Please cite the Published Version

Niepsch, Daniel, Clarke, Leon J, Tzoulas, Konstantinos and Cavan, Gina (2021) Distinguishing atmospheric nitrogen compounds (nitrate and ammonium) in lichen biomonitoring studies. Environmental Science: Processes & Impacts, 23 (12). pp. 2021-2036. ISSN 2050-7887

DOI: https://doi.org/10.1039/d1em00274k

Publisher: Royal Society of Chemistry (RSC)

Version: Accepted Version

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:

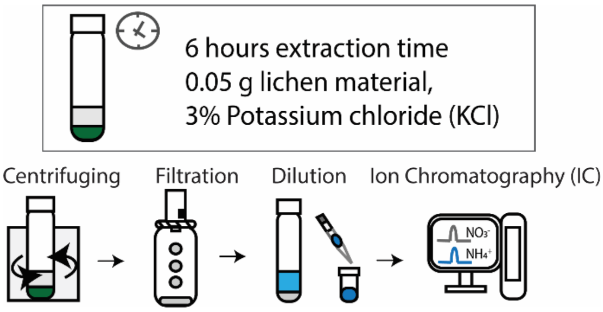
Daniel Niepsch - https://orcid.org/0000-0002-0177-1996

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

Table of contents



Nitrogen speciation (NO_3^- and NH_4^+) method from lichen material, using 0.05 g lichen, 3 mL of 3% KCl and 6 hours extraction.

Abstract

Nitrogen speciation, i.e. distinguishing nitrate (NO_3^-) and ammonium (NH_4^+), is commonly undertaken in soil studies, but has not been conducted extensively for lichens. Lichen total nitrogen contents (N wt%) reflect airborne atmospheric nitrogen loadings, originating from anthropogenic sources (e.g. vehicular and agricultural/ livestock emissions). Albeit nitrogen being an essential lichen nutrient, nitrogen compound (i.e. NO_3^- and NH_4^+) concentrations in the atmosphere can have deleterious effects on lichens. Moreover, N wt% do not provide information on individual nitrogen compounds, i.e. NO_3^- and NH_4^+ which are major constituents of atmospheric particulate matter (e.g. PM_{10} and $PM_{2.5}$). This study presents a novel method to separate and quantify NO_3^- and NH_4^+ extracted from lichen material. An optimal approach was identified by testing different strengths and volumes of potassium chloride (KCl) solutions and variable extraction times, i.e. the use of 3% KCl for 6 hours can achieve a same-day extraction and subsequent ion chromatography (IC) analysis for reproducible lichen nitrate and ammonium concentration determinations. Application of the method was undertaken by comparing urban and rural *Xanthoria parietina* samples to investigate the relative importance of the two nitrogen compounds in contrasting environments. Findings presented showed that lichen nitrogen compound concentrations varied in rural and urban *X. parietina* samples, suggesting different atmospheric nitrogen loadings from potentially different sources (e.g. agricultural and traffic) and varied deposition patterns (e.g. urban layout impacts). Despite potential impacts of nitrogen compounds on lichen metabolism, the approach presented here can be used for quantification of two different nitrogen compounds in lichen biomonitoring studies that will provide specific information on spatial and temporal variability of airborne NO_3^- and NH_4^+ concentrations that act as precursors of particulate matter, affecting air quality and subsequently human health.

Keywords: Air quality, nitrogen speciation (NO_3^- and NH_4^+), *Xanthoria parietina*, Ion chromatography (IC)

1. Introduction

Reactive nitrogen in the atmosphere comprises nitrogen oxides (NO_x) and reduced nitrogen compounds (NH_x), which originate from different sources, including fossil fuel combustion and agricultural processes[1,2]. Nitrate (NO_3^-) is a key plant nutrient and is released into the environment by anthropogenic sources, such as the production and use of fertilisers, and via fossil fuel combustion as nitric oxides[3,4]. Anthropogenic emissions of ammonia (NH_3) into the atmosphere are mainly derived from animal waste, chemical fertilizers and biomass burning, accounting for 277 kilotons of atmospheric emissions in the UK[5]. Atmospheric ammonia (NH_3) is rapidly deposited within 4 to 5 km of its source, but can be converted to ammonium (NH_4^+) in the atmosphere and then be transported distances of 100–1,000 km[4,6,7]. However, road traffic emissions release both nitrate and ammonia compounds[4,8] and it is suggested that NH_3 is now the dominant nitrogen species emitted by vehicles in urban environments[9–12]. NH_3 may now be the dominant nitrogen species emitted by vehicles, due to improved NO_x reduction efficiency of three-way catalysts, varied gradients of roads, varied traffic speeds (i.e. stop-and-go), and vehicle age[9–12].Humans are exposed to airborne pollutants via inhalation, ingestion and to a minor extent through dermal, or skin, contact[13]. Health impacts induced by exposure to nitrate and ammonium specifically are reported for the cardiovascular and respiratory systems[14–16]. Because these nitrogen compounds are important precursors of secondary particulates (i.e. $PM_{2.5}$ and PM_{10})[17,18], it is crucial to obtain information on the spatial distribution of airborne NO_3^- and NH_4^+ within urban environments, as these pollutants negatively affect air quality and human health[5,11,18]. The current EU/UK Air Quality Directive (2008/50/EC) is set out to monitor airborne pollutant levels and to minimise human health risks from particulate matter ($PM_{2.5/10}$), sulphur dioxide and nitrogen oxides (SO_x and NO_x), ozone (O_3) and carbon monoxide (CO). Hence, local authorities in the UK use urban air quality monitoring stations to continuously record these airborne pollutants, including NO_x and nitrogen dioxide (NO_2). However, other nitrogen compounds, i.e. nitrate (NO_3^-), ammonia (NH_3) and ammonium (NH_4^+), are not continuously measured by common air quality monitoring stations, due to their significant spatial heterogeneity and the requirement for costly equipment[19]. It is therefore important to apply different approaches, to provide additional information about airborne NO_3^- and NH_4^+ pollution across different environments (e.g. urban and rural).

A biomonitoring approach, using organisms that are part of an ecosystem, can be used to provide quantitative information (i.e. a measured concentration of a compound) that reflect the environmental conditions in a region[20]. Lichens have been shown to be useful organisms for biomonitoring of atmospheric pollution and air quality[20] and distributions of specific lichen species can reflect varying levels of atmospheric pollution, because different lichens (e.g. crustose, foliose and fruticose) exhibit differential sensitivity to specific airborne pollutants[21,22]. They consist of fungal (mycobiont: Ascomycetes), basidiomycete yeasts and photosynthetic (photobiont) partners, the latter either a green-algae and/or cyanobacteria, and lack roots and cuticle compared to higher plants[21,23,24]. Recent studies also suggest the importance of lichen-associated bacteria (more than 800 types of bacteria on single lichen individual) and their ability to fix nutrients (e.g. nitrogen)[25–28]. Due to these morphological features, lichens take up nutrients and also airborne pollutants directly from the surrounding atmosphere, by dry and/or wet deposition[20,29]. Lichens are commonly used as biomonitors where costly equipment is not viable, because they are (a) widely distributed, (b) perennial, (c) long-living, (d) slow growing and (e) able to bioaccumulate air pollutants[20,30]. Thus, they provide an integrated pollution profile [20]. For example, the nitrophytic lichen *Xanthoria parietina* flourishes in nitrogen-rich habitats, e.g. areas enriched in NO_x and NH_3, showing less sensitivity to high nitrogen compounds than acidophytic lichens[4,22,31–33]. It is found ubiquitously across urban environments, as well as in proximity to domestic livestock, due to increased atmospheric nitrogen concentrations in such areas[31,34,35]. Consequently, *X. parietina* is ideally suited for development and application of a lichen nitrate and ammonium extraction method, across both urban and rural settings.

Lichen total nitrogen contents (N wt%) can reflect airborne nitrogen loads from anthropogenic impacts (e.g. vehicular and agricultural/livestock emissions) and can be used to investigate spatial variability in the atmospheric burden of total nitrogen compound pollutants. However, such an approach cannot distinguish between the different nitrogen compounds that can be present in the atmosphere, e.g. NO_3^- and NH_4^+. Albeit nitrogen being an essential nutrient for lichens, nitrogen compounds such as NO_3^- and NH_3/NH_4^+ reportedly impact on lichen species distribution and metabolism[4,35–39]. Moreover, both nitrogen compounds can be constituents of atmospheric particulate matter (e.g. PM_{10} and $PM_{2.5}$) that are closely linked to human health risks, contributing to approximately 29,000 premature deaths in the UK[40,41]. Hence, additional monitoring of individual nitrogen compounds, e.g. by applying an easy-to-use lichen biomonitoring approach, will provide further insights into deteriorated air quality, potential identification of areas that are more likely to have elevated levels of PM and associated human health risks.

Distinguishing nitrate and ammonium compounds within environmental samples has previously been restricted to soil analyses and has not been applied to the same extent for lichens. Soil nitrate can be extracted using concentrated salt solutions, whilst ammonium can be extracted using potassium (K) solutions, with 1 mol L^{-1} and 2 mol L^{-1} potassium chloride (KCl) solutions widely used[42,43]. In contrast, there is little published data on the extraction and separation of nitrate and ammonia compounds from lichens. Naeth and Wilkinson (2008) applied a water extraction and ion chromatography analysis method to extract nitrate and ammonium from lichens sampled proximal to a diamond mine in Canada[44]. Sims *et al.* (2017) measured solubilised NO_3^- using a nitrate ion selective electrode (NO_3^- ISE) for the crustose lichen *Buellia dispersa* to assess nitrate pollution in the Las Vegas Valley[45]. However, these methods used solvents, i.e. deionised water, that might be insufficient to extract fully all nitrate and ammonium from lichens, or suffer from analytical limitations such as interfering anions (e.g. bicarbonate, carbonate and phosphate for NO_3^- ISE)[46]. Therefore, there is need for a new method to simultaneously extract both nitrogen compounds from biomonitors (i.e. lichens), in air quality assessment studies.

This study aims: (i) to evaluate, if an optimal nitrate (NO_3^-) and ammonium (NH_4^+) chemical extraction protocol for application to lichen material, using KCl solutions of different strengths that potentially increase extraction efficiency, with subsequent analysis by ion chromatography (IC) can be developed; (ii) if the method can be applied to separate the established lichen total nitrogen content measurement into two constituent nitrogen compounds, i.e. nitrate (NO_3^-) and ammonium (NH_4^+), as well as to quantify the concentrations of the two different nitrogen compounds; and (iii) if the developed extraction method can be applied to lichen samples collected from urban

(City of Manchester, UK) and rural (a poultry farm near Shrewsbury, UK) environments, in order to complete a test investigation of the relative importance of nitrate and ammonium compounds to the atmospheric nitrogen loading and atmospheric deposition in these two contrasting areas. The developed chemical extraction technique extends the scope of lichen biomonitoring studies by enabling quantification of different atmospheric nitrogen compounds, particularly in areas not continuously monitored by automated air quality stations. However, further method validation is necessary, to fully identify potential impacts of nitrogen compounds on lichen N wt%. For instance, temporal variability (monthly or seasonal changes) could be recorded, to investigate N-deposition (i.e. wet and dry) in more detail. Additionally, applying stable-nitrogen isotopes (e.g. $\delta^{15}N$)[47–49] in lichens could aid the understanding of potential NO_3^- and/or NH_4^+ impacts on lichens and identify potential sources[48,49].

2. Materials and Methods

2.1 Case study areas – urban and rural environments

Greater Manchester is a major urban conurbation in the northwest of England, UK, with 2.7 million inhabitants[50]. The City of Manchester (hereafter Manchester), is the centre of this conurbation and covers an area of about 11,500 hectares, with ca. 550,000 inhabitants[51]. Local airborne pollutant data for Manchester reveal particular problems regarding particulate matter (PM) and nitrogen dioxide (NO_2), and associated human health problems (i.e. cardiovascular and respiratory diseases), caused by deteriorated air quality and air pollution[41]. The contrasting rural environment investigated (**Fig. S1**) is located near the small town Wem and covers an area of 366 hectares with 6,100 inhabitants[52], north of Shrewsbury, Shropshire, UK. It was chosen for comparison because of its remote and rural location, with atmospheric NO_3^- and NH_4^+ being primarily influenced by the agricultural surroundings, such as farm land and livestock, e.g. poultry[53], whereas the Manchester urban environment is characterised by predominance of industrial, domestic and vehicular nitrogen emissions[54]. No continuous recording data on individual nitrogen compound pollution (i.e. nitrate and ammonia/ammonium) are available for either location.

2.2 Lichen sampling and preparation

In the context of a larger biomonitoring study to assess spatial variability of air quality in Manchester[47], urban *X. parietina* lichen samples (N=87; **Fig. 1**) were sampled from available and accessible street trees (e.g. *Acer* sp., *Fraxinus* sp. and *Tilia* sp.) following a general north-east/south-west transect across the centre of Manchester, during two sampling periods in 2016/2017 and 2018.

Potential sampling location across Manchester city centre were explored in March/April 2016 (prior to lichen sampling), based on tree species, tree abundance, site accessibility and visible lichen growth on twigs and branches (i.e. *X. parietina*). It is obvious (**Fig. 1**) that sampling locations within the city centre were limited, due to low tree cover. It was not possible to sample lichens from one tree species only, because of diverse ornamental and planted trees across the city centre area; however, tree species with similar bark substrate acidity[23], were sampled for lichens.

Environmental factors, such as light availability and illumination, precipitation, humidity, bark age, corrugation and acidity are important factors for lichen community development[20,30,55]. For instance, light availability is a crucial parameter for the growth photobiont (during thallus hydration), which consequently is vital for the lichen-forming fungus[30,56]. However, less acidic conditions are generally found in upper parts of a tree[55] and the presence of nitrophtytic species on twigs and branches suggest the presence of nitrogen compounds and similar environmental conditions (e.g. eutrophication) across Manchester that impact on lichen colonisation and succession, e.g. favouring the growth of nitrogen-preferring species such as *X. parietina*[57]. Small branches and twigs (2–4 m above ground) were sampled from trees, using a tree pruner (locations in **Fig. 1**), from the cardinal direction facing the closest road. Depending on lichen coverage (and accessibility) on individual trees, one or more cardinal directions (clockwise rotation) were sampled and lichen samples were combined into a single sample. Collected twigs with lichens were stored in paper bags and returned to the laboratory; lichen material then was carefully scraped off the bark using a stainless-steel scalpel, non-lichen detritus was removed under an illuminated magnifying glass and dry lichen samples were ground into powder and homogenised using an agate pestle and mortar and stored in glass vials (in the dark at 20°C – room temperature, separated from chemicals) until chemical analyses. Due to the long initial sampling period (June 2016 to October 2017), certain sites (N=17, **Fig. 1**) were re-sampled in 2018, from the exact same trees for *X. parietina*, to investigate any temporal variability in lichen nitrogen chemistry. Re-visited sites were chosen based on measured total nitrogen contents (wt% N)[47], because different wt% N potentially indicate varying N-compound inputs.

A single urban *X. parietina* lichen sample, of which sufficient lichen material (~6.5 g) was sampled, was used for methodological development (**Fig. 1**, red cross). Only one site was used for method development to allow for comparison of extracted NO_3^- and NH_4^+ concentrations between experiments. This 'test site' was located close to a major 'A road' leading into Manchester city centre, which is used by ~30,000 vehicles daily[58] thereby indicating that road traffic emissions most likely influences airborne NO_3^- and NH_4^+ at this location.

X. parietina lichens (N=12; **Fig. S1**) also were sampled from a rural environment, around a rural poultry farm in May 2018. These lichens were collected from oak (*Quercus* spp.) and hawthorn (*Crataegus* spp.) trees, using the same procedure as undertaken in the urban environment. *Crataegus* spp. is a more shrub-like, smaller tree[59], which was sampled for *X. parietina*, requiring conscientious comparison. However, it was primarily sampled in proximity to the poultry farm, generally completely covered with lichens, suggesting a surplus of atmospheric nitrogen. Although different tree species were sampled for twigs and branches, compared to the urban environment, ammonia (NH_3) emissions in rural environments are reportedly related to increases in bark pH[60].

Thus, suggesting bark eutrophication that favours the growth of nitrogen-preferring *X. parietina* and subsequently allow for comparison between environments. Rural lichens were sampled on a general north-east/south-west transect away from the poultry farm, both in close proximity (i.e. 50 to 500 m) and at greater distances (i.e. 1–3 km). Sampling locations were selected where there was public access, i.e. fenced and hedged agricultural fields and private property could not be sampled. Site-specific data for urban and rural sampling sites are displayed in **Tab. S1** and **S2**.

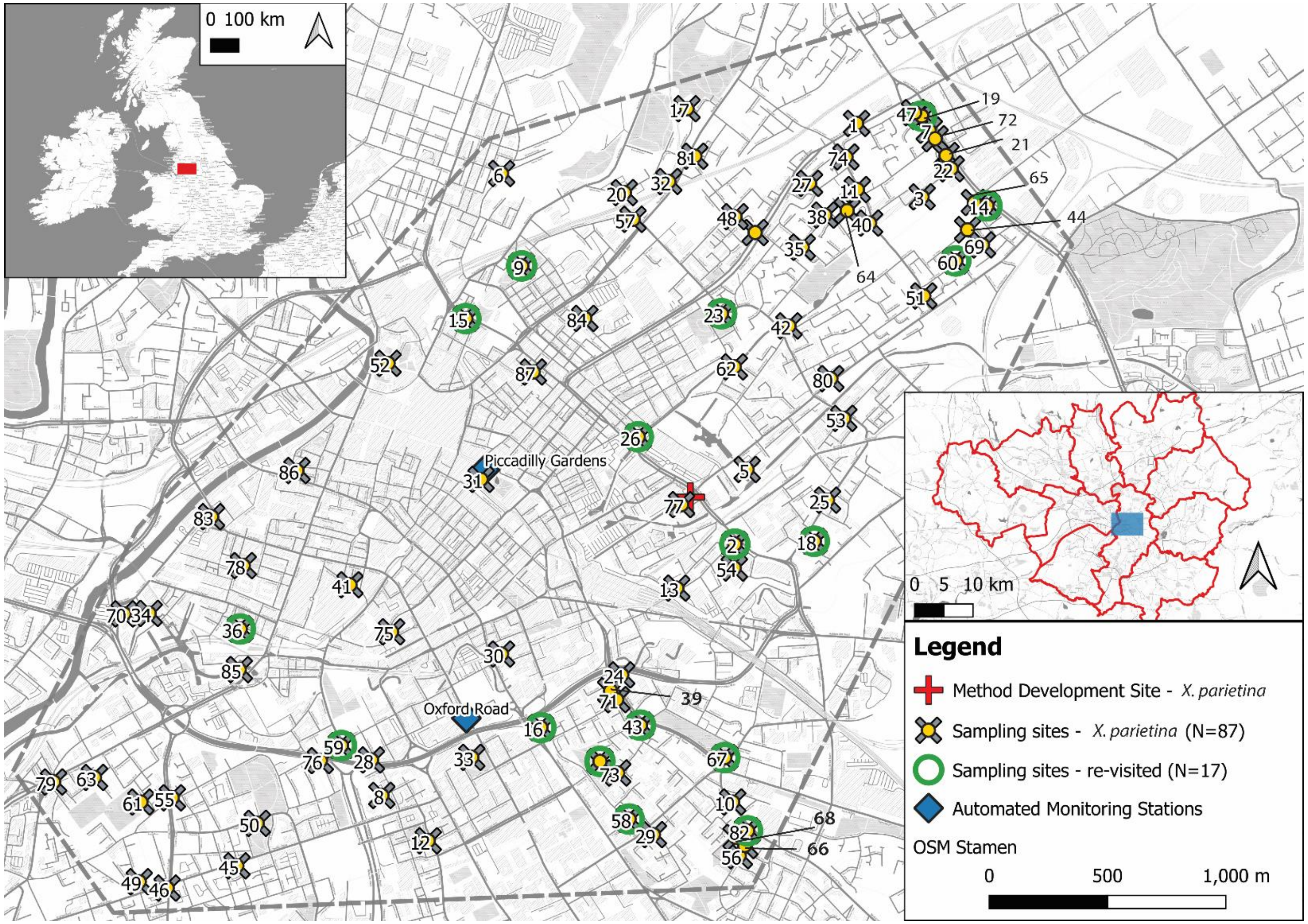


Fig. 1: *X. parietina* lichen sampling locations sites (i.e. trees, displayed with site-IDs; N=87; sampled between June 2016 and October 2017), including method development site (red cross), within Manchester city centre; Circled sampling sites (green, N=17) were re-sampled in 2018. Automated air-quality monitoring stations (Oxford Road and Piccadilly Gardens) and location of study area within Greater Manchester and the UK also are shown (contains Ordnance Survey data © Crown copyright and database right 2010-21. OpenStreetMap® is open data, licensed under the Open Data Commons Open Database License (ODbL) by the OpenStreetMap Foundation (OSMF).

2.3 Experimental setup to extract NO_3^- and NH_4^+ from lichen material

To assess extractability of NO_3^- and NH_4^+ from lichen material, methodological development was based on soil extraction studies for inorganic nitrogen that have applied potassium chloride (KCl) solutions, with 2M KCl being most commonly applied[43]. A KCl-based extraction protocol was also considered appropriate for lichens. Influences of extraction solvent concentration (in % KCl), solvent volume to lichen mass ratio (in m/v), time of extraction (in hours), and mixing (vortexing and non-vortexing), were all tested to determine an optimal extraction procedure, i.e. to obtain reproducible NO_3^- and NH_4^+ concentrations from lichen material (**Tab. S3**). A low sample mass-to-extraction solvent volume ratio (1:100 m/v) was used to begin methodological development, i.e. 0.05 g lichen material and 5 mL solvent was chosen to effectively extract NO_3^- and NH_4^+ and minimise potential solution saturation when using a higher m/v-ratio. Experimental set-up and test variables and associated rationale are summarised in **Tab. S3**.

About 0.05 g of lichen was weighed into 12 mL plastic tubes (101 x 16.5 mm, Sarstedt), in triplicate for each extraction procedure variable tested, and extraction solvent was added to the tubes (**Fig. 2**). Potassium chloride (KCl, ≥99%, Sigma-Aldrich) extraction solutions were prepared fresh before each sample extraction. Tubes containing lichen material and solvent were cautiously shaken (non-vortexing), to release lichen material stuck to the bottom of the tube, or were vortexed (30 sec. at 1000 rpm) utilizing a digital vortex mixer (VWR) to potentially fully mix and disperse lichen powder material. Vortexing was included to investigate potential cell wall breakdown and release of intra-cellular nitrogen compounds, in addition to nitrogen compounds adsorbed to the lichen surface. Lichen samples were left for the specified extraction times (6 h or 24 h) and then centrifuged for 20 minutes at 4000 rpm. Subsequently, 0.5 mL supernatant was pipetted out and passed through 0.2 µm nylon syringe filters (Fisher Scientific) into new tubes (**Fig. 2**). Filtered extraction solvents were diluted to 1% KCl strength (using 18.2 MΩ ultrapure water) and the concentrations of the two individual nitrogen compounds determined using a Thermo Scientific (Dionex) ICS-5000 ion chromatography (IC) system. Blanks were included during each extraction experiment, these were handled in exactly the same way as the lichen samples (i.e., vortexing and filtering) and used for blank subtractions before further data analysis.

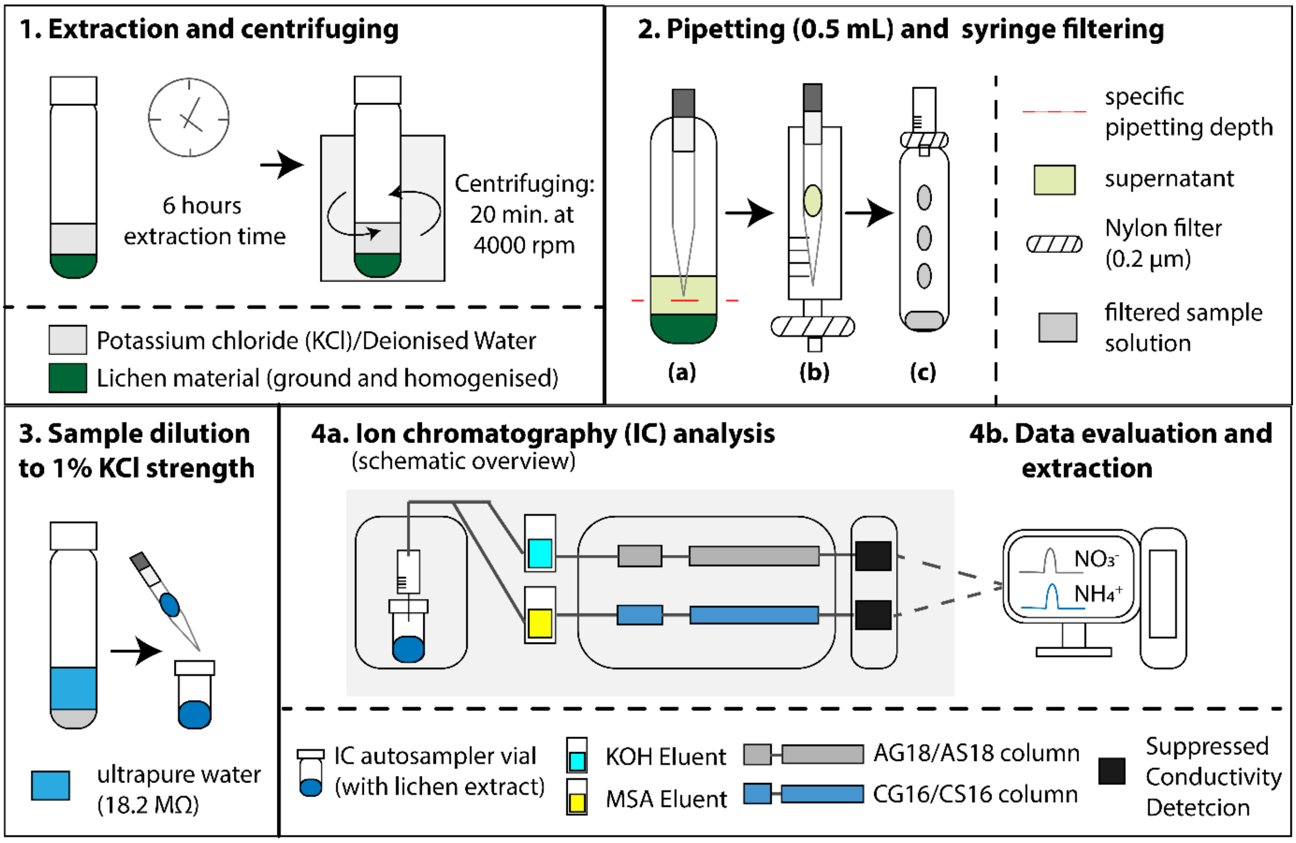


Fig. 2: Schematic overview of lichen extraction procedure, including extraction and centrifuging (1), pipetting of supernatant (1a) and syringe filtering (2b and 2c), and dilution of extracted sample solution (3) prior to IC analysis (4a) and data evaluation and extraction (4b)

2.4 Quantification of NO_3^- and NH_4^+ by ion chromatography (IC)

Ion chromatography is one commonly used method to determine anions (NO_3^-) and cations (NH_4^+), and is particularly recommended for speciation analyses, due to simultaneous determination, short measurement time, good reproducibility and high sensitivity[61]. This study used a Dionex-ICS 5000 (Thermo Fisher Scientific, UK) in the Department of Natural Sciences, Manchester Metropolitan University.

Calibration standards were made up in DI or 1% KCl, to ensure matrix matching with the final diluted (where necessary) lichen solvent extracts. Calibration standards (six point) were made up from ‘Dionex Seven Anion Standard II’ (Thermo Scientific, UK) and ‘Dionex Six Cation Standard I’ (Thermo Scientific, UK). Anions (nitrate; NO_3^-) were analysed using an AG18 guard column (2 mm x 50 mm) and an AS18 separation column (2 mm x 250 mm). A potassium hydroxide eluent gradient was generated electrolytically using an EGC III KOH cartridge, starting at 18 mM KOH with a slope of 1.96 mM/min for 16 minutes. For cations (ammonium; NH_4^+) a CG16 guard column (3 mm x 50 mm) and CS16 separation column (3 mm x 250 mm) were used. Methanesulfonic acid (MSA) at 39 mM was pumped isocratically during the analysis. Both signals, for anion: NO_3^- and cation: NH_4^+, were measured using suppressed conductivity.

Certified reference material (CRM, 'Simple Nutrients – Whole volume', Sigma-Aldrich, UK) for anions (certified as NO_3-N: 10.5±0.187 mg L^{-1}, converted to NO_3^-: 46.48 mg L^{-1} using IUPAC atomic weights for nitrogen and oxygen), and a diluted calibration standard (3.125 mg L^{-1}; Dionex Six Cation Standard I) for cations, were included within each measurement batch (i.e. separate extraction experiments) to check accuracy and precision (both instrumental and between extraction experiments). Overall accuracy (N=62; **Tab. S4; Fig. S2**) of reference material concentration determinations was 98% for NO_3^- and 105% for NH_4^+, with individual sample batch accuracy ranging between 93% and 104% (NO_3^-), and from 94% to 116% (NH_4^+). Overall precision (N=62; **Tab. S4**; **Fig. S2**) was <5 %RSD (relative standard deviation in %) for NO_3^- and <8 %RSD for NH_4^+, with individual batch precision ranging between 1% and 6% (for NO_3^-) and 1% and 9% (for NH_4^+). Batch-to-batch correction for any variability in IC-determined NO_3^- and NH_4^+ concentrations in lichen material extracts was not undertaken, because of good repeatability for each analytical batch.

Lower limits of detection (LLD), calculated as three times the standard deviation of replicate procedural blank measurements, were calculated separately for each analytical batch and ranged between 0.09 and 0.41 mg L^{-1} for NO_3^-, and from 0.01 to 0.13 mg L^{-1} for NH_4^+. Average procedural blank concentrations of NO_3^- and NH_4^+ were used for blank subtraction, sometimes resulting in measured analyte concentrations below LLD for some extracted lichen material, these measurements were excluded from further analysis (for experiments undertaken and extracted lichen NO_3^- and NH_4^+, which are displayed as N/A in **Tab. S1**).

2.5 *Statistical analysis*

Data visualisation and statistical testing were performed using Minitab 19 (State College, PA, USA), Origin (OriginLab Corporation, MA, USA) and open-source software R (R Foundation for Statistical Computing, AUT) and jamovi (The jamovi project, AUS)[62–65]. Mapping of data was performed using QGIS 3.12 (QGIS Association, CH)[66].

Dataset normality was tested using Shapiro-Wilk, because of its higher statistical power compared to other statistical tests, e.g. Kolmogorov-Smirnoff, irrespective of sample size[67]. Outcomes of the Shapiro-Wilk test for dataset normality informed the use of parametric or non-parametric tests for dataset comparisons. Comparison of nitrogen compound concentrations resulting from different lichen sampling months (temporal variability) was undertaken using Wilcoxon test statistics (NO_3^-; non-parametric) and paired t-test (NH_4^+; parametric), respectively. Nitrogen compound concentrations extracted from urban and rural lichen samples were compared using a Mann-Whitney test (two-tailed; non-parametric).

3. Results and Discussion

3.1 Influence of solvent type, concentration and volume on extractability of lichen NO_3^- and NH_4^+

During experiment 1, extracted NO_3^- concentrations showed higher replicate variability with increasing solvent strength, i.e. 7.5% and 15% KCl (**Fig. 3a**). For the single lichen test sample, the lowest extracted NO_3^- concentration was found for deionised water (DI; 1.03 mg kg^{-1}), whereas the highest was for 15% KCl (94.85 mg kg^{-1}; **Fig. 3a**). Furthermore, application of 15% KCl resulted in highly variable and inconsistent NO_3^- concentrations for all tested solvent volumes (**Fig. 3a**). More reproducible extracted NO_3^- concentrations were recorded using 1% KCl for all different extraction solvent volumes (although recorded NO_3^- for 1 mL and 2 mL showed concentrations below the limit of detection, <LLD). Deionised water extractions showed a slight increase of minimum extracted NO_3^- concentration with increasing extraction volumes, an observation comparable to results for 1% KCl (**Fig. 3a**).

Minimum and maximum extracted ammonium concentrations generally increased with increasing solvent volume in experiment 1 (**Fig. 3c**). However, extracted NH_4^+ concentrations were more variable for the higher concentration 7.5% and 15% KCl solvents (**Fig. 3c**), an outcome comparable to the extracted NO_3^- concentrations. Hence, a potential saturation of smaller extraction volumes and potential impact, i.e. dissolution of the lichen material, on NH_4^+ concentrations when using more concentrated solvents (e.g. 15% KCl; **Fig. 3c**) is suggested. Replicate extracted NH_4^+ concentrations for less concentrated KCl solutions (3% and 6% KCl) were more reproducible in experiment 2, albeit with the highest extracted NH_4^+ concentration (49.93 mg kg^{-1}; **Fig. 3d**) using 5 mL 1% KCl being an outlier with no plausible explanation.

Overall, extracted NO_3^- and NH_4^+ concentrations were most variable for replicates using the higher concentration KCl solutions (i.e. 7.5% and 15% KCl), suggesting that these solvents are too concentrated for a reproducible extraction of the target nitrogen compounds from lichen material. Reducing KCl solvent concentrations to 6% and 3% improved the repeatability of the more variable (cf. ammonium) NO_3^- extractions, with

2 mL and 3 mL volumes of 3% KCl resulting in the most reproducible concentrations (for 0.05 g of lichen material).

Using concentrated salt solutions (e.g. KCl) with a high soil weight-to-solution ratio (e.g. 1:10 m/v) to remove biologically available nutrients from soil samples has been found to be preferable over deionised water[42]. The primary aim of this study was to identify, if KCl solutions, rather than deionised water, are beneficial for extracting nitrogen compounds and findings presented demonstrate that a low sample mass-to-solution ratio (e.g. 1:40 and 1:60 m/v) is appropriate for the most reproducible extraction of NO_3^- and NH_4^+ from lichen material. Interestingly, in contrast to the expected increased extraction efficiency by high strengths KCl solutions, reduced KCl strengths were found preferable (i.e. 3% KCl). However, the single lichen test sample used for methodological development was collected from a potentially highly polluted urban environment, such that higher sample mass-to-extraction solution ratios might be more appropriate for lichen samples from less polluted environments. Nonetheless, because of the potential for saturation when using smaller solvent volumes, a 1:60 ratio (0.05 g of lichen material and 3 mL KCl) has been identified as the optimal analytical protocol. But, given the limited number of studies that have attempted to quantify separately lichen NO_3^- and NH_4^+ contents there is scope to test other solvents (e.g., $BaCl_2$, $NaHCO_3$ and NH_4Cl, that have also been applied in soil studies)[42] to improve further extraction of nitrogen compounds from lichen material.

3.2 Influences of extraction time and mixing procedure on extractability of lichen NO_3^- and NH_4^+

Because of variable concentrations for replicates for both nitrogen compounds with a 24-hour extraction of lichen material (Experiments 1 and 2 were extracted for 24 hours; **Fig. 3**), a considerably reduced extraction time was investigated. A 6-hour approach was assessed as a means of minimising variability in extracted NO_3^- and NH_4^+ and to facilitate same-day extraction and quantification of the two nitrogen compounds (Experiment 3, **Tab. S3**). Overall, highest extracted NO_3^- concentration was recorded using a 6-hour extraction, ranging from 95.7 to 105.1 mg kg^{-1} (**Fig. 4**), whereas 24-hour extraction NO_3^- concentrations were below the analytical run LLD (2 mL: <0.18 mg L^{-1}; 3 mL: <0.11 mg L^{-1}; missing bars in **Fig. 4**) for the tested solvent volumes, which is comparable to some NO_3^- concentrations from experiment #1 and

#2. In contrast, reproducible ammonium concentrations were recorded for replicates for both 24- and 6-hour extraction times, with slightly higher concentrations when left for 24 hours using 3 mL of 3% KCl (**Fig. 4**). However, these higher NH_4^+ concentrations were considered negligible in comparison to 6-hour extracted NH_4^+ concentrations (using 2 mL or 3 mL of solvent; **Fig. 4**), because of results obtained for NO_3^- concentrations that were <LLD in this experiment (**Fig. 4**).

Gradual increases of NO_3^- and NH_4^+ concentrations with increasing extraction time have been reported for soil extraction studies[42,43]. Damage to lichen cells (i.e. algal and fungal) over time and subsequent release of intra-cellular nitrogen compounds could explain the differences between tested extraction times, which could also be evident when using an aggressive mixing procedure (i.e. vortexing; discussed below). Also, microbial reduction of NO_3^- in aqueous solutions (e.g. soil extracts) when organic matter is present has been reported[46] and could be an explanation for the 24-hour extraction NO_3^- concentrations being below the LLD (e.g. excluded values in **Fig. 3** and **Fig. 4**). NO_3^- in solutions can reportedly be reduced to nitrite and ammonium by UV light and can form metal-nitrate complexes[68–70]. Lichen extracts for 24 hours were extracted in the laboratory at room temperature, suggesting potential UV-light reduction. Further, metal ions in the lichen extracts (e.g. Mg, Ni, Cu and Zn)[69] could have formed complexes with present NO_3^- over the prolonged extraction period. Lichens are associated with bacteria (i.e. from their substrate they grow on and on their surface)[25,27,28] that most likely acted as nitrate reducers (i.e. oxidation of nitrate by bacteria in the presence of organic matter)[46] during the completed KCl extraction experiments. Boric acid (1 mol L^{-1}) could have been used as a preservation solution for 24-hour extraction times[46], to minimise potential NO_3^- reduction in the lichen-KCl-solution, but testing this approach was not within the scope of this study. However, this study has identified an optimal extraction method (first aim), using a reduced extraction time (i.e. 6 hours) to achieve same-day extraction and subsequent IC analysis. Thus, the developed method was able to extract (and quantify) the two target nitrogen compounds from lichen material (second aim).

Potential release of intra-cellular nitrogen through aggressive mixing (vortexing cf. non-vortexing) of the lichen-solvent mixture also was investigated; this test did not show any significant statistical differences in extracted nitrate or ammonium concentrations (**Fig. 4**), suggesting that the sample state, i.e. lichen ground to a fine powder, is suitable to extract the two different nitrogen compounds. Therefore, when lichen material is ground to a fine powder, the “total” amount of nitrogen compounds, i.e. a combination of adsorbed (i.e. particulate) and intra-cellular NO_3^- and NH_4^+, is likely extracted.

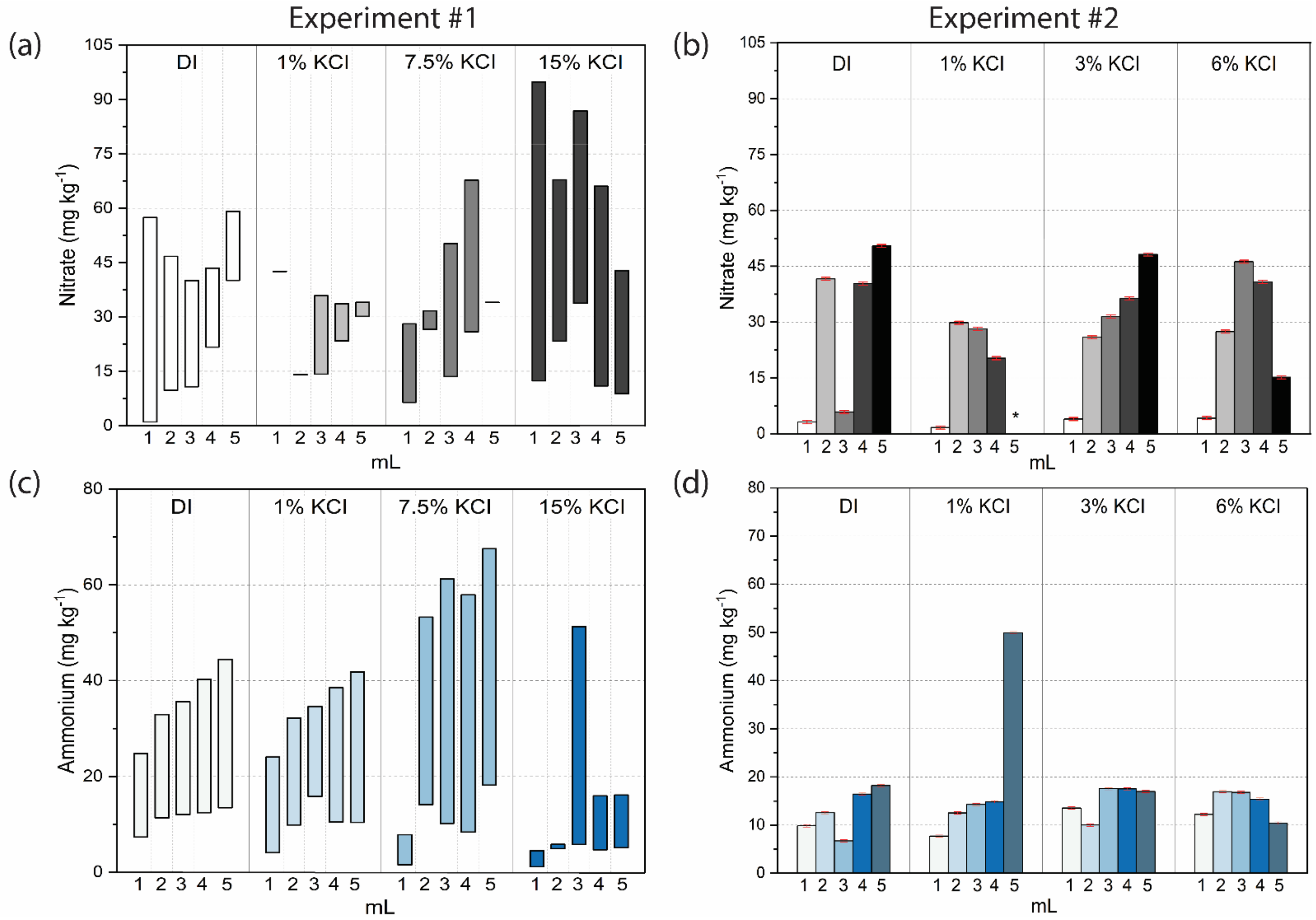


Fig. 3: (a) NO_3^- and (c) NH_4^+ extracted concentrations [mg kg^{-1}] for experiment #1 (triplicate extractions) and (b) NO_3^- and (d) NH_4^+ extracted concentrations [mg kg^{-1}] for experiment #2 (triplicate extractions for 3% and 6% KCl only, due to sample mass restrictions for further method testing and results obtained by experiment #1), error-bars represent IC-CRM %RSD: NO_3^-: 6.42% and NH_4^+: 6.30%); experiment #1 presented as range plot (minimum to maximum), due to high variability of recorded concentrations using more concentrated solvents (7.5% and 15% KCl), experiment #2 presented as bar graphs (showing proportionately extracted NO_3^- and NH_4^+) due to more reproducible concentrations obtained for replicate extractions for lower concentration KCl solutions (3% and 6% KCl); both grouped by solvent and volume used [in mL].Values <LLD (*) for analytical runs were excluded.

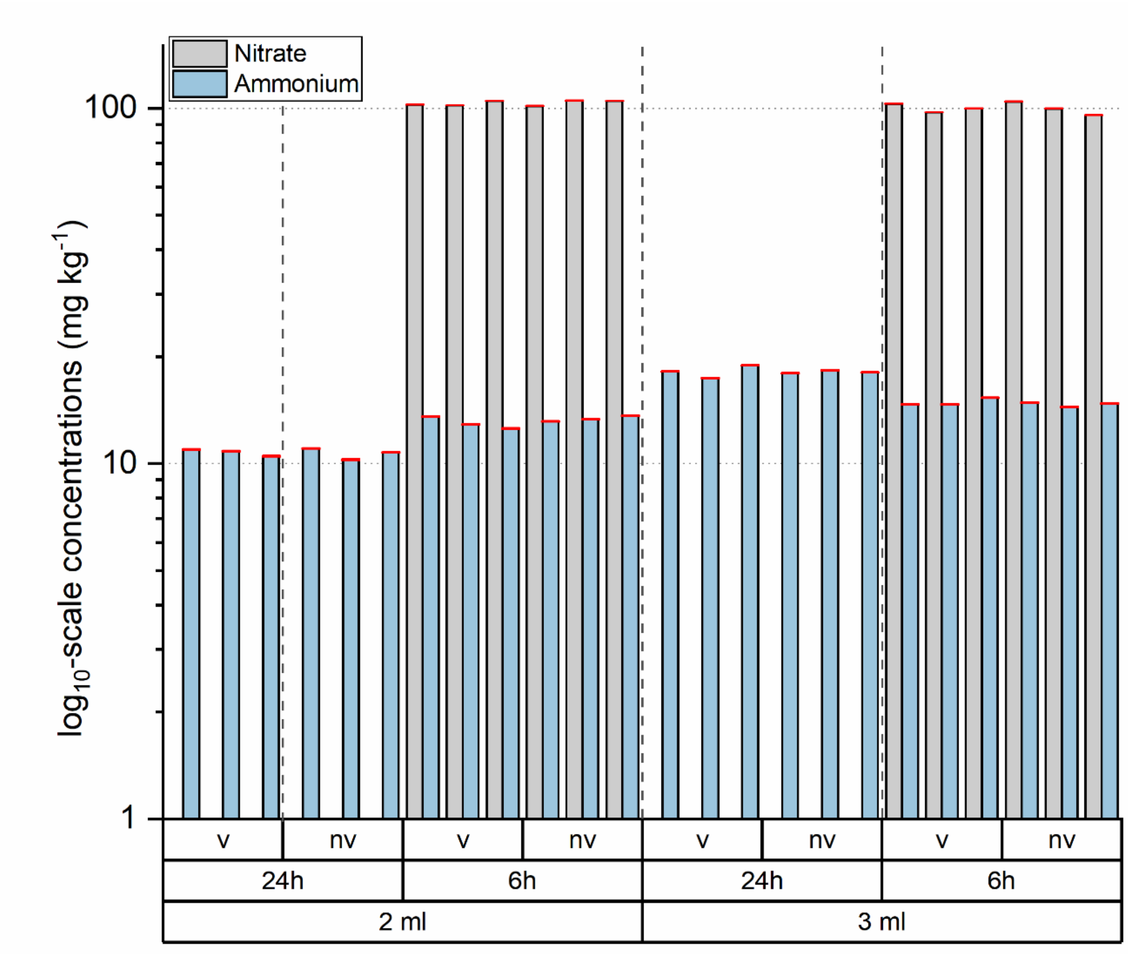


Fig. 4: Nitrate and ammonium extracted concentrations [mg kg^{-1}] (log$_{10}$-scale; triplicate extractions for tested variables) for 2 mL and 3 mL volumes of 3% KCl, for 24- (24h) and 6-hour (6h) extraction times and mixing technique: vortexing (v) and non-vortexing (nv); presented as bar chart, error-bars represent IC-CRM %RSD: NO_3^-: ±0.91%; NH_4^+: ±0.76% (24 h) and NO_3^-: ±1.63% NH_4^+: ±1.07% (6 h); no bars for NO_3^- represent excluded values, because concentrations were below the analytical run detection limits (<0.18 mg L^{-1} for 2 mL and 24 hours; <0.11 mg L^{-1} for 3 mL and 24 hours)

3.3 Optimised extraction method to compare rural and urban lichen nitrate and ammonium concentrations

The optimised chemical extraction method (3 mL of 3% KCl and a 6-hour extraction time) was applied to urban and rural lichen samples, to test the method and compare the relative contributions of different airborne nitrogen compounds to the lichen nitrogen content, in contrasting environments. Generally, NO_3^- and NH_4^+ concentration ranges in urban *X. parietina* samples were higher compared to rural counterparts (**Fig. S3**). NO_3^- was from 9.96–143 mg kg^{-1} in urban (N=87) and from 1.07–46 mg kg^{-1} in rural (N=12) lichen samples. NH_4^+ concentrations ranged from 3.18–28 mg kg^{-1} (urban) and 8.54–13 mg kg^{-1} (rural), respectively. Mann-Whitney test statistics showed significant ($p<0.01$) differences between rural and urban *X. parietina* samples for nitrate concentrations, but not for ammonium ($p=0.27$).

Highest extracted NO_3^- concentration in the rural environment was recorded in close proximity (<150 m) to the poultry farm. Poultry manure contains nitrate that is bound to particulates or occurs as nitric acid[71–73] most likely influencing extracted lichen NO_3^- concentrations. Comparably, NH_4^+ concentrations in rural *X. parietina* also were highest in the vicinity of the poultry farm (>12 mg kg^{-1}) suggesting primarily agricultural and livestock pollutant sources, e.g. from animal waste and chemical fertiliser[4,53]. Poultry farm emissions directly deposited on the lichen surface apparently affected recorded lichen nitrogen compound concentrations. Certainly, agricultural practices (e.g. fertilizer use) could have also influenced recorded lichen NO_3^- and NH_4^+. For instance, nitrogenous fertilisers in the form of ammonium nitrate (NH_4NO_3) are commonly used in the UK and agricultural activities are responsible for 88% of annual (2016) UK NH_3 emissions, and its reaction product NH_4^+, in rural environments; the latter is primarily emitted at ground level[48,53].

By comparison, higher and more variable concentration ranges for the urban environment *X. parietina*, for both NO_3^- and NH_4^+, suggest a more complex mixture of airborne nitrogen compounds (oxidised – NO_x and reduced NH_x) and indicate the importance of both atmospheric nitrogen compounds in an urban surrounding, i.e. from varying potential pollution sources such as traffic, industrial and domestic emissions[74], when compared to the rural environment. For instance, Longley *et al.* (2005) reported that traffic is a significant source of ammonia (and NO_x) in the city centre of

Manchester[75], which could explain elevated NH_4^+ concentrations recorded in urban lichens. However, NH_4^+ concentrations were elevated in both environments (no statistically significant difference), although likely linked to different sources, whereas NO_3^- concentrations are potentially related to increased NO_x emissions in the urban environment. Comparably, Niepsch *et al.* (2021) reported elevated NO_2 concentrations across Manchester[76].

Overall, this study was able to identify the relative importance of 'speciated' atmospheric nitrogen compounds (third aim) in diverse environments. Nonetheless, additional research focussing on NO_3^- and NH_4^+ concentrations in more N-sensitive lichens could provide further information on "critical" levels of atmospheric concentrations and deposition. Such an approach could be further combined with stable-nitrogen ($\delta^{15}N$) isotope ratios of lichens for source apportionment studies[47].

3.4 Temporal variability of NO_3^- and NH_4^+ in urban X. parietina samples in Manchester

Lichen sampling, from the exact same tree, was repeated in 2018 to investigate potential temporal variability superimposing on spatial variability of both nitrogen compounds. Extracted NO_3^- concentrations were not statistically significantly (Wilcoxon test; $p=0.06$) different at re-visited sites (in 2018; **Fig. S4**, **Tab. S5**), whereas ammonium concentrations (**Fig. S4**) were significantly different (paired t-test; $p<0.01$) between sampling periods 2016/2017 and re-visited in 2018.

Seasonal variability of atmospheric NO_3^- and NH_4^+ concentrations and particulate matter (e.g. containing NH_4NO_3) in urban environments has been reported, being particularly elevated during cold seasons[77–82]. Such seasonal trends for particulate matter (PM_{10} at Oxford Road and $PM_{2.5}$ at Piccadilly Gardens) were also recorded at automated air quality monitoring stations[83,84] during this study's sampling intervals, with lichen NO_3^- and NH_4^+ concentrations also suggesting temporarily variable nitrogen compound concentrations across the Manchester city centre area. Temporal variation in NO_3^- and NH_4^+ due to atmospheric process i.e. volatilization of particulate-NH_4^+ during warmer periods[78,82,85] could not be accounted for and were out of scope for investigation by this study. Further, a potential wash-off effect during precipitation events cannot be fully excluded and the recorded temporal variation could be related to removal of adsorbed NO_3^- and NH_4^+ during a rainy period prior to sampling. Hence, potential differences from dry and wet deposition of NO_3^- and NH_4^+ during lichen sampling periods cannot be fully accounted for in this study.

Nevertheless, to minimise such an effect, sampling was undertaken during dry days (throughout the sampling intervals) only. Additionally, prior to grinding and chemical extraction, this study's *X. parietina* samples were not washed, such that deposited NO_3^-/NH_4^+-containing particles and/or nitric acid adsorbed to the lichen surface were included during the extraction process[73,86]. To successfully investigate seasonal patterns of nitrate and ammonium concentrations, and to minimise effects of atmospheric reactions and removal of NO_3^- and NH_4^+ containing particulates during wetter seasons, this study's findings suggest a more frequent lichen sampling can yield a higher density temporal dataset. Additionally, incorporation of meteorological parameters that affect ambient NO_3^- and NH_4^+ concentrations (e.g. air temperature and relative humidity), as well as speciation of particulate matter for nitrogen compounds[77] into a lichen biomonitoring approach could be used to explain further any temporal variability of lichen-extracted NO_3^- and NH_4^+. Thus, using lichens to extract different nitrogen compounds can provide information on temporal variability of particulate pre-cursors, specifically at sampling locations that are not covered by continuous airborne pollutant measurement stations.

3.5 Spatial variability of NO_3^- and NH_4^+ in X. parietina samples in Manchester

Spatially variable NO_3^- concentrations (9.96–143 mg kg^{-1}) were evident across the Manchester study area, with elevated NO_3^- generally measured at roadside/road junction locations in the north-east and south-east of the study area, whereas lower NO_3^- concentrations were recorded within Manchester city centre (**Fig. 5a**). In contrast, lichen NH_4^+ (3.18–27.7 mg kg^{-1}) was highest within the city centre area and along major roadside locations (**Fig. 5b**).

Varying lichen NO_3^- concentrations have been reported around a diamond mine in Canada and for the Los Angeles Valley (USA), the latter with more comparable (14–564 mg kg^{-1}) NO_3^- concentrations to those recorded in this study[44,45]. However, comparison between studies is difficult, because of the different lichen species utilised (e.g. terricolous, crustose and foliose [this study])[44,45] methodologies applied for extraction of nitrogen compounds (e.g. deionised water, Naeth and Wilkinson, 2008; KCl solution, this study) and analysis (e.g. ion-selective electrode, Sims *et al.*, 2017; ion chromatography, Naeth and Wilkinson, 2008 and this study). Nonetheless, spatial variability of lichen-extracted NO_3^- and NH_4^+ across Manchester suggest site-specific influences on airborne nitrogen compounds. NO_3^- is an important constituent of NO_x (i.e. primarily sourced from vehicular emissions in Manchester)[41] and could have been deposited as fine particulates on the lichen surface in close proximity to major roads[60]. Indeed, elevated lichen NO_3^- concentrations along the road network (**Fig. 5**) suggest rapid deposition (as HNO_3 and/or particulate-NO_3) in the vicinity of "localised" sources[45,73]. Slightly elevated lichen-NO_3^- (>29 mg kg^{-1}) in more residential surroundings (e.g. in the northeast of the research area; **Fig. 5a**) suggest possible additional NO_x sources, e.g. from domestic combustion and energy generation[74].

Lichen-NH_4^+ concentrations (**Fig. 5b**) suggest traffic-related NH_x as the main cause of ammonium pollution, e.g. from three-way catalyst cars and connected increases in airborne NH_3 and subsequently NH_4^+[9,11,12]. Elevated ammonia/ammonium concentrations from vehicular sources (i.e. diesel vehicles) have been reported for urban areas and higher vehicular nitrogen emissions have been linked to slow traffic flow and driving speed[9–11,78,85,87]. Thus, higher lichen NH_4^+ concentrations within Manchester city centre could be related to driving patterns (i.e. slow driving speed and stop-and-go traffic), which are particularly slow during peak hours (i.e. AM and PM peaks)[88]. Vehicular emitted NH_3 greatly enhances the formation and growth of secondary aerosols[11,89] and elevated lichen NH_4^+ concentrations could indicate predominantly adsorption of secondary particulates (e.g. NH_4NO_3 and/or $(NH_4)_2SO_4$). Notably NH_4^+ primarily occurs in the fine particulate fraction and higher lichen-NH_4^+ within the city centre suggest 'canyoning' effects (i.e. by higher buildings within the city centre area) and potential ineffective removal of particulates (by wind and rain) within the densely built-up area[6,87,90].

The presence of other nitrogen species (e.g. NO and NO_2), as well as ozone (O_3), and atmospheric reactions between airborne pollutants[81] within Manchester's city centre also could explain spatial (and temporal) differences recorded for lichen extracted NO_3^- and NH_4^+ concentrations. Indeed, an effect of sampling location (i.e. distance to potential pollution source) and sampling direction has been reported[44,45].

Local pollution sources (e.g. industrial and/or vehicular) and urban characteristics (e.g. open spaces, building heights and building density) are important considerations when applying a lichen biomonitoring approach.

In this study, lichens were sampled from street trees across Manchester, within more densely built-up, open and residential surroundings and varying distances to roads. Although the findings of this study suggest vehicular emissions as the primary cause of recorded NO_3^- and NH_4^+ concentrations, a more complex mixture of “local” pollution sources (NO_x and NH_x) and potential urban layout effects are also suggested (**Fig. 5**).

Ecological indicators, such as lichens, are influenced by environmental factors on different spatial scales (locally and regionally) as well as by multiple pollutants[91]. Cytotoxic effects of high pollutant concentrations (e.g. NO_3^- and NH_4^+) on lichens have been reported and molecular mechanisms of lichen nitrogen metabolism are not yet fully understood, but need to be considered when using lichens in air quality assessment studies[4,39,92]. However, it has been hypothesised that lichen fruiting bodies (apothecia) and nitrogen-fixating, free-living or vascular plant associated bacteria could function as nitrogen sinks and fixators on the lichen surface, when exposed to high pollutant concentrations[4,26,39,93]. In this study, the N-tolerant lichen *X. parietina* was utilised, whose tolerance to high nitrogen deposition rates is potentially linked to a low cation exchange capacity and subsequently limited cell wall binding sites[35,94]. Potential impacts of elevated nitrogen compounds and other pollutants (e.g. airborne metals)[44,95] on lichens, meteorological parameters (e.g. wind, temperature and precipitation)[95,96], as well as potential effects by the urban surrounding and subsequent pollutant dispersion patterns[8,97,98] were out of scope for this study, but should be considered for inclusion in further research on spatial distribution of lichen extracted NO_3^- and NH_4^+.

Notwithstanding some limitations of this study, lichen-extracted (*X. parietina*) NO_3^- and NH_4^+ concentrations, using the novel approach, provide information on spatial variability of two important airborne nitrogen compounds. However, little standardisation for lichen sample pre-extraction preparation (i.e. washing procedure) is available[29], and comparison of washed and unwashed lichen samples could allow for determination of deposited against intra-cellular nitrogen compounds. However, Gaio-Oliveira *et al.* (2001) reported no difference for total nitrogen between washed and unwashed *X. parietina* samples and that organic nitrogen in lichens in the ranges of 20–30 mg (per gram of dry weight)[99], whereas NO_3^- and NH_4^+ on the thallus surface are in the µg ranges[94,99]. Thus, they suggested no significant contribution of dry deposited nitrogen to the total lichen nitrogen. Further Munzi *et al.* (2017) reported a pH alkalinisation of lichen surfaces when exposed to elevated NO_3^- concentrations, whereas an acidification was observed during NH_4^+ exposure[100]. Hence, a pH-dependent competition of pollutants (e.g. metals) and nutrients (e.g. nitrogen) for binding sites[101], that may affect elemental uptake, is suggested.

Due to the importance of NO_3^- and NH_4^+ in particulate formation[40], “total” concentrations obtained by a lichen extraction approach applied in this study, provide crucial insights into atmospheric nitrogen compound concentrations. Hence, a lichen biomonitoring approach can provide a simple and easy-to-use strategy to identify areas of human health concern, because airborne pollutant concentrations (e.g. $PM_{2.5/10}$) are only measured by two automated monitoring stations within the city centre of Manchester. Subsequently, poor air quality and associated human health impacts by nitrogen compounds are potentially underestimated across Manchester and affects a larger area than covered by continuously monitored monitoring locations (e.g. at Piccadilly Gardens and at Oxford Road). Such an approach can be further investigated and supported using passive and/or active monitoring programmes and of course, the exact same lichen biomonitoring approach can easily be applied to other urban environments.

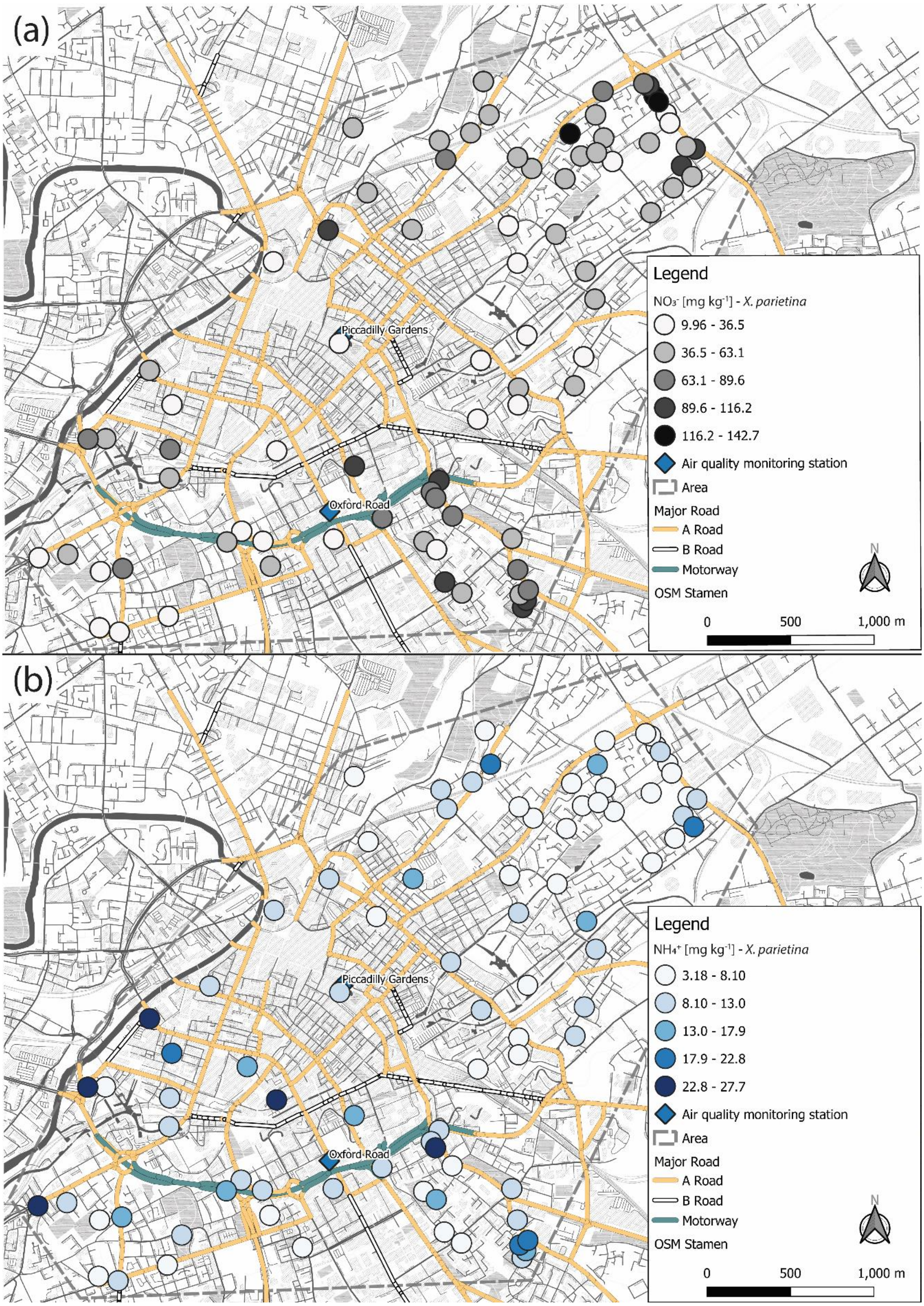


Fig. 5: Colour-graduated maps of urban *X. parietina* extracted nitrate (a) and ammonium (b) concentrations [mg kg^{-1}]; displayed with major road network (motorway, A- and B- roads) and automated air quality monitoring stations across Manchester city centre (Contains Ordnance Survey data © Crown copyright and database right 2010-21. OpenStreetMap® is open data, licensed under the Open Data Commons Open Database License (ODbL) by the OpenStreetMap Foundation (OSMF)

3.6 Nitrogen compounds and potential impacts on lichens

In principle, lichens are able to use inorganic nitrogen (e.g. NO_3^- and NH_4^+) to fulfil their nitrogen demand[4]. Although this study recorded spatial and temporal variability of nitrogen compounds (NO_3^- and NH_4^+) across Manchester, it is worth noting that both compounds can be incorporated into the lichen (e.g. in proteins), but also may have toxic effects on lichens. However, little is known about lichen nitrogen species assimilation, with nitrate assimilation being less effective than ammonium assimilation under laboratory conditions[4,39,102,103]. Pavlova and Maslov (2008) reported that the mycobiont is responsible for nitrate assimilation in green-algal (*Trebouxia* sp.) lichens[104]. Lichens (*X. parietina)* analysed in this study also contain the green-algal species *Trebouxia*[4,35], suggesting that the mycobiont in this lichen is also responsible for nitrate assimilation. Moreover, it has been reported that different nitrogen compounds cause changes in the protein profile of lichens, specifically induced by the fungal partner[100]. For instance, the algal partner in *X. parietina* (*Trebouxia* spp.) was found to respond to increased NO_3^- by changes in photosynthetic proteins[100].

On the contrary, NH_4^+ is known for its toxicity to lichens and needs to be rapidly converted to its non-toxic storage form[4,39,92]. However, it is known that green-algal lichens have a higher affinity for ammonium and nitrophytic lichens (i.e. *X. parietina*) increase their photosynthetic capacity even at high NH_4^+ concentrations[4,35,102,103,105]. Gaio-Oliveira *et al.* (2005b) further hypothesised that the presence of apothecia (lichen fruiting bodies) could function as nitrogen sinks, when exposed to high NH_4^+[39]. Cation exchange capacity and polyamine production have also been proposed to oppose detrimental effects by NH_4^+[36,94,106]. For instance, a reduction in K^+ and Mg^{2+} in *X. parietina* was proposed as an avoidance mechanism for ammonium-induced membrane damage or solute leaching[36].

Despite the potential influences of elevated NO_3^- and NH_4^+ concentrations on lichens, inorganic nitrogen fractions are most likely linked to environmental sources[94]. Hence, spatial and temporal variability recorded in this study are potentially linked to (locally) variable concentrations of anthropogenically emitted NO_x and NH_x. Nonetheless, further research is necessary to incorporate lichen metabolism into biomonitoring studies.

4. Conclusion

Although lichen total N contents (N wt%) can be used to investigate spatial variability in the total atmospheric burden of nitrogen compound pollutants, such an approach does not distinguish between different nitrogen compounds. The main aim of this study was to develop an optimised method for extracting and quantifying NO_3^- and NH_4^+ from lichen material, to extend and improve airborne nitrogen lichen biomonitoring studies by distinguishing two important constituents in particulate matter formation for improved air quality assessment studies.

The favoured extraction method consists of 3 mL of 3% (~0.5 mol L^{-1}) KCl solution, using a same-day (6 hour) extraction and subsequent analysis (by ion chromatography) approach, which allows for reproducible extraction of NO_3^- and NH_4^+ from lichen material. This new method was applied to urban and rural lichen samples (*Xanthoria parietina*) to compare contrasting environments and to assess temporal and spatial variability of nitrogen compounds in Manchester (UK) city centre. This study's results suggest NH_x as the major pollutant across the rural environment, whereas both NO_x and NH_x were recorded for the urban environment. Thus, the new nitrogen speciation method can be used for determination and quantification of airborne nitrogen compounds via a lichen biomonitoring approach, providing additional information on airborne NO_3^- and NH_4^+ pollution. Moreover, both nitrogen compounds are not commonly covered by continuous air quality monitoring programmes (e.g. in the UK and the EU) and are important precursors for airborne particulate matter. Elevated NO_3^- and NH_4^+ across Manchester (UK) suggest poor air quality and potential human health impacts on a larger scale than covered by continuous air quality measurement stations (e.g. at Piccadilly Gardens and at Oxford Road).

Combining a lichen biomonitoring approach for analysis of different nitrogen compounds, e.g. total N wt% and separation of two constituent nitrogen chemical species, will allow for improved assessment of the relative importance of these different compounds in diverse environments. Analysis of additional environmental compartments (e.g. nitrate and ammonium in soil) in combination with speciation of airborne particulate matter for NO_3^- and NH_4^+ will extend air quality assessment and human health risk studies. Using lichen stable-isotope-ratio signatures (e.g. $\delta^{15}N$

values) alongside the nitrogen speciation approach presented here also will aid in the identification of potential airborne nitrogen pollution sources in greater detail.

Author contributions

Daniel Niepsch: Resources, investigation, validation, methodology, formal analysis, writing – original draft; **Leon J. Clarke:** project formulation and administration**,** methodology, funding acquisition, supervision, writing – review & editing; **Konstantinos Tzoulas:** writing – review & editing; **Gina Cavan:** writing – review & editing

Conflict of Interest

There are no conflicts of interest to declare

Acknowledgements

DN was supported by a Manchester Metropolitan University Environmental Science Research Centre (MMU-ESRC 2015-02) PhD studentship. The authors would like to thank Dave McKendry for assistance during IC analysis. The authors also would like to thank the independent reviewers and associate editor for their valuable feedback to improve the manuscript.

References

1 H. B. Olsen, K. Berthelsen, H. V. Andersen and U. Søchting, Xanthoria parietina as a monitor of ground-level ambient ammonia concentrations, *Environ. Pollut.*, 2010, **158**, 455–461.

2 M. A. Sutton, C. E. R. Pitcairn and C. P. Whitfield, *Bioindicator and biomonitoring methods for assessing the effects of atmospheric nitrogen on statutory nature conservation sites*, 2004.

3 ATSDR, *Toxicological Profile: Nitrate and Nitrite*, Atlanta, GA, 2015.

4 M. Hauck, Ammonium and nitrate tolerance in lichens, *Environ. Pollut.*, 2010, **158**, 1127–1133.

5 NAEI, Pollutant Information: Ammonia, http://naei.beis.gov.uk/overview/pollutants?pollutant_id=21, (accessed 1 May 2018).

6 S. V. Krupa, Effects of atmospheric ammonia (NH3) on terrestrial vegetation: A review, *Environ. Pollut.*, 2003, **124**, 179–221.

7 J. Webb, H. Menzi, B. F. Pain, T. H. Misselbrook, U. Dämmgen, H. Hendriks and H. Döhler, Managing ammonia emissions from livestock production in Europe, *Environ. Pollut.*, 2005, **135**, 399–406.

8 S. R. Gadsdon and S. A. Power, Quantifying local traffic contributions to NO_2 and NH_3 concentrations in natural habitats, *Environ. Pollut.*, 2009, **157**, 2845–2852.

9 G. A. Bishop and D. H. Stedman, Reactive Nitrogen Species Emission Trends in Three Light-/Medium-Duty United States Fleets, *Environ. Sci. Technol.*, 2015, **49**, 11234–11240.

10 F. Stritzke, O. Diemel and S. Wagner, TDLAS-based NH3 mole fraction measurement for exhaust diagnostics during selective catalytic reduction using a fiber-coupled 2.2-µm DFB diode laser, *Appl. Phys. B Lasers Opt.*, , DOI:10.1007/s00340-015-6073-5.

11 K. Sun, L. Tao, D. J. Miller, D. Pan, L. M. Golston, M. A. Zondlo, R. J. Griffin, H. W. Wallace, Y. J. Leong, M. M. Yang, Y. Zhang, D. L. Mauzerall and T. Zhu, Vehicle Emissions as an Important Urban Ammonia Source in the United States and China, *Environ. Sci. Technol.*, 2017, **51**, 2472–2481.

12 J. N. Cape, Y. S. Tang, N. Van Dijk, L. Love, M. A. Sutton and S. C. F. Palmer, Concentrations of ammonia and nitrogen dioxide at roadside verges, and their contribution to nitrogen deposition, *Environ. Pollut.*, 2004, **132**, 469–478.

13 M. Kampa and E. Castanas, Human health effects of air pollution, *Environ. Pollut.*, 2008, **151**, 362–367.

14 WHO, *Health risks of air pollution in Europe – HRAPIE project*, Copenhagen, 2013.

15 S. Y. Kim, J. L. Peel, M. P. Hannigan, S. J. Dutton, L. Sheppard, M. L. Clark and S. Vedal, The temporal lag structure of short-term associations of fine particulate matter chemical constituents and cardiovascular and respiratory hospitalizations, *Environ. Health Perspect.*, 2012, **120**, 1094–1099.

16 J. Y. Son, J. T. Lee, K. H. Kim, K. Jung and M. L. Bell, Characterization of fine particulate matter and associations between particulate chemical constituents and mortality in Seoul, Korea, *Environ. Health Perspect.*, 2012, **120**, 872–878.

17 A. H. Berner and J. D. Felix, Investigating ammonia emissions in a coastal urban airshed using stable isotope techniques, *Sci. Total Environ.*, 2020, **707**, 134952.

18 B. Gu, L. Zhang, R. Van Dingenen, M. Vieno, H. J. Van Grinsven, X. Zhang, S. Zhang, Y. Chen, S. Wang, C. Ren, S. Rao, M. Holland, W. Winiwarter, D. Chen, J. Xu and M. A. Sutton, Abating ammonia is more cost-effective than nitrogen oxides for mitigating PM 2.5 air pollution, *Science (80-.).*, 2021, **374**, 758–762.

19 P. Pinho, C. Barros, S. Augusto, M. J. Pereira, C. Máguas and C. Branquinho, Using nitrogen concentration and isotopic composition in lichens to spatially assess the relative contribution of atmospheric nitrogen sources in complex landscapes, *Environ. Pollut.*, 2017, **230**, 632–638.

20 P. B. C. Forbes, L. van der Wat and E. M. Kroukamp, in *Comprehensive Analytical Chemistry*, ed. P. Forbes, Elsevier B.V., 2015, vol. 70, pp. 53–108.

21 T. H. Nash III, *Lichen Biology*, Cambridge University Press, Cambridge, New York, Melbourne, Madrid, Cape Town, Sangapore, Sao Paulo, Delhi, 2nd ed., 2008.

22 F. S. Dobson, *Lichens - An Illustrated Guide to the British and Irish Species*, The Richmond Publishing Co. Ltd., Slough, 6th ed., 2011.

23 U. Kirschbaum and V. Wirth, *Flechten erkennen - Umwelt bewerten*, Hessisches Landesamt für Umwelt und Geologie, Wiesbaden, 3. edition., 2010.

24 T. Spribille, V. Tuovinen, P. Resl, D. Vanderpool, H. Wolinski, M. C. Aime, K. Schneider, E. Stabentheiner, M. Toome-heller, G. Thor, H. Mayrhofer and J. P. Mccutcheon, Basidiomycete yeasts in the cortex of ascomycete macrolichens, , DOI:10.1126/science.aaf8287.

25 M. Grimm, M. Grube, U. Schiefelbein, D. Zühlke, J. Bernhardt and K. Riedel, *Front. Microbiol.*, 2021, 12.

26 N. Desnoues, M. Lin, X. Guo, L. Ma, R. Carreño-Lopez and C. Elmerich, *Microbiology*, 2003.

27 S. Fernández-Brime, L. Muggia, S. Maier, M. Grube and M. Wedin, Bacterial communities in an optional lichen symbiosis are determined by substrate, not algal photobionts, *FEMS Microbiol. Ecol.*, 2019, **95**, 1–11.

28 K. Almendras, J. García, M. Carú and J. Orlando, Nitrogen-fixing bacteria associated with Peltigera cyanolichens and Cladonia chlorolichens, *Molecules*, , DOI:10.3390/molecules23123077.

29 P. B. C. Forbes, in *Comprehensive Analytical Chemistry*, ed. P. B. C. Forbes, Elsevier B.V., Amsterdam, Boston, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo, 2015, vol. 70, pp. 239–267.

30 V. Shukla, U. D.K. and R. Bajpai, *Lichens to Biomonitor the Environment*, Springer India, New Delhi, 2014.

31 L. B. Sparrius, Response of epiphytic lichen communities to decreasing ammonia air concentrations in a moderately polluted area of The Netherlands, *Environ. Pollut.*, 2007, **146**, 375–379.

32 C. M. Van Herk, E. A. M. Mathijssen-Spiekman and D. De Zwart, Long distance nitrogen air pollution effects on lichens in Europe, *Lichenologist*, 2003, **35**, 347–359.

33 P. Pinho, C. Branquinho, C. Cruz, Y. S. Tang, T. Dias, A. P. Rosa, C. Máguas, and M. A. S. Martins-Loução, M.-A. Parto and M. A. Sutton, in *Atmospheric Ammonia: Detecting Emission Changes and Environmental Impacts*, eds. M. A. Sutton, S. Reis and S. Baker, Springer Netherlands, Dordrecht, 2009, pp. 109–123.

34 C. M. Van Herk, Bark pH and susceptibility to toxic air pollutants as independent causes of changes in epiphytic lichen composition in space and time, *Lichenologist*, 2001, **33**, 419–441.

35 G. Gaio-Oliveira, L. Dahlman, K. Palmqvist and C. Máguas, Ammonium uptake in the nitrophytic lichen *Xanthoria parietina* and its effects on vitality and balance between symbionts, *Lichenol.*, 2004, **36**, 75–86.

36 S. Munzi, C. Branquinho, C. Cruz and S. Loppi, Nitrogen tolerance in the lichen Xanthoria parietina: The sensitive side of a resistant species, *Funct. Plant Biol.*, 2013, **40**, 237–243.

37 S. Munzi, C. Cruz, C. Branquinho, P. Pinho, I. D. Leith and L. J. Sheppard, Can ammonia tolerance amongst lichen functional groups be explained by physiological responses?, *Environ. Pollut.*, 2014, **187**, 206–209.

38 L. Paoli, I. Maslaňáková, A. Grassi, M. Bačkor and S. Loppi, Effects of acute NH3 air pollution on N-sensitive and N-tolerant lichen species, *Ecotoxicol. Environ. Saf.*, 2015, **122**, 377–383.

39 G. Gaio-Oliveira, L. Dahlman, K. Palmqvist, M. A. Martins-Loução and C. Máguas, Nitrogen

uptake in relation to excess supply and its effects on the lichens *Evernia prunastri* (L.) Ach and *Xanthoria parietina* (L.) Th. Fr., *Planta*, 2005, **220**, 794–803.

40 M. Wang, W. Kong, R. Marten, X. C. He, D. Chen, J. Pfeifer, A. Heitto, J. Kontkanen, L. Dada, A. Kürten, T. Yli-Juuti, H. E. Manninen, S. Amanatidis, A. Amorim, R. Baalbaki, A. Baccarini, D. M. Bell, B. Bertozzi, S. Bräkling, S. Brilke, L. C. Murillo, R. Chiu, B. Chu, L. P. De Menezes, J. Duplissy, H. Finkenzeller, L. G. Carracedo, M. Granzin, R. Guida, A. Hansel, V. Hofbauer, J. Krechmer, K. Lehtipalo, H. Lamkaddam, M. Lampimäki, C. P. Lee, V. Makhmutov, G. Marie, S. Mathot, R. L. Mauldin, B. Mentler, T. Müller, A. Onnela, E. Partoll, T. Petäjä, M. Philippov, V. Pospisilova, A. Ranjithkumar, M. Rissanen, B. Rörup, W. Scholz, J. Shen, M. Simon, M. Sipilä, G. Steiner, D. Stolzenburg, Y. J. Tham, A. Tomé, A. C. Wagner, D. S. Wang, Y. Wang, S. K. Weber, P. M. Winkler, P. J. Wlasits, Y. Wu, M. Xiao, Q. Ye, M. Zauner-Wieczorek, X. Zhou, R. Volkamer, I. Riipinen, J. Dommen, J. Curtius, U. Baltensperger, M. Kulmala, D. R. Worsnop, J. Kirkby, J. H. Seinfeld, I. El-Haddad, R. C. Flagan and N. M. Donahue, Rapid growth of new atmospheric particles by nitric acid and ammonia condensation, *Nature*, 2020, **581**, 184–189.

41 D. Regan, *Manchester Public Health Annual Report 2018*, Manchester, 2018.

42 D. L. Jones and V. B. Willett, Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil, *Soil Biol. Biochem.*, 2006, **38**, 991–999.

43 D. G. Maynard and Y. P. Kalra, in *Soil sampling and Methods of Analysis*, ed. M. R. Carter, Lewis Publishers, Boca Raton, 1st editio., 1993, pp. 25–38.

44 M. A. Naeth and S. R. Wilkinson, Lichens as Biomonitors of Air Quality around a Diamond Mine, Northwest Territories, Canada, *J. Environ. Qual.*, 2008, **37**, 1675.

45 D. Sims, A. Hudson, J. Park, V. Hodge, H. Porter and W. Spaulding, Buellia dispersa (Lichens) Used as Bio-Indicators for Air Pollution Transport: A Case Study within the Las Vegas Valley, Nevada (USA), *Environments*, 2017, **4**, 94.

46 EPA, *Method 9210A - Potentiometric Determination of Nitrate*, 2007.

47 D. Niepsch, Manchester Metropolitan University, 2019.

48 S. H. Boltersdorf and W. Werner, Source attribution of agriculture-related deposition by using total nitrogen and δ15N in epiphytic lichen tissue, bark and deposition water samples in Germany, *Isotopes Environ. Health Stud.*, 2013, **49**, 197–218.

49 S. H. Boltersdorf and W. Werner, Lichens as a useful mapping tool?—an approach to assess atmospheric N loads in Germany by total N content and stable isotope signature, *Environ. Monit. Assess.*, 2014, **186**, 4767–4778.

50 GMCA, *2017 Air Quality Annual Status Report (ASR) for Greater Manchester*, Manchester, 2018.

51 Manchester City Council, State of the City Report 2019, 2019, 1–68.

52 Shropshire Council, 2018, 1–46.

53 AQEG, *Air Pollution from Agriculture*, 2018.

54 NAEI, Data Selector - Nitrogen dioxides (NO_x), http://naei.beis.gov.uk/data/data-selector?view=air-pollutants.

55 K. Broad, *Lichens in southern woodlands*, 1989.

56 L. Dahlman and K. Palmqvist, Growth in two foliose tripartite lichens, Nephroma arcticum and Peltigera aphthosa: Empirical modelling of external vs internal factors, *Funct. Ecol.*, 2003, **17**, 821–831.

57 R. Larsen Vilsholm, P. A. Wolseley, U. Søchting and P. J. Chimonides, Biomonitoring with lichens on twigs, *Lichenologist*, 2009, **41**, 189–202.

58 DfT, Road traffic statistics, https://www.gov.uk/government/publications/road-traffic-estimates-

great-britain-jan-to-mar-q1-2014%5Cnhttps://www.gov.uk/government/collections/road-traffic-statistics.

59 P. Sterry, *Collins Complete Guide to British Trees*, HarperCollins Publisher Ltd., London, 2007.

60 C. M. Van Herk, in *Lichens in a changing pollution environment: English Nature Research Reports*, eds. P. Lambley and P. A. Wolseley, English Nature, Peterborough, 2003, pp. 13–20.

61 R. Michalski and I. Kurzyca, Determination of nitrogen species (Nitrate , Nitrite and Ammonia Ions) in Environmental Samples by Ion Chromatography, *Polish J. Environ. Stud.*, 2014, **15**, 5–18.

62 Minitab, 2019.

63 OriginLab, 2018.

64 R Core Team, 2019.

65 The jamovi project, 2020.

66 QGIS Development Team, 2019.

67 N. M. Razali and Y. B. Wah, Power comparisons of Shapiro-Wilk , Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests, *J. Stat. Model. Anal.*, 2011, **2**, 21–33.

68 J. C. Fanning, The chemical reduction of nitrate in aqueous solution, *Coord. Chem. Rev.*, 2000, **199**, 159–179.

69 I. Panas, A. Snis, F. Acke and T. Johnson, Transition metal complexes with $(NO)_3$- anions, *Chem. Phys. Lett.*, 1999, **302**, 431–436.

70 C. C. Addison, N. Logan, S. C. Wallwork and C. D. Garner, Structural aspects of co-ordinated nitrate groups, *Q. Rev. Chem. Soc.*, 1971, **25**, 289–322.

71 K. H. Nahm, Evaluation of the nitrogen content in poultry manure, *Worlds. Poult. Sci. J.*, 2005, **59**, 77–88.

72 M. M. Twigg, C. F. Di Marco, S. Leeson, N. van Dijk, M. R. Jones, I. D. Leith, E. Morrison, M. Coyle, R. Proost, A. N. M. Peeters, E. Lemon, T. Frelink, C. F. Braban, E. Nemitz and J. N. Cape, Water soluble aerosols and gases at a UK background site – Part 1: Controls of PM2.5 and PM10 aerosol composition, *Atmos. Chem. Phys.*, 2015, **15**, 8131–8145.

73 J. Riddell, P. E. Padgett and T. H. Nash, Physiological responses of lichens to factorial fumigations with nitric acid and ozone, *Environ. Pollut.*, 2012, **170**, 202–210.

74 DEFRA, *Clean Air Strategy 2019*, 2019.

75 I. D. Longley, J. D. Whitehead, H. Coe and M. W. Gallagher, Winter and summer ammonia emissions in the City of Manchester, UK, related to traffic and background sources measured using an Aerodyne Quantum Cascade-TDLAS, *WIT Trans. Ecol. Environ.*

76 D. Niepsch, L. J. Clarke, K. Tzoulas and G. Cavan, Spatiotemporal variability of nitrogen dioxide (NO_2) pollution in Manchester (UK) city centre (2017–2018) using a fine spatial scale single-NOx diffusion tube network, *Environ. Geochem. Health*, , DOI:10.1007/s10653-021-01149-w.

77 K. Juda-Rezler, M. Reizer, K. Maciejewska, B. Błaszczak and K. Klejnowski, Characterization of atmospheric PM2.5 sources at a Central European urban background site, *Sci. Total Environ.*, , DOI:10.1016/j.scitotenv.2020.136729.

78 J. D. Whitehead, I. D. Longley and M. W. Gallagher, Seasonal and diurnal variation in atmospheric ammonia in an urban environment measured using a quantum cascade laser absorption spectrometer, *Water. Air. Soil Pollut.*, 2007, **183**, 317–329.

79 M. A. H. Khan, W. C. Morris, L. A. Watson, M. Galloway, P. D. Hamer, B. M. A. Shallcross, C. J. Percival and D. E. Shallcross, Estimation of Daytime NO_3 Radical Levels in the UK Urban Atmosphere Using the Steady State Approximation Method, *Adv. Meteorol.*, 2015, **2015**, 1–9.

80 A. Geyer, B. Alicke, R. Ackermann, M. Martinez, M. Harder, W. Brune, P. di Carlo, E. Williams, T. Jobson, S. Hall, R. Shetter and J. Stutz, Direct observations of daytime NO_3 : Implications for urban boundary layer chemistry, *J. Geophys. Res.*, 2003, **108**, 4368.

81 A. K. Benton, J. M. Langridge, S. M. Ball, W. J. Bloss, M. Dall'Osto, E. Nemitz, R. M. Harrison and R. L. Jones, Night-time chemistry above London: Measurements of NO3 and N2O5 from the BT Tower, *Atmos. Chem. Phys.*, 2010, **10**, 9781–9795.

82 H. S. Lee, C. M. Kang, B. W. Kang and H. K. Kim, Seasonal variations of acidic air pollutants in Seoul, South Korea, *Atmos. Environ.*, , DOI:10.1016/S1352-2310(98)00382-3.

83 Air Quality England, *Air Pollution Report - Manchester Oxford Road (MAN1)*, Manchester, 2018.

84 Air Quality England, *Air Pollution Report - Manchester Piccadilly (MAN7)*, Manchester, 2018.

85 C. Perrino, M. Catrambone, D. Menno Di Bucchianico and I. Allegrini, Gaseous ammonia in the urban area of Rome, Italy and its relationship with traffic emissions, *Atmos. Environ.*, , DOI:10.1016/S1352-2310(02)00469-7.

86 L. Paoli, A. Vannini, Z. Fačkovcová, M. Guarnieri, M. Bačkor and S. Loppi, One year of transplant: Is it enough for lichens to reflect the new atmospheric conditions?, *Ecol. Indic.*, 2018, **88**, 495–502.

87 T. Tzamkiozis, L. Ntziachristos and Z. Samaras, Diesel passenger car PM emissions: From Euro 1 to Euro 4 with particle filter, *Atmos. Environ.*, 2010, **44**, 909–916.

88 Highway Forecasting and Analytical Services, *HFAS Report 1843 - Transport Statistics Manchester 2014, Appendix 3: Traffic flow, road accidents and congestion plots*, Manchester, 2015.

89 T. Liu, X. Wang, W. Deng, Y. Zhang, B. Chu, X. Ding, Q. Hu, H. He and J. Hao, Role of ammonia in forming secondary aerosols from gasoline vehicle exhaust, *Sci. China Chem.*, , DOI:10.1007/s11426-015-5414-x.

90 B. J. Finlayson-Pitts and J. N. Pitts, *Chemistry of the Upper and Lower Atmosphere - Theory, Experiments, and Applications*, Academic Press, New York, 1st edn., 1999.

91 M. C. Ribeiro, P. Pinho, E. Llop, C. Branquinho, A. J. Sousa and M. J. Pereira, Multivariate geostatistical methods for analysis of relationships between ecological indicators and environmental factors at multiple spatial scales, *Ecol. Indic.*, 2013, **29**, 339–347.

92 B. Neuhauser, M. Dynowski, M. Mayer and U. Ludewig, Regulation of NH4+ Transport by Essential Cross Talk between AMT Monomers through the Carboxyl Tails, *PLANT Physiol.*, , DOI:10.1104/pp.106.094243.

93 I. Martinez-Argudo, R. Little, N. Shearer, P. Johnson and R. Dixon, Nitrogen fixation: key genetic regulatory mechanisms, *Biochem. Soc. Trans.*, , DOI:10.1042/bst0330152.

94 G. Gaio-Oliveira, C. Branquinho, C. Maguas and M. A. Martins-Loucao, The concentration of nitrogen in nitrophilous and non-nitrophilous lichen species, *Symbiosis*, 2001, **31**, 187–199.

95 D. F. Levia, Nitrate sequestration by corticolous macrolichens during winter precipitation events, *Int. J. Biometeorol.*, 2002, **46**, 60–65.

96 EEA, *Air quality in Europe — 2015 report*, 2015.

97 B. Loubet, W. A. H. Asman, M. R. Theobbald, O. Hertel, Y. S. Tang, P. Robin, M. Hassouna, U. Dammgen, S. Genermont, P. Cellier and M. A. Sutton, in *Atmospheric Ammonia*, 2009, pp. 205–263.

98 M. Kurppa, A. Hellsten, M. Auvinen, S. Raasch, T. Vesala and L. Järvi, Ventilation and air quality in city blocks using large-eddy simulation-urban planning perspective, *Atmosphere (Basel).*, 2018, **9**, 1–27.

99 A. L. Capelao, C. Maguas, C. Branquinho, C. Cruz and M. A. Martins-Loucao, *Lichens as*

biomonitors of atmospheric ammonium/ammonia deposition in Portugal (IAEA-TECDOC--1152), 2000.

100 S. Munzi, L. J. Sheppard, I. D. Leith, C. Cruz, C. Branquinho, L. Bini, A. Gagliardi, G. Cai and L. Parrotta, The cost of surviving nitrogen excess: energy and protein demand in the lichen Cladonia portentosa as revealed by proteomic analysis, *Planta*, 2017, **245**, 819–833.

101 L. Paoli, A. Vannini, F. Monaci and S. Loppi, Competition between heavy metal ions for binding sites in lichens: Implications for biomonitoring studies, *Chemosphere*, 2018, **199**, 655–660.

102 K. Palmqvist and L. Dahlman, Responses of the green algal foliose lichen *Platismatia glauca* to increased nitrogen supply., *New Phytol.*, 2006, **171**, 343–56.

103 L. Dahlman, J. Persson, K. Palmqvist and T. Näsholm, Organic and inorganic nitrogen uptake in lichens., *Planta*, 2004, **219**, 459–67.

104 E. A. Pavlova and A. I. Maslov, Nitrate uptake by isolated bionts of the lichen Parmelia sulcata, *Russ. J. Plant Physiol.*, 2008, **55**, 475–479.

105 G. Gaio-Oliveira, L. Dahlman, K. Palmqvist and C. Máguas, Responses of the lichen Xanthoria parietina (L.) Th. Fr. to varying thallus nitrogen concentrations, *Lichenologist*, 2005, **37**, 171–179.

106 S. A. Pirintsos, S. Munzi, S. Loppi and K. Kotzabasis, Do polyamines alter the sensitivity of lichens to nitrogen stress?, *Ecotoxicol. Environ. Saf.*, 2009, **72**, 1331–1336.

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

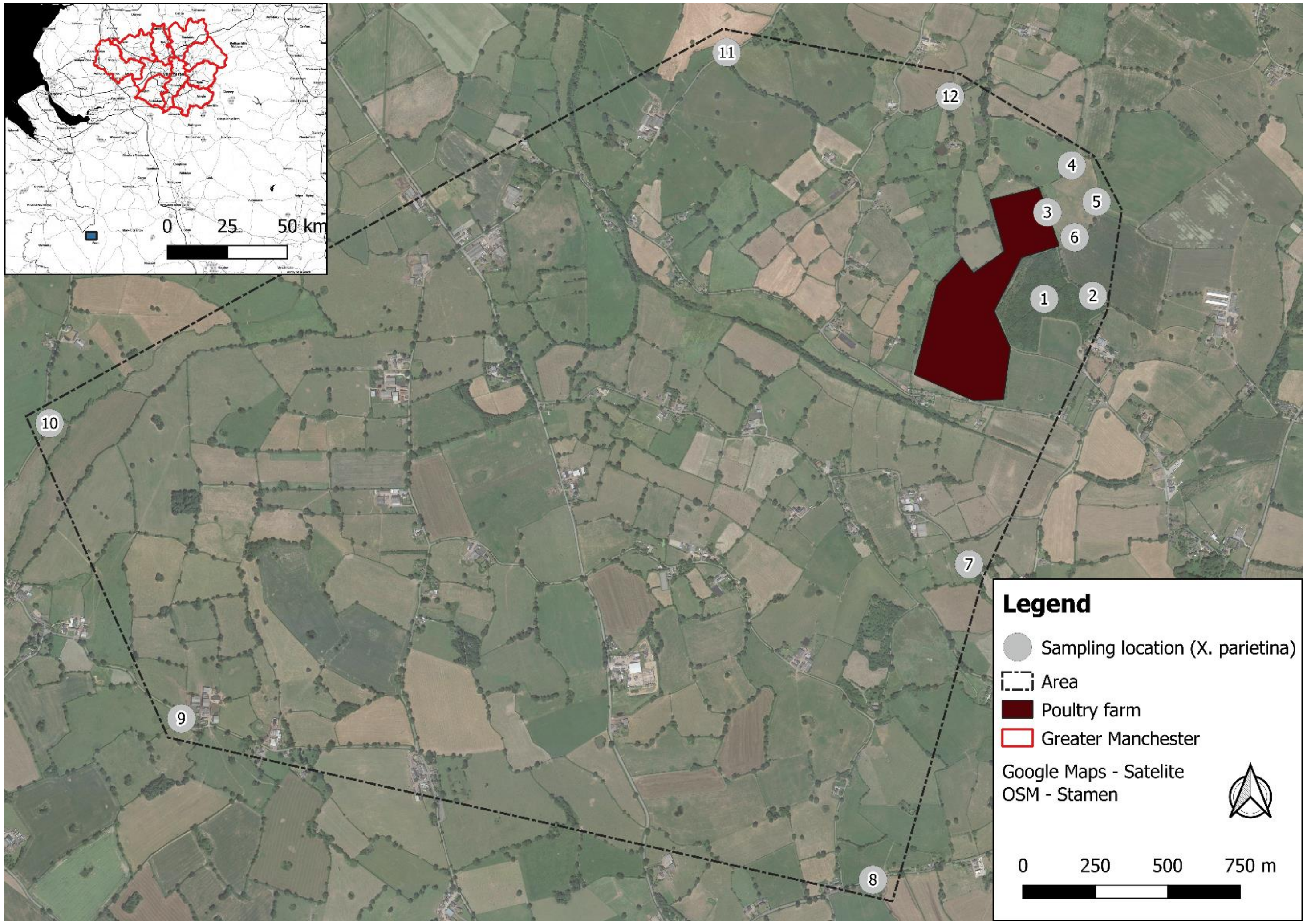


Fig. S1: Lichen sampling sites of *Xanthoria parietina* collected from around a poultry farm near Shrewsbury, UK; displayed with OSGB 1936 XY-coordinates [see Tab. S2] and its location (blue rectangle in inset map) in relation to Greater Manchester (UK) (base map: Google Satellite, 2018), OpenStreetMap® 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

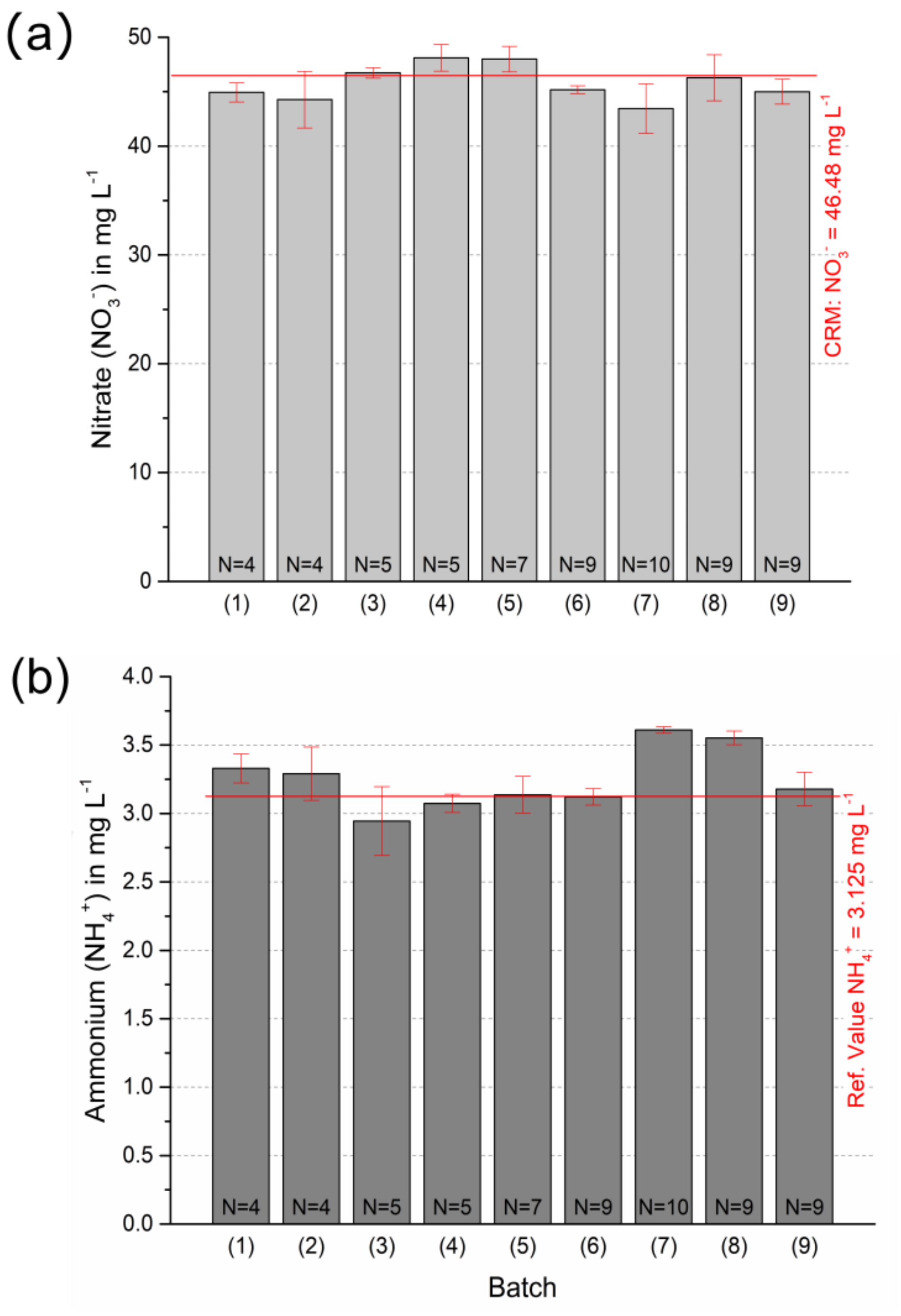


Fig. S2: Nitrate (a) and ammonium (b) concentrations determined for the CRM solutions Table S3) for each KCl extraction batch (N=62 individual measurements in N=9 batches), displayed with number of individual analyses per batch (error bars displayed as 1x standard deviation); red reference lines represent 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})

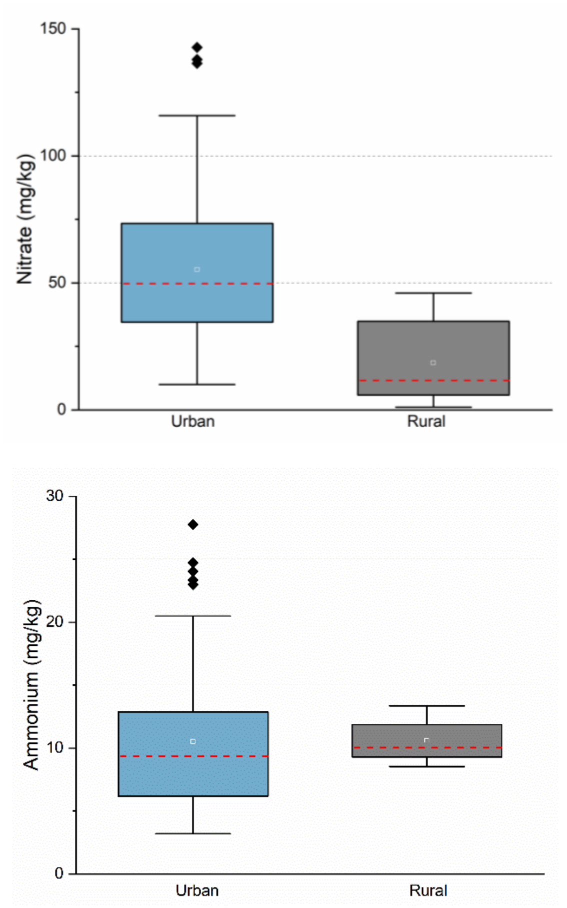


Fig. S3: Comparison of 3% KCl extracted nitrate (NO_3^-) [top] and ammonium (NH_4^+) [bottom] concentrations recorded in *X. parietina*, sampled in Manchester (urban) and around a poultry farm (rural); displayed as box-plots (25th to 75th quartile, 1.5x IQR whiskers, median line [red], mean value [white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

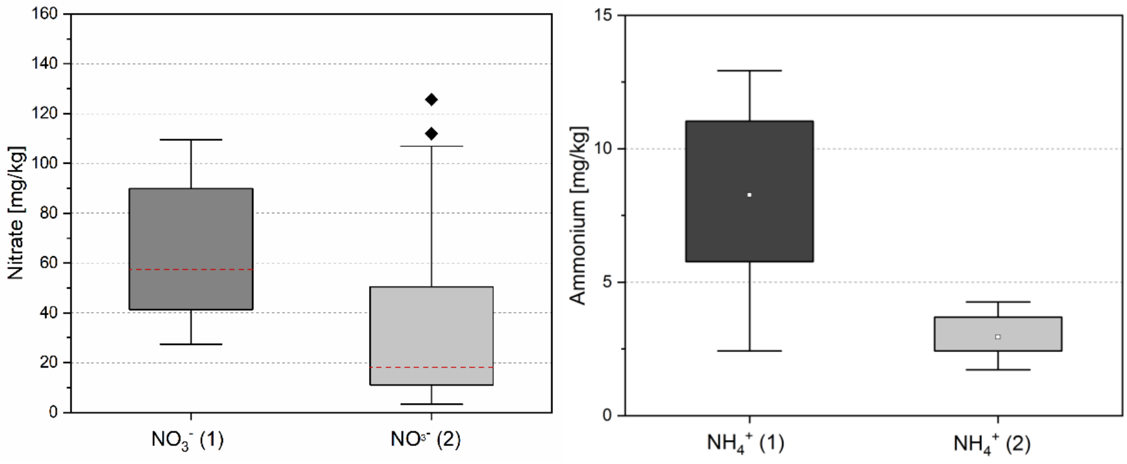


Fig. S4: Boxplots (25th to 75th percentile, 1.5x IQR whiskers, median line [red dotted], mean value [white square] and outliers [black diamonds]) of nitrate [left] and ammonium [right] concentrations for the same sites at two different sampling periods: (1) 2016/17 and (2) 2018.