APPENDICES

Appendix A. Climatic Conditions at Esholt

The climatic conditions for the experimental timeframe are reported in Table A1. Data are provided from the Met Office UK weather station located in Bradford, West Yorkshire (Location 4149E 4352N), and from readings taken in situ.

Table A1. Climatic conditions at Esholt

Month	Net evaporation, mm	Rainfall, Mm	Temperature, °C						Sun hours
			Air			Water			
			Mean	Max.	Min	Mean	Max.	Min	
OCT-04	71.6	122.2	9.5	12.9	7.1	9.2	11.8	7.0	93.5
NOV-04	-12.0	35.3	6.9	10.0	4.9	7.2	10.0	4.3	44.8
DEC-04	3.5	49.9	4.8	8.2	2.6	4.9	7.0	3.0	51.3
JAN-05	21.4	84.8	5.2	8.8	3.0	4.9	7.5	3.0	34.8
FEB-05	2.9	51.6	3.8	6.7	1.9	4.9	7.8	2.8	69.0
MAR-05	1.6	22.9	6.4	9.7	4.1	4.5	5.5	3.5	65.1
APR-05	24.6	96.7	7.8	12.4	4.4	9.7	11.0	8.5	130.2
MAY-05	-12.1	37.8	10.3	15.2	6.6	14.6	16.0	13.3	208.7
JUN-05	-14.7	35.8	14.3	19.3	10.7	15.7	19.5	11.8	172.7
JUL-05	7.8	65.2	15.6	20.3	12.5	16.7	20.5	14.3	154.9
AUG-05	3.6	47.5	15.1	20.1	11.6	15.4	16.5	13.8	168.6
SEP-05	16.8	66.4	13.9	18.3	10.6	15.1	17.3	13.0	134.4
OCT-05	47.7	103.8	11.9	15.1	9.9	12.1	14.5	8.5	52.7
NOV-05	7.0	59.8	5.6	9.4	2.9	3.7	5.5	3.3	94.4
DEC-05	9.1	53.8	3.9	7.2	1.3	4.0	6.0	3.0	41.4
JAN-06	-30.6	16.3	3.6	6.3	1.9	4.0	6.0	2.8	29.0
FEB-06	-0.9	40.3	3.5	6.5	1.5	3.8	5.5	3.0	71.3
MAR-06	75.2	113.2	3.7	7.4	1.1	4.0	6.0	3.0	87.2
APR-06	2.3	52.5	7.4	11.5	4.6	5.9	8.5	3.8	160.9
MAY-06	14.2	62.8	10.9	17.4	8.7	12.3	15.3	10.0	163.0
JUN-06	-99.0	11.6	14.8	20.4	11.0	15.7	20.3	11.5	164.4
JUL-06	-83.1	29.5	18.3	24.8	13.6	18.9	21.8	15.0	265.4
AUG-06	-16.5	92.7	15.0	19.1	12.3	15.8	17.8	14.0	121.4
SEP-06	30.4	82.7	15.6	19.6	12.3	15.1	17.3	13.0	131.6
OCT-06	43.1	89.6	11.8	15.4	9.3	12.1	14.5	8.5	89.8
NOV-06	70.4	78.6	7.3	11.0	4.8	5.9	8.5	3.8	85.7
DEC-06	95.8	133.5	5.4	8.5	4.1	4.7	7.8	2.5	42.9
JAN-07	85.6	142.4	5.9	9.3	3.8	4.8	7.0	3.0	58.5
FEB-07	72.2	103.4	5.0	8.6	2.9	4.9	7.3	2.8	80.3
MAR-07	-1.2	49.4	6.3	10.3	3.5	6.5	8.8	3.8	125.9
APR-07	-41.9	4.2	10.1	15.1	6.5	10.0	13.0	6.5	194.8
MAY-07	28.7	68.8	10.8	15.3	7.8	14.6	16.0	13.3	130.5

Appendix B. Water Quality Statistics in the Maturation Ponds M1and M2 from Data Collected Over the Experimental Timeframe

Table B1. M1 influent

Parameter	Mean	S.D.	S.E.	95-percentile
Ammonium, mg N/l	5.9	4.1	±0.5	14.0
Alkalinity, mg $CaCO_3$/l	105	36	±5	160
Bacteriochlorophyll, mg/l	46	95	±12	212
BOD_5, mg/l	33	20	±3	78
BOD_5 (filtered), mg/l	10	6	±1	18
Chlorophyll-*a*, µg/l	388	407	±50	1276
COD, mg/l	112	59	±7	218
COD (filtered), mg/l	49	22	±3	87
E, coli, cfu/100 ml	8.81E+04	1.14E+05	±1.65E+04	3.19E+05
Nitrite, mg N/l	ND	ND	ND	ND
Nitrate, mg N/l	0.09	0.08	±0.02	0.18
SS, mg/l	45	31	±4	101
TKN, mg N/l	11.6	5.4	±0.6	19.3
TKN (filtered), mg N/l	7.8	4.3	±0.5	15.3
VSS, mg/l	41	31	±4	97

Key: S.D. = standard deviation; S.E. = standard error; ND= no detectable (< 0.03 mg N/l)
Number of samples = 70

Table B2. M1 effluent

Parameter	Mean	S.D.	S.E.	95-percentile
Ammonium, mg N/l	3.5	3.2	±0.4	9.4
Alkalinity, mg CaCO3/l	92	25	±3	132
Bacteriochlorophyll, mg/l	31	41	±5	104
BOD5, mg/l	25	16	±2	53
BOD5 (filtered), mg/l	5	2	±0.5	10
Chlorophyll-*a*, µg/l	397	425	±52	1270
COD, mg/l	102	62	±8	221
COD (filtered), mg/l	39	17	±2	66
E. coli, cfu/100 ml	5.46E+03	9.02E+03	±1.30E+03	2.38E+04
Nitrite, mg N/l	ND	ND	ND	ND
Nitrate, mg N/l	0.11	0.09	±0.02	0.24
SS, mg/l	50	44	±5	139
TKN, mg N/l	9.5	4.6	±0.5	17.5
TKN (filtered), mg N/l	5.4	3.4	±0.4	11.6
VSS, mg/l	52	42	±6	130

Key: S.D. = standard deviation; S.E. = standard error; ND= no detectable (< 0.03 mg N/l)
Number of samples = 70

Table B3. M2 effluent

Parameter	Mean	S.D.	S.E.	95-percentile
Ammonium, mg N/l	2.9	2.6	±0.3	8.0
Alkalinity, mg $CaCO_3$/l	88	22	±3	124
Bacteriochlorophyll, mg/l	36	58	±7	180
BOD_5, mg/l	23	17	±2	62
BOD_5 (filtered), mg/l	5	4	±1	13
Chlorophyll-*a*, µg/l	330	431	±53	1178
COD, mg/l	95	65	±8	211
COD (filtered), mg/l	44	28	±3	105
E. coli, cfu/100 ml	8.20E+02	1.29E+03	±1.87E+02	3.58E+03
Nitrite, mg N/l	ND	ND	±ND	ND
Nitrate, mg N/l	0.25	0.37	±0.07	0.96
SS, mg/l	40	38	±4	115
TKN, mg N/l	8.1	3.8	±0.5	15.5
TKN (filtered), mg N/l	4.7	2.5	±0.3	9.0
VSS, mg/l	41	35	±5	114

Key: S.D. = standard deviation; S.E. = standard error; ND= no detectable (< 0.03 mg N/l)
Number of samples = 70

Table B4. M1 water column

Parameter	Mean	S.D.	S.E.	95-percentile
Ammonium, mg N/l	3.8	3.0	±0.4	9.5
Alkalinity, mg CaCO3/l	94	24	±3	130
Bacteriochlorophyll, mg/l	32	49	±6	139
Chlorophyll-*a*, µg/l	403	422	±52	1179
SS, mg/l	49	40	±5	132
VSS, mg/l	52	38	±5	123

Key: S.D. = standard deviation; S.E. = standard error
Number of samples = 70

Table B5. M2 water column

Parameter	Mean	S.D.	S.E.	95-percentile
Ammonium, mg N/l	2.9	2.5	±0.3	7.5
Alkalinity, mg $CaCO_3$/l	88	21	±3	120
Bacteriochlorophyll, mg/l	32	41	±5	116
Chlorophyll-*a*, µg/l	348	414	±51	1381
SS, mg/l	43	33	±4	106
VSS, mg/l	41	32	±4	103

Key: S.D. = standard deviation; S.E. = standard error
Number of samples = 70

Appendix C. Nitrogen Mass Balance

C.1 Net nitrogen mass balance

Total nitrogen fluxes over M1 pond were calculated according to the diagram showed in Figure C.1.

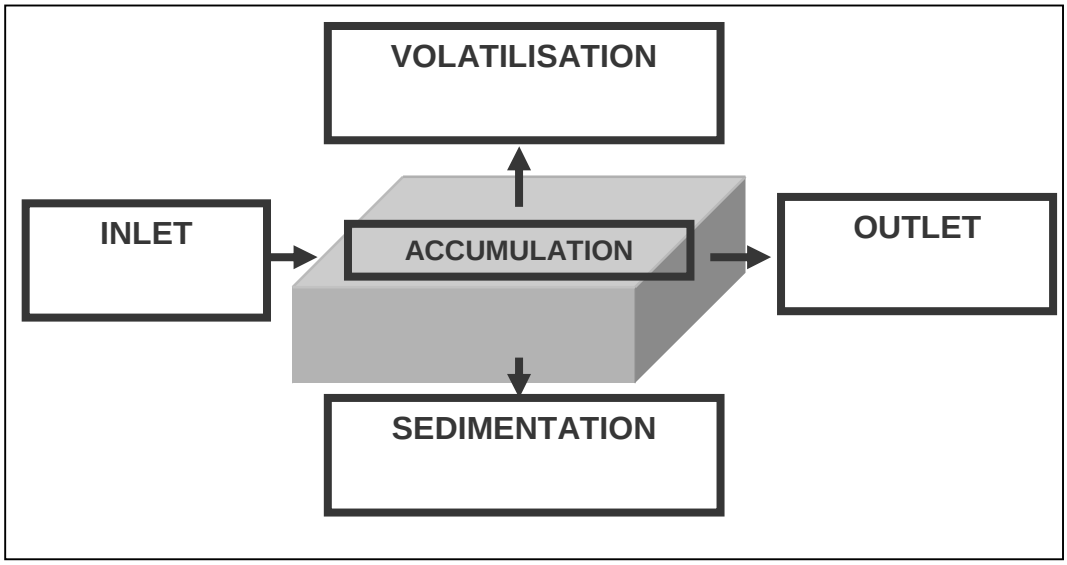

Figure C.1 Diagramme for a net nitrogen mass balance in M1 pond

Corresponding equations for each flux are shown as follows:

INLET:

$$No = 10{,}000 \times \left(\frac{Qo \times Co}{A} \right) \qquad \textbf{(C.1)}$$

where *No* is the mean total nitrogen inlet loading (g N/ha d), *Qo* is the mean inlet pond flow rate (m^3/d), *Co* is the mean total nitrogen concentrations from pond influent (mg N/l) and A is the surface are of the pond (m^2).

OUTLET:

$$Ne = 10{,}000 \times \left(\frac{Qe \times Ce}{A} \right) \qquad \textbf{(C.2)}$$

where *Ne* is the mean total nitrogen outlet loading (g N/ha d), *Qe* is the mean inlet pond flow rate (m^3/d), *Ce* is the mean total nitrogen concentrations from pond effluent (mg N/l) and *A* is the surface area of the pond (m^2).

VOLATILISATION:

$$N_{vol} = 10 \times f \times \left(\frac{\sum_{i=1}^{i=4} c_i \times V_i}{a \times t} \right) \quad \textbf{(C.3)}$$

where N_{vol} is the ammonia volatilisation rate (g N/ha d), f is the absorption recovery factor (1.27), C_i is the concentration of ammonium (mg N/l) in the absorption container i (Absorption column (i = 1), flask 1 (2), flask 2 (3), flask 3 (4)), V_i is the volume (l) of the boric acid solution from the absorption container i, a is the area of the capture chamber (m^2) and t is the duration time of the experiment (d).

ACCUMULATION:

$$N_{acc} = 10{,}000 \times \left(\frac{(Cf - Ci) \times V}{A \times t} \right) \quad \textbf{(C.4)}$$

where N_{acc} is the mean total nitrogen accumulation rate in the water column (g N/ha d) over the experimental timeframe (t, d); Cf and Ci are the initial (t=0) and final (t=t) total nitrogen concentration in the water column (mg N/l), respectively; V is the net water volume in the pond (m^3); and A is the surface area of the pond (m^2).

SEDIMENTATION:

Nitrogen sedimentation rates (N_{sed}, g N/ha d) were calculated by using the following equation:

$$N_{sed} = 10{,}000 \times \left(\frac{V_{TS} \times TS \times N}{A \times t} \right) \quad \textbf{(C.5)}$$

where V_{TS} and TS are the volume (l) and the content of total solids (g/l) from thickened sediment samples, respectively; N is the nitrogen content (fraction) in dry sediment samples, A is the sum of the top area of each bucket (m^2) and t is the time of sampling (d).

C.2 ^{15}N mass balance

For tracer experiments with ^{15}N stable isotopes, net content of ^{15}N was calculated from δ^{15}N values and nitrogen concentrations from each fraction in M1 pond effluent and water column (soluble organic nitrogen, suspended organic nitrogen, ammonium, oxidised nitrogen), from sediment samples and from volatilised gases (ammonia and NOx). The equations used to calculate net ^{15}N concentrations are shown as follows:

$$^{15}N = N_{total} \times {}^{15}N_{fraction} \quad \textbf{(C.6)}$$

$$^{15}N_{fraction} = \left(\frac{^{15}N/^{14}N_{sample}}{1 + {}^{15}N/^{14}N_{sample}} \right) \quad \textbf{(C.7)}$$

$$\delta\,^{15}N\,,\,^{0}/_{00} = \left[\frac{\left(^{15}N/^{14}N\right)_{sample} - \left(^{15}N/^{14}N\right)_{std}}{\left(^{15}N/^{14}N\right)_{std}} \right] \times 1000 \quad \textbf{(C.8)}$$

where ^{15}N is the net concentration or content of ^{15}N in the sample; N_{total} is the total nitrogen concentration (mg N/l) or content (g N/kg, dry base) in the sample; $^{15}N_{fraction}$ is the fraction of ^{15}N in the sample, assuming that ^{15}N and ^{14}N are the only two nitrogen isotopes in the sample; $\delta^{15}N$ is the isotopic composition of nitrogen in any sample (‰); and $^{15}N/^{14}N_{sample}$ and $^{15}N/^{14}N_{std}$ are the ^{15}N:^{14}N ratios in the sample and atmospheric N_2 gas (0.0036765), respectively.

The calculation procedure includes: (1) the calculation of $^{15}N/^{14}N_{sample}$ values from equation C.8 using δ^{15}N readings; (2) the calculation of the $^{15}N_{fraction}$ from equation C.7 using $^{15}N/^{14}N_{sample}$ values calculated previously; and (3) the calculation of ^{15}N content or concentration in the sample from equation C.6 using previous results from step 2.

Appendix D. Water Quality Characteristics in the Effluent of the Maturation Pond M1 During Tracer Experiments with ^{15}N Stable Isotopes

Table D1. Tracer experiment with ^{15}N-labelled ammonium in summer 2005

Day	SS	Sus Org N	Sol Org N	NH_4^+	NO_3^-	Day	SS	Sus Org N	Sol Org N	NH_4^+	NO_3^-
1	97	8.9	1.4	2.0	0.09	26	99	7.8	1.4	4.2	0.03
2	100	7.3	2.8	1.7	0.08	27	88	7.4	0.8	4.2	0.07
3	110	8.4	1.7	1.7	0.09	28	108	9.0	3.4	2.2	0.11
4	119	8.4	3.1	1.4	0.08	29	115	9.5	3.9	1.7	0.11
5	121	9.5	2.5	1.4	0.07	30	130	8.4	3.6	3.1	0.09
6	103	9.0	2.8	2.2	0.09	31	157	14.2	0.9	1.7	0.08
7	100	7.8	3.7	2.5	0.09	32	142	13.6	1.5	1.7	0.07
8	97	7.8	3.6	2.0	0.09	33	139	11.3	2.4	1.4	0.05
9	103	8.4	3.3	1.7	0.07	34	126	12.5	0.9	1.7	0.11
10	106	8.4	3.0	2.0	0.09	35	124	13.4	1.1	3.4	0.05
11	89	9.6	3.0	2.0	0.12	36	106	10.2	3.2	1.7	0.08
12	100	9.1	1.3	2.5	0.12	37	111	11.9	1.5	1.7	0.08
13	97	8.4	2.2	2.8	0.13	38	96	11.7	0.9	2.0	0.09
14	97	9.6	2.8	2.2	0.10	39	106	11.4	1.5	1.7	0.07
15	98	9.5	2.8	1.7	0.07	40	103	12.0	0.6	2.0	0.06
16	101	7.9	3.0	2.0	0.08	41	102	13.6	0.7	0.8	0.10
17	91	8.4	3.0	2.0	0.09	42	103	10.2	2.1	1.1	0.03
18	106	7.3	4.5	2.2	0.06	43	118	12.3	1.4	1.4	0.03
19	102	8.4	3.4	2.2	0.23	44	82	8.9	3.7	0.8	0.07
20	100	8.9	4.0	2.2	0.12	45	109	11.2	1.6	0.6	
21	97	8.9	4.2	2.0	0.10	46	104	9.0	2.0	0.8	
22	107	9.6	4.2	0.8	0.07	47	129	14.0	0.5	0.6	
23	113	11.2	2.8	1.1	0.07	48	91	8.4	1.1	0.6	
24	116	10.0	3.1	2.5	0.10	49	113	11.2	1.6	0.6	
25	113	8.9	3.7	2.5	0.08						

SS = suspended solids, mg/l; NH_4^+ = ammonium, mg N/l; NO_3^- = nitrate, mg N/l;
Sus Org N = suspended organic nitrogen, mg N/l; Sol Org N = soluble organic nitrogen, mg N/l

Table D2. Tracer experiment with ^{15}N-labelled algae in summer 2006

Day	SS	Sus Org N	Sol Org N	NH_4^+	NO_3^-
1	80	6.16	2.52	0.84	0.50
2	87	3.92	1.96	0.84	0.25
3	88	4.48	2.24	0.56	0.14
4	98	4.48	1.96	0.84	0.08
5	98	3.36	2.52	0.84	0.12
6	90	4.48	2.52	0.84	0.09
7	110	6.16	1.68	0.56	0.07
8	53	5.60	1.96	0.84	0.07
13	79	4.48	2.52	0.84	0.10
14	67	2.80	3.64	0.84	0.08
15	48	2.80	3.36	0.56	0.10
16	55	3.92	3.36	0.56	0.07
17	69	3.36	2.52	0.84	0.08
18	60	3.36	3.36	0.56	0.09
19	69	4.48	3.36	0.56	0.10
20	108	7.28	3.36	0.56	0.29
21	66	3.36	2.80	0.56	0.46
22	66	2.80	2.52	0.84	0.43
23	67	4.48	0.84	0.28	0.14
24	73	5.04	0.84	0.28	0.04
25	57	3.36	1.12	0.56	0.09
26	52	3.36	1.40	0.28	0.11
27	50	2.80	1.96	0.28	0.11
28	59	3.36	1.96	0.28	0.09
29	47	1.12	1.12	0.56	0.12
30	49	1.12	1.12	0.56	0.13
31	46	0.56	1.96	0.28	0.15
32	68	0.56	2.52	0.28	0.09
33	44	1.12	1.96	0.28	0.17
34	45	0.56	2.52	0.28	0.16
35	47	2.80	0.00	0.56	0.18
36	47	3.92	0.56	0.56	0.16
37	49	3.36	0.28	0.84	0.18
38	50	2.24	0.28	0.84	0.17
39	44	2.80	0.56	0.56	0.19
40	47	3.36	0.56	0.56	0.17
43	46	3.92	0.28	0.28	0.18
49	61	3.42	0.28	0.28	0.22
58	80	5.04	0.56	0.56	0.15
65	91	6.16	0.28	0.28	0.13
72	79	5.04	0.84	0.28	0.16
106	53	5.04	0.84	0.28	0.17
134	24	1.68	0.56	1.68	0.23
168	14	0.56	0.56	3.92	0.16

SS = suspended solids, mg/l; NH_4^+ = ammonium, mg N/l; NO_3^- = nitrate, mg N/l;
Sus Org N = suspended organic nitrogen, mg N/l; Sol Org N = soluble organic nitrogen, mg N/l

Table D3. Tracer experiment with ^{15}N-labelled ammonium in winter 2006/2007

Day	SS	Sus org N	Sol org N	NH4+	NO3-
0	6	1.12	0.00	5.04	0.45
1	6	1.12	0.00	6.16	0.88
2	8	0.84	0.00	6.16	0.91
3	6	0.56	0.00	6.16	0.76
4	6	0.56	0.28	5.88	0.65
5	6	0.56	0.56	5.60	0.54
6	7	0.56	0.56	5.60	0.39
7	7	0.56	0.56	5.60	0.48
8	8	0.56	0.28	5.88	0.49
9	8	0.56	0.00	6.16	0.48
10	8	0.56	0.28	6.16	0.47
11	8	0.56	0.56	6.16	0.49
12	8	0.56	0.84	6.16	0.46
13	7	0.56	1.12	6.16	0.42
14	7	0.56	0.98	6.30	0.44
15	6	0.56	0.84	6.44	0.40
16	8	0.56	0.98	6.30	0.74
17	8	0.56	1.12	6.16	1.12
18	12	0.56	1.26	6.86	0.79
19	8	0.56	1.40	7.56	0.73
20	8	1.96	1.12	7.28	0.48
21	7	3.36	0.84	7.00	0.97
22	8	1.96	0.98	6.58	0.96
23	8	0.56	1.12	6.16	0.95
24	10	0.56	0.84	5.88	0.84
25	8	0.56	0.56	5.60	0.73
26	10	1.12	0.56	5.60	0.64
27	9	1.68	0.56	5.60	0.55
28	7	2.24	0.56	5.04	0.46
29	11	2.80	0.56	4.48	0.36
30	8	1.68	0.70	4.34	0.41
31	7	0.56	0.84	4.20	0.46
32	10	0.56	0.84	3.92	0.64
33	8	0.56	0.84	3.64	0.82
34	7	0.42	0.84	3.64	0.49
35	8	0.28	0.84	3.64	0.17
36	7	0.42	0.70	3.50	0.33
37	8	0.56	0.56	3.36	0.49
38	9	0.56	0.42	3.50	0.45
39	8	0.56	0.28	3.64	0.41
40	7	0.56	0.28	3.36	0.40
41	8	0.56	0.28	3.08	0.38
42	9	0.84	0.28	2.80	0.40
43	9	1.12	0.28	2.52	0.42
44	11	0.84	0.14	2.66	0.54
45	11	0.56	0.00	2.80	0.66

SS = suspended solids, mg/l; NH_4^+ = ammonium, mg N/l; NO_3^- = nitrate, mg N/l;
Sus Org N = suspended organic nitrogen, mg N/l; Sol Org N = soluble organic nitrogen, mg N/l

Table D4. Tracer experiment with ^{15}N-labelled nitrite in winter 2006/2007

Day	SS	Sus Org N	Sol Org N	NH_4^+	NO_3^-	Day	SS	Sus Org N	Sol Org N	NH_4^+	NO_3^-
46	12	0.56	0.28	2.52	0.61	75	26	1.12	0.56	2.24	0.38
47	12	0.56	0.56	2.24	0.69	76	34	1.96	2.38	1.54	0.34
48	12	0.56	0.42	2.10	0.60	77	31	2.80	4.20	0.84	0.35
49	10	0.56	0.28	1.96	0.50	78	33	1.96	3.50	0.98	0.26
50	13	1.12	0.14	1.82	0.77	79	34	1.12	2.80	1.12	0.26
51	12	1.68	0.00	1.68	0.85	80	34	1.96	2.24	1.12	0.15
52	15	1.12	0.70	1.54	0.63	81	34	2.80	1.68	1.12	0.31
53	18	0.56	1.40	1.40	0.49	82	35	2.52	1.96	0.84	0.18
54	9	0.84	0.84	1.12	0.66	83	36	2.24	2.24	0.56	0.47
55	24	1.12	0.28	0.84	0.86	84	37	1.96	2.38	0.42	0.22
56	29	1.40	0.28	0.84	0.57	85	37	1.68	2.52	0.28	0.21
57	31	1.68	0.28	0.84	0.54	86	34	1.96	2.52	0.28	0.34
58	31	2.24	0.14	0.70	0.62	87	30	2.24	2.52	0.28	0.38
59	34	2.80	0.00	0.56	0.46	88	33	2.24	2.38	0.42	0.34
60	38	2.24	0.28	0.56	0.57	89	36	2.24	2.24	0.56	0.35
61	33	1.68	0.56	0.56	0.58	90	36	2.24	1.40	1.40	0.26
62	36	1.96	0.70	0.42	0.32	91	36	2.24	0.56	2.24	0.26
63	33	2.24	0.84	0.28	0.38	93	34	1.96	1.40	1.68	0.15
64	31	2.52	0.70	0.42	0.40	95	32	1.68	2.24	1.12	0.31
65	35	2.80	0.56	0.56	0.46	97	32	2.24	1.82	0.98	0.18
66	33	1.96	2.10	0.70	0.18	99	32	2.80	1.40	0.84	0.47
67	32	1.12	3.64	0.84	0.25	101	36	2.80	1.54	1.26	0.22
68	33	1.12	2.80	0.56	0.15	103	40	2.80	1.68	1.68	0.21
69	33	0.56	3.08	0.28	0.31	105	38	3.08	1.96	1.68	0.34
70	33	0.84	2.94	0.42	0.18	107	35	3.36	2.24	1.68	0.38
71	32	1.12	2.80	0.56	0.47	110	30	2.80	3.08	0.84	0.34
72	32	1.40	2.66	0.42	0.22	113	30	3.08	2.66	1.26	0.35
73	31	1.68	2.52	0.28	0.21	116	33	3.36	2.24	1.68	0.26
74	34	1.40	1.54	1.26	0.34						

SS = suspended solids, mg/l; NH_4^+ = ammonium, mg N/l; NO_3^- = nitrate, mg N/l;
Sus Org N = suspended organic nitrogen, mg N/l; Sol Org N = soluble organic nitrogen, mg N/l

Appendix E. Results from the calibration of the stable isotope ratio mass spectrophotometer with SRM

Instrument calibration for the determination of ^{15}N:^{14}N ratios by delta ^{15}N readings ($\delta^{15}N$, ‰) was carried out with two standard reference materials (SRM) of labelled ammonium sulphate: IAEA-USGS26 ($\delta^{15}N = +53.7$) and IAEA-USGS25 ($\delta^{15}N = -30.4$), provided by the U.S. Geological Service (Denver, CO) and certified by the Section of Isotope Hydrology, International Atomic Energy Agency (Vienna). Three catalyser columns were used (Column 9, 10 and 11) and results from instrumental calibration of the elemental analyzer coupled with a stable isotope ratio mass spectrophotometer (EA-IRMS; EuroEA3000-Micromass Isoprime, Eurovector, Milan) are reported as follows:

Table E1. Results for $\delta^{15}N$ readings from SRM (Column 9A)

USGS-26 (53,0, ‰)			**USGS-25** (-31,8, ‰)		
No.	**Sample**	**($\delta^{15}N$, ‰)**	**No.**	**Sample**	**($\delta^{15}N$, ‰)**
8	OCN9-USGS26-1.raw	53.0	11	OCN9-USGS25-1.raw	-30.4
9	OCN9-USGS26-2.raw	52.9	12	OCN9-USGS25-2.raw	-30.2
10	OCN9-USGS26-3.raw	53.3	25	OCN9-USGS25-4.raw	-31.3
37	OCN9-USGS26-6.raw	52.7	38	OCN9-USGS25-5.raw	-30.5
50	OCN9-USGS26-7.raw	52.7	39	OCN9-USGS25-6.raw	-31.3
63	OCN9-USGS 26-8.raw	52.9	51	OCN9-USGS25-7.raw	-30.6
64	OCN9-USGS 26-9.raw	53.3	65	OCN9-USGS 25-8.raw	-31.6
77	OCN9-USGS 26-10.raw	52.3	66	OCN9-USGS 25-9.raw	-31.5
83	OCN9-USGS 26- 11.raw	52.7	78	OCN9-USGS 25-10.raw	-30.2
84	OCN9-USGS 26- 12.raw	53.0	85	OCN9-USGS 25-11.raw	-31.3
97	OCN9-USGS-26- 13.raw	53.4	86	OCN9-USGS 25-12.raw	-31.2
104	OCN9-USGS 26- 14.raw	52.5	98	OCN9-USGS-25- 13.raw	-30.9
111	OCN9-USGS 26- 15.raw	52.5	105	OCN9-USGS 25-14.raw	-30.9
112	OCN9-USGS 26- 16.raw	52.6	113	OCN9-USGS 25-15.raw	-31.1
125	OCN9-USGS 26- 17.raw	52.7	114	OCN9-USGS 25-16.raw	-31.7
137	OCN9-USGS 26- 18.raw	52.9	126	OCN9-USGS 25-17.raw	-31.7
138	OCN9-USGS 26- 19.raw	52.1	139	OCN9-USGS 25-18.raw	-31.7
			140	OCN9-USGS 25-19.raw	-31.7
	STANDARD DEV.	0.36		STANDARD DEV.	0.52
	AVERAGE	52.8		AVERAGE	-31.1

Table E2. Results for $\delta^{15}N$ readings from SRM (Column 9B)

USGS-26 (53,0, ‰)			**USGS-25** (-31,8, ‰)		
No.	**Sample**	**($\delta^{15}N$, ‰)**	**No.**	**Sample**	**($\delta^{15}N$, ‰)**
157	OCN9-USGS 26- 21.raw	53.3	160	OCN9-USGS 25-22.raw	-31.3
158	OCN9-USGS 26- 22.raw	53.3	172	OCN9-USGS 25-23.raw	-31.5
171	OCN9-USGS 26- 23.raw	52.6	188	OCN9-USGS 25-24.raw	-31.7
186	OCN9-USGS 26- 24.raw	52.4	214	OCN9-USGS 25-26.raw	-31.5
187	OCN9-USGS 26- 25.raw	52.9	227	OCN9-USGS 25-27.raw	-32.2
213	OCN9-USGS 26- 26.raw	53.1	228	OCN9-USGS 25-28.raw	-32.4
225	OCN9-USGS 26- 27.raw	53.0	240	OCN9-USGS 25-29.raw	-31.5
226	OCN9-USGS 26- 28.raw	53.0	266	OCN9-USGS 25-30.raw	-31.8
239	OCN9-USGS 26- 29.raw	53.1	293	OCN9-USGS 25-31.raw	-32.0
254	OCN9-USGS 26- 30.raw	52.9	325	OCN9-USGS 25-33.raw	-32.3
278	OCN9-USGS 26- 31.raw	53.3	326	OCN9-USGS 25-34.raw	-32.3
311	OCN9-USGS 26- 32.raw	53.1	341	OCN9-USGS 25-35.raw	-31.6
323	OCN9-USGS 26- 33.raw	52.4	357	OCN9-USGS 25-36.raw	-31.9
324	OCN9-USGS 26- 34.raw	52.7	358	OCN9-USGS 25-37.raw	-32.2
340	OCN9-USGS 26- 35.raw	53.1	373	OCN9-USGS 25-38.raw	-31.3
355	OCN9-USGS 26- 36.raw	53.3			
356	OCN9-USGS 26- 37.raw	53.6			
372	OCN9-USGS 26- 38.raw	53.2			
	STANDARD DEV.	0.32		STANDARD DEV.	0.38
	AVERAGE	53.0		AVERAGE	-31.8

Table E3. Results for $\delta^{15}N$ readings from SRM (Column 10)

USGS-26 (53,0, ‰)			**USGS-25** (-31,8, ‰)		
No.	**Sample**	**($\delta^{15}N$, ‰)**	**No.**	**Sample**	**($\delta^{15}N$, ‰)**
9	OC10-USGS26-2.raw	53.3	11	OC10-USGS25-1.raw	-29.9
10	OC10-USGS26-3.raw	53.4	12	OC10-USGS25-2.raw	-30.0
25	OC10-USGS26-4.raw	53.5	13	OC10-USGS25-3.raw	-29.6
38	OC10-USGS26-5.raw	53.5	26	OC10-USGS25-4.raw	-29.9
39	OC10-USGS26-6.raw	53.4	40	OC10-USGS25-5.raw	-30.1
56	OC10-USGS26-7.raw	53.1	41	OC10-USGS25-6.raw	-30.1
71	OC10-USGS26-8.raw	53.1	57	OC10-USGS25-7.raw	-30.4
72	OC10-USGS26-9.raw	53.5	73	OC10-USGS25-8.raw	-30.1
88	OC10-USGS26-10.raw	53.1	89	OC10-USGS25-10.raw	-30.3
105	OC10-USGS26-11.raw	52.7	106	OC10-USGS25-11.raw	-29.7
155	OC10-USGS26-15.raw	53.6	159	OC10-USGS25-16.raw	-30.1
156	OC10-USGS26-16.raw	52.9	160	OC10-USGS25-17.raw	-30.3
157	OC10-USGS26-17.raw	53.1	185	OC10-USGS25-19.raw	-30.2
171	OC10-USGS26-18.raw	53.3	186	OC10-USGS25-20.raw	-30.3
183	OC10-USGS26-19.raw	53.4	199	OC10-USGS25-21.raw	-30.2
184	OC10-USGS26-20.raw	53.0	215	OC10-USGS25-22.raw	-30.2
198	OC10-USGS26-21.raw	53.0	216	OC10-USGS25-23.raw	-30.1
213	OC10-USGS26-22.raw	53.0	248	OC10-USGS25-25.raw	-30.6
214	OC10-USGS26-23.raw	53.4	264	OC10-USGS25-26.raw	-30.4
230	OC10-USGS26-24.raw	53.2	265	OC10-USGS25-27.raw	-30.7
247	OC10-USGS26-25.raw	53.2	280	OC10-USGS25-28.raw	-30.4

Table E3. Results for $\delta^{15}N$ readings from SRM (Column 10) (cont.)

USGS-26 (53,0, ‰)			**USGS-25** (-31,8, ‰)		
No.	**Sample**	**($\delta^{15}N$, ‰)**	**No.**	**Sample**	**($\delta^{15}N$, ‰)**
262	OC10-USGS26-26.raw	52.8	297	OC10-USGS25-29.raw	-30.4
263	OC10-USGS26-27.raw	53.1	313	OC10-USGS25-30.raw	-30.0
279	OC10-USGS26-28.raw	53.0	314	OC10-USGS25-31.raw	-30.5
296	OC10-USGS26-29.raw	53.1	329	OC10-USGS25-32.raw	-30.7
311	OC10-USGS26-30.raw	52.7			
312	OC10-USGS26-31.raw	52.9			
328	OC10-USGS26-32.raw	53.0			
	STANDARD DEV.	0.25		STANDARD DEV.	0.28
	AVERAGE	53.2		AVERAGE	-30.2

Table E4. Results for $\delta^{15}N$ readings from SRM (Column 11)

USGS-26 (53,0, ‰)			**USGS-25** (-31,8, ‰)		
No.	**Sample**	**($\delta^{15}N$, ‰)**	**No.**	**Sample**	**($\delta^{15}N$, ‰)**
10	OCN11-USGS26-2.raw	52.77	42	OCN11-USGS25-5.raw	-29.86
11	OCN11-USGS26-3.raw	53.31	43	OCN11-USGS25-6.raw	-30.19
41	OCN11-USGS26-6.raw	52.51	82	OCN11-USGS25-8.raw	-29.92
65	OCN11-USGS26-7.raw	53.09	83	OCN11-USGS25-9.raw	-30.13
80	OCN11-USGS26-8.raw	52.72	98	OCN11-USGS25-10.raw	-30.27
81	OCN11-USGS26-9.raw	53.50	115	OCN11-USGS25-11.raw	-29.96
97	OCN11-USGS26-10.raw	52.72	131	OCN11-USGS25-12.raw	-30.35
114	OCN11-USGS26-11.raw	53.30	132	OCN11-USGS25-13.raw	-29.79
129	OCN11-USGS26-12.raw	52.71	147	OCN11-USGS25-14.raw	-31.02
130	OCN11-USGS26-13.raw	53.40	200	OCN11-USGS25-28.raw	-30.68
146	OCN11-USGS26-14.raw	53.02	201	OCN11-USGS25-29.raw	-30.80
169	OCN11-USGS26-31.raw	52.84	216	OCN11-USGS25-30.raw	-30.43
183	OCN11-USGS26-27.raw	53.34	233	OCN11-USGS25-23.raw	-30.22
198	OCN11-USGS26-28.raw	53.32	249	OCN11-USGS25-24.raw	-31.13
199	OCN11-USGS26-29.raw	53.53	265	OCN11-USGS25-26.raw	-30.76
215	OCN11-USGS26-30.raw	52.79	282	OCN11-USGS25-15.raw	-30.77
232	OCN11-USGS26-23.raw	53.61	331	OCN11-USGS25-19.raw	-30.08
247	OCN11-USGS26-24.raw	53.29	347	OCN11-USGS25-20.raw	-29.99
248	OCN11-USGS26-25.raw	53.31	348	OCN11-USGS25-21.raw	-29.93
264	OCN11-USGS26-26.raw	52.98	363	OCN11-USGS25-22.raw	-29.99
281	OCN11-USGS26-15.raw	53.12			
330	OCN11-USGS26-19.raw	53.10			
345	OCN11-USGS26-20.raw	53.30			
346	OCN11-USGS26-21.raw	53.08			
362	OCN11-USGS26-22.raw	53.75			
	STANDARD DEV.	0.31		STANDARD DEV.	0.42
	AVERAGE	53.1		AVERAGE	-30.4

Appendix F. Culturing ^{15}N-labelled algae in the labortory

The ^{15}N-labelled algae were produced by culturing *Chlorella vulgaris* (CCAP 211/11B; SAMS Research Services Ltd, Oban, Scotland) in 10 litres of Bold's basal medium (BBM) (Andersen *et al*., 2005), substituting the nitrogen source ($NaNO_3$) with $^{15}NH_4Cl$. The culturing medium was made up according to the following recipe:

STOCK	STOCK SOLUTION	ml/litre
1. KH_2PO_4	8.75 g/500 ml	10 ml
2. $CaCl_2.2H_2O$	1.25 g/500 ml	10 ml
3. MgSO4.$7H_2O$	3.75 g/500 ml	10 ml
4. $NaNO_3$	12.5 g/500 ml	10 ml
5. K_2HPO_4	3.75 g/500 ml	10 ml
6. NaCl	1.25 g/500 ml	10 ml
7. Na_2EDTA	10 g/l	
KOH	6.2 g/l	1 ml
8. $FeSO_4.7H_2O$	4.98 g/l	
H_2SO_4 (conc.)	1 ml/l	1 ml
9. Trace Metal Solution (see below)		1 ml
10. H_3BO_3	5.75 g/500 ml	0.7 ml

Trace Metal Solution:

Substance	g/litre
1. H_3BO_3	2.86 g
2. $MnCl_2.4H_2O$	1.81 g
3. $ZnSO_4.7H_2O$	0.222 g
4. Na $MoO_4.5H_2O$	0.390 g
5. $CuSO_4.5H_2O$	0.079 g
6. $Co(NO_3)_2.6H_2O$	0.0494 g

Each of the above substances for the Trace Metal solution was dissolved separately, prior to adding the next on the list. pH of the medium was adjusted to 6.8 with NaOH or HCl and autoclave.

Figure F1. Laboratory set-up for culturing ^{15}N-labelled algae