Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supplementary Information

Distinguishing atmospheric nitrogen compounds (nitrate and ammonium) in lichen biomonitoring studies

Daniel Niepsch[1], Leon J. Clarke[1], Konstantinos Tzoulas[1], Gina Cavan[1]

[1]Department of Natural Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, M1 5GD, Manchester, UK

Corresponding author: Daniel.Niepsch@gmx.de

ORCIDs:

Daniel Niepsch - https://orcid.org/0000-0002-0177-1996

Leon J. Clarke – https://orcid.org 0000-0002-2278-4139

Konstantinos Tzoulas – https://orcid.org/0000-0001-5363-2050

Gina Cavan - https://orcid.org/0000-0002-8429-870X

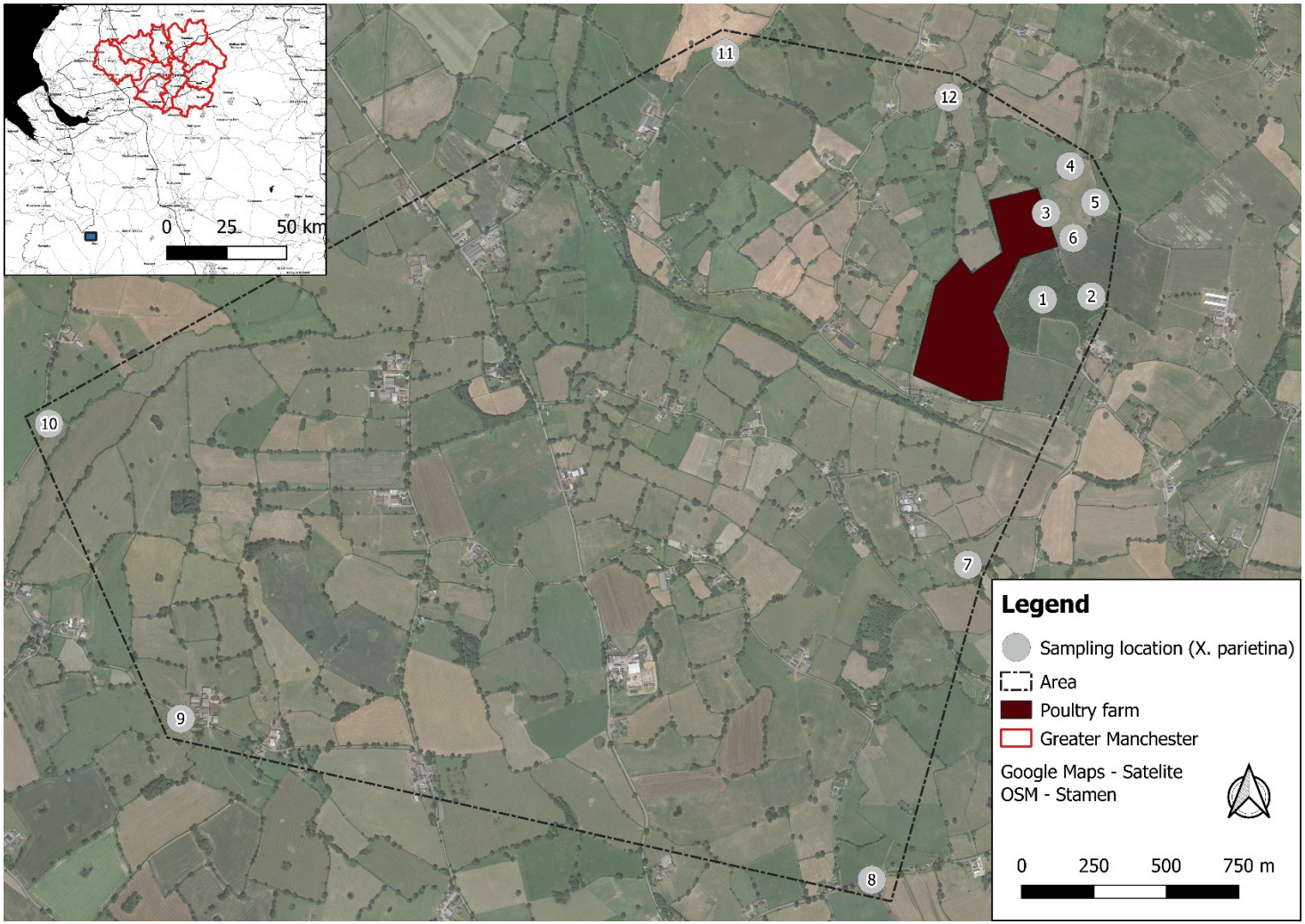

Fig. S1: Lichen sampling sites of *Xanthoria parietina* collected from around a poultry farm near Shrewsbury, UK; displayed with OSGB 1936 XY-coordinates [see Tab. S2] and its location (blue rectangle in inset map) in relation to Greater Manchester (UK) (base map: Google Satellite, 2018), OpenStreetMap® is open data, licensed under the Open Data Commons Open Database License (ODbL) by the OpenStreetMap Foundation (OSMF).

Tab. S1: Extracted nitrate (NO_3^-) and ammonium (NH_4^+) concentrations (mg kg^{-1}) using 3% KCl for urban (Manchester, UK) lichen sampling sites (as displayed in main article Figure 1; N=87; *Xanthoria parietina*); British National Grid (OSGB1936) coordinates included; N/A – indicating no data available

Site ID	Coordinates OSGB1936	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	Site ID	Coordinates OSGB1936	NO_3^- (mg/kg)	NH_4^+ (mg/kg)
1	X: 385883 Y: 399803	87.15	5.53	**46**	X: 382968 Y: 396568	33.88	12.89
2	X: 385368 Y: 398023	37.57	5.77	**47**	X: 386128 Y: 399848	73.40	6.10
3	X: 386160 Y: 399492	40.62	6.18	**48**	X: 385369 Y: 399408	62.03	7.00
4	X: 384798 Y: 397104	50.42	5.94	**49**	X: 382850 Y: 396595	28.11	5.26
5	X: 385420 Y: 398339	12.56	7.44	**50**	X: 383349 Y: 396842	*N/A*	11.84
6	X: 384382 Y: 399591	43.36	5.19	**51**	X: 386167 Y: 399073	45.85	3.99
7	X: 386190 Y: 399774	142.73	5.68	**52**	X: 383902 Y: 398788	12.87	11.69
8	X: 383876 Y: 396960	45.73	7.10	**53**	X: 385829 Y: 398555	46.77	12.86
9	X: 384467 Y: 399200	36.75	5.61	**54**	X: 385366 Y: 397922	24.86	7.69
10	X: 385359 Y: 396932	76.08	10.41	**55**	X: 382990 Y: 396950	64.36	15.69
11	X: 385886 Y: 399523	37.66	6.96	**56**	X: 385387 Y: 396705	96.65	10.49
12	X: 384072 Y: 396768	*N/A*	3.18	**57**	X: 384938 Y: 399397	66.30	10.19
13	X: 385123 Y: 397837	9.96	7.44	**58**	X: 384921 Y: 396860	100.04	8.04
14	X: 386434 Y: 399454	91.29	9.00	**59**	X: 383705 Y: 397171	27.40	9.35
15	X: 384231 Y: 398977	94.34	11.86	**60**	X: 386302 Y: 399221	45.18	7.99
16	X: 384548 Y: 397247	88.52	11.03	**61**	X: 382857 Y: 396932	25.59	6.59
17	X: 385166 Y: 399865	54.01	7.39	**62**	X: 385366 Y: 398773	33.06	12.37
18	X: 385704 Y: 398035	49.68	11.28	**63**	X: 382661 Y: 397036	43.52	9.35
19	X: 386158 Y: 399834	109.51	5.06	**64**	X: 385843 Y: 399434	37.94	4.43
20	X: 384901 Y: 399510	54.00	8.59	**65**	X: 386381 Y: 399468	57.85	5.77
21	X: 386261 Y: 399667	18.88	4.28	**66**	X: 385410 Y: 396748	97.72	17.57
22	X: 386283 Y: 399611	35.44	4.17	**67**	X: 385326 Y: 397120	46.71	12.92
23	X: 385312 Y: 398998	35.57	5.11	**68**	X: 385373 Y: 396782	47.44	18.98
24	X: 384890 Y: 397470	90.59	8.80	**69**	X: 386415 Y: 399289	50.67	18.22
25	X: 385760 Y: 398209	31.30	11.91	**70**	X: 382786 Y: 397728	77.03	24.03

Site ID	Coordinates OSGB1936	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	Site ID	Coordinates OSGB1936	NO_3^- (mg/kg)	NH_4^+ (mg/kg)
26	X: 384960 Y: 398477	*N/A*	11.11	**71**	X: 384869 Y: 397365	88.59	23.35
27	X: 385684 Y: 399549	136.43	5.96	**72**	X: 386215 Y: 399738	137.97	10.19
28	X: 383832 Y: 397110	26.22	10.89	**73**	X: 384873 Y: 397055	34.49	16.56
29	X: 385024 Y: 396794	59.61	7.14	**74**	X: 385838 Y: 399661	44.82	16.58
30	X: 384382 Y: 397558	93.59	14.84	**75**	X: 383917 Y: 397652	25.03	27.74
31	X: 384295 Y: 398297	21.89	12.65	**76**	X: 383616 Y: 397107	50.51	17.65
32	X: 385089 Y: 399557	56.82	8.13	**77**	X: 385143 Y: 398190	10.60	11.82
33	X: 384256 Y: 397121	20.30	10.83	**78**	X: 383290 Y: 397932	19.53	18.76
34	X: 382892 Y: 397730	38.65	5.58	**79**	X: 382488 Y: 397017	19.52	22.98
35	X: 385653 Y: 399279	62.31	5.32	**80**	X: 385774 Y: 398723	49.57	13.79
36	X: 383277 Y: 397664	75.80	10.59	**81**	X: 385199 Y: 399664	59.54	18.50
37	X: 385454 Y: 399341	53.51	5.33	**82**	X: 385422 Y: 396810	85.03	20.49
38	X: 385749 Y: 399415	62.48	6.64	**83**	X: 383156 Y: 398139	58.93	24.71
39	X: 384843 Y: 397402	78.99	9.58	**84**	X: 384734 Y: 398978	62.95	14.87
40	X: 385938 Y: 399381	24.65	4.97	**85**	X: 383273 Y: 397492	52.09	9.31
41	X: 383741 Y: 397851	*N/A*	13.29	**86**	X: 383517 Y: 398334	*N/A*	9.87
42	X: 385597 Y: 398946	38.01	6.17	**87**	X: 384516 Y: 398751	*N/A*	7.27
43	X: 384969 Y: 397257	64.43	7.48				
44	X: 386352 Y: 399353	115.85	9.65				
45	X: 383263 Y: 396662	30.39	7.69				

Tab. S2: Extracted nitrate (NO_3^-) and ammonium (NH_4^+) concentrations (mg kg^{-1}) using 3% KCl of rural lichen samples (as displayed in Fig. S1; N=12, *Xanthoria parietina*); British National Grid (OSGB1935) coordinates included; N/A – no data available

Site ID	Coordinates OSGB1936	NO_3^- (mg kg^{-1})	NH_4^+ (mg kg^{-1})
1	X: 351324 Y: 332751	15.60	9.18
2	X: 351492 Y: 332760	5.81	9.46
3	X: 351338 Y: 333049	34.84	12.17
4	X: 351423 Y: 333210	45.96	13.06
5	X: 351508 Y: 333084	11.62	13.37
6	X: 351431 Y: 332962	38.46	11.38
7	X: 351055 Y: 331839	24.93	10.24
8	X: 350712 Y: 330755	3.85	9.82
9	X: 348328 Y: 331336	9.99	11.55
10	X: 347883 Y: 332358	1.07	8.54
11	X: 350236 Y: 333611	*N/A*	8.99
12	X: 351006 Y: 333452	11.38	9.41

Tab. S3: Summary of experiment setups and variables tested for nitrate and ammonium extractions from *X. parietina* lichens; Solvents: DI – ultrapure water (18.2MΩ); 15% KCl – ~2M; 7.5% KCl – ~1M; 6% KCl – ~0.75M; 3% KCl – ~0.5M and 1% KCl M ~0.2M

Experiment	Variables	Rationale
#1	**Solvents:** DI, 1%, 7.5% and 15% KCl **Solvent volume [mL]:** 1, 2, 3, 4, 5 **Extraction time [hours]:** 24 (non-vortexing)	KCl concentrations (2M – 15% and 1M – 7.5%) as commonly applied in soil-N studies and their applicability to lichen material. Reduction of KCl concentration to 1% KCl to reduce necessary dilutions prior to IC analysis (max. concentration of 1% KCl on instrument) and reduce potential matrix effects. DI used for comparison (as applied in Naeth & Wilkinson 2018). Testing of lower solvent volumes to concentrate extracted NO_3^-/NH_4^+ from lichen material and investigate potential saturation of smaller volumes.
#2	**Solvents:** DI, 1%, 3% and 6% KCl **Solvent volume [mL]:** 1, 2, 3, 4, 5 **Extraction time [hours]:** 24 (non-vortexing)	Lowered KCl concentrations to 3% and 6% (together with DI and 1% KCl) to evaluate extractability of NO_3^- and NH_4^+ from 'weaker' KCl solutions and to potentially reduce variability of extracted concentrations, as seen in Experiment #1.
#3	**Solvents:** 3% KCl **Volume [mL]:** 2, 3 **Extraction time [hours]:** 6, 24 (vortexing and non-vortexing)	Consistent results obtained for 3% KCl using 2 mL and 3 mL of solvent. Therefore, 'same-day' (6 hours) extraction and analysis by IC conducted to assess data variability (and potential in-tube reactions, i.e. by bacteria). Vortexing included to investigate potential cell wall breakdown and intra-cellular release of N-compounds.

Tab. S4: Certified and measured nitrate (in CRM – Simple Nutrients – Whole Volume; Sigma-Aldrich) and ammonium (material made up from Dionex Six Cation Standard I calibration standard (serial dilution to 3.125 mg L^{-1} – mid-range) concentrations for all analytical batches (N=62 individual measurements, in N=9 batches), with overall accuracy (%), overall precision (coefficient of - %CV) and lower limits of detection (LLD; expressed as three times standard deviation of replicate procedural blank measurements) ranges for procedural blanks (N=46). *CRM NO_3-N converted to NO_3^- using the IUPAC atomic weights (N=14.007 and O=15.999), using a conversion factor of 4.426644 and resulting in 46.48 mg L^{-1} NO_3.

	Certified value [mg L^{-1}] ± 1xSD	**Measured value [mg L^{-1}] ± 1xSD**	**Accuracy (%) - overall**	**Precision (%CV) - overall**	**LLD [mg/l] (Min-Max)**
Nitrate (NO_3^-)*	10.50 ± 0.187 (NO_3^- as N) *Converted to 46.48 mg L^{-1} NO_3*	45.6 ± 2.12	98%	4.64	0.09 – 0.41
Ammonium (NH_4^+)	400 ± 3 Diluted to: 3.125	3.28 ± 0.25	105%	7.52	0.01 – 0.13

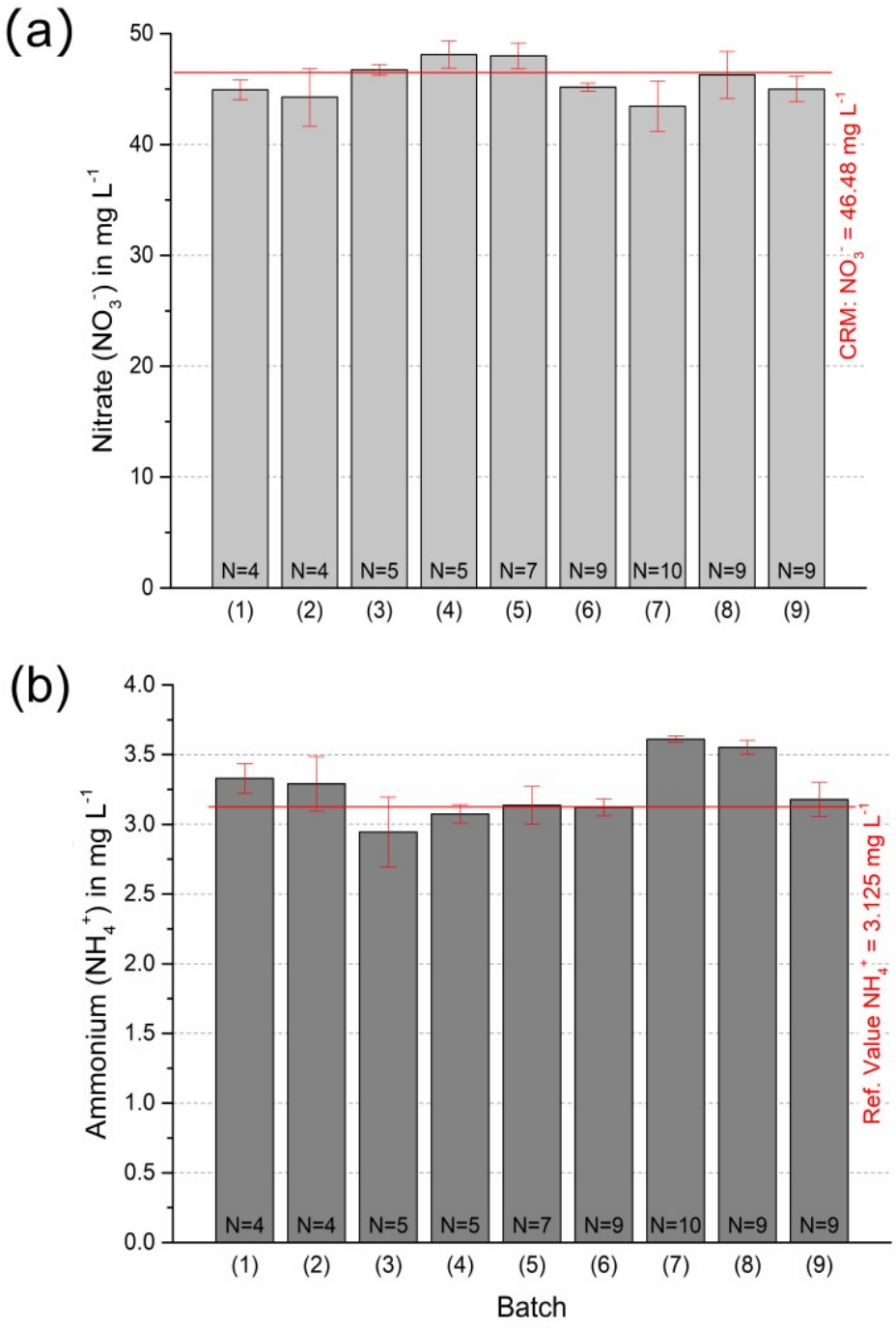

Fig. S2: Nitrate (a) and ammonium (b) concentrations determined for the CRM solutions Table S3) for each KCl extraction batch (N=62 individual measurements in N=9 batches), displayed with number of individual analyses per batch (error bars displayed as 1x standard deviation); red reference lines represent certified values (nitrate – NO_3^-: 46.48 mg L^{-1}, certified as NO_3-N: 10.5 mg L^{-1} and ammonium – NH_4^+: 3.125 mg L^{-1})

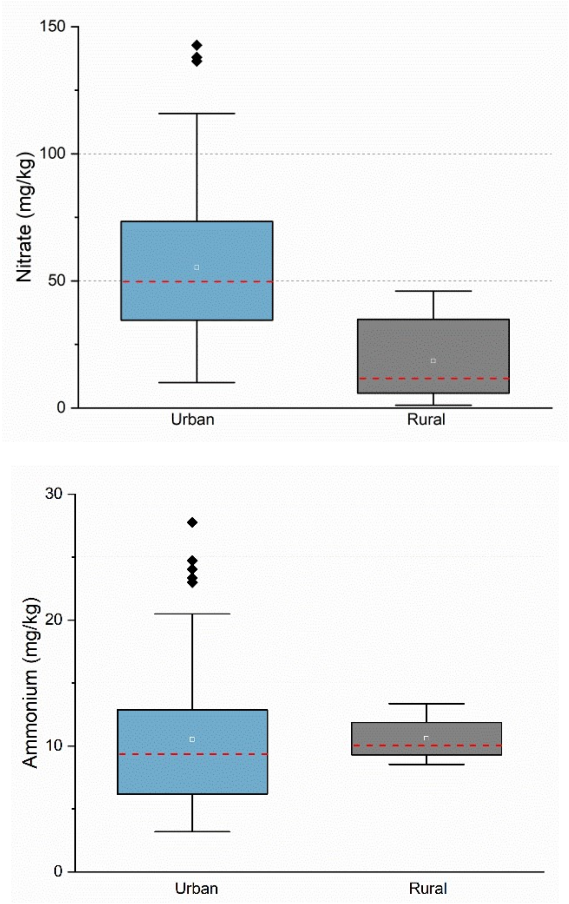

Fig. S3: Comparison of 3% KCl extracted nitrate (NO_3^-) [top] and ammonium (NH_4^+) [bottom] concentrations recorded in *X. parietina*, sampled in Manchester (urban) and around a poultry farm (rural); displayed as box-plots (25th to 75th quartile, 1.5x IQR whiskers, median line [red], mean value [white square] and outliers [black diamonds])

Tab. S5: Comparison of nitrate (NO_3^-) and ammonium concentrations (NH_4^+) [in mg kg^{-1}] in *X. parietina* (N=17) for different sampling periods undertaken in 2016/17 (1) and the same sites re-visited in 2018 (2) [see site ID – **Tab. S1**; **Fig. S4** for graphical comparison] to investigate any temporal variability.

	NO_3^- (1) [mg kg^{-1}]	NO_3^- (2) [mg kg^{-1}]	NH_4^+ (1) [mg kg^{-1}]	NH_4^+ (2) [mg kg^{-1}]
1 (ID: 58)	100	107	8.04	2.41
2 (ID: 23)	35.6	5.94	5.11	2.72
3 (ID: 43)	64.4	112	7.48	2.90
4 (ID: 18)	49.7	3.36	11.3	2.70
5 (ID: 16)	88.5	17.6	11.03	2.73
6 (ID: 19)	110	4.43	5.06	1.96
7 (ID: 26)	*N/A*	29.1	11.1	3.72
8 (ID: 36)	75.8	26.9	10.6	4.26
9 (ID: 14)	91.3	18.5	9.00	3.69
10 (ID: 67)	46.7	12.4	12.9	2.57
11 (ID: 2)	37.6	126	5.77	3.80
12 (ID: 9)	36.8	N/A	5.61	1.90
13 (ID: 60)	45.2	16.5	7.99	3.34
14 (ID: 82)	85.03	71.8	2.43	2.43
15 (ID: 59)	27.4	29.3	9.35	3.36
16 (ID: 4)	50.4	9.9	5.94	1.72
17 (ID: 15)	94.3	16.2	11.9	3.89

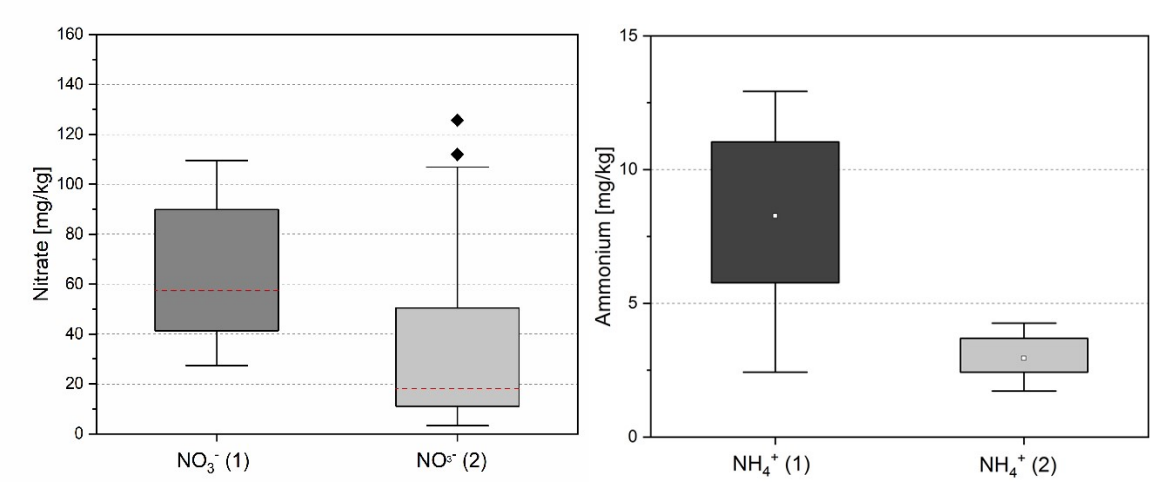

Fig. S4: Boxplots (25th to 75th percentile, 1.5x IQR whiskers, median line [red dotted], mean value [white square] and outliers [black diamonds]) of nitrate [left] and ammonium [right] concentrations for the same sites at two different sampling periods: (1) 2016/17 and (2) 2018.