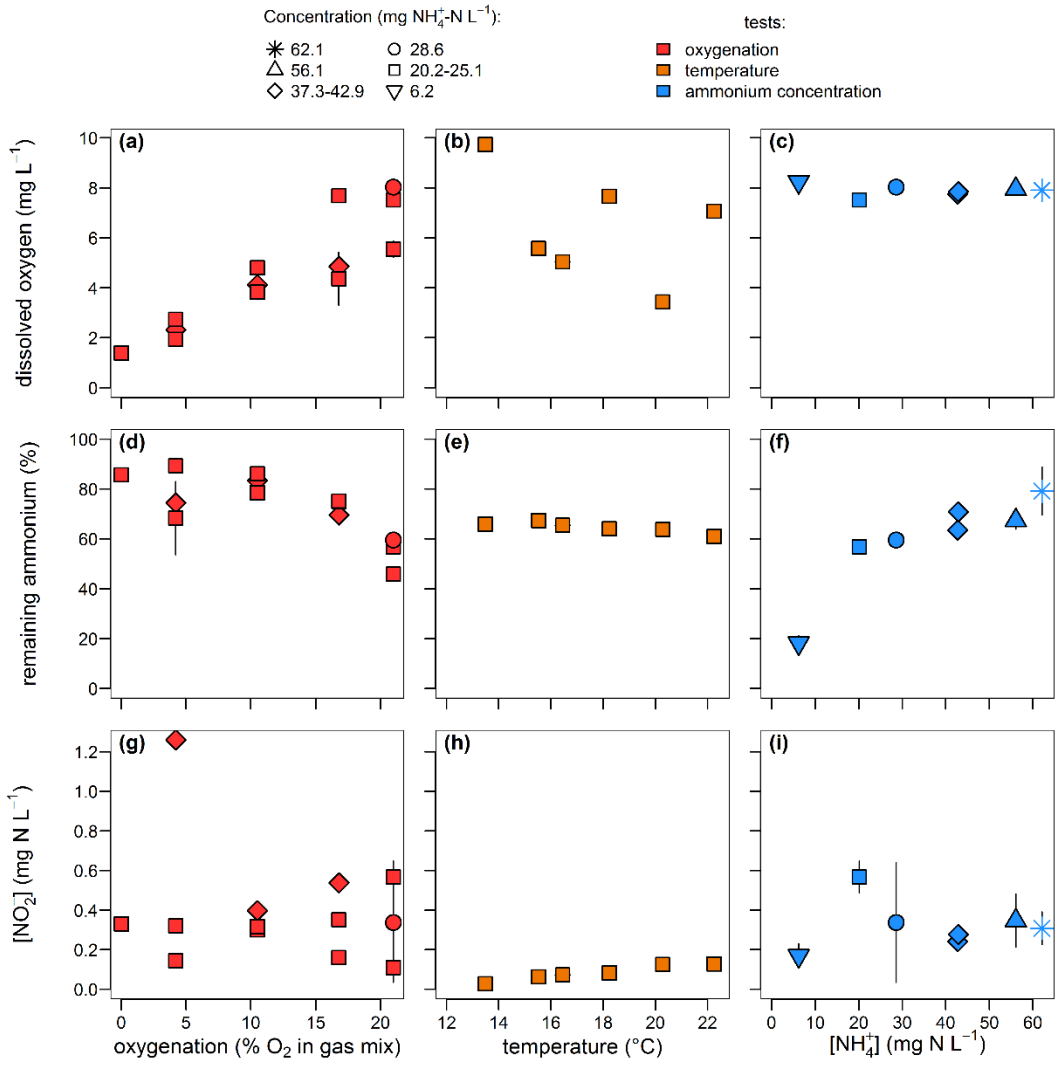

Figure S1. Effect of oxygenation, temperature and ammonium concentration on (a-c) dissolved oxygen concentration, (d-f) the remaining ammonium, and (g-i) the nitrite concentration.

28

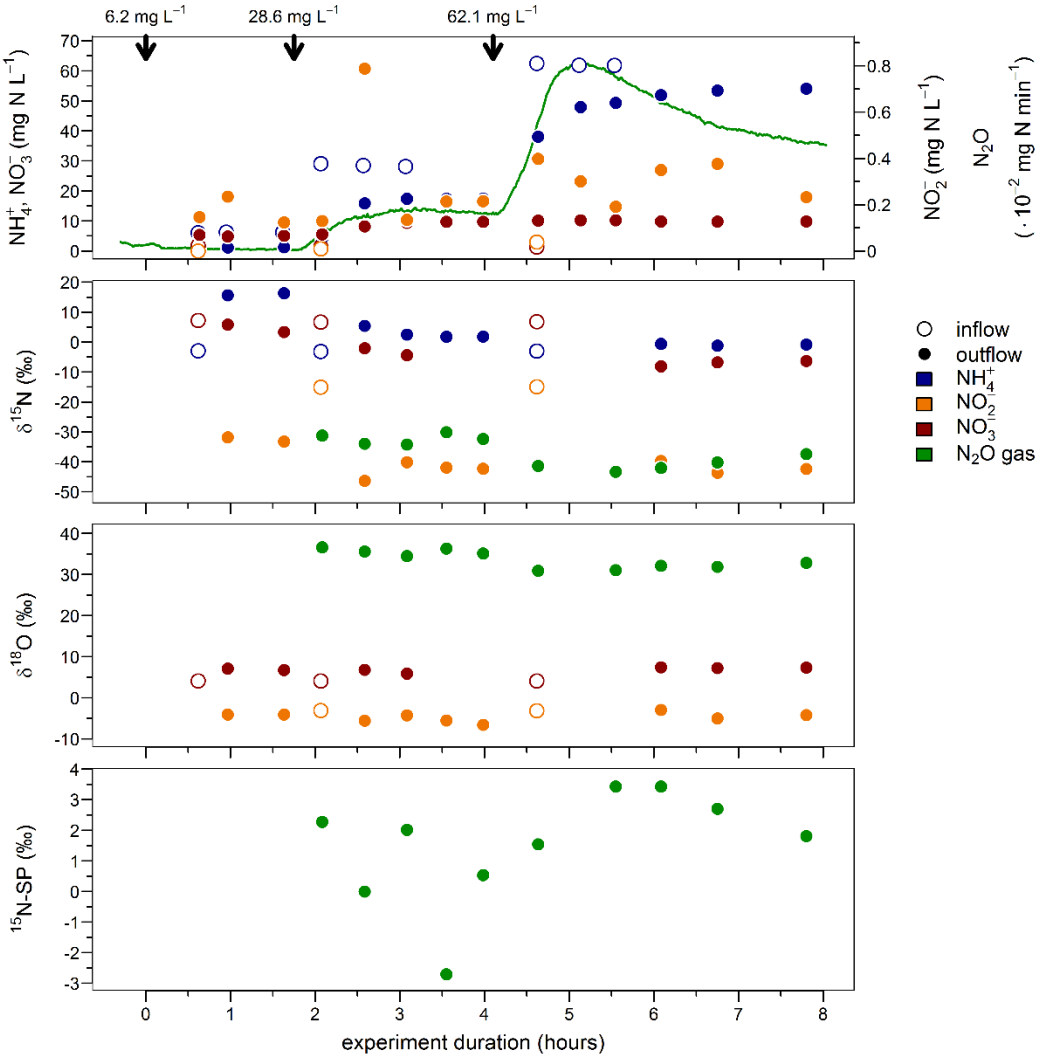

Figure S2. Concentrations, and nitrogen and oxygen isotope ratios of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and nitrous oxide (N_2O) measured during the increasing ammonium concentration experiment. Nitrogen isotopomer site-preference (^{15}N-SP) was also calculated.

29