AHDB

AGRICULTURE & HORTICULTURE DEVELOPMENT BOARD

Student Final Report No. 7788 **December 2015**

Developing in-situ and real-time methods of soil nitrogen determination

December 2015

Student Final Report No. 7788

Developing in-situ and real-time methods of soil nitrogen determination

Rory Shaw

School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd, LL55 3BW

Supervisors: Dr. A.P. Williams[1], Prof. D.L. Jones[1], Prof. A.J. Miller[2]

[1]School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd, LL55 3BW

[2]John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK,

This is the final report of a PhD project (AHDB Beef & Lamb Ref: 7788) that ran from Dec 2011 to Dec 2015. The work was funded by AHDB Beef & Lamb, AHDB Dairy, HCC and QMS.

CONTENTS

1. Abstract

The efficient use of nitrogen (N) fertilisers in agriculture is of great concern as diffuse losses, where N has been applied in excess of crop demand, may lead to significant environmental pollution and contribute to global warming. There is also direct economic cost to the agricultural sector. This project investigated a range of novel and emerging techniques to better enable the real-time and in-situ determination of soil N levels, in order to increase our fundamental understanding of soil N dynamics and improve management of agricultural soils. Microdialysis is an emerging technique that has been used for in-situ and minimally-invasive sampling of soil solution solutes. In study 1, the use of microdialysis for the assessment of soil N status was investigated. Diffusive-flux measurements of eight contrasting soils were compared to conventional soil core batch extractions (using 0.5 M K_2SO_4 or distilled H_2O). The percentage contribution that amino acids, NH_4^+, and NO_3^- made to total plant-available N were most similar to distilled water extractions. However, the relative magnitude of the diffusive-flux measurements did not always reflect the pool sizes as estimated by the soil extractions, which indicates the role that differing chemical and physical soil properties have in the control of plant N availability. In study 2, microdialysis was used for the in-situ sampling of amino acids, NH_4^+, and NO_3^- from the rhizospheres of maize (*Zea mays* L.) seedlings grown in soil filled rhizotubes. The results showed a significant decrease in soil solution [NO_3^-] as the root tip grew past the probe. Net amino acid exudation from root tips had been identified using direct sampling from root surfaces of seedlings grown in a sterile nutrient solution but this exudation was not evident in the microdialysis sampling, which was attributed to rapid microbial uptake. Study 3 investigated the use of commercially available NO_3^- ion-selective electrodes (ISEs) and dual-wavelength UV spectroscopy for the rapid on-farm measurement of soil N. Our results showed that manual extraction using distilled H_2O, combined with either NO_3^- ISEs or UV spectroscopy could accurately determine the NO_3^- concentration of the extracts. As such, both of these methods have the potential to be used as on-farm quick tests. In study 4, the use of novel NO_3^- ISEs for in-situ and real-time monitoring of an agricultural soil, both in a field trial and under controlled conditions in the laboratory, was demonstrated. Results from the ISEs were found to be statistically similar to conventional laboratory analysis of contemporaneous soil samples on 16 out of 19 occasions. These novel NO_3^- ISEs provide a new opportunity for in-situ and real-time measurement of soil N dynamics, which represents a significant step forward for analytical soil science and environmental monitoring. Study 5 investigated the spatial variation of soil N in a grazed grassland field. It was established that at least 61% of the total accumulated variance in amino acids, NH_4^+ and NO_3^- occurred at scales < 2 m, with significant variation occurring at the sub 1-cm scale. This data was used to demonstrate how an in-situ sensing network could be optimised on a cost-accuracy basis. Future work needs to focus on how data derived from in-situ soil N sensors can be used to improve fertiliser recommendations and the efficiency of N-use in agriculture.

2. Industry messages

This project aimed to develop methods for the in-situ and real-time analysis of soil nitrogen. These methods have the potential to be used in the future, both on-farm and as research tools, to improve management of fertiliser nitrogen. This is likely to result in reduced input costs and a reduction in the environmental impact derived from the loss of fertiliser nitrogen from agricultural land. The key messages from this project are listed below.

- A more dynamic approach to fertiliser management, facilitated by the use of real-time data, may allow more accurate fertiliser application in both space and time.
- Microdialysis sampling of soil solution allows the in-situ assessment of soil nitrogen, with excellent spatial and temporal resolution. This method is likely to offer new insights into the complexities of soil nitrogen supply. However, in its current form it is not suitable for on-farm use.
- Current technology, in the form of commercially available ion-selective electrodes and UV-based sensors can be used for the on-farm rapid analysis of soil samples.
- This project developed a new sensor that can be used for real-time and in-situ monitoring of soil nitrate.
- This project demonstrated how a network of such sensors may be optimally designed to enable the monitoring of soil nitrate within a field or management unit for a given budget or precision requirement.
- It is likely, at least initially, that use of senor networks will only be economically feasible for high-value agriculture (i.e. horticulture). As the price of technology continues to fall and further developments are made, uptake by grassland-based agriculture maybe possible.
- Further research is required to determine how results from networks of in-situ soil nitrogen sensors can be used to optimise fertiliser management.

3. Introduction

Agriculture faces a challenging future, where increasing production to meet demand from an ever-growing global population is set against the need to reduce its environmental impact. Of particular concern is the diffuse loss of reactive nitrogen (N) from agricultural land where N fertilisers and manures are frequently applied in excess of crop demand. These losses have resulted in perturbation of natural ecosystems and enrichment of the atmosphere, hydrosphere and biosphere (Vitousek et al., 1997). It is currently estimated that on average, 50% of manure and fertiliser N applied to agricultural land in Europe is lost to the environment, resulting in an economic cost in the range of €13 - €65 billion per year (Sutton et al., 2011). As such, improving the efficiency of N-use represents a major goal of sustainable farming systems from both an economic and environmental standpoint.

Improving our knowledge of both spatial and temporal variation in soil N availability and embracing the precision agriculture paradigm may bring about improvements in fertiliser N management and N-use efficiency (NUE). However, current methods of soil testing are time-consuming and expensive and there is an over reliance on semi-official fertiliser recommendations and modelling approaches which have limited accuracy (Cuttle and Jarvis, 2005; Sylvester-Bradley et al., 2008). Significant improvements in NUE may be realised by the development of methods and sensors that allow on-site or in-situ monitoring of soil N in real time (Sylvester-Bradley et al., 1999; Adamchuk et al., 2004; Kim et al., 2009). Furthermore, our fundamental understanding of soil N dynamics, and hence, our ability to infer accurate fertiliser recommendations from soil measurements, is frequently limited by a lack of non-destructive and minimally-invasive in-situ techniques.

This project addresses these issues by investigating and developing a range of emerging and novel techniques for the determination of plant-available forms of soil N. The project focuses principally on nitrate (NO_3^-), as it is generally most readily-available form of nitrogen for plant uptake, but also investigates both ammonium and amino acids. The project can be split into 5 separate studies which are briefly introduced below.

3.1. Study 1

Assessing soil nitrogen availability using microdialysis-derived diffusive flux measurements.
Published article: *Shaw R. et al., 2014*. *Soil Science Society of America Journal, 78: 1797 – 1803*

Microdialysis is an emerging technique that enables a minimally invasive assessment of soil N availability to be made in-situ (Inselsbacher et al., 2011). Its unique diffusion-based system of sampling the soil solution may better reflect the plant-soil system that it attempts to evaluate and therefore, assessment of soil N status using microdialysis may offer substantial advantages over conventional soil extractions. In study 1, microdialysis sampling and conventional soil extractions (H_2O and 0.5 M K_2SO_4) were performed to make an assessment of the soil N status of 8 grassland soils along an altitudinal gradient. The results from the different methods were compared within the context of the plant-availability of soil N. The potential and limitations of the microdialysis method are also discussed.

3.2. Study 2

Nitrogen dynamics in the rhizosphere
Conference paper: R. Shaw, A.P. Williams, D.L. Jones. (2014). Nitrogen dynamics in the rhizosphere. In: C. Cordovil (ed.). Proceedings of the 18th nitrogen workshop – The nitrogen challenge: building a blueprint for nitrogen use efficiency and food security. Lisbon, Portugal. pp 179 – 180.

The rhizosphere represents the zone of soil immediately surrounding a plant root. It is a location of intense competition between plants and microbes for nutrients and these complex interactions may control the availability of nitrogen (N) for plant uptake. As such, gaining a better understanding of these interactions is important for determining optimal management of plant nutrition and efficient use of N inputs. However, quantification of rhizosphere N dynamics are difficult to achieve without causing significant disturbance to the system being evaluated and has been limited by a lack of non-destructive sampling methods (Oburger et al., 2013). To this end, in-situ microdialysis sampling was used to assess the concentrations of amino acids, ammonium (NH_4^+) and nitrate (NO_3^-) in the rhizosphere of an actively growing maize seedling.

3.3. Study 3

Assessing the potential for ion-selective electrodes and dual-wavelength UV spectroscopy as a rapid on-farm measurement of soil nitrate concentration.
Published article: *Shaw R. et al., 2013. Agriculture, 3: 327 – 341.*

On-farm and rapid analysis of soil N status using simple field-based assessment tools may allow farmers to perform soil testing at a higher spatial and temporal frequency. Previous work, using NO_3^- test strips with hand held reflectometers and nitrate ion-selective electrodes (ISEs) has previously been described as semi-quantitative (Schmidhalter, 2005). New, more quantitative methods are therefore required. This study aimed to evaluate the use of commercially-available nitrate ISEs and UV spectroscopy for rapid on-farm testing of soil NO_3^-.

3.4. Study 4

In-situ monitoring of soil nitrate in real time using ion-selective electrodes
Article submitted to Computers and Electronics in Agriculture

Obtaining real-time information from in-situ monitoring, could provide high resolution information on the spatial and temporal dynamics of plant-available nutrients such as nitrogen (N). Incorporation of such techniques with existing precision agriculture approaches may improve N management with subsequent reductions in environmental and economic costs. However, soil provides a very challenging sensing environment and currently there is a lack of sensors that have the capability for in-situ monitoring of soil N. Ion-selective electrodes (ISEs) are simple, cheap and accurate sensors and have previously been used for the direct ex-situ measurement of soil NO_3^- (Ito et al., 1996; Adamchuck et al., 2005). In this study we demonstrate the use of a novel ISE for in-situ, real time

monitoring of NO_3^- in a grassland agricultural soil over a 7 day period in both a laboratory and field trial.

3.5. Study 5

Characterising the within-field scale spatial variation of different N forms in a grassland soil and the implications for in-situ N sensor technology and precision agriculture
Article in review with Agriculture, Ecosystems and Environment

The use of in-situ sensors capable of real-time monitoring of soil nitrogen (N) may facilitate improvements in agricultural N-use efficiency through better fertiliser management. Optimising the deployment of in-situ sensors for both accuracy and cost requires consideration of the spatial variation of N forms at within-field scales. In this study, a geo-statistical nested sampling approach was used to characterise the spatial variability of amino acids, ammonium (NH_4^+) and nitrate (NO_3^-) in the soil of a grazed grassland field (1.9 ha). The results of the sampling were then used to explore how a network of in-situ soil NO_3^- sensors may be optimally designed on the basis of cost and accuracy.

4. Materials and methods

4.1. Study 1

Soil Sampling

Soils were collected from eight contrasting agricultural grazed grasslands, along an altitudinal gradient, within an 8 km^2 region close to Abergwyngregyn, UK (53°14′ N, 4°01′ W). Prior to sampling, the vegetation and litter layer were removed from an area 15 cm × 15 cm, and the top 15 cm of the soil collected for experimentation.

Microdialysis sampling of soil solution

A syringe pump, holding two 20 ml polypropylene syringes (Terumo-Europe NV, Leuven, Belgium), filled with high purity deionized water (the perfusate), were connected to two CMA 20 microdialysis probes (CMA Microdialysis AB, Kista, Sweeden), with a 100 kDa molecular weight cut off, polyethersulfone membrane (4 mm long, 500 µm external diameter). For each replicate sample, between 50-70 g of pre-sieved (8 mm) soil was placed in a 100 cm^3 plastic beaker and packed to the bulk densities, which were representative of field conditions. A microdialysis probe was inserted into the soil, using the needle and introducer supplied by the manufacturers. The flow rate of the pump was set to 5 µl min^{-1} in accordance with Inselsbacher et al. (2011). Dialysate was collected over a time period of 1-2 h in 1.5 ml microfuge tubes and stored at -18°C. The diffusive fluxes of amino acids, NH_4^+ and NO_3^- were determined by calculating the amount of each N-form that diffused across the microdialysis probe membrane during each sampling period and are expressed in units of nmol cm^{-2} h^{-1} (Inselsbacher et al., 2012).

Traditional soil N extraction

All sieved soil samples were extracted following standard procedures (Jones and Willett, 2006). Briefly, field-moist, 8 mm sieved soil (5 g) was extracted (175 rev min^{-1}, 1 h) using 0.5 M K_2SO_4 - to determine exchangeable and free N, or distilled water - to determine free N, at a soil: extractant ratio of 1:5 (w:v), the extracts centrifuged (4,000 *g*, 15 min), and the resulting supernatant collected and frozen (-18°C) to await chemical analysis.

Chemical analysis of soil extractions and microdialysis samples

Amino acids were determined by the *o*-phthadialdehyde spectrofluorometric method of Jones et al. (2002). NH_4^+ was determined by the nitroprusside colorimetric method of Mulvaney (1996) and NO_3^- by the colorimetric Griess reaction of Miranda et al. (2001).

Calculations and statistical analysis

In order to determine the relative importance of each N pool for plant nutrition, the percentage contribution of each N form to the total plant-available N (amino acids, NH_4^+ and NO_3^-) was calculated

for each of the three sampling methods (K_2SO_4 extraction, H_2O extraction and microdialysis diffusive flux) for each soil type. Statistical analysis was carried out using one way ANOVA followed by Fishers LSD *post-hoc* test using SPSS v.20 (IBM Ltd., Portsmouth, UK) with $P < 0.05$ used as the cut-off for statistical significance.

4.2. Study 2

Soil sampling

Four replicate soil samples (n = 4) were collected from the Henfaes Research Station, (Bangor University), Abergwyngregyn, Gwynedd, North Wales (53°14′N, 4°01′W). The soil is classified as a Eutric Cambisol which has a sandy clay loam texture and a fine crumb structure.

Microdialysis monitoring of rhizosphere N dynamics

Monitoring of rhizosphere soil N dynamics was achieved by growing the axial root of maize (*Zea mays* L.) seedlings within a soil filled rhizotube microcosm. Maize was chosen as the study plant as its large root structure facilitates such studies. A microdialysis probe was placed within the rhizotube. The primary root axis was allowed to grow towards and then past the microdialysis probe enabling repeated and non-destructive sampling of soil solution chemistry to be made as shown in Figure 1.

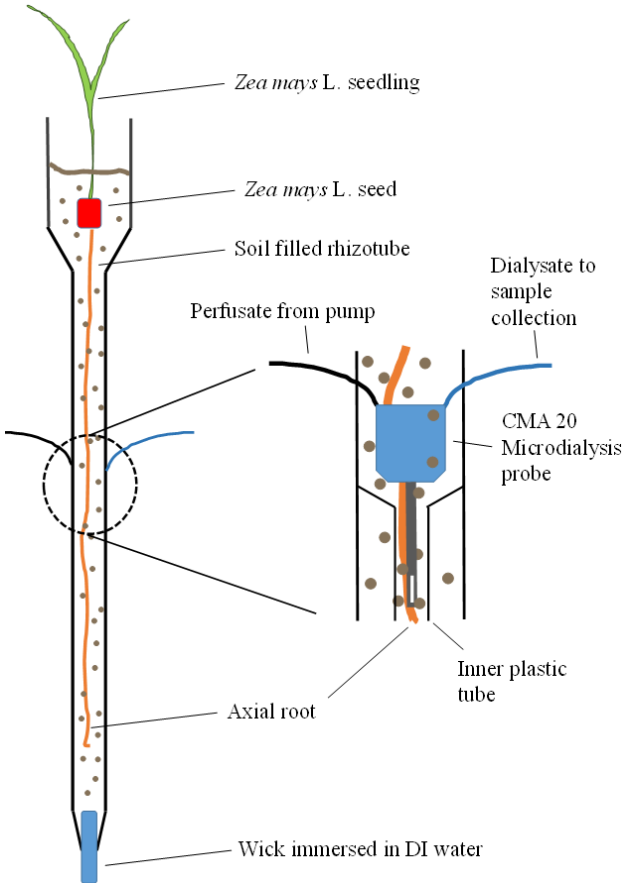

Figure 1. Equipment set-up for microdialysis monitoring of rhizosphere N dynamics in soil filled microcosms.

A peristaltic pump (Watson Marlow 205u, Watson Marlow, Falmouth, UK) was used to perfuse high purity DI water through CMA 20 microdialysis probes (CMA Microdialysis AB, Kista, Sweeden), with a 20 kDa molecular weight cut off, polyethersulfone membrane (4 mm long, 500 µm external diameter). The flow rate of the pump was set to 5 µl min^{-1} in accordance with Inselsbacher et al. (2011). Prior to and following the rhizosphere sampling, the microdialysis probes were calibrated to determine the relative recovery of amino acids, NH_4^+ and NO_3^- (Inselsbacher 2011). Replicate microcosms were placed in a climate-controlled chamber and samples recovered from the microdialysis probes every 4 h for a total of 68 h. Estimations of the intrinsic soil solution concentration of amino acids, NH_4^+ and NO_3^- for each 4 h sampling period were calculated using the respective relative recoveries of the 3 N forms (Inselsbacher 2011).

Statistical analysis

Differences in microdialysis derived soil N concentrations between specific time points or between the planted and unplanted treatments were assessed with two-sample t tests using SPSS v.20 (IBM Ltd., Portsmouth, UK) with $p < 0.05$ used as the cut-off for statistical significance.

4.3. Study 3

Soil sampling

Three contrasting soils were collected from Henfaes Research Station. Soil 1 is a lowland, clay loam textured Eutric Cambisol collected from an area of no vegetation cover, which had been used for potato production the previous season. Soil 2 is a lowland, silty loam textured Dystric Gleysol collected from a poorly draining area of an intensively sheep grazed field (ca. >10 ewe ha^{-1}) receiving regular fertiliser inputs (120 kg N ha^{-1} yr^{-1}) and dominated by *Lolium perenne* L. Soil 3 is a sandy loam textured Haplic Podzol collected from an upland, extensively grazed (<0.1 ewe ha^{-1}) unimproved acid grassland (*Pteridium aquilinum* L. Kuhn. and *Festuca ovina* L.).

NO_3^- Determination Using Ion Selective Electrode Rapid Test Method

10 g soil (n = 3 for each soil type) was placed in a 50 cm^3 polypropylene tube and spiked with 1 ml of NO_3^- solution (2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100 or 0 mg l^{-1} (in addition the Eutric Cambisol was spiked with 20 and 10 mg l^{-1})) to achieve a range of intrinsic NO_3^- concentrations reflective of those that might occur in the field. Extraction was then performed by the addition of 20 ml of double distilled (DD) H_2O followed immediately by manual shaking by hand for 2 min. This extraction procedure is referred to as the rapid extraction method. A pre-calibrated NO_3^- ISE (ELIT 8021 & ELIT 003n, Electro Analytical Instruments, Wembley, UK) was placed into the resulting soil slurry and a reading taken after 3 min. Between each measurement, the electrodes were rinsed with H_2O and dried with paper tissue. The soil slurry was subsequently centrifuged and the supernatant decanted for NO_3^- analysis by the colorimetric Griess reaction method of Miranda et al. (2001) - referred to as the standard lab method.

Nitrate Extraction and Determination by the Standard Lab Method

NO_3^- was extracted from the soil using 1 M KCl or DD H_2O (10 g soil:20 ml) by mechanical shaking at 150 rev min^{-1} for 30 min. The resulting mixture was then centrifuged and analysed by the colorimetric Griess reaction method of Miranda et al. (2001). This is referred to as the standard lab method with KCl/H_2O extraction.

NO_3^- Determination by Dual Wavelength UV Spectroscopy

NO_3^- in the standard KCl/ H_2O extracts was also analysed with dual wavelength UV spectroscopy at 205 nm and 300 nm using the method described in Edwards et al (2001).

Statistical analysis

Linear regression was used to compare the NO_3^- concentration results obtained using the methods described above.

4.4. Study 4

Field site and soil characteristics

The field site used for this study is located within the Henfaes Research Station, Abergwyngregyn, UK (53°14′N 4°01′W). The site has a temperate oceanic climate, receives an average annual rainfall of 1250 mm and has a mean annual soil temperature at 10 cm depth of 11 °C. The field is roughly rectangular with a perimeter of 559 m and an area of 1.91 ha. It has an average altitude of 12.1 m and slope of 1.5 % with a northerly aspect. It is a semi-permanent sheep-grazed grassland, dominated by *Lolium perenne* L. The current ley was seeded by direct drill in April 2009 using a perennial and hybrid ryegrass mix. The field has been used for both all year round grazing and silage production since 2009, receiving an inorganic fertiliser input of between 100 – 130 kg N ha^{-1} in addition to potassium (K), phosphate (P) and sulphur (S) at recommended rates. Lime has also been applied when necessary to increase the pH. In 2014, inorganic fertiliser was applied on 12/5/14 and 11/7/14 at a rate of N:P:K 50:10:10 and 60:4:0 kg ha^{-1} respectively. The soil at the field site is a free draining Eutric Cambisol with a sandy clay loam texture and a fine crumb structure.

Chemical analysis

NO_3^- in soil extractions and soil solution samples was determined using the colorimetric Griess reaction of Miranda et al. (2001).

NO_3^- ISE construction

NO_3^- ISEs were constructed in our laboratory using a simple and reproducible protocol. A NO_3^- sensing membrane cocktail was created using the method described in Miller and Zhen (1991). High

density polyethylene pipette tips (1250 µl, graduated; TipOne®, StarLab, Milton Keynes, UK) were dipped into the membrane cocktail so that the tip filled via capillary action to a depth of 3 mm. The tips were then left in a fume hood for 24 h to allow the THF to evaporate and the membrane to harden. The tips were subsequently back filled with a 100 mM KNO_3/KCl solution into which an $Ag/AgCl_2$ wire was inserted. The pipette tip was then sealed and a standard electrical wire was attached. Throughout this study the NO_3^- ISEs were coupled with a commercially available double junction lithium acetate reference electrode (ELIT 003n; NICO2000 Ltd., Harrow, Middlesex, UK).

Effect of temperature on the ISE-datalogging system

To investigate the effect of temperature on the ISEs and datalogger, a simple experiment was performed. Pre-calibrated NO_3^- ISEs (n = 5) were placed in individual 20 ml sample vials containing 10 ml of 1 mM NO_3^- and sealed with Parafilm®. The NO_3^- ISEs were connected, using a 1 m long piece of standard electrical wire, to a multi-channel data logger (DL2e, Delta-T Devices Ltd, Cambridge, UK). The data logger was programmed to make and record differential DC voltage measurements in the range 0 – 500 mV every 0.5 h. Initially, the data logger was placed in a temperature-controlled incubator which was programed to ramp the temperature from 9 to 45 and back to 9 °C, with the rate of change set to 3 °C h^{-1}. The NO_3^- ISEs were placed in an adjacent incubator, which was set to a constant 20 °C. NO_3^- ISE output was recorded over a 48 h period. Following this, the data logger and the NO_3^- ISEs were swapped so that the data logger was exposed to a constant temperature and the NO_3^- ISEs to the variable temperature. The NO_3^- ISE output was recorded for a further 62 h.

Trial of NO_3^- ISE for in-situ soil monitoring under controlled environmental conditions

Replicate turfs (n = 5) of size 50 × 15 × 10 cm (length × width × depth) were cut from the experimental field and taken immediately to the laboratory. Here, each turf was cut into 3 sections. The first section was 10 × 15 × 10 cm and used for soil extractions (Rousk and Jones, 2010). The extracts were centrifuged (4,000 *g*, 15 min), and the resulting supernatant collected and frozen (-18°C) to await chemical analysis. Soil solution was also obtained by the centrifugal-drainage method of Giesler and Lundström (1993). Soil extracts and solutions were analysed for NO_3^-. In addition, approximately 3 g of soil was used for moisture content analysis.

The second and third sections of turf had equal dimensions of 20 × 15 × 10 cm, and were used for the experimental procedure. The turfs were placed on top of horticultural capillary matting in plastic containers, which had roughly the same dimensions as the turfs, and moved to a climate controlled chamber. The ends of the capillary matting were placed in a reservoir of distilled H_2O placed 4 cm below the base of the soil to ensure the soil stayed moist throughout the experimental period. In the second set of turfs, a hole of approximately 1 cm diameter was made to a depth of 6 cm in the center of each turf. A pre-calibrated NO_3^- ISE was placed into this hole. The hole was then backfilled and a gentle downward pressure was applied to the NO_3^- ISE to ensure good membrane-soil contact. The NO_3^- ISEs were then connected to the data logger using a 1 m length of standard

electrical wire. The data logger was programmed to make and record differential DC voltage measurements in the range 0 – 500 mV every 1 h. The third set of turfs were used for destructive sampling and subsequent NO_3^- analysis throughout the experimental period in order to determine the performance of the NO_3^- ISE. This was performed in the middle of the day and night of the programmed diurnal cycle in order to try and identify any diurnal or temperature related variation in soil NO_3^- concentration. Soil cores were taken in triplicate from each turf between depths of 3 – 8 cm using a soil corer with a diameter of 5 mm. The triplicate cores were bulked prior to being hand crumbled and mixed. Soil extractions were performed as described above, with the resulting extracts frozen to await NO_3^- analysis. In addition, approximately 1 g of the bulked sample was used for moisture content analysis. The data from the soil extractions were used to calculate the NO_3^- concentration of the bulk soil, expressed on a dry weight basis (mg N kg^{-1}). The extractions were also used to estimate the NO_3^- concentration in soil solution (mg N l^{-1}). The calculation assumes that all the NO_3^- in the soil extracts came from the soil solution pool so a simple soil solution dilution factor can be calculated using the soil moisture content. The climate chamber was programmed to run a 24 h diurnal cycle with conditions similar to that which may occur during a summer's day. The cycle had a 12 h day/night period with a temperature max of 25 °C after 6 h and a temperature minimum of 10 °C at 18 h. Photosynthetically active radiation (PAR) was set to 0 % for the 12 h night period. At the commencement of the day period the PAR was set to 50 % with a peak in the middle of the day of 100 % before returning to 50 % at the end of the day period. Relative humidity was set to 50 % for the day and 70 % for the night. This program was run for 3 whole cycles. Following this, the program was adjusted so that the temperature remained constant at 20 °C whilst the other variables remained unchanged. The program was then run for a further 4 diurnal cycles. At the end of the experimental period the NO_3^- ISEs were removed from the turfs. The vegetation and top 1 cm of the soil was removed and the soil extracted and soil solution sampled as described above. Extracts and soil solutions were subsequently frozen to await NO_3^- analysis. The NO_3^- ISEs were rinsed briefly in distilled H_2O and then soaked in 100 mM NO_3^- prior to being recalibrated to assess changes in calibration parameters.

Field trial of ISEs for in-situ monitoring of soil NO_3^-

A 2 × 2 m block was chosen at random from within the experimental field. Within this block, four 30 cm^2 sections of turf and topsoil were removed to a depth of 5 cm. Three3 holes at a gradient of approximately 20° below the horizontal were made into the sides of these holes, into which the NO_3^- ISEs were inserted. Gentle pressure was applied to the NO_3^- ISEs to ensure good soil-membrane contact. The holes were back filled and the turfs replaced to ensure that the NO_3^- ISEs were completely buried with the exception of the electrical cable. In total, 12 NO_3^- ISEs were implanted into the soil (n = 12). The NO_3^- ISEs were connected, using a 1 m long piece of standard electrical cable, to the data logger, which was housed in a waterproof container. The data logger was programmed to make and record differential DC voltage measurements in the range 0 – 500 mV

every 2 h. The NO_3^- ISEs were deployed during the afternoon of 6/8/2014 and logging commenced at 16:30h on the same day. Logging ceased at 08:30h on 12/8/14, giving a total logging time of 136 h. Failure of 1 NO_3^- ISE occurred immediately and it was assumed that this was caused by membrane damage during insertion into the soil. Results presented are means ± SEM (n = 11). At the end of the monitoring period the NO_3^- ISEs were removed from the soil and taken back to the laboratory for recalibration.

To make an assessment of the accuracy of the NO_3^- ISEs, soil samples were taken for conventional laboratory analysis. In an adjacent 2 × 2 m block, a soil corer with a diameter of 1 cm was used to take replicate soil samples (n = 4) from a depth of 5 – 10 cm. In a third block, larger volumes of soil (approx. 300 g field moist) were sampled (n = 4) from the same depth using a trowel. The soil was placed in gas-permeable plastic bags and transferred immediately to the laboratory, were they were refrigerated at 4 °C. Extractions were performed on the soil cores on the same day as sampling as previously described. The larger soil samples from the third block were used to obtain soil solution by centrifugal-drainage as previously described. Soil extracts and solutions were analysed for NO_3^-.

Statistical analysis of data

Significance testing was performed using one-sample t-tests, two-sample t-tests and one-way ANOVA as appropriate.

4.5. Study 5

Field site and soil characteristics

The field site and soil characteristics were the same as for study 4.

Sampling design and protocol

The aim of the sampling was to characterize the variability of plant-available N forms – amino acid-N, NH_4^+ and NO_3^- – at a range of spatial scales relevant to planning the design of an in-situ sensor network. In particular, it was necessary to examine the relative importance of variance between and within local regions each of which might be represented by a cluster of soil N sensors deployed around a single data logger such that the maximum distance between any two sensors is about 2 m. In a livestock-grazed grassland environment, it was expected that one of the main sources of variation in soil N would be the uneven and relatively random distribution of urine patches of linear dimensions about 40 cm (Bogaert et al., 2000; Selbie et al., 2015). Variation at larger scales may also be may also be important due to preferential use of certain areas of the field such as tracks, areas of shade and around drinking troughs (Bogaert et al., 2000), which may be reflected in local gradients in soil chemistry. The study field is broadly homogenous in terms of its topography and soil type. Furthermore, a visual inspection of the field revealed no obvious large-scale gradients in vegetation condition which is likely to reflect the broadly homogenous nature of the soil. Previously, the field has received uniform management in terms of its fertiliser and lime inputs and grazing

regime. Because of these factors, it was decided to treat the field as singular management unit with a singular mean rather than subdivide the field into separate management zones.

Given these considerations, a nested sampling protocol was designed with length scales within each mainstation of 1 cm, 10 cm (intermediate between the fine scale and urine patch scale), 50 cm (urine patch scale) and 2 m (upper bound on the "within-region served by a sensor cluster" scale). To assess spatial variation at larger scales, mainstations were distributed by stratified random sampling with the target field divided into four quarters (strata) of equal area. Four mainstations were established at independently and randomly-selected locations within each quarter (stratum), giving a total of 16 mainstations. The design of the sampling scheme within each mainstation was obtained by the optimization procedure of Lark (2011) on the assumption of a fractal or quasi-fractal process in which the variance is proportional to the log of the spatial scale. The objective function was the mean estimation variance of the variance components. With 12 samples per mainstation the total sample size was 192. The sample sites were then selected at each mainstation by randomizing the direction of the vectors between the substations at each level of the design shown in Figure 2, while keeping the lengths of the vectors fixed. For practical purposes, sampling was split over 2 successive days, with 2 strata sampled on day 1 and two on day 2, giving a total of 8 mainstations and 96 samples per day. No duplicate sampling took place as each sample site was visited only once over the 2 day period.

An initial nested sampling campaign was performed over 2 days on the 4th and 5th June, 2014. Following this, all sheep were removed and the field remained ungrazed until 2nd September, 2015. A further nested sampling campaign was performed on the 31st July and 1st August, 2014, 3 weeks after the field received a N fertiliser input of 60 kg N ha^{-1}. These are subsequently referred to as the June nested sampling and the July nested sampling respectively. Sample site locations were set up the day before sampling took place. At each sampling location a soil corer, of diameter 1 cm, was used to sample soil. A 5 cm soil core from between depths of 5 -10 cm was sampled and placed in gas-permeable plastic bags, and stored in a refrigerated box. This depth was chosen as it represents the middle of the rooting zone and would make installation of any in-situ sensor a straight forward process. Following the sampling event the samples were transferred immediately to the laboratory where they were refrigerated at 4 °C. Extraction of soluble N from soil was performed on the soil cores on the same day as sampling as described below. During the second nested sampling event, duplicate sub-sampling and chemical analysis were performed on 4 out of the 12 samples from each mainstation in order to make an assessment of in order to make an estimate of the error variance attributable to subsampling and analytical error.

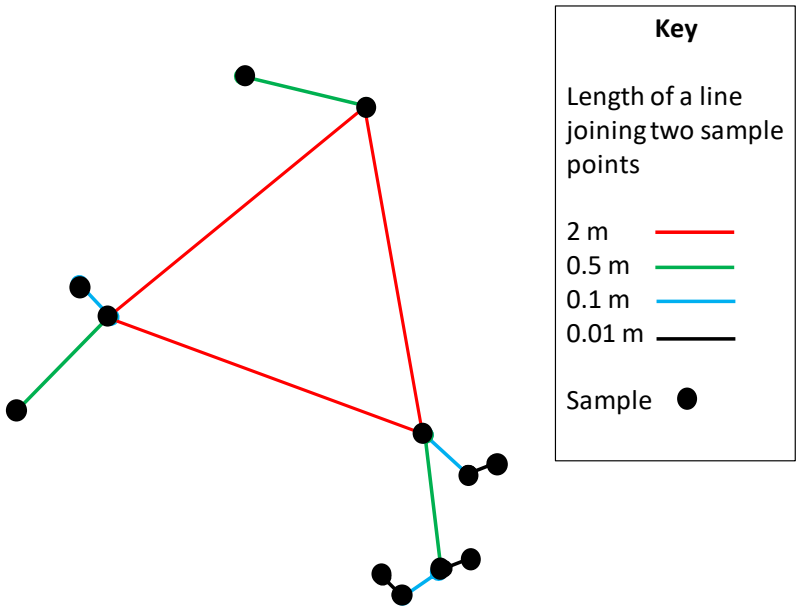

Figure 2. The optimised sampling design of a mainstation. Distances between sampling points were fixed but angles were randomized, with the exception of the 2 m vectors.

Soil properties are also likely to vary at sub-core (< 1 cm) scales (Parkin, 1987; Stoyen et al., 2000). As such, a further sampling design and protocol was developed and performed on the 25th June, 2014 to investigate how this micro-heterogeneity affected the spatial variability of N forms at the "aggregate scale" (< 1 cm). Two sampling locations were chosen at random within each of the 4 strata. At each location, a pair of samples were taken, using the protocol described above, with a distance of 1 cm between each sample. This resulted in a total of 16 core samples. On return to the laboratory, the cores where broken apart and 4 "aggregates" of weight 60 – 80 mg were collected (diameters ca. 1-2 mm). These aggregates were then extracted for soluble N and analysed using the protocol described below.

Extraction and chemical analysis of soil samples

All soil extractions were performed on the same day as sample collection, according to the following protocol (Jones and Willett, 2006; Rousk and Jones, 2010; Inselsbacher, 2014). Sub-samples of field-moist soil (2 g) were extracted on ice (175 rev min^{-1}, 15 min) using cooled (5 °C) 0.5 M K_2SO_4 at a soil: extractant ratio of 1:5 (w:v). The extracts were then centrifuged (4,000 *g*, 15 min), and the resulting supernatant collected and frozen (-18°C) to await chemical analysis. The protocol differed slightly for the soil aggregate samples. Each aggregate, of weight 60 – 80 mg, was placed in a 1.5 ml Eppendorf micro-centrifuge tube and crumbled gently using a metal spatula. The soil was then extracted in 500 µl of 0.5 M K_2SO_4 as described above. Total free amino acid-N was determined by the *o*-phthaldialdehyde spectrofluorometric method of Jones et al. (2002). NH_4-N was determined by the nitroprusside colorimetric method of Mulvaney (1996) and NO_3-N by the colorimetric Griess reaction of Miranda et al. (2001).

Statistical analysis

The aim of the statistical analysis was to compute the variance components attributable to each of the spatial-scales in order to inform the optimisation of the sensor network design. After Box-Cox transformations the data from the nested sampling was analysed according to Webster and Lark (2013). The variance components are reported in 2 forms. Firstly the variance components calculated from the Box-Cox transformed data were calculated. These results are needed for optimising the design of an in-situ network (see below), however, as the units are not on the original scales of measurement, general interpretation is difficult. Because of this, variance components for the spatial scales were calculated for the original measurement scale using the mean absolute deviation from the mean (MAD) procedure.

Optimising the design of an in-situ sensor network

The transformed variance components derived from the nested sampling and subsequent statistical analysis were used to examine the theoretical performance of different designs of in-situ soil NH_4^+ and NO_3^- sensor networks. When considering the optimal design, two factors must be considered. Firstly, what is the required level of precision for the estimation of the field mean and how many sensors and data loggers are required to achieve this? Secondly, how can the design be optimised in-terms of achieving a desired level of precision at minimum cost? Alternatively, it may be useful to explore how to design the network to achieve the highest precision possible given a certain budget restriction.

To estimate the level of precision associated with a particular sensor network design, the between-sensor within-logger component of variance, where a cluster of n_e sensors are randomly located within a region of 2 m diameter around each of n_l data logging hubs, which are located by simple random sampling, can be approximated by

$$\sigma^2_{sensor} = \sigma^2_{2} + \sigma^2_{0.5} + \sigma^2_{0.1} + \sigma^2_{0.01}, \quad (1)$$

and the between-logger variance by

$$\sigma^2_{logger} = \sigma^2_{s} + \sigma^2_{m}. \quad (2)$$

As such, the standard error of the field mean soil N concentration derived from the sensor network can be estimated as follows:

$$\sigma_{mean} = \{(\sigma^2_{logger} / n_l) + (\sigma^2_{sensor} / n_l n_e)\}^{½} \quad . \quad (3)$$

This allows the 95% confidence interval of the field mean estimations to be calculated, given the variance components calculated from the nested sampling, for particular combinations and numbers of data loggers (n_l) and sensors (n_e). These calculations were performed on the transformed scale prior to back-transformation of the 95 % confidence interval to the original scale of measurement.

In order to demonstrate how the design may be optimised on a cost basis it was necessary to decide on a unit cost for a data logger and a sensor. Given the potential of electrochemical sensors for in-situ monitoring, it was decided that the unit cost for the sensor would be £200, based on the cost of a commercially available NH_4^+ or NO_3^- ISE (ELIT 8021, ELIT 003, Nico2000, Harrow, UK) and £2000 for the data logger, based on the cost of a commonly used data logger (DL2e DeltaT,

Cambridge, UK). Whilst these costs are somewhat arbitrary, it does allow useful comparison between designs to be made. It would also be possible to change these unit costs to explore how using different sensors and loggers may affect the optimisation of the network.

The 95% confidence intervals were computed for sensor network designs that consisted of 1 to 10 data loggers with 2 to 15 sensors distributed equally among the loggers, 15 being the maximum number of sensor ports on the data logger (DL2e DeltaT, Cambridge, UK). This allowed construction of graphs (Fig. 12) which illustrate the total cost for each design plotted against the resulting 95% confidence interval of the estimated field mean.

5. Results

5.1. Study 1

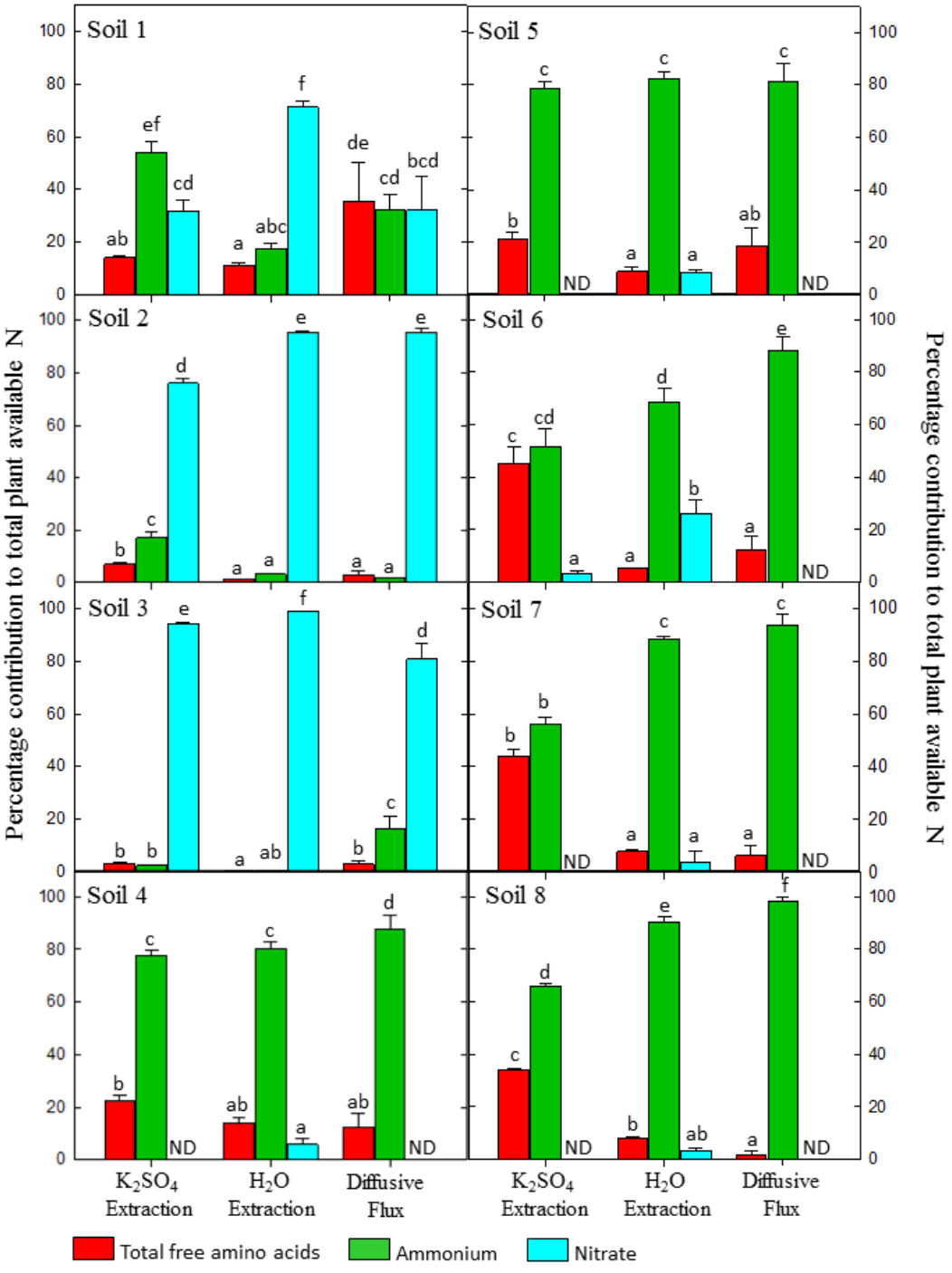

Figure 3. Percentage contribution of total free amino acids, NH_4^+ and NO_3^- to total plant-available N in eight contrasting agricultural grassland soils, estimated by 0.5 M K_2SO_4 and H_2O soil extractions, and microdialysis-derived, diffusive flux measurements. Values represent means ± standard errors (soils 1-3 (n=4), soils 4-8 (n=3)). Letters show statistical differences between the percentage contribution made by each N form, within each soil, for the three different methods of assessing soil N status.

The percentage contribution of amino acids, NH_4^+ and NO_3^-, to total plant-available N for the soils, as estimated with the three different extraction techniques, is shown in Figure 3. Overall, in soils 2-8, the dominant N-form was the same for the three different extraction methods. For soils 4-8, NH_4^+ was the dominant N-form, with percentage contributions, estimated by K_2SO_4 extractions, in the range of 51.6 – 78.7%. For soils 2 and 3, NO_3^- was dominant, contributing respectively, 94.3 and 76.0% of plant-available N, estimated by K_2SO_4 extractions. The results for soil 1 shows a much more complicated picture, with the 3 methods giving very different results. Here, NH_4^+ and NO_3^-, were the largest contributors to total plant-available N, as determined, respectively, by K_2SO_4 and H_2O extractions, whereas the contribution of each N-form, as determined by microdialysis, were not significantly different. For soils 2-8, K_2SO_4 extractions gave the largest percentage contributions and absolute concentrations, shown in Figure 4, of Amino acids, with an increase in AA_{K2SO4}, from 54 µmol AA kg^{-1} in soil 3, to 526 µmol AA kg^{-1} in soil 8. In contrast, the inverse was the case for microdialysis sampling, with the lowest AA_{DFLUX} of 0.07 nmol Amino acids cm^{-2} h^{-1}, in soil 8, and the highest of 0.68 nmol Amino acids cm^{-2} h^{-1}, in soil 1. Soil concentrations of AA_{H2O}, were lower than AA_{K2SO4} and less varied across the 8 soils, with the highest concentration of 116 µmol AA kg^{-1} in soil 4. $NH_4^+{}_{K2SO4}$ soil concentrations were always higher than $NH_4^+{}_{H2O}$ soil concentrations, although the relative magnitude was similar across all the soils. The magnitude of the $NH_4^+{}_{K2SO4}$ and $NH_4^+{}_{H2O}$ soil concentrations, were not always reflected in diffusive flux results. The highest $NH_4^+{}_{DFLUX}$ of 3.94 nmol NH_4^+ cm^{-2} h^{-1} was in soil 6, which had the fifth lowest $NH_4^+{}_{K2SO4}$ and $NH_4^+{}_{H2O}$ concentration. However, there were no significant differences in $NH_4^+{}_{DFLUX}$ between soils 2 and 4-8. NO_3^- was detected in all the H_2O extracts but not in the microdialysis samples for soils 4-8 or in the K_2SO_4 extracts for soils 4-5 and 7-8. Soil 2 had significantly the largest $NO_3^-{}_{K2SO4}$ and $NO_3^-{}_{H2O}$ pool sizes but the second largest $NO_3^-{}_{DFLUX}$.

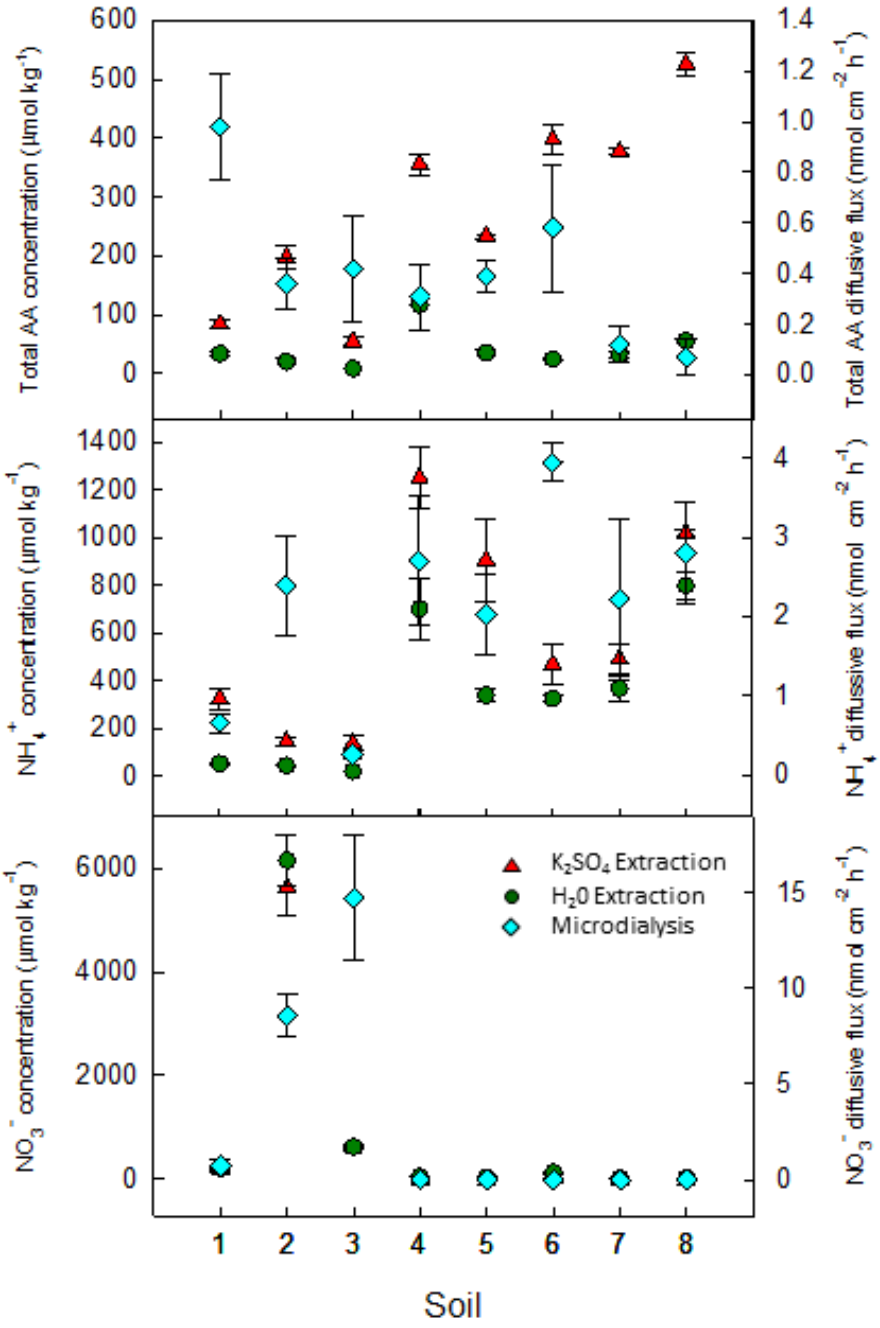

Figure 4. Concentrations of total amino acids, NH_4^+ and NO_3^- (µmol N kg^{-1}) in eight contrasting agricultural grassland soils, estimated by 0.5 M K_2SO_4 and H_2O soil extractions, and microdialysis-derived, diffusive flux measurements (nmol N cm^{-2} h^{-1}), from 8 temperate grassland soils. Values represent means ± standard errors (soils 1-3 (n=4), soils 4-8 (n=3)).

5.2. Study 2

As shown in Figure 5, the concentration of amino acids in the soil solution from the control (unplanted) treatment decreased significantly ($p < 0.001$) over the course of the experiment from 10.6 ± 1.5 µM N at 4 h to 4.2 ± 0.2 µM N at 68 h. The concentration transiently peaked in the control treatment at 36 h with 14.1 ± 1.5 µM N and then decreased rapidly to 4.3 ± 0.4 µM N. The concentration of amino acids for the planted treatment also fell over the course of the experiment,

but by a smaller amount, from 11.5 ± 1.2 µM N at 4 hr to 9.5 ± 4.4 µM N at 68 h, although this not statistically significant. The concentration peaked in the planted treatment at 48 h with 12.0 ± 4.6 µM N. Unlike the control, the concentration in the planted root treatment did not appear to stabilise, although none of the observed fluctuations proved statistically significant.

The concentration of NH_4^+ in the unplanted treatment varied throughout the experiment and showed no clear trend. Concentrations at 4 and 68 h were 22.4 ± 4.1 µM and 37.3 ± 12.4 µM respectively and were not significantly different from each other. Conversely, for the planted treatment, a statistically significant decreasing trend was observed with time as the NH_4^+ concentration dropped from 54.1 ± 10.9 µM at 4 h to 7.7 ± 0.3 µM by the end of the experiment (P = 0.013). Between 28 and 40 h, the NH_4^+ concentration decreased by over 50 % from 29.3 ± 13.2 µM to 14.0 ± 4.9 µM, which corresponded to passage of the root tip past the microdialysis probe, although the difference here was not statistically significant. Furthermore the concentration of NH_4^+ prior to the passage of the root was variable and there also was a small peak at 36 h after the root had grown past the probe. As such, this noise makes attributing the observed decrease in NH_4^+ concentration to plant uptake difficult. However, the concentration does appear to flatline after 40 h.

The NO_3^- soil solution concentration in the control at 4 h was 301.0 ± 34.2 µM, which was statistically similar to the treatment of 203.8 ± 10.8 µM. However, between 30 and 36 h there was a large divergence in the two treatments as the unplanted soil increased by 67% to 505.7 ± 256.6 µM while the planted treatment decreased by 28% to 96.80 ± 45.4 µM. The mean concentration in the unplanted control after 36 h remained above 350 µM, although large inter-replicate was evident, resulting in no significant difference in NO_3^- concentration between the start and end of the experiment. In contrast, NO_3^- concentrations in the treatment containing roots continued to decline to a low point of 19.2 ± 3.9 µM at 52 h. This concentration was significantly different to the 32 h time point and coincides with growth of the root past the microdialysis probe (p = 0.003). The NO_3^- concentration then increased slightly to 32.4 ± 17.0 µM by the end of the experiment. No significant difference was found between the initial NO_3^- concentration at 4 h and the final concentration, however, a significant difference (p = 0.042) was found between the NO_3^- concentration at 8 h and 68 h despite the concentration at 8 h being lower than at 4 h. The difference between the control and root treatments at the end of the experiment was also significant.

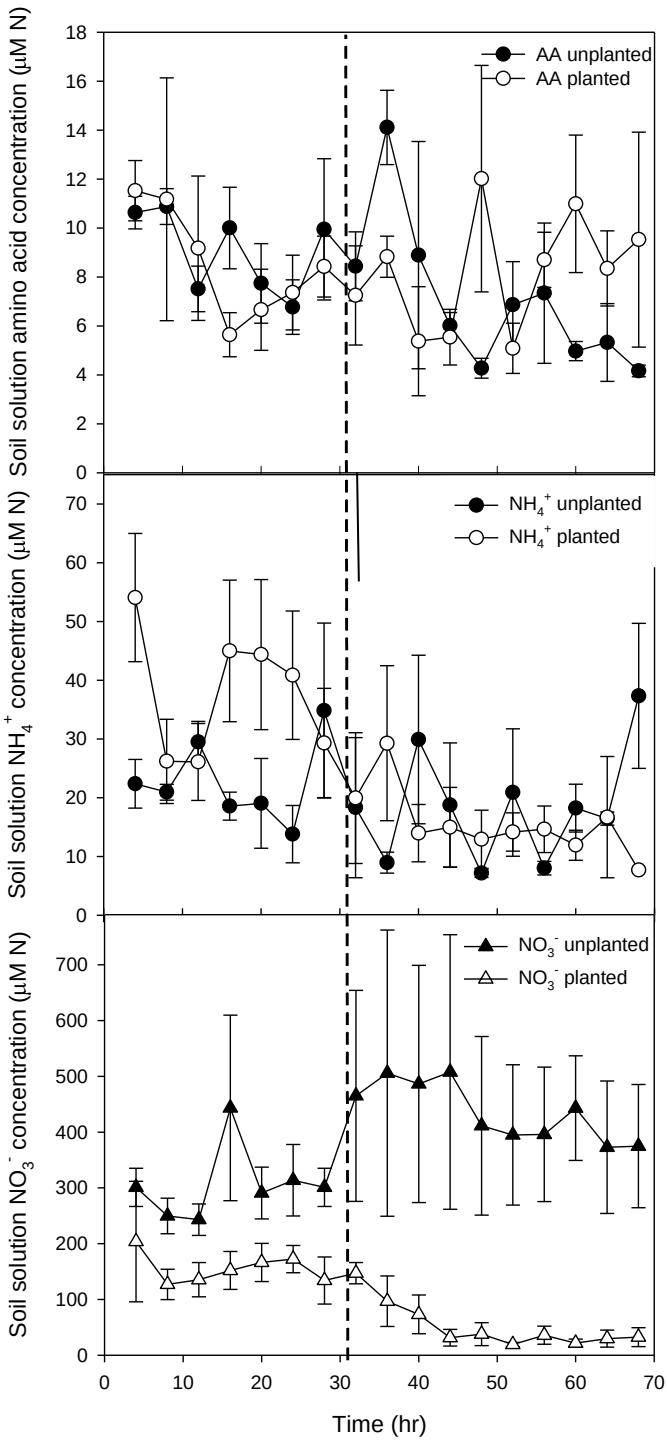

Figure 5. Soil solution amino acid, NH_4^+ and NO_3^- concentrations estimated by in-situ microdialysis sampling from root-free (unplanted control) and rhizospheric soil (planted). In the planted treatment the axial root of a maize seedling passed the membrane of the in-situ microdialysis probe at 31.0 ± 1.2 h (indicated with vertical dotted line). Microdialysis samples were collected over 4 h periods. Values represent means ± SEM (planted: n = 3, unplanted control: n = 4).

5.3. Study 3

Comparison of the ISE Rapid Test with the Standard Lab Method

The ISE rapid test method was compared to the standard lab method with KCl extraction. Figure 6 shows a good correlation between the ISE rapid test and the standard lab method with KCl extraction for the determination of soil NO_3^- in all three soil types. However, significant differences were found between the two methods when applied to the Eutric Cambisol and Dystric Gleysol. Analysis of the rapid extraction extracts with the standard lab method for these soils showed no significant differences when compared to the ISE rapid test method (data not shown). This suggests that the significant differences between the ISE rapid test method and the standard lab method with KCl extraction was due to either differences in extraction efficiency or natural soil variation, but not the performance of the ISE.

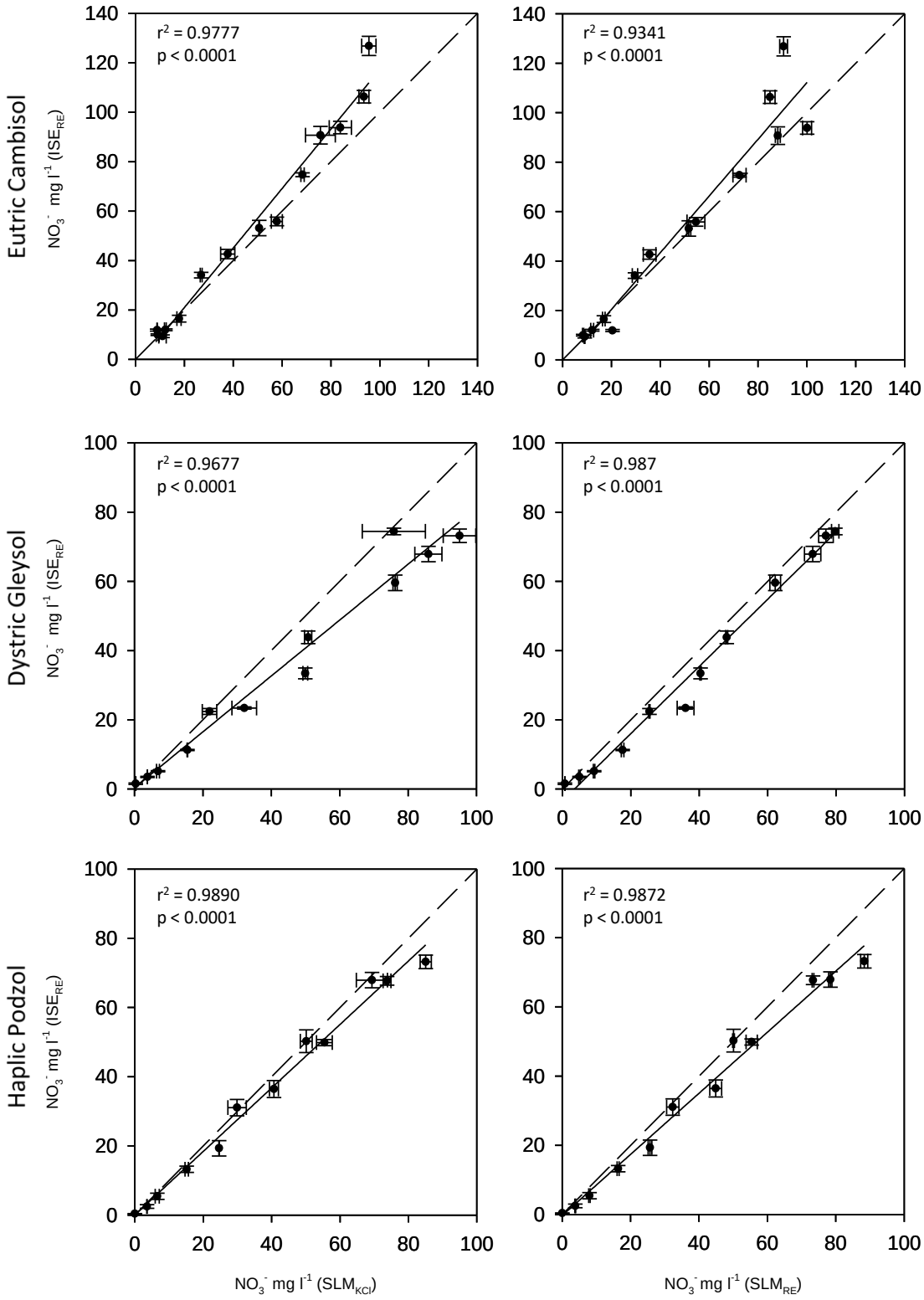

Figure 6. Comparison of the ion selective electrode (ISE) rapid test (ISE_{RE}) with the standard lab method—extractions in KCl (SLM_{KCl}) and rapid extraction procedure (SLM_{RE})—for NO_3^- determination in three soils amended with increasing amounts of NO_3^-. The dotted line represents the theoretical 1:1 line for the two methods whilst the solid line represents the linear regression line describing the actual relationship between the two methods. Values represent means ± SEM (n = 3). The r^2 and p value from the regression analysis are shown for each graph.

Comparison of UV Spectroscopy with the Standard Lab Method

Figure 7 shows an excellent correlation and a near 1:1 response between the standard lab method and UV spectroscopy method for the determination of NO_3^- in H_2O and KCl soil extracts. The response of UV spectroscopy to pure solutions of NO_3^- was linear from 0.05–12.5 mg l^{-1} (compared to 0.2–50 mg l^{-1} with the standard lab method). Consequently, most of the extracts required a 1:10 (v/v) dilution prior to NO_3^- determination. No significant differences were found between the methods using H_2O extraction for all three soil types and for KCl extraction with the Haplic Podzol. However, significant differences were found between the standard lab method and the UV spectroscopy for KCl extractions from the Eutric Cambisol and Dystric Gleysol. A closer look at Figure 4 shows that it is only the three lowest concentrations that appear to deviate significantly from the 1:1 regression line. These were the only samples, extracted in 1 M KCl which were not diluted prior to UV analysis, which suggests that the error is due to interference from the 1 M KCl. Edwards et al. (2001), found no interference from saline constituents although they did not use solutions as strong as 1 M.

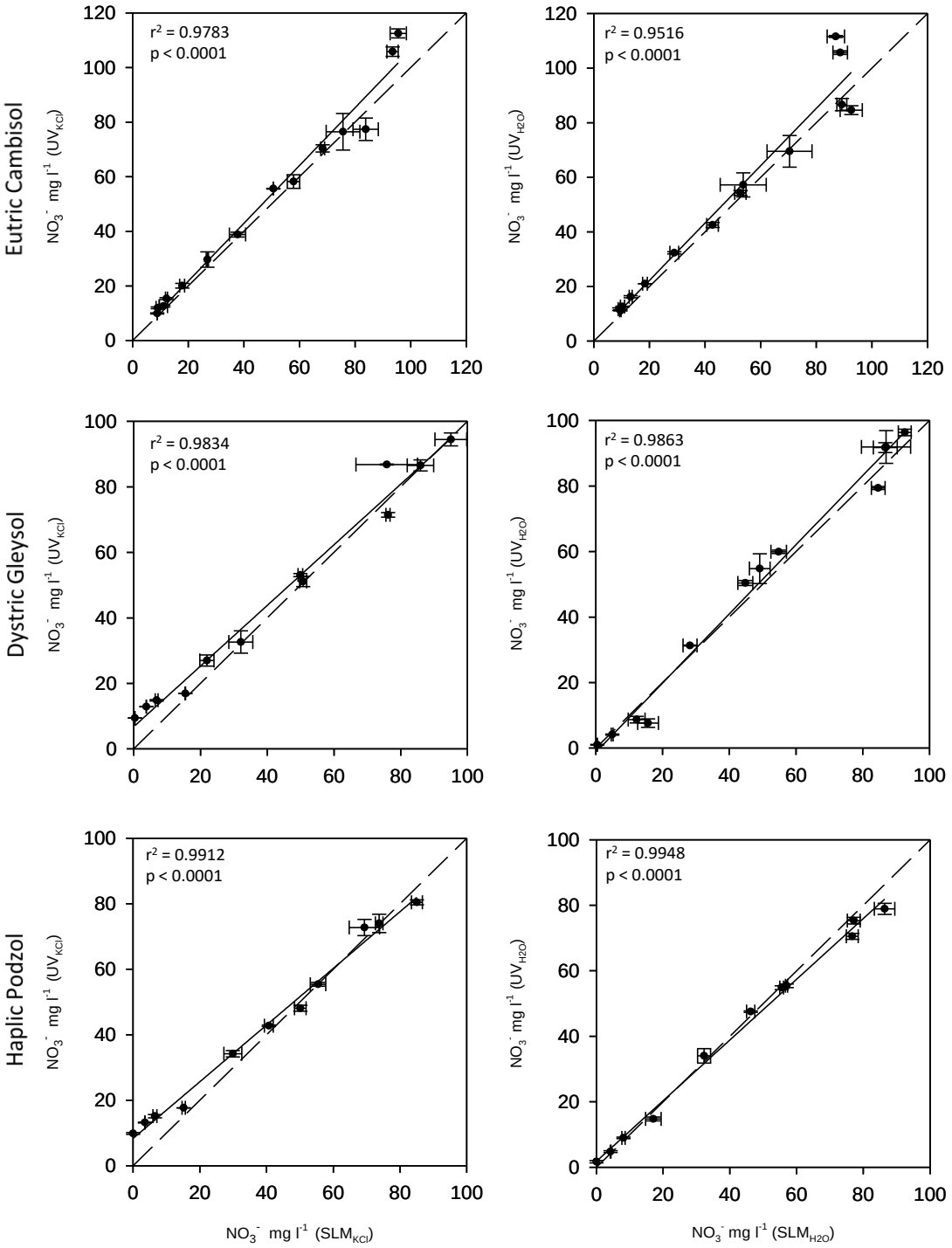

Figure 7. Comparison of UV spectroscopy—extractions in KCl (UV_{KCl}) and H_2O (UV_{H2O})—with the standard lab method—extractions in KCl (SLM_{KCl}) and H_2O (SLM_{H2O})—for NO_3^- determination in three soils amended with increasing amounts of NO_3^-. The dotted line represents the theoretical 1:1 line for the two methods whilst the solid line represents the linear regression line describing the actual relationship between the two methods. Values represent means ± SEM (n = 3). The r^2 and p value from the regression analysis is shown for each graph.

5.4. Study 4

Effect of temperature on the ISE-datalogging system

The effect of temperature on the NO_3^- ISEs was considered similar to theoretical norms and was compensated for as described in Le Goff et al (2002). A temperature effect on the datalogger was also evident. Inspection of these data revealed that the effect was channel specific. The mV output of 2 channels covaried positively, 2 channels covaried negatively with temperature and 1 showed little response. Furthermore, the magnitude of the response, both positive and negative, was not consistent between the electrodes. The reason for this effect is not known. This presented a challenge for developing an accurate temperature compensation calculation. It was therefore necessary to calculate the maximum and minimum effect of temperature so that the real value would likely exist between the 2 extremes. As the mV output of one of the data logger channels did not appear to be affected by the variable temperature, the minimum effect was assumed to be no effect. Results of the NO_3^- ISE monitoring are plotted twice in figures 8 and 9, to show data which has not been adjusted for datalogger temperature and data which has been normalized to a datalogger temperature of 25 °C.

In-situ soil NO_3^- monitoring under controlled environmental conditions

NO_3^- ISEs were used for the in-situ monitoring of soil solution NO_3^- activity in replicate turfs over a 160 h period under controlled environmental conditions in the laboratory and the results are presented in Figure 8. For the first 72 h, during which the temperature varied diurnally from 10 to 25 °C, the NO_3^- ISE estimates of soil solution NO_3^- activity showed a gradual decrease from 3.9 ± 1.4 to 1.8 ± 0.5 mg N l^{-1} (means ± SEM, n = 5). The NO_3^- ISE results also showed a cyclical variation during this time, which appeared to be positively correlated with both soil and data logger temperature and the diurnal cycle. For the remaining experimental time, during which the air temperature was set to a constant 20 °C, the NO_3^- ISE results increased slightly from 1.8 ± 0.5 to 2.6 ± 1.9 mg N l^{-1}, although this was largely due to the increase in 1 replicate from 2.9 to 10.2 mg N l^{-1}. There was still a small diurnal temperature variation (≈ 4 °C) apparent for the soil and the data logger, which was attributed to radiative heating. The NO_3^- ISE results exhibited a very small cyclical variation during this time period which corresponded to the above temperature variation and the day/night cycle. The soil solution NO_3^- concentration, estimated from 0.5 M K_2SO_4 extractions of soil cores, were statistically similar to the NO_3^- ISE estimations (p > 0.05) for all but 2 (132 & 156 h) of the 13 sampling time points. In general, the soil core derived estimates of soil solution NO_3^- were larger than the NO_3^- ISE results. There was no evidence of the temperature/diurnal related variation that was seen in the NO_3^- ISE results. The initial soil core derived estimates of soil solution NO_3^- were 6.2 ± 2.3 mg N l^{-1} (means ± SEM, n = 5). This increased to the experimental maximum of 16.9 ± 6.9 at 47 h. The concentration at the end of the experiment (156 h) was 9.9 ± 2.4 mg N l^{-1}. Once the ISE experiment had ended, soil solution was recovered from the NO_3^- ISE turfs using centrifugal-drainage. The resulting NO_3^- concentration was 2.2 ± 0.1 mg N l^{-1}, which was not significantly different from the NO_3^- ISE estimated

activity (p > 0.05). Recalibration of the NO_3^- ISEs was attempted at the end of the trial to assess changes in calibration parameters over the course of the monitoring periods. Despite rinsing with distilled H_2O and subsequent soaking in 100 mM NO_3^- it was not possible to remove all the soil from the membrane. Because of this, the ISE would not stabilise, therefore calibration was not possible.

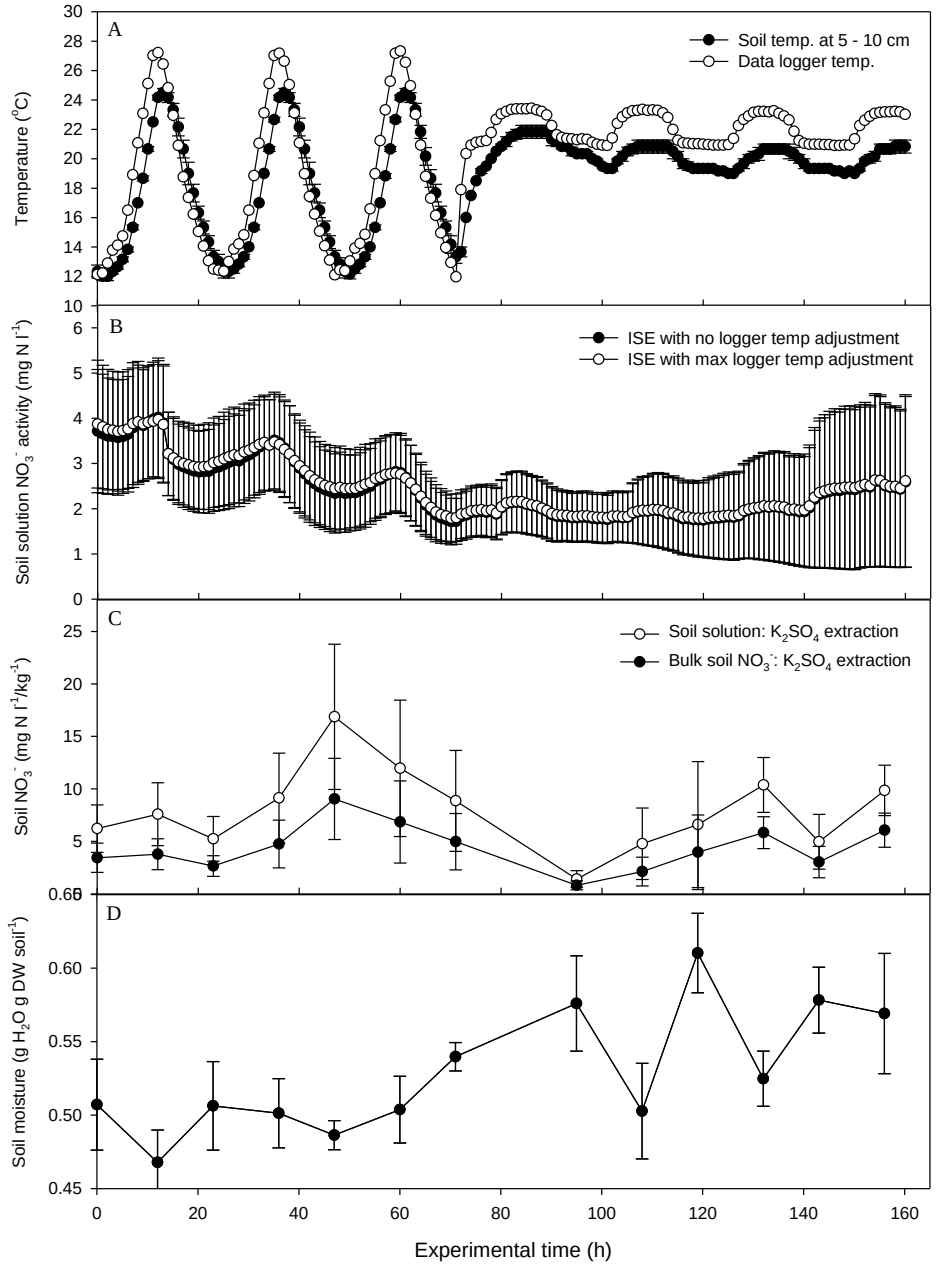

Figure 8. Results of in-situ soil NO_3^- monitoring under controlled environmental conditions over a 160 h period. Panel A shows the temperature (°C) of the data logger and soil at 5–10 cm depth (means ± SEM, n = 3). Panel B shows the estimation of soil solution NO_3^- activity by the in-situ NO_3^- ISE with (open circles) and without (closed circles) adjustment for data logger temperature (means ± SEM, n = 5). Panel C shows estimations of soil solution (mg N l^{-1}) and bulk soil NO_3^- concentration from soil core extractions and chemical analysis (mg kg^{-1}) (means ± SEM, n = 5). Panel D shows soil moisture content (means ± SEM, n = 5).

Field trial of ISEs for in-situ monitoring of soil NO_3^-

NO_3^- ISEs were deployed in-field over a 7 d period for in-situ monitoring of soil solution NO_3^- activity and the results are presented in Figure 9. During the monitoring period the environmental conditions were variable. A period of rain (8.8 mm) on the first day of the monitoring period meant that the soil moisture content was fairly high at the start of the experiment (0.28 ± 0.01 g H_2O g soil DW^{-1}, mean ± SEM, *n* = 4). During the monitoring period the mean daily temperature max and min was 19.4 °C and 12.9 °C respectively. On 10th August there was a further rainfall event (7.3 mm) and as a consequence, air temperature was much lower than previous days. There was also a resulting increase in the soil moisture content from 0.16 ± 0.01 g H_2O g soil DW^{-1} on 8th August to 0.23 ± 0.01 g H_2O g soil DW^{-1} on Sunday 10th. Due to the sunny and warm conditions on 4 of the days, the data logger temperature showed a large diurnal variation. For example, the data logger temperature decreased from a maximum of 44.2 °C at midday on August 10th to a minimum of 12.1 °C at midnight on August 11th. Soil temperature also showed a diurnal variation, although the variation was much lower than the data logger temperature and the air temperature. It remained between a maximum and a minimum of 22.8 °C and 17.7 °C for the duration of the monitoring period. 12 NO_3^- ISEs were deployed and of these one failed immediately on insertion into the soil. A noticeable feature of the NO_3^- ISE results are the peaks that occur during the day, with [NO_3^-] maximums occurring at 14:30h. These correspond to maximum daily soil and logger temperature. Ignoring these spikes, the NO_3^- ISE results showed a general increasing trend from 8.28 ± 2.25 mg N l^{-1} at 06:30h on August 7th to 13.09 ± 3.66 mg N l^{-1} at 06:30h on Sunday 10th. Following this, a gradual decline was observed. This decline occurred after the above-mentioned significant rainfall event and resulting soil moisture increase. The inter-replicate range of NO_3^- ISEs was large with differences of up to 57 mg N l^{-1} occurring between the lowest and highest replicate. Compared to the estimation of soil solution NO_3^- by conventional sampling and lab analysis, the NO_3^- ISEs estimations were between 2 to 5 times greater, although this was only significantly different for the Sunday 10th sampling event. The conventional soil sampling was not performed at a fine enough temporal resolution to determine whether the diurnal variation observed by the NO_3^- ISEs was occurring. The adjustment made to the NO_3^- ISEs results for the temperature effect on the data logger caused a slight reduction in the maximum of each spike and a very small increase at data logger temperatures below 25 °C. Recalibration of the NO_3^- ISEs was attempted to assess changes in calibration parameters over the course of the monitoring periods. Despite rinsing with distilled H_2O and subsequent soaking in 100 mM NO_3^-, it was not possible to remove all the soil from the membrane. Because of this, the ISE would not stabilise so calibration was not possible.

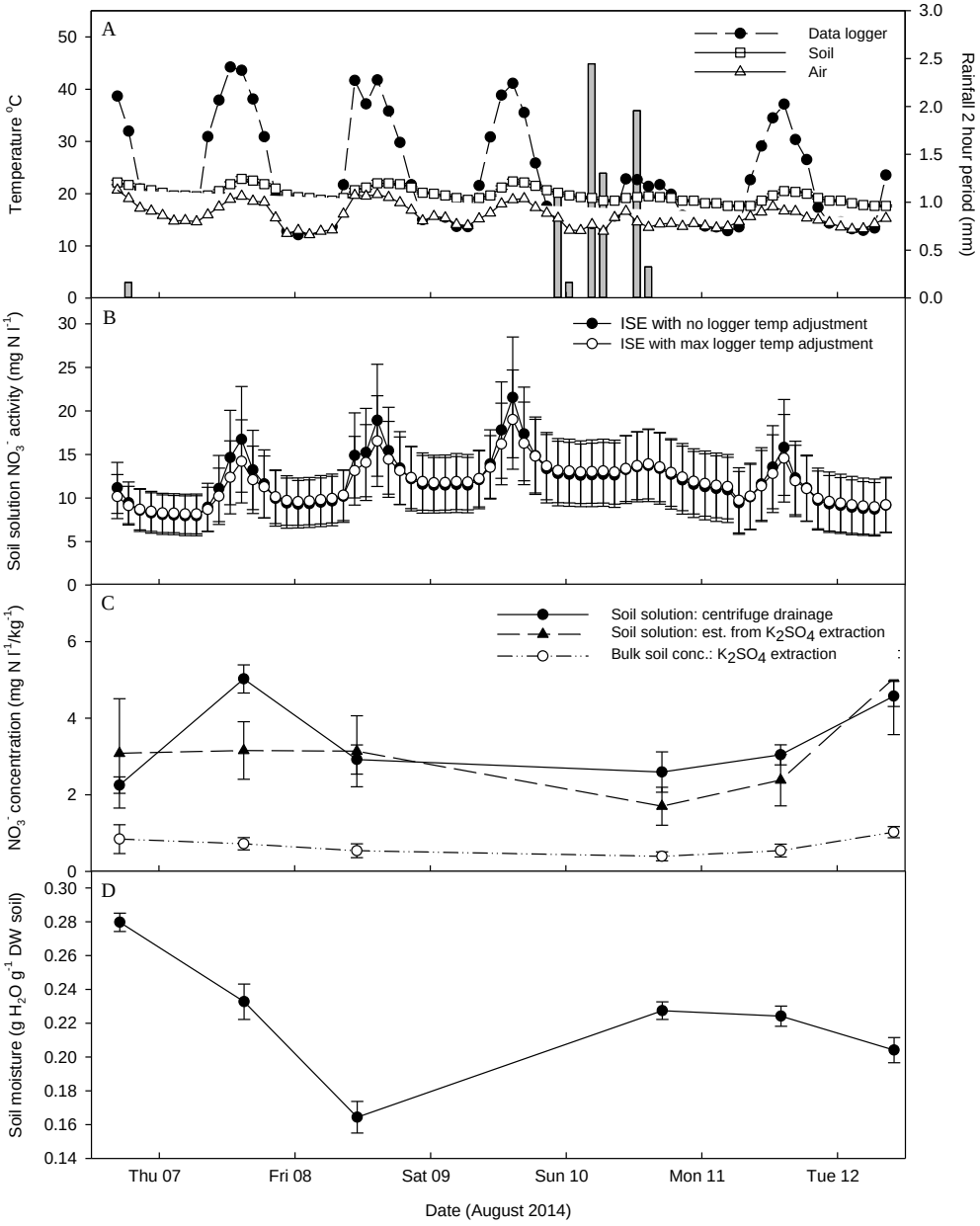

Figure 9. Results of in-situ soil NO_3^- monitoring in an agricultural grassland field over a 7 day period. Panel A shows the temperature (°C) of the air, data logger and soil at 5–10 cm depth (means ± SEM, n = 3). Rainfall totals (mm) are for 2 h time periods. Panel B shows the estimation of soil solution NO_3^- activity by the in-situ NO_3^- ISE with (open circles) and without (closed circles) adjustment for data logger temperature (means ± SEM, n = 11). Panel C shows estimations of soil solution and bulk soil NO_3^- concentration from soil core extractions and centrifugal-drainage followed by chemical analysis (means ± SEM, n = 4). Panel D shows soil moisture content (means ± SEM, n = 4).

Influence of temperature on ISE monitoring results

One obvious feature of the NO_3^- ISEs results from both the laboratory and field monitoring was the covariance with both logger and soil temperature and the diurnal cycle. In the laboratory monitoring experiment the temperature was variable for the first 3 diurnal cycles, then set to a constant 20 °C

for the subsequent 4 diurnal cycles, although some small variation in data logger and soil temperature was still evident. The observed variation in the NO_3^- ISE results decreased markedly after the first 3 diurnal cycles. This suggests that temperature rather than the diurnal cycle was the cause of the observed variation. The fact that the results, which have been adjusted for temperature effects on both the data logger and the ISEs, still show co-variation with temperature could suggest that the measurements reflect a real soil phenomenon. However, a closer look at the results from the field trial would suggest otherwise. The diurnal variation in the NO_3^- ISE results from the field experiment was much larger than in the laboratory experiment despite variation in soil temperature being much lower. Temperature variation in the data logger conversely was much larger. The NO_3^- ISEs results increased dramatically as the temperature of the data logger exceeded 25 °C in a manner that was consistent with an exponential relationship between temperature and the ISE output. As such, it is likely that the observed diurnal variation was mainly an experimental artifact caused predominantly by a temperature effect on the data logger. Although, due to the uncertainty over the size of the temperature effect, there may also be some temperature and diurnal related changes to the intrinsic soil NO_3^- concentration (Delhon et al., 1996; Marhan et al., 2015).

5.5. Study 5

Nested sampling to evaluate the spatial distribution of soluble N in soil prior to application of N fertilizer

The different forms of N showed slightly different scale-dependencies, although in general, short-range variance dominated (Fig. 10.) NO_3^- was found to have the largest total accumulated variance of 0.92 µg N g^{-1}, followed by NH_4^+ with 0.75 µg N g^{-1}, and amino acids with 0.51 µg N g^{-1}. For amino acids, the 1-cm scale had the largest variance component, constituting 58.6% of the total accumulated variance. The 10-cm and the between-mainstations within-strata term were also considered important spatial components. For NH_4^+, the 1-cm scale had the largest variance component, constituting 63.0% of the total accumulated variance. However, for spatial scales greater than 1 cm, only the between-mainstations within-strata term was considered important. For NO_3^-, there was more variance at larger scales compared to the other forms of N, with the 10-cm scale having the largest variance component, constituting 28.0% of the total accumulated variance. Furthermore, all the spatial scales, with the exception of the 2-m scale, exhibited variance that was considered important. Short-range scale variation still dominated though, with 70.4% of the variance occurring at spatial scales up to 50 cm. It should be noted that the 1-cm scale component will also include any measurement error.

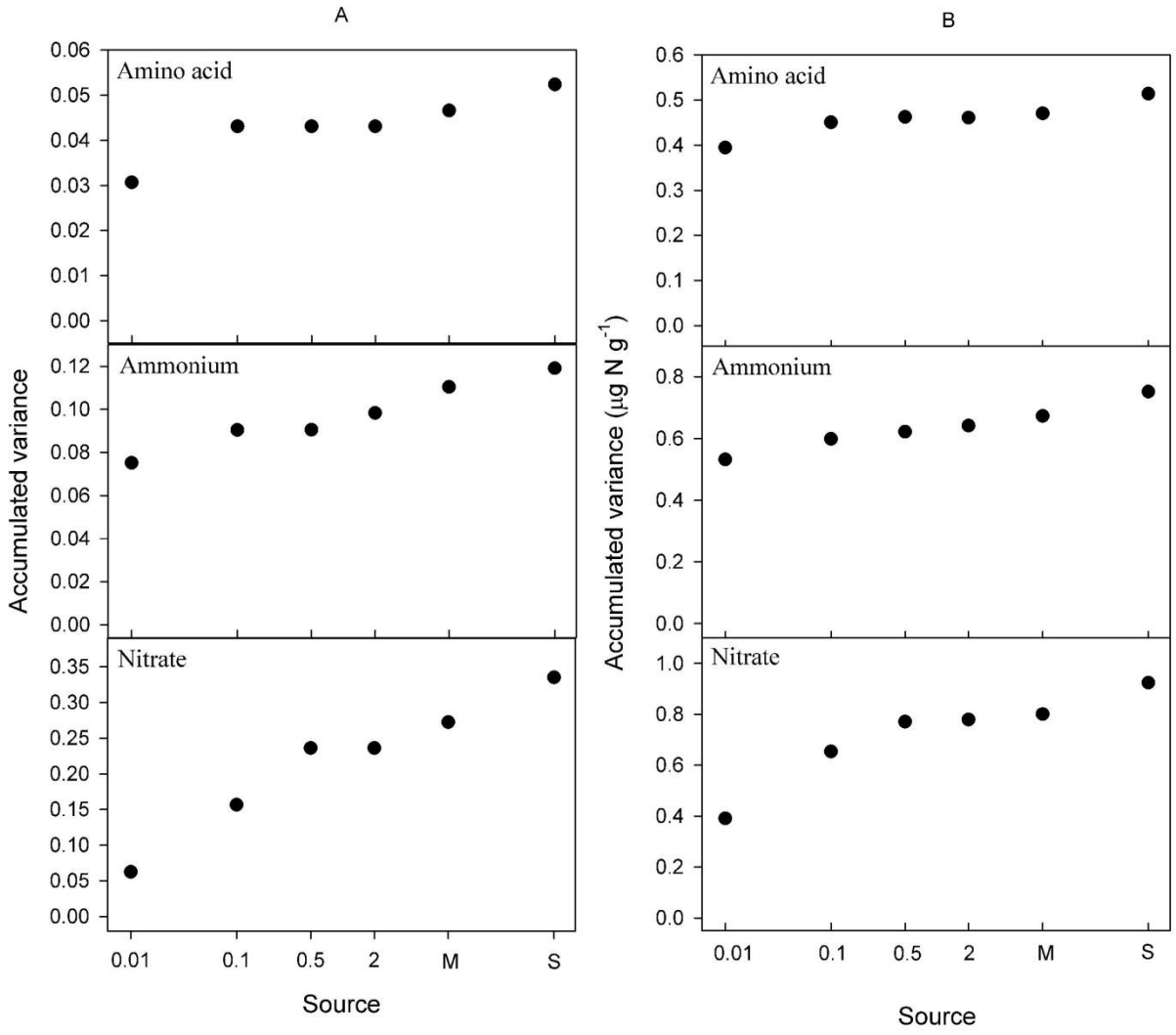

Figure 10. Accumulated variance components from the finest to coarsest spatial scale, derived from the June nested sampling results (before fertiliser addition). Panel A uses the Box-Cox transformed units and panel B uses the units of the original scale of measurement computed by the MAD (median absolute deviation from the median) procedure. Source is the spatial-component in meters, with M and S representing the between-mainstation and between-strata components respectively.

Nested sampling to evaluate the spatial distribution of soluble N in soil after application of N fertiliser

The different forms of N showed slightly different scale-dependencies, although in general short-range variance dominated (Fig. 11). NH_4^+ was found to have the largest total accumulated variance of 0.88 µg N g^{-1}, followed by NO_3^- and amino acids with 0.63 and 0.42 µg N g^{-1} respectively. For amino acids, the between mainstations within-strata had the largest variance component, constituting 35.8% of the total accumulated variance, although 57.7% of the total accumulated variance occurred at scales up to 10 cm. The 1-cm, 10-cm and the between-mainstations within-strata term were considered important spatial components. For NH_4^+, the 1-cm scale had the largest

variance component, constituting 55.1% of the total accumulated variance. Spatial scales greater than 10 cm accounted for only 13.3% of the total accumulated variance. Only the 1-cm and the between-mainstations within-strata terms were considered important spatial components. For NO_3^-, the between-mainstations within strata scale was the largest variance component, constituting 38.7% of the total accumulated variance. The 1-cm, 10-cm and the between-mainstations within-strata term were considered important spatial components. Short-range scale variation still dominated though, with 61.2% of the variance occurring at spatial scales up to 50 cm.

Duplicate measurements on 4 samples from each mainstation allowed the 1-cm spatial variance component to be resolved from the subsampling and measurement error. As this residual term formed the ultimate term in the model, it allowed an assessment of the importance of the 1-cm spatial component. For all of the N forms, the 1-cm scale was considered an important spatial component, and was larger than the residual variance. However, the residual variance, which was similar for all N forms, constitutes a substantial component of the accumulated variance and was, for all N forms, larger than the variance at 50 cm and 2 m.

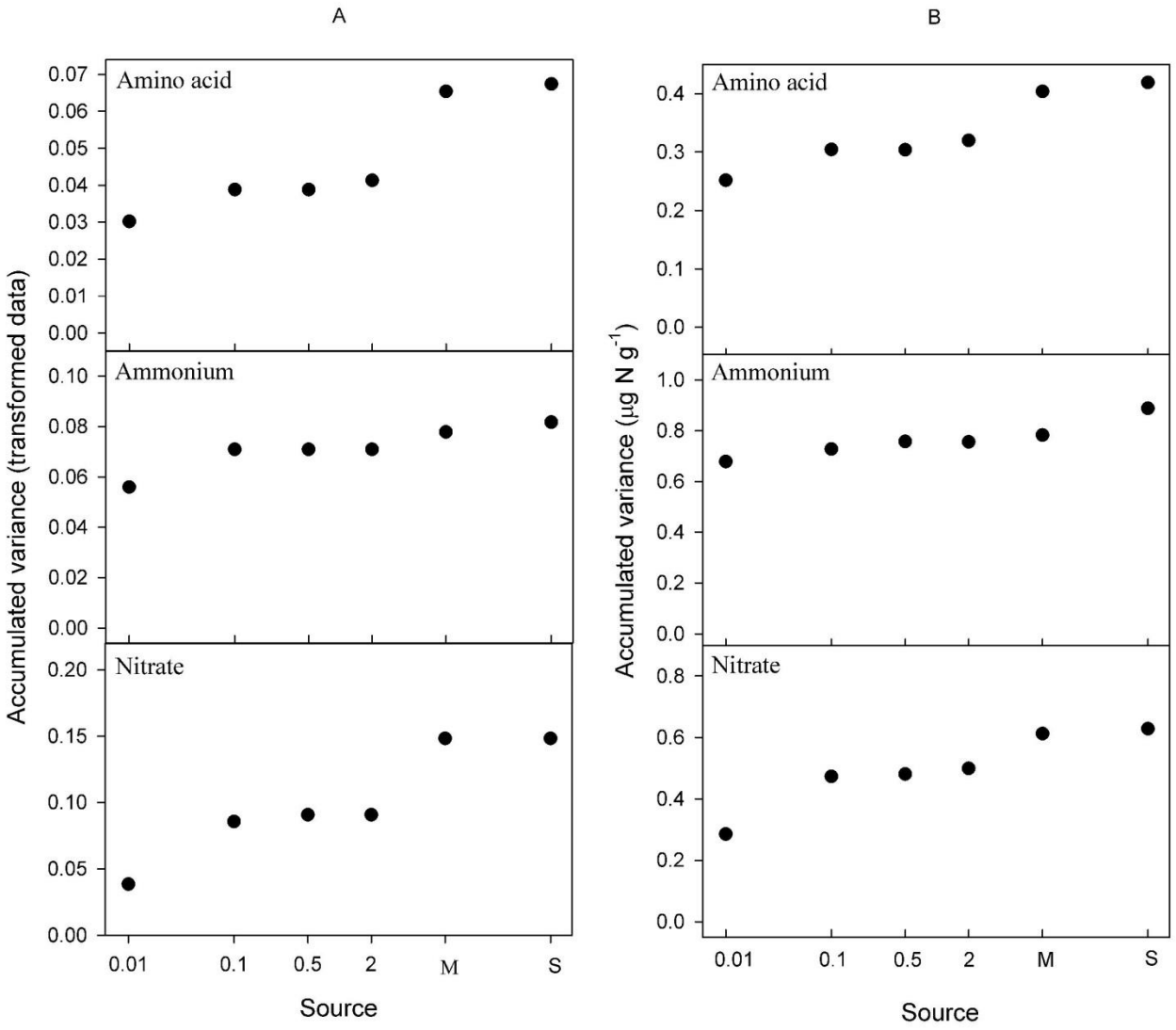

Figure 11. Accumulated variance components from the finest to coarsest scale, derived from the July nested sampling results (after fertiliser addition). Panel A uses the Box-Cox transformed units and panel B uses the units of the original scale of measurement computed by the MAD (median absolute deviation from the median) procedure. Source is the spatial-component in meters, with M and S representing the between-mainstation and between-strata components respectively.

Aggregate-scale variability of soluble N in soil

In all cases, the largest variance component was found to be the between-aggregate within-core scale (Table 1). For NH_4^+ and NO_3^-, 91.3% and 80.1% respectively of the total accumulated variance occurred at this scale, which was an order of magnitude higher than the variance at the between core scale. The variance at the aggregate scale for amino acid-N was slightly lower at 69.2%. The between-core component, which represents the 1-cm spatial scale, was considered important for amino acids and NO_3^-, but not NH_4^+. Neither the between-pair component, which is similar to the between-mainstations scale, nor the between-strata component, were considered to be important

spatial components. However, the stratum and mainstation scale in this analysis were based on limited replication. The focus of this particular sampling exercise was on the aggregate and core scale, so general conclusions from these results about the importance of coarser-scale variation were not made.

Table 1. Variance components for the (Box-Cox transformed) variables describing the aggregates-scale spatial variability of soluble N in a grassland soil.

Variable	Variance component			
	σ^2_s	σ^2_p	σ^2_c	σ^2_a
Nitrate-N	0.0	0.0	0.072	0.289
Ammonium-N	0.0	0.0311	0.005	0.3766
Amino acid-N	0.0	0.0055	0.0167	0.0499

Optimisation of a within-field sensor network for monitoring soluble N in soil

The optimisation of the design of an in-situ network of NO_3^- and NH_4^+ sensors can be explored using the graphs in Fig. 12. The graphs show how increasing both the number of sensors per data logger, and increasing the number of data loggers, reduces the width of the 95% confidence interval of the estimated field mean derived from the sensor network. There are differences in the results between NO_3^- and NH_4^+ and between sampling events. For example, to achieve a 95% confidence interval width of no more than 1 µg N g^{-1} for a NO_3^- sensor network, given the spatial variation observed in the June sampling event, would require 3 data loggers each with 11 sensors at a cost of £8200. For the July sampling, 2 data loggers each with 9 sensors would be sufficient, at a lower cost of £5800. For a NH_4^+ sensor network, given the spatial variation observed in the June sampling event, 2 data loggers each with 6 sensors, at a cost of £5200 would be required to achieve a 95% confidence interval width of no more than 1 µg N g^{-1}. For the July sampling, 2 data loggers each with 8 sensors, at a slightly higher cost of £5600, would be required. Reducing the width of the 95% confidence interval substantially below 1 µg N g^{-1} dry soil would result in a large cost increase, with small marginal improvement on increasing the size of the network. For a NO_3^- sensor network, given the spatial variation observed in the June sampling event, reducing the width of the confidence interval < 0.5 µg N g^{-1} would require 10 loggers each with 12 sensors, at a cost of £22400.

An alternative approach is to optimise the sensor network design within the constraints of a fixed budget. A budget of £5000 for a NO_3^- sensor network could provide a single data logger with 15 sensors or 2 data loggers each with 5 sensors. This could be used to provide a single logger with 15 sensors on each, or two loggers with 5 sensors on each. The width of the confidence interval for these two options is ± 2.12 and ± 1.70 µg N g^{-1} dry soil respectively, so the second option is the rational choice.

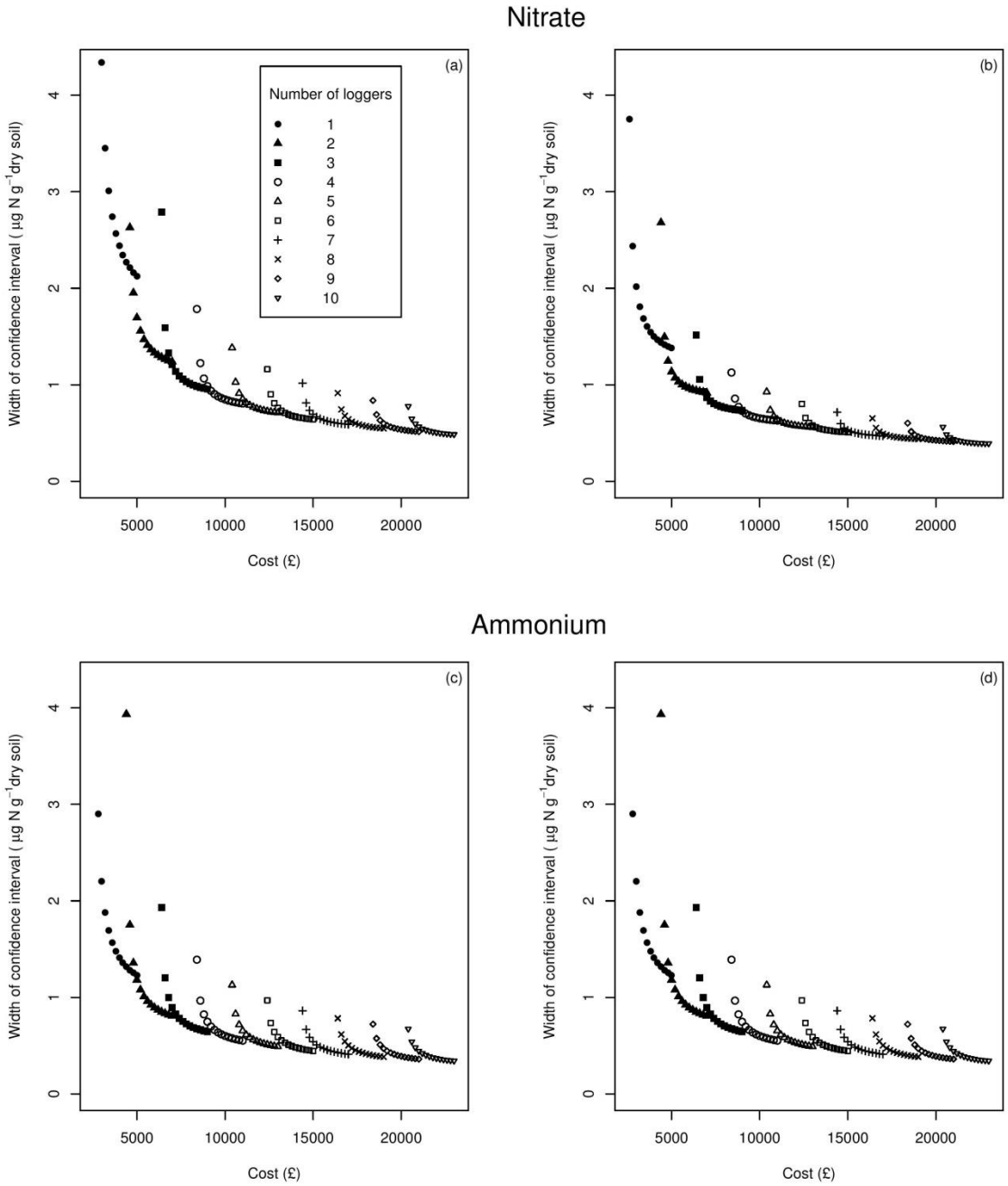

Figure 12. Width of the 95% confidence interval for alternative sensor network designs of different cost computed to facilitate monitoring of soil N in a 1.9 ha grassland field. Values are computed from variance components from nested sampling of nitrate in (a) June (before fertiliser addition) and (b) July (after fertiliser addition) and of ammonium in (c) June (before fertiliser addition) and (d) July (after fertiliser addition) and on the basis of unit costs for a sensor and a data logger of £200 and £2000 respectively. Note that the arrays comprise 1-10 loggers and a maximum of 15 sensors per logger. To allow a common range of values on the ordinates of these graphs, and to facilitate interpretation, arrays with fewer than five sensors in total have been excluded from Figure 4(a) and arrays with fewer than three sensors have been excluded from Figures 4(b-d).

6. Discussion

6.1. Discussion of experimental work

This PhD aimed to develop novel methods to enable real-time and in-situ measurement of soluble N in soil. Better quantification of soil N status is required to improve management of agricultural land receiving N fertilisers and manures, and increase our fundamental understanding of soil N processes. However, soil scientists, farmers, and agronomists are currently limited by a lack of non-destructive and user-friendly techniques that allow real-time and in-situ soil N determination. The research undertaken here set the following aims in order to address this issue:

- To investigate the use of microdialysis-based sampling for the determination of plant-available N and in-situ monitoring of soil N dynamics (studies 1 & 2);
- To develop farmer-operated tools and methodologies which are user-friendly and could be used for the on-farm determination of soil N (studies 3 & 4).
- To construct, develop and test a NO_3^- ISE for the real-time, in-situ monitoring of soil N (study 4).
- To investigate how to optimise the field-scale configuration of an in-situ sensor network to facilitate both accurate and economical soil N monitoring (study 5).

Below, the results and implications of the experimental work undertaken to satisfy the above aims are discussed.

Microdialysis is a technique that has been widely used in pharmacological research for the in-situ sampling of biological fluids (Nesbitt et al., 2013). Recently, several research papers have utilised this approach for the in-situ sampling of soil solution (Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012a). In study 1, we explored how microdialysis could be used to determine soil N availability. Diffusive-flux measurements of eight soils along a catena sequence were compared to conventional soil core batch extractions (using 0.5 M K_2SO_4 or distilled H_2O). The percentage contribution that amino acids, NH_4^+, and NO_3^- made to total plant-available N, were most similar to conventional distilled water extractions. However, the relative magnitude of the diffusive flux measurements did not always reflect the pool sizes as estimated by the soil extractions. Microdialysis was also used for in-situ sampling of amino acids, NH_4^+, and NO_3^- from the rhizospheres of *Zea mays* L. seedlings (study 2) grown in soil-filled rhizotubes. The microdialysis sampling showed a significant decrease in soil solution NO_3^- concentration, which corresponded to the time the root tip grew past the probe and this was attributed to plant uptake.

These two studies highlight both the advantages of microdialysis sampling and its associated problems. It has been suggested that such a sampling procedure will better inform the availability of N for plant uptake as diffusion through the membrane is dependent not only on the concentration of the target solute but also its mobility through the soil, which, in turn, is dependent upon a range of

physical and chemical variables (Inselsbacher and Näsholm, 2012b; Shaw et al., 2014). This is exemplified in study 1, where clear differences in N availability measured using pool size or diffusive flux were apparent across a wide range of soils differing in their chemical and physical soil properties. The other main advantages of the microdialysis approach is the small size of the probes and the ability to take multiple samples over an extended period time with minimal perturbation to the system being evaluated. This enables an assessment of soil N dynamics to be made with excellent spatial and temporal resolution as demonstrated in our study of rhizosphere N dynamics (study 2). This excellent spatial resolution can also be an issue as soil is an inherently heterogeneous medium, especially at small scales (Parkin, 1987; Nunan et al., 2002). This is often manifested in our microdialysis results as large errors around means, despite pre-sieving and mixing. This spatial variability has also been demonstrated in a previous microdialysis study (Inselsbacher et al., 2011).

The results of the microdialysis sampling were presented differently in study 1 and 2. In study 1, the diffusive-flux measurement was used, and in study 2 absolute soil solution concentrations were calculated. Deriving soil solution concentrations from microdialysis relies on the application of a correction factor (i.e. the percentage recovery of N from a standard solution). It further assumes that this correction factor remains constant across a wide range of soils. It is highly likely that, due to inherent and obvious differences between soil and a standard solution, that the percentage recovery will vary between these mediums and between different soils (e.g. due to surface contact, inherent moisture content, etc.). However, results from study 2 showed that the initial soil solution concentration of amino acids and NO_3^-, as assessed by centrifugal-drainage and microdialysis, were statistically similar. Whether this is true for other soils requires further investigation. The diffusive-flux measurement simply describes the rate of solute diffusion into the microdialysis probe, which will be affected, not just by the absolute concentration, but also by a range of physical and chemical soil properties. As such, this method may better reflect N which is available for plant uptake. However, this measurement is biased towards solutes with a lower molecular weight, as these will diffuse most quickly across the microdialysis membrane creating a larger concentration gradient around the probe, and hence resulting in a faster rate of diffusion through the soil. The diffusive-flux is also dependent upon the type of microdialysis probe used, its molecular weight cut-off, pore size and the speed at which the perfusate is pumped through the probe. As such, absolute comparisons between different studies are difficult to make. Drawing conclusions for plant nutrition from the diffusive-flux measurement is also confounded by the modifying rhizosphere effect and active root uptake mechanisms (Shaw et al., 2014).

Whilst microdialysis may continue to offer new insights into soil N dynamics, using this approach for agronomic purposes will require considerable development prior to commercial adoption. Currently, the microdialysis samples need subsequent chemical analysis which precludes its on-farm use. It may be possible to combine microdialysis with an on-line measuring system but this will add further complexity and expense to a system that already requires a water reservoir and pump.

As it became apparent that microdialysis is currently unsuitable for this application, the use of commercially available NO_3^- ISEs and UV spectroscopy for a soil NO_3^- rapid-test was investigated (study 3). Our results showed that manual extraction using distilled H_2O, combined with either NO_3^- ISEs or UV spectroscopy could accurately determine the NO_3^- concentration of the extracts. As such, both of these methods have the potential to be used as on-farm quick tests. Whilst UV spectroscopy has not previously been used in this context, the concept of on-farm rapid-tests are not new (Jemison & Fox, 1988; Hartz, 1994), but despite this, on-farm use is thought to be low. Using UV spectroscopy may require filtering of extracts prior to testing and using ISEs requires some pre-calibration. In addition, an assessment of soil moisture content is needed to calculate an accurate soil NO_3^- concentration. These issues, when combined with a lack of a suitable decision support system to generate fertiliser recommendations, perceptions of cost-benefit and farmer attitudes to new technologies may partially explain low uptake.

ISEs have many properties that are advantageous for in-situ soil monitoring. Previous work has demonstrated their use for direct soil measurements (Ito et al., 1996; Adamchuk et al., 2005) but until now there has been no evidence that they have been successfully used in-situ and real-time monitoring of soil NO_3^-. In study 4, we demonstrate the use of a novel NO_3^- ISE for in-situ and real-time monitoring of an agricultural soil, both in a field trial and under controlled conditions in the laboratory. Results from the ISEs were found to be statistically similar to conventional laboratory analysis of contemporaneous soil samples on 16 out of 19 occasions. These novel NO_3^- ISEs provide a new opportunity for in-situ and real-time measurement of soil N dynamics, which represents a significant step forward for analytical soil science and environmental monitoring. In our study, we found that temperature had a significant effect on the ISEs and the datalogger, which could not be fully compensated for. Therefore, further work is required to better understand the effects of temperature on the ISE-datalogging system and develop improved compensation calculations. As ISEs measure the soil solution, it will also be important to look at how differing soil moisture contents affect the ISE performance. It is likely that the ISEs may not operate in very dry conditions, which may limit their usefulness for long-term monitoring. Furthermore, interpreting how soil moisture-related changes in soil solution NO_3^- concentration affects the availability of NO_3^- for plant uptake requires further investigation, which may be achieved using microdialysis. In addition, the NO_3^- ISE gives no consideration for other plant-available N forms, especially those which may be predominantly held on the solid phase (i.e. NH_4^+).

Results from the microdialysis experiments (studies 1 & 2) and the in-situ NO_3^- ISE testing (study 4) showed large variability around means, which may reflect inherent spatial variation at small scales. Using in-situ methods to estimate soil N status at field-scale may be confounded by variation at range of scales. In study 5, we investigated the spatial variation of soil N in a grazed grassland field in order to optimise the spatial and economic configuration of an in-situ sensor network. Our work established that at least 60% of the variance in amino acids, NH_4^+ and NO_3^- occurred at scales < 2 m, with significant variation occurring at the sub 1-cm scale. This data was used to demonstrate how

an in-situ sensing network could be optimised on a cost-accuracy basis. Given the unit costs of £2000 and £200 respectively for a data logger and NO_3^- ISE, the field mean for NO_3^--N concentration could be estimated with a 95 % confidence interval no wider than ± 1 µg N g^{-1} for a cost of £8,200. However, these calculations are based on a sensing support size of around 1 cm. Sensors, such as the NO_3^- ISEs developed in this project, that operate at sub 1-cm scales will be exposed to further variation, and hence more local replication will be required at the sub 1-cm scale to achieve similar levels of accuracy, with a resulting cost increase. It is therefore clear that in-situ monitoring is likely to incur significant costs, and future work must focus on assessing the cost-benefit and determining the most effective way to use the real-time data to inform fertiliser management.

The novel NO_3^- ISEs and microdialysis sampling have some considerable advantages but also some disadvantages when compared to conventional destructive soil testing, and these are summarised briefly below and in Table 2. Both methods allow an in-situ assessment of soil N with minimal disturbance to the system that is being evaluated. The main advantage of the novel ISEs is that soil NO_3^- can be quantified in-situ and in real-time at a fine temporal resolution, without the need for any destructive sampling and laboratory analysis. This make them ideal tools for on-farm monitoring use, as once they have been set up they require no further input. One drawback of this approach is the potentially high start-up costs, although for long-term monitoring at a high temporal and spatial resolution it is likely that total costs would be lower than performing conventional soil sampling and analysis at the same resolution. A further disadvantage is that the novel ISEs are only capable of sensing NO_3^-, so information on other forms of plant-available N is not captured. Microdialysis also has the advantage over conventional soil testing that sampling can be performed in-situ, with minimal disturbance. However, currently microdialysis samples requires subsequent analysis in a laboratory resulting is both an economic and time cost. Furthermore, running the microdialysis probes is a more active process compared to the ISEs due to the need for a pump system and sample collection. Microdialysis is able to assess a large range of soil solutes, including all forms of plant-available N. It also has the advantage over both the other methods that its unique method of sampling via passive diffusion may better reflect the availability of N forms for plant uptake.

Table 2. Comparison of microdialysis and novel NO_3^- ISEs with conventional soil extractions for the assessment of soil N

	Conventional salt extraction (1 M KCl/0.5 M K_2SO_4)	**Novel NO_3^- ISE**	**Microdialysis**
Sampling requirement	Destructive sampling of soil and transport to laboratory	Can be used in-situ. ISEs are sensors	Soil solution solutes sampled in-situ via passive diffusion
Analytical requirement	Vigorous shaking with strong salt solution followed by filtering/centrifuging and chemical analysis in laboratory	Analysis performed in-situ and stored on data logger. Calibration required to convert mV output to [NO_3^-]	Chemical analysis of samples in laboratory
N pool assessed	Exchangeable soluble N pool. Both organic and inorganic N forms	NO_3^- activity of the soil solution	Time integrated concentration/diffusive flux. Both organic and inorganic soluble N forms
Level of disturbance	High – soil is removed from system	Very low following initial placement into soil	Low – only small quantities of soil solutes removed
Temporal resolution	Poor. Each sampling event requires significant extra cost/labour	Excellent. ISE output can be recorded at < 1 Hhz	Good. Probes can be run continuously. Resolution limited by sample volume required for analysis
Spatial resolution	Large range of sample sizes possible i.e. > 1 kg to < 1 g. Samples can be homogenised/bulked to reduce small scale spatial heterogeneity	Excellent. Diameter of sensing membrane < 1 mm. Subject to micro-scale heterogeneity	Excellent. Linear dimension of membrane 4 mm. Subject to micro-scale heterogeneity
Cost	High in terms of labour, especially if sampling is carried out at fine spatial and temporal resolution	High start-up costs, but value increases as temporal resolution increase	Medium start-up costs as pump also required. Costs increase with number of samples due to requirement for subsequent analysis
Relevance of results to plant nutrition	Possible changes in N pool sizes during sampling and analysis. Concentration may not equal plant availability	Soil solution NO_3^- activity may be what is 'sensed' by plants. Importance of interaction between variable soil moisture content/ N activity and plant availability needs further investigation	Results may better reflect plant availability as they are affected by many soil and environmental variables. However, N transformations occur in rhizosphere and plant uptake is selective/active

6.2. Limitations of this project

The vast majority of the experimentation performed in this project was carried out using grassland soils local to Bangor University. Whilst microdialysis diffusive flux measurements (study 1), the novel NO_3^- ISEs were tested solely on a Eutric Cambisol. As such, the transferability of these approaches to other, particularly, arable soils, is unknown. This is particularly concerning with regard to the NO_3^- ISEs. It is likely, given the high costs associated with using the novel ISEs for real-time in-situ monitoring, that uptake of this approach will be limited initially to more profitable forms of agriculture, such as arable cropping and horticulture. These soils are often different to grassland soils in many aspects, which may affect the performance of the novel ISEs and the design of in-situ sensor arrays. For example, soils under arable cultivation are often drier, especially in the upper profile, have lower organic content and have much less variance at small-scales when compared to grassland soils. Furthermore, soils that have a high proportion of clay, such as those used for arable agriculture in central and eastern England, are prone to cracking during the summer months. The performance of the novel ISEs in such soil remains very much an unknown. It is also possible that microdialysis may not function effectively in very dry soils as the some of the perfusate may be lost across the membrane and into the soil. Future testing of these methods must focus on a wider range of soils, particularly those that are used for arable cropping and horticulture.

A further limitation to the study is that the novel ISE is only capable of sensing NO_3^- and not other forms of plant-available N. Whilst NO_3^- is often considered the most important N form in high-input arable soils, the contribution of NH_4^+ and organic forms may be significant, especially under low-input and grassland systems. The extent to which fertiliser recommendations can be improved from NO_3^- measurements only requires further investigation.

6.3. Future work

Method/technological development of microdialysis and novel ISEs

This body of research represents the early stages in the development of microdialysis and in-situ ISEs. Further work on both of these techniques is required to fully optimise their performance and increase their usefulness to both scientists and the agricultural industry. One of the disadvantages of the microdialysis technique is the need to collect samples at regular intervals for subsequent analysis. To overcome this, automated sample analysis using systems such as ‘lab-on-chip’ (Beaton et al., 2012) or flow-injection analysis using electrochemical sensors (Kim et al., 2007) could be adapted for use with microdialysis. One further problem with the microdialysis sampling is that equilibrium between the perfusate and the soil solution is never achieved and extremely low flow rates are required to ensure concentrations in the perfusate are detectable. Significantly increasing the length of the membrane would increase the time for diffusion and hence improve recovery rates. The length of the membrane used in this project was 4 mm. Increasing this length may reduce its usefulness for investigating systems at fine spatial scales – such as the rhizosphere – but it may

help to reduce the impact of micro-scale heterogeneities which may hinder field research. Another option is to operate microdialysis as a circulatory system with in-line analysis. This would avoid the need for sampling and may further boost recovery of soil solutes into the perfusate. However, whether this would enable equilibrium to be reached and how quickly the system would respond to changes in the intrinsic solution require further investigation.

As discussed above, considerable further testing of the novel ISEs on a range of soil types and environmental conditions is required. The effect of soil moisture on both the performance of the ISEs and the interpretation of the results is particularly important. For the ISEs to function, the membrane needs to be able to interact with the soil solution, which may be impossible in very dry conditions. Consideration of how changes in moisture content may affect the interpretation of the ISE results is also required. Assuming no change in the amount of NO_3^- in any given volume of soil, a decrease in soil moisture content will result in results in an increase in soil solution NO_3^-. With knowledge of the soil moisture content it is possible to convert the soil solution concentration in to a bulk soil concentration, however, water matric potentials will differ between different soils with the same moisture content, which further complicates interpretation. Plants access soil nutrients by a combination of root interception, mass flow and diffusion (Barber, 1984). How the concentration/moisture content/matric potential interaction affects the availability of NO_3^- for plant uptake and the relative importance of root interception, mass flow and diffusion is poorly defined and requires further investigation. Microdialysis may be an ideal tool to assess how soil moisture contents control the availability of soluble N forms for plant uptake via both diffusion and mass flow (Oyewole et al., 2014).

6.4. Using in-situ methods to improve fundamental understanding of soil N processes

Gaining a better understanding of soil N processes is often limited by a lack of in-situ and non-destructive techniques. As has been demonstrated in this project, microdialysis can be used to determine how soil N dynamics vary over time and space. However, whilst microdialysis estimations of pool size and diffusive-fluxes may be affected by the balance of consumptive and additive processes, they have not yet been used to determine absolute fluxes between pools. This could be done indirectly using an incubation approach or directly using isotopic labelling. Repeating the rhizosphere study (study 2) using ^{14}C-labelled plants may allow the degree of root exudation of amino acids in soil to be determined.

Whilst microdialysis may better inform on the availability of soil N, drawing conclusions for plant nutrition is confounded by a number of factors including rhizosphere priming effects. Exudation of labile C from root tips stimulates microbial growth, which in turn leads to a reduction in the availability of N for plant uptake (Kuzyakov, 2002). Microdialysis could be used to assess the effect of this C exudation on N availability by using a low molecular weight C substrate (such as glucose) as the perfusate. This would diffuse out through the microdialysis membrane into the soil, simulating root exudation and creating a 'rhizosphere effect'. Varying the composition, concentration and C:N ratio of the LMW C substrate with a range of soils may enable new insights into rhizosphere priming to be made.

The scope for using NO_3^- ISEs with the capability for in-situ measurements for research into soil N dynamics is significant. Staying within an agricultural context, reducing emissions of N_2O from soils receiving N inputs is of great importance (Mosier et al., 1998). There has been much research into the biogeochemical controls of emissions and potential mitigation options, but the research is limited by the ability to make continuous soil NO_3^- measurements inside gas sampling chambers. Destructive sampling within chambers causes disturbance to the soil which will affect gaseous emissions. As such, it is currently difficult to directly relate the concentration of soil NO_3^- to N_2O emissions. In-situ measurements of soil NO_3^- using ISEs could be used to address this issue and improve our understanding of the relationship between soil N dynamics and N_2O emissions. One potential mitigation option is the use of nitrification inhibitors to retard the production of NO_3^- from NH_4^+ (Zhang et al., 2015). Investigating the use of these inhibitors using both NO_3^- ISEs and microdialysis would likely result in an improved understanding of how they affect soil N cycling and enable optimisation of their use.

6.5. Using in-situ sensors for precision agriculture

We have demonstrated in-situ monitoring of soil NO_3^- using a novel ISE and have also explored how networks of these sensors could be spatially configured to provide accurate and economic data. Such real-time data may enable a shift away from predetermined and empirically-derived fertiliser recommendations based upon N requirements over a growing season and potential crop yields, to a more dynamic system that responds rapidly to changes in crop N demand and soil N availability. This would have the benefit of minimising N surpluses in the soil hence decrease losses therefore potentially resulting in increased NUE. However, using this data to improve fertiliser recommendations presents a significant future challenge. N management is confounded by the multiple biotic and abiotic variables that ultimately control the final yield of the crop and the efficiency at which N is used. It is important to understand the reasons for any observed variation of soil N availability, and how that will affect cop growth at that specific location. The observed variation may be due to differences in crop uptake rates, N inputs or N cycling dynamics as determined by the biogeochemistry of the soil. Changes in NO_3^- concentration may also simply reflect recent rainfall events (i.e. dilution or leaching) rather than biological uptake. Further, sensors are frequently deployed in the topsoil which may not reflect N availability at depth. This is of particular relevance in arable cropping systems where roots can penetrate to >1.5 m in the soil profile and where soil moisture often constrains N uptake from dry topsoils. It is also important to determine whether the concentration of NO_3^- is growth-limiting or whether other agronomic factors are limiting (e.g. pH, other nutrients, plant pathogens, bulk density). It may be the case that in certain areas, improving NUE comes not from adjusting N fertiliser application rates but improving other factors, such as the status of other key nutrients, soil drainage or soil compaction. Currently, there are no sensors for the in-situ determination of soil NH_4^+ and plant-available forms of organic N, such as amino acids. Whilst in many high-input arable systems, NO_3^- is the most dominant N form, the importance of NH_4^+ and organic N should not be underestimated in grassland, low-input and organic farming systems. It is clear that as well as monitoring soil N it is important to determine plant N status in order to estimate crop N requirement as it has been shown that plant N uptake is controlled by both plant growth and availability of soil N (Devienne-Barret et al., 2000; Gastal and Lemaire, 2002). Such technology is now in commercial use in the form of tractor-mounted crop canopy scanners which can be coupled with variable rate fertiliser spreaders (Diacono et al., 2013).

Given the complexity of the plant-soil system, it is likely that modelling approaches will be the best way forward for generating fertiliser recommendations. The aim of a dynamic approach to fertiliser management should be to maintain the pool of plant-available N at a level that matches plant uptake. Modelling approaches using real-time data may optimise both the timing and amount of fertiliser needed. Take for example, a study conducted by van Alphen (2002), who used soil N modelling combined with real-time weather data to monitor soil N status. Spatial variation was incorporated through the use of management zones, which were defined in terms of water regimes and N dynamics. Early warning was provided when soil mineral N concentrations dropped below a

critical threshold. Used as a trigger, this information served to optimise the timing of four consecutive N fertilisations. Compared to conventional management, fertiliser input was reduced by 15–27%, without affecting grain yield. This approach could be improved by incorporating real-time soil NO_3^- measurements using the novel ISEs. However, calculating a 'trigger' NO_3^- concentration will be very challenging and the following points must be considered:

How does soil moisture content interact with soil solution NO_3^- to control the availability of NO_3^- for plant uptake? Using soil moisture sensors in combination with NO_3^- ISE would allow the calculation of NO_3^- concentration on a per kg of soil or per ha basis.

Crop demand for N is variable during the growing season. For winter wheat, most N uptake occurs during a 2 month period in spring. Therefore, any trigger value will depend upon crop N demand at any given point in the growing season. Using crop canopy sensing techniques (Diacono et al., 2013) to assess plant N status may allow N demand, and hence a trigger concentration, to be determined.

Presuming that within each management zone there are multiple sensors as part of an array, how many of these need to drop below the 'trigger' value before an application of fertiliser should be made? Assuming that any management zone is fairly homogenous in terms of its N dynamics, then the mean value of the sensors would be used as the 'trigger' value. However, if variance in NO_3^- concentration within a management zone becomes significant then a more dynamic approach to spatial variation may be needed.

Soil solution NO_3^- concentration will fluctuate depending upon soil moisture content but also the balance of nitrification, denitrification and plant uptake. As such, the NO_3^- concentration may drop below the 'trigger' value for a short period of time before recovering. How long must the value remain below the 'trigger value' before a fertiliser event is initiated? Monitoring soil moisture content will enable fluctuations in soil solution NO_3^- concentration due to soil moisture dynamics to be determined. Fluctuations in the intrinsic supply of NO_3^- may be accounted for by monitoring plots with no N inputs.

In addition, data gained from the in situ sensors could be supported by a tractor-mounted near-infra red scanning of the soil matrix which provides additional estimates of soil organic matter quality which can be linked to rates of N mineralization/supply (Gomez et al., 2008). This will rely on developing sensitive algorithms to support the "SMART" farming approach, where real-time data on plant/soil conditions are gathered from numerous sources and integrated using an "Internet of Things" approach. Ultimately, this may enable a step change towards a more dynamic approach of nutrient management. Based on the rate of recent advances in sensor technology, networks and data processing platforms, realistically, this approach could be commercially implemented on UK farms in the next 25 years.

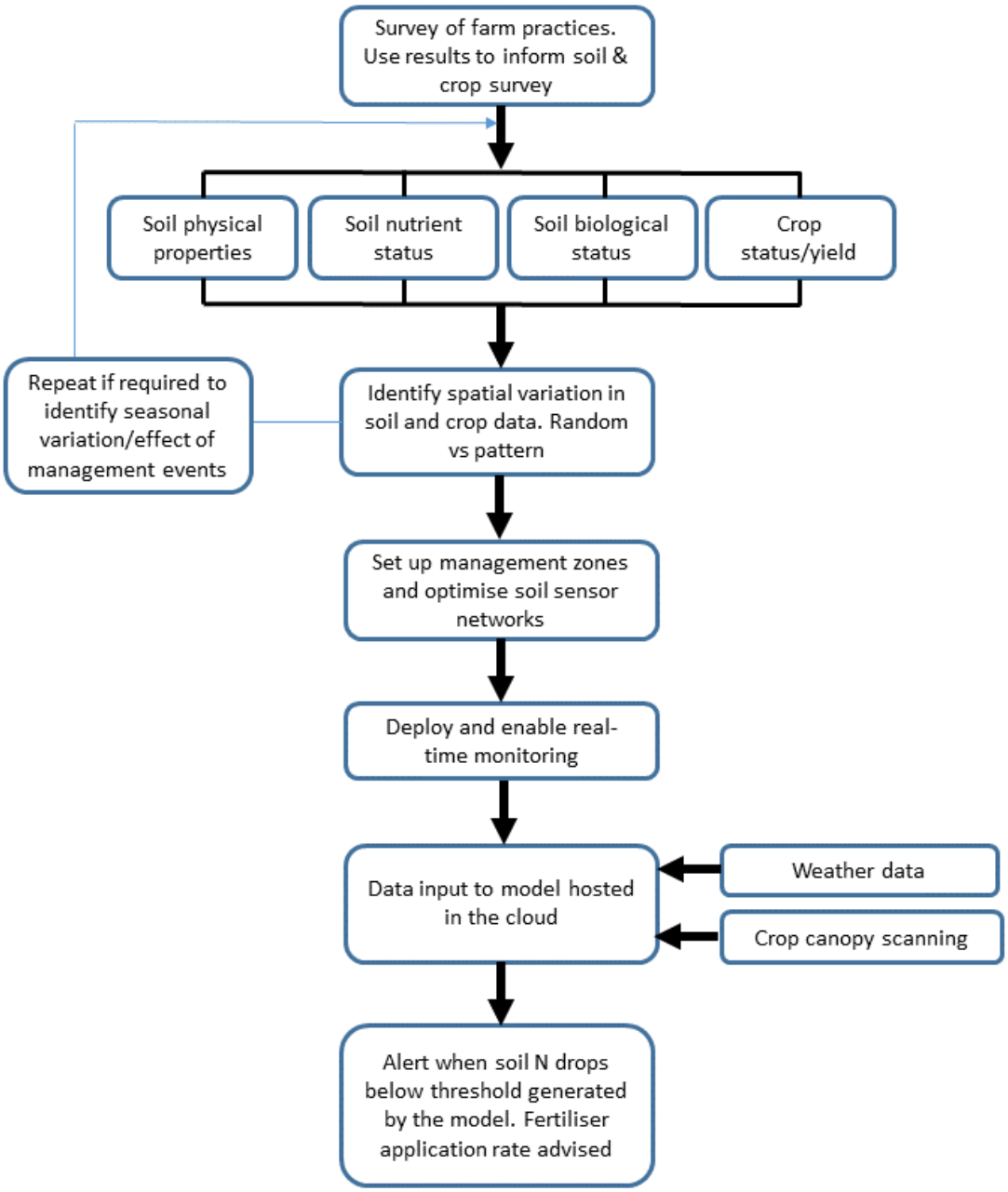

Figure 13. Flow diagram describing a new approach to N fertiliser management based on real-time data input into cloud based models.

6.6. Conclusions

This project aimed to develop in-situ and real-time methods of soil N determination to enable continuous monitoring of agricultural soil and improve understanding of soil N dynamics. The research was carried out with the ultimate aim that the techniques developed may eventually result in an improvement in the NUE of agriculture. The project has demonstrated the use of microdialysis as a novel in-situ method that better reflects the availability of N for plant uptake than conventional destructive sampling and soil extractions. Use of microdialysis to assess N dynamics in the rhizosphere also proved successful. It is likely that microdialysis will continue to offer new insights

into the functioning of soil N processes and the factors which control the availability of N for plant uptake. The project has also developed a novel NO_3^- ISE that was used successfully for the in-situ monitoring of a grassland agricultural soil. In addition, the project demonstrated, using a geo-statistical approach, how the ISEs could be deployed to optimise field-scale monitoring of soil NO_3^-. This represents a significant step forward for analytical soil science and agricultural management. However, due to the significant cost of field-scale monitoring, it is likely that use of such an approach will be limited initially to agricultural land used for growing high value arable and horticultural crops. Further work is required to test the ISEs in a wide range of soil types and environmental conditions. Considerable research is also needed to determine how data generated from in-situ sensors can be used to improve fertiliser recommendations.

7. References

Adamchuk, V. I.; Hummel, J. W.; Morgan, M. T.; Upadhyaya, S. K. 2004. On-the-go soil sensors for precision agriculture. Comput. Electron. Agric. 44: 71-91.

Adamchuk, V. I.; Lund, E. D.; Sethuramasamyraja, B.; Morgan, M. T.; Dobermann, A.; Marx, D. B. 2005. Direct measurement of soil chemical properties on-the-go using ion-selective electrodes. Comput. Electron. Agric. 48: 272-294.

Barber, S. A. 1984. Soil nutrient bioavailability. A mechanistic approach. John Wiley, New York, USA.

Beaton, A. D.; Cardwell, C. L.; Thomas, R. S.; Sieben, V. J.; Legiret, F.; Waugh, E. M.; Statham, P. J.; Mowlem, M. C.; Morgan, H. 2012. Lab-on-chip measurement of nitrate and nitrite for in situ analysis of natural waters. Environ. Sci. Technol. 46: 9548–9556.

Bogaert, N., Salomez, J., Vermoesen, A., Hofman, G., Van Cleemput, O., Van Meirvenne, M., 2000. Within-field variability of mineral nitrogen in grassland. Biol. Fertility Soils. 32: 186-193.

Cuttle, S. P.; Jarvis, S. C. 2005. Use of a systems synthesis approach to model nitrogen losses from dairy farms in south-west England. Grass Forage Sci. 60: 262-273.

Delhon, P.; Gojon, A.; Tillard, P.; Passama, L. 1996. Diurnal regulation of NO_3^- uptake in soybean plants. 4. Dependence on current photosynthesis and sugar availability to the roots. J. Exp. Bot. 47: 893-900.

Devienne-Barret, F.; Justes, E.; Machet, J. M.; Mary, B. 2000. Integrated control of nitrate uptake by crop growth rate and soil nitrate availability under field conditions. Ann. Bot-London. 86: 995-1005.

Diacono, M.; Rubino, P.; Montemurro, F. 2013. Precision nitrogen management of wheat. A review. Agron. Sustain. Dev. 33: 219-241.

Edwards, A.C.; Hooda, P.S.; Cook, Y. 2001. Determination of nitrate in water containing dissolved organic carbon by ultraviolet spectroscopy. Int. J. Environ. Anal. Chem. 80: 49–59.

Gastal, F.; Lemaire, G. 2002. N uptake and distribution in crops: an agronomical and ecophysiological perspective. J. Exp. Bot. 53: 789-799.

Giesler, R. Lundström U. S. 1993. Soil solution chemistry – The effects of bulking soil samples and spatial variation. Soil Sci. Soc. Am. J. 57: 1283–1288.

Gomez, C.; Viscarra Rossel, R. A.; McBratney, A. B. 2008. Soil organic carbon prediction by hyperspectral remote sensing and field vis-NIR spectroscopy: An Australian case study. Geoderma. 146: 403-411.

Hartz, T.K. 1994. A quick test procedure for soil nitrate-nitrogen. Commun. Soil Sci. Plant Anal. 25: 511–515.

Inselsbacher, E., 2014. Recovery of individual soil nitrogen forms after sieving and extraction. Soil Biol. Biochem. 71: 76-86.

Inselsbacher, E.; Näsholm, T. 2012. The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. New Phytol. 195: 329-334.

Inselsbacher, E.; Näsholm, T. 2012b. A novel method to measure the effect of temperature on diffusion of plant-available nitrogen in soil. Plant Soil. 354: 251-257.

Inselsbacher, E.; Ohlund, J.; Jamtgard, S.; Huss-Danell, K.; Näsholm, T. 2011. The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. Soil Biol. Biochem. 43: 1321-1332.

Ito, S.; Baba, K.; Asano, Y.; Takesako, H.; Wada, H. 1996. Development of a nitrate ion-selective electrode based on an Urushi matrix membrane and its application to the direct measurement of nitrate-nitrogen in upland soils. Talanta. 43: 1869-1881.

Jemison, J.M.; Fox, R.H. 1988. A quick-test procedure for soil and plant-tissue nitrates using test strips and a hand-held reflectometer. Commun. Soil Sci. Plant Anal.19:1569–1582.

Jones, D. L.; Owen, A. G.; Farrar, J. F. 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biol. Biochem. 34: 1893-1902.

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

Kim, H.; Sudduth, K. A.; Hummel, J. W. 2009. Soil macronutrient sensing for precision agriculture. J. Environ. Monit. 11: 1810-1824.

Kim, H-J.; Hummel, J. W.; Sudduth, K. A.; Motavalii, P. P. 2007. Simultaneous analysis of soil macronutrients using ion-selective electrodes. Soil Sci. Soc. Am. J. 71: 1867-1877.

Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. J. Plant Nutr. Soil. Sc. 165: 382-396.

Lark, R.M., 2011. Spatially nested sampling schemes for spatial variance components: Scope for their optimization. Comput. Geosci. 37: 1633-1641.

Le Goff, T.; Braven, J.; Ebdon, L.; Chilcott, N.; Scholefield, D.; Wood, J. 2002. An accurate and stable nitrate-selective electrode for the in situ determination of nitrate in agricultural drainage waters. Analyst. 127: 507-511.

Marhan, S.; Auber, J.; Poll, C. 2015. Additive effects of earthworms, nitrogen-rich litter and elevated soil temperature on N_2O emission and nitrate leaching from an arable soil. Appl. Soil Ecol. 86: 55-61.

Miller, A. J.; Zhen, R. G. 1991. Measurement of intracellular nitrate concentrations in *Chara* using nitrate-selective microelectrodes. Planta. 184: 47-52.

Miranda, K. M.; Espey, M. G.; Wink, D. A. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide-Biol. Ch. 5: 62-71.

Mosier, A.; Kroeze, C.; Nevison, C.; Oenema, O.; Seitzinger, S.; van Cleemput, O. 1998. Closing the $N_{(2)}O$ budget: nitrous oxide emissions through the agricultural nitrogen cycle - OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. Nutr. Cycl. Agroecosys. 52: 225-248.

Mulvaney, R. L. 1996. Nitrogen - Inorganic forms. Sparks, D. L., Ed. Methods of soil analysis. Part 3. Chemical methods. Soil Science Society of America. Madison, WI, USA. pp 1123-1184.

Nesbitt, K. M.; Jaquins-Gerstl, A.; Skoda, E. M.; Wipf, P.; Michael, A. C. 2013. Pharmacological mitigation of tissue damage during brain microdialysis. Anal. Chem. 85: 8173-8179.

Nunan, N.; Wu, K.; Young, I.; Crawford, J.; Ritz, K. 2002. In situ spatial patterns of soil bacterial populations, mapped at multiple scales, in an arable soil. Microb. Ecol. 44: 296-305.

Oburger, E.; Dell'mour, M.; Hann, S.; Wieshammer, G.; Puschenreiter, M.; Wenzel, W. W. 2013. Evaluation of a novel tool for sampling root exudates from soil-grown plants compared to conventional techniques. Environ. Exp. Bot. 87: 235-247.

Oyewole, O. A.; Inselsbacher, E.; Näsholm, T. 2014. Direct estimation of mass flow and diffusion of nitrogen compounds in solution and soil. New Phytol. 201: 1056-1064

Parkin, T. B. 1987. Soil microsites as a source of denitrification variability. Soil Sci. Soc. Am. J. 51: 1194-1199.

Rousk, J.; Jones, D. L. 2010. Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in H_2O and 0.5 M K_2SO_4 soil extracts. Soil Biol. Biochem. 42: 2331-2335.

Selbie, D.R., Buckthought, L.E., Shepherd, M.A., 2015. The challenge of the urine patch for managing nitrogen in grazed pasture systems. Adv. Agron. 129: 229-292.

Schmidhalter, U. 2005. Development of a quick on-farm test to determine nitrate levels in soil. J. Plant Nutr. Soil Sc.168: 432–438.

Shaw, R.; Williams, A. P.; Jones, D. L. 2014. Assessing soil N availability using microdialysis-derived, diffusive flux measurements. Soil Sci. Soc. Am. J. 78: 1797-1803.

Stoyan, H., De-Polli, H., Böhm, S., Robertson, G.P., Paul, E.A., 2000. Spatial heterogeneity of soil respiration and related properties at the plant scale. Plant Soil. 222: 203-214.

Sutton, M. A.; Oenema, O.; Erisman, J. W.; Leip, A.; van Grinsven, H.; Winiwarter, W. 2011. Too much of a good thing. Nature 472: 159-161.

Sylvester-Bradley, R.; Kindred, D., R.; Blake, J.; Dyer, C., J.; Sinclair, A., H. 2008. Optimising fertiliser nitrogen for modern wheat and barley crops. Project Report no. 438. HGCA. London, UK.

Sylvester-Bradley, R.; Lord, E.; Sparkes, D. L.; Scott, R. K.; Wiltshire, J.; Orson, J. 1999. An analysis of the potential of precision farming in Northern Europe. Soil Use Manage. 15: 1-8.

van Alphen, B. 2002. A case study on precision nitrogen management in Dutch arable farming. Nut. Cycl. Agroecosys. 62: 151-161.

Vitousek, P. M.; Aber, J. D.; Howarth, R. W.; Likens, G. E.; Matson, P. A.; Schindler, D. W.; Schlesinger, W. H.; Tilman, D. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecol. Appl. 7: 737-750.

Webster, R., Lark, R.M., 2013. Field sampling for environmental science and management. Routledge, Abingdon, UK.

Zhang, M.; Fan, C. H.; Li, Q. L; Li, B.; Zhu, Y. Y.; Xiong, Z. Q. 2015. A 2-yr field assessment of the effects of chemical and biological nitrification inhibitors on nitrous oxide emissions and nitrogen use efficiency in an intensively managed vegetable cropping system. Ag. Eco. Env. 201: 43-50.