Development and Application of the Diffusive Gradients in Thin Films Technique for the Measurement of Nitrate in Soils

Development and application of the DGT technique for the measurement of nitrate in soils

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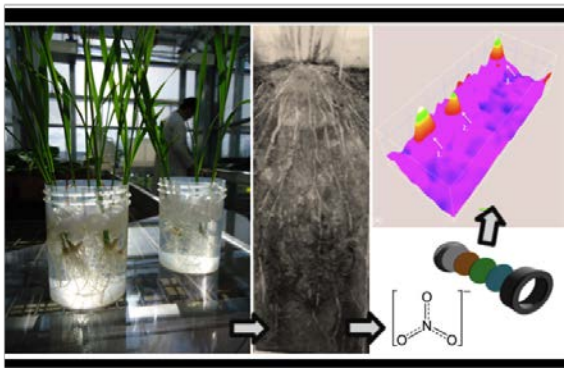

Graphic Abstract

ABSTRACT

Nitrate (NO_3-N), the main plant/microbial nitrogen source, has a fast turnover in soil driven by species transformation (nitrification/denitrification) and phyto/microbiota-assimilation. The technique of diffusive gradients in thin films (DGT) is capable of a robust, low disturbance measurement of NO_3-N, but it has not been implemented due to the absence of a binding layer suitable for deployment in soils. In this study a new, styrene divinylbenzene based absorbent with amine functional groups (SIR-100-HP) was cast into an agarose gel support. The NO_3-N ion selectivity of the SIR-100-HP/agarose binding layer was retained in the presence of high multivalent ion concentrations and was used successfully to acquire *in situ* NO_3-N measurements in bulk soil. The kinetics of binding and the maximum binding capacity were determined. The total capacity of the DGT containing the SIR-100-HP/agarose binding phase was 667 μg NO_3-N. The performance of DGT was not affected by varying pH (3-8) and ionic strength (0-0.018 mol L^{-1}), while anion competition effects at concentrations reflecting those in common agricultural soils were found to be negligible. Complete elution (100% efficiency) of NO_3-N from the binding phase was achieved using a solution of 5% NaCl. This technique was validated in three contrasting soils. C_{DGT} measurements were in excellent agreement with porewater NO_3-N values. Two-dimensional NO_3-N mapping of a profile of flooded rice paddy soil demonstrated the potential of this novel methodology for improved characterisation of *in situ* N speciation for further understanding of bioavailability and biogeochemical processes of NO_3-N in soils.

INTRODUCTION

Nitrogen (N), an essential mineral nutrient, is present in aerobic soil in several forms, with the oxyanion NO_3-N being the primary species and hence the main available N source for plant/microbial growth. The NO_3-N demand by crops is greater than the natural supply from

most soils[1,2]. Typically plant tissue concentrations are >1000 times higher than that of their corresponding soil porewaters, therefore the diffusive supply of NO_3-N to the roots is a major limiting factor for production yields[1-3]. In order to sustain and enhance crop production, NO_3-N fertilizers have been widely and heavily applied to agricultural soils, but N-use efficiency remains low and over application of N fertilizers is common; an issue both of environmental and economic concern[4-8].

Fertiliser applications to agricultural soils are largely determined by the measurements of the exchangeable NO_3-N pool. Salt extractions such as 2M KCl are the worldwide standard, but although of low cost, for many soils they provide a poor prediction of plant-N uptake[9]. Common difficulties associated with these assays include: i) a non-selectivity for NO_3-N, with the extraction also targeting all inorganic-N species. Therefore changes in speciation during sample collection, transfer, storage and analysis need to be considered. ii) a failing to encompass the rapid turnover of NO_3-N in soil; a dynamic process governed by many factors including microbial/plant/redox mediated species change, biotic uptake and abiotic immobilisation[10]. Changes in the NO_3-N pool with time are not accounted for by individual soil extraction measurements because only a single temporal time point is assessed. Furthermore, collection and processing of the soils invariably disturb the dynamics of the system and hence introduce additional measurement discrepancies. Transporting the collected field soils quickly to the laboratory is another consideration and challenge.

Quantitative determination of NO_3-N concentrations can be achieved by other methods such as ion-selective electrodes (ISE), but even without considering the detector drift, problems with self-calibrations, a lack of sensitivity and selectivity (especially in complex soil matrixes), the development of organic films and biofouling on the sensors, the high set-up

and running costs preclude these methods as a widespread soil screening tool. Further, the efficacy of ISE's in agricultural field soils has yet to be fully validated[11].

In situ passive sampling techniques that collect analytes in a low disturbance/quantitatively well-defined manner such as DGT (Diffusive Gradients in Thin-films) can potentially overcome many of the limitations of both the salt extraction and ISE methods. The DGT measurement is an effective proxy/surrogate for plant uptake because it can successfully mimic the diffusive supply processes near root surfaces, while integrating a wider range of key soil properties that impact on release/adsorption than other single measurement approaches[12,13]. Further, devices can be deployed cost effectively in sufficient numbers to obtain good spatial coverage, while critically in the case of NO_3-N assessment, providing a time integrated measurement[14]. *In situ* deployment provides a more realistic measure of the soil NO_3-N pool as any disturbance to the system during sampling is minimised.

A NO_3-N selective DGT would greatly simplify the process of N measurement. The preconcentration of NO_3-N by the DGT binding gel not only improves the sensitivity of the method, reduces measurement bias but also protects against N-speciation changes associated with sample collection, transfer and storage. Very recently, a NO_3-N DGT method based on a Purolite A520E anion exchange resin was developed for freshwaters[15]. However to date, there has been no validation of the method in soils, where the technique has perhaps it's most merit and yet is most challenged by interferences caused by competing ions. In the present study a new amine functionalised, styrene divinylbenzene, strong base anion exchange resin candidate was investigated for the development of a novel SIR-100-HP/agarose DGT and its suitability for NO_3-N measurements in soils.

MATERIALS AND METHODS

Apparatus and chemicals. The anion exchange resin (SIR-100-HP) was purchased from Resintech™ (West Berlin, New Jersey, USA) and milled to a particle size of 200 mesh (Globe Mill, Retch, Germany). Agarose powder was bought from Fisher Scientific (UK). The other reagents were of analytical-reagent grade and were purchased from Sigma (USA). High-purity demineralized water (18.2 ΩM cm^{-1}) provided by a Milli-Q (MQ) Plus filter apparatus (Millipore, USA) was used in this experiment.

Preparation of DGT. The presented method of preparing agarose gel is a modification of the procedure described by Docekalova & Divis[16] and Menegario *et al.*[17]. A diffusive gel containing 1.5% (m/v) agarose was prepared by dissolving the agarose in MQ water. The mixture was placed in an oven at 100ºC for 2 hr and gently stirred until all the agarose was dissolved. The hot gel solution was mixed well and immediately cast between two preheated glass plates (100ºC) separated by a 0.8 mm plastic spacer. The gel-mould was left to cool down to room temperature, and dismantled. Discs with a diameter of 2.5 cm were cut from the cooled gel and stored in MQ water.

A binding gel was prepared following a similar procedure, 4 g of resin (SIR-100-HP), drained of excess water, was transferred to a beaker with 11 ml of 1.5% hot agarose solution. The solution with the resin was mixed vigorously to make sure the resin was fully dispersed. Then the solution was cast into the glass plate mould with 0.5 mm spacers and cooled at room temperature as described above for diffusive gels.

NO_3-N determination. NO_3-N was measured colorimetrically by a spectrophotometer (Thermo Scientific) based on the Griess Reagent method according to Thabano *et al.*[18]. NO_3-N was reduced to NO_2-N using copper coated cadmium granules. This experimental step operated at >95% efficiency. The NO_2-N produced reacts with sulfanilamide solution, then the resulting solution is coupled with N-(1-Naphtyl)-ethylemediamine dihydrochloride to form a coloured azo dye.

Kinetics and the Elution Efficiency of the Binding Gel. A binding layer (resin + gel support) must immobilise the target analyte effectively and efficiently in order to satisfy the requirements of the DGT principle. Mass balance experiments were conducted to investigate the kinetic properties of the binding gels for NO_3-N over a time range spanning 1 min. to 24 hr. In each treatment, performed in triplicate, a gel disc was immersed in a 10 ml solution of 50 mg L^{-1} NO_3-N. The solutions were gently shaken continuously (SSL1 orbital, Stuart, at 50 rpm) with sub-samples collected before disc immersion and after retrieval.

NO_3-N must be quantifiably and consistently eluted from the binding layer to enable analytical measurement. Mineral acids are the most common DGT eluents, but are not suitable for a NO_3-N specific DGT because many of the methods for determining NO_3-N are pH sensitive. NaCl is an effective ion exchange resin eluent[19,20] of neutral pH and widely available at low cost. To optimise the elution of NO_3-N from the gels, discs were immersed in 10 ml of 10 mg L^{-1} NO_3-N for 20 hr, and then eluted for 4, 8, 20 hr in 5 ml of either 1%, 2%, 3% or 5% (m/v) NaCl.

Calculating DGT labile NO_3-N. The DGT solute concentration/activity (see equation 1) can be simply derived from 5 parameters.

$$C_{DGT} = M \times \Delta g / (D \times A \times t) \quad \textbf{(eq. 1)}$$

The diffusive layer thickness (Δg) and sampling window area (A) are specific to the geometry of the DGT device, but consistent amongst samplers.

Diffusion coefficient (D). As no published diffusion coefficient for NO_3-N in agarose gel was available this had to be experimentally determined. This was achieved using a previously described diffusion cell[21], which consisted of two compartments (A and B) joined by a 1.5 cm diameter circular connecting window. A 0.8 mm thick diffusive gel was placed across the window and the sections secured with clips. Compartment A was filled with 50 ml of 200 mg L^{-1} $NaNO_3$ solution and compartment B was filled with MQ water. The solution in each compartment/section was well stirred during the experiments. Subsamples (1 ml) were collected every 10 min. over a time series, ranging from 30 to 120 min.. The diffusion coefficient was calculated from the slope of the linear plot of the mass of $NaNO_3$ in compartment B versus time.

Time (*t*). The NO_3-N binding layer must be functional within a DGT device over suitable time frames that match possible changes in N concentrations within the environment (i.e. diurnal cycles). To test the time dependence of the NO_3-N DGT, the devices (n = 21) were exposed to a stirred solution containing 10 mg L^{-1} NO_3-N over a time series of 4 to 48 hr. At each sampling time point, three DGT units were collected for analysis.

Mass (*M*). Binding layers have a finite capacity, which governs the DGT performance. Determining this upper threshold is critical for successful measurements. DGT's (n = 21)

were deployed in a series of solutions with different NO_3-N concentrations, spanning a concentration range from 0 to 240 mg L^{-1} for 4 hr. Each concentration treatment was performed in triplicate.

Characteristics of DGT performance in solutions.

Standard piston-type DGT holders with a 2 cm diameter exposure window (DGT Research Ltd.) were used for the DGT devices. A 0.5 mm thick binding gel was placed on the bottom of the holder, which was covered in order by a 0.8 mm thick diffusion gel and a 0.13 mm thick cellulose filter membrane (Whatman, 0.45 µm pore size). Unless otherwise stated all the tests were carried out in a deployment tank containing 2.8 L of 50 mg L^{-1} NO_3-N solution and eluted in 5% NaCl (m/v) for 8 hrs.

DGT Detection Limits, were calculated as three times the standard deviation of the DGT blanks (gels used in the devices were derived from the same gel batches used for the DGT experiments) (n = 12). Blank analyses were assessed as follows: DGT devices (three replicates per experiment) were assembled and placed in a deployment tank with 2.8 L MQ water for 4 hr.

Effect of pH and Ionic Strength. In order to investigate the effect of pH on DGT responses, DGT assemblies were immersed in $NaNO_3$ solutions prepared to cover a pH range from 3.1 to 8.1. The pH of the solutions was adjusted using dilute 1% H_2SO_4 (v/v) or 2 mol L^{-1} NaOH. . To test DGT performance at low ionic strengths, DGT assemblies were exposed to $NaNO_3$ solution with appropriate additions of Na_2SO_4 to give an ionic strength range of 0 to 0.018 mol L^{-1}. For both deployment campaigns (*pH and ionic strength*) and at each treatment, three SIR-100-HP/agarose DGT devices were deployed in two separate time series

experiments (4 and 24 hr), one following the standard DGT testing procedure and the other for being consistent with the soil deployment time of 24 hr.

Competition. The effect of potential competitive anions in solutions at concentrations that reflect the porewater of most common soils was studied[22]. DGT devices (in triplicate) were deployed separately for both 4 and 24 hr in various well stirred solutions containing both 50 mmol L^{-1} $NaNO_3$ and: (a) no other amendment/control, (b) Cl^- [250 mg L^{-1}], (c) NO_2-N [1 mg L^{-1}], (d) $CaCO_3$ [13 mg L^{-1}], (e) HCO_3^- [50 mg L^{-1}], (f) HPO_4^{2-} [10 mg L^{-1}].

Characteristics of DGT performance in soils.

Three contrasting agricultural soils (0-20cm depth), two from the UK and one from South China were tested. The two soils from the UK have been characterized previously[23,24], the first being a humic rendzina with a clay loam texture (*Rendzina*) and the second a brown sandy loam (*Brown sand*). The final sample was a clay/silt paddy soil (*Paddy soil*). Prior to DGT deployment the soils were air-dried and then passed through a 2 mm sieve to ensure homogeneous soil samples for comparison of porewater and DGT-measured concentrations.

For DGT deployment, 80 g of each air-dried soil sample was brought to 60% maximum water holding capacity (MWHC) and incubated for 2 days. The moisture content was then raised to 80% MWHC for 24 hr[13]. DGT devices (n=3) were placed on the soil paste and twisted gently a few times to ensure complete contact between the filter membrane of the device and the soil. They were deployed for 20 hr at 16.5°C.

On retrieval, DGT devices were jet-washed with MQ water to remove soil particles and then disassembled. The binding gels were removed from the DGT device and immersed in 10 mL of 5% NaCl (m/v) for at least 8 hr prior to analysis. After completing the DGT deployments, soil solution was collected by centrifuging the soil at 5000 g for 15 min. [23]. The supernatants were filtered through a 13 mm diameter, 0.45 μm, polysulfone filter. Total concentrations of NO_3-N in DGT elutes and in soil pore water were determined by using the method stated previously.

Two dimensional mapping of NO_3-N distribution in paddy soil

In this experiment perspex rhizotrons with removable front plates[25,26] (inner dimensions. HxWxD: 40x20x3cm) were filled with dry, sieved (< 2 mm) paddy soil, which was set in layers to achieve an even soil structure. Soils were carefully re-wetted with a water spray until saturation. A nuclepore membrane (0.2 μm pore size, thickness ~ 10 μm) then overlaid the soil, and was secured to the outer walls of the rhizotron with water-proof tape. A rice seedling was transplanted into the rhizotron and the whole system was transferred into a water tank in the greenhouse for three months. The spatial heterogeneity of NO_3-N distribution in soil would have been developed with time due to biogeochemical processes in both bulk soil and in the rhizosphere. At grain maturation, a 12.5 x 6.5 cm NO_3-N DGT was deployed within the root zone, attached to the inner-side of the detachable front plate of the rhizotron with waterproof tape. Ingress of oxygen into the anaerobic soils was minimal during deployment as this operation was performed in aquarium water that had previously been deoxygenated with nitrogen.

After deployment, the NO_3-N DGT was rinsed with MQ water, cut into 5x5 mm squares and transferred into 1.5 ml micro-centrifuge tubes. Gel pieces were eluted in 1 ml 5% NaCl and

shaken for 8 hr at room temperature. The samples were then centrifuged at 5000g for 5 min. and the recovered solutions transferred to 15 ml tubes and diluted 6 times. NO_3-N concentrations were analyzed by flow injection analysis (Manufacturer: Lachat Inc., USA).

RESULTS AND DISCUSSION

Kinetic performance of the SIP-100-HP binding gel

Figure 1a demonstrates the adsorption of NO_3-N by the resin gel with time. The initial steepness of the uptake curve (0-10 min.) demonstrates that binding is sufficiently rapid to ensure the NO_3-N concentration at the resin surface is effectively zero. The resin was able to scavenge all the NO_3-N from the solution, accumulating more than 50% of the total mass within 30 min. of immersion. The maximum amount of N accumulated by a DGT device in a 60 sec. deployment time can be calculated for a solution of 50 mg L^{-1}; a typical solution concentration employed in performance testing[27]. The result shows that the amount theoretically taken-up by DGT (1.2×10^{-3} mg) is less than the amount of N taken up by the resin gel in the kinetic experiment for the same 60 sec. time period (Figure 1a and b). The binding rate is therefore more than sufficient to satisfy the DGT demand.

Elution efficiency

In addition to having both a high and consistent elution efficiency[28], the eluting solution for the DGT binding phase elutes needs to be safe to work with, cost effective, and not interfere with the analytical measurement. A series of experiments demonstrate NaCl to be an excellent candidate elute for a SIP-100-HP/agarose binding layer, fulfilling all the above criteria. Table 1. shows the effect of NaCl concentration (1-5% m/v) and extraction time (4-20 hr) on the elution efficiency. In summary, the recovery of NO_3-N increased both with increasing eluent concentration and/or elution time, with complete desorption of the SIP-100-

HP bound NO_3-N. In a compromise between elute concentration and extraction time, the optimal method finally adopted, for all the further experiments, was a 5% NaCl (m/v) solution with gel being eluted for 8 hr.

DGT blanks and detection limits

The regression line from the calibration data for the NO_3-N measurements were used directly to calculate the limit of detection (C_{LOD}) and quantification (C_{LOQ}), according to eq. (2) and (3) respectively. Where S_i was calculated from the standard deviation of the y/x intercept and b the slope.

$$C_{LOD} = 3\ S_i\ /\ b \quad \textbf{(eq. 2)}$$

$$C_{LOQ} = 10\ S_i\ /\ b \quad \textbf{(eq. 3)}$$

The C_{LOD} and C_{LOQ} for NO_3-N were 0.07 μg L^{-1} and 0.2 μg L^{-1}, respectively. For each batch of binding layers synthesised and used in the DGTs，a blank measurement was made to monitor the possible contamination during experiment process. As DGT is an accumulation technique, the method detection limit (MDL) varies with deployment time and solution concentration. For this study, binding gel blanks were collected and the method detection limit (MDL) of the DGT technique was calculated as three times the standard deviation of the blank value. The average MDLs achieved in the laboratory experiments for a DGT deployment of 4 hr and 1 day were 3.9 μg L^{-1}, 0.7 μg L^{-1}, respectively. The method precision for DGT's deployed in solutions (n = 6) of $NaNO_3$ for 4 hr were <3%.

Diffusion Coefficient in the gel

The masses of NO_3-N that diffused from compartment A of the diffusion cell to compartment B, through the agarose gel with time were positively correlated (regression coefficient, 0.98) (Figure 2a),. Diffusion coefficients, D, for NO_3-N were calculated using eq. 4[29]. For a temperature of 24ºC, D equalled 11.1×10^{-6} cm^2 s^{-1}. Where s is the slope of the regression line derived from mass *vs*. time, and C is the NO_3-N concentration in compartment A (source compartment). The diffusion coefficients for the temperature series 1 to 35ºC are provided in Table S1 (Supporting Information).

$$D=s\Delta g/(CA) \quad \textbf{(eq. 4)}$$

These diffusion coefficients were used for all calculations in this study. To date, there is still no relevant data available to compare with this value. However, it is 22% lower compared to the diffusion coefficient, 14.2×10^{-6} cm^2 s^{-1} (24ºC) published for NO_3-N in polyacrylamide diffusive gel[15] calculated from the mass accumulation in DGT devices in time series experiments.

Capacity of DGT

There is a potential for the binding gel to become saturated because of the high accumulation of analytes when the measurement is performed over the long-term (weeks or months) or in environmental matrixes with high analyte concentrations. The DGT capacity may thus be a limiting factor for such applications. A linear and theoretically predictable response was obtained with deployment solution concentrations up to 120 mg L^{-1} NO_3-N for 4 hr (Figure 2b). In these linear regions, the measurements were close to the theoretical line calculated

from the known solution concentrations. The linear response between accumulated mass by DGT and the increasing solution concentrations showed that the capacity of a single DGT device is 667 mg NO_3-N, thereby validating the quantitative use of DGT below mass loading of 377 mg cm^{-2}. This is comparable to the capacity of the only other NO_3-N DGT.

Effect of deployment time

According to DGT theory, the mass of analyte accumulated by DGT devices should increase linearly with time, providing the capacity of the adsorbent has not been exceeded and the uptake kinetics are rapid enough to ensure the concentration of analyte at the interface between the binding gel and diffusive gel is zero. In this study, the mass of NO_3-N loaded onto the binding gel increased linearly in a deployment solution of 50 mg L^{-1} NO_3-N, with time within 36 hr and fitted the theoretical line calculated from the known solution concentrations (Figure 1b). However, the accumulated mass deviated below the theoretical line at 48 hr (Figure 1b) as the accumulated amount was close to the capacity limit.

Effect of pH

The effect of pH on the DGT performance is demonstrated by the ratio of DGT measured NO_3-N to actual concentration in solution (Table 2). The ratio of NO_3-N varied between a minimum of 0.9 and a maximum of 1.1 when the pH changed from 3.1 to 8.1, which is within acceptable performance parameters for DGT measurements and in agreement with other commonly used resin layers[31, 32]. Rarely is the pH of a soil outside this range so pH limitations are not a significant consideration with this new method.

Effect of ionic strength

In soil solution/porewaters, ionic strength and composition can vary greatly. To evaluate the

effect of ionic strength on operational performance, the DGT assemblies were exposed to test solutions containing 10 mg L^{-1} NO_3-N over a range of ionic strengths (0-36 mmol L^{-1}). Table 2 shows the ratio between the DGT measured NO_3-N against the NO_3-N concentration in solution. At ionic strengths of 0.3, 3, 9 and 18 mmol L^{-1}, DGT measurements agreed well with the actual concentrations in the deployment solutions (Table 2). Whereas a lower DGT measurement was found at ionic strengths of 36 mmol L^{-1}， as the ratio of C_{DGT} to C_{soln} decreased to 0.78 ± 0.02 . To place this in perspective, it is commonplace in many soil studies to use 5 mmol L^{-1} Ca^{2+} solutions for equilibration with soil to mimic typical soil pore water ionic strength and composition[33].

Competition Effects

To test the potential competition effects from the major anions in soil solution and how they impact on NO_3-N DGT performance, different exposure scenarios designed to reflect environmentally relevant conditions were trialled. The results (Table 2) showed that the impact on DGT performance from potentially competing anions is likely to be negligible in typical soil porewaters. ANOVA analysis, revealed no statistically significant difference ($p > 0.05$) between the concentrations measured in the control and the other anion treatments. Furthermore, all the C_{DGT}/C_{soln} ratios were generally in the range 0.90 to 1.10, which is considered acceptable for DGT measurements (Table 2). However, some elements in natural media vary extensively. Concentrations of Cl^- in some extreme case, such as alkaline saline soils, can for example exceed 500 mg L^{-1} [34]. When the NO_3-N DGT was trialled in deployment solutions of 500 mg L^{-1}, binding efficiency was slightly impaired causing the C_{DGT}/C_{soln} to decline from 0.99±0.02 (at 250 mg NO_3^- L^{-1}; 4 hr deployment) to 0.90±0.03. When HPO_4^{2-} exceed 25 mg L^{-1}, which is comparably infrequent [3], the ratio of C_{DGT}/C_{soln} fell to 0.71 (4 hr deployment, Table 2). These results suggest that there is competition between

389 NO_3-N and other ions in solution for binding sites on the binding resin under extreme
390 conditions.
391
392 **Application in soils**
393 DGT devices were deployed in three types of soils for 20 hr. The concentrations of NO_3-N in
394 DGT elution and soil solution were determined. DGT measured concentrations, $C_{DGT,}$ were
395 calculated from the mass of NO_3-N accumulated using equation 1. The C_{DGT} measurements
396 were in good agreement (<10% error) with those for C_{soln} (Table 2), indicating NO_3-N supply
397 is well buffered. Nitrate transfer from porewater to the SIR-100-HP/agarose DGT devices,
398 follows the same principals that govern all DGT measurements. However, interpretation of
399 the change in solute concentration in the porewaters as NO_3-N is continuously removed by
400 the DGT samplers, differs slightly from that of other elements/species. This is because
401 desorption of NO_3^-N from binding sites on the soil-solid phase is less important in re-
402 establishment of equilibrium, than is typical for other moieties. Nitrate is not readily retained
403 by soil particles, unless they possess a positive charge (e.g. low pH soils)[22]. Therefore, the
404 NO_3-N reserve/store available for exchange, in the majority of unfertilised soils is relatively
405 small, deriving primarily from amino acids bound to soil particle surfaces[35]. Mineralisation,
406 the microbial conversion of organic-N to mineral-N, therefore contributes more to the solute
407 supply flux, and hence controls the buffering characteristics of the soil. Mineralization rates
408 are highest in warm, moist, organic soils[22]. The next method development step, would be the
409 validation of SIR-100-HP/agarose DGT either *in-situ* or on moist, field soils cored and
410 preserved for analysis in the laboratory, as robust predictors of plant uptake/tissue content; as
411 is the case for using DGT to predict bioavailable P[36] and trace metals in plants[13].
412
413

Mapping of the distribution of DGT labile NO_3-N in a paddy soil

To extend the application of DGT in soils, a two-dimensional distribution of labile NO_3-N in a paddy soil was obtained. Clear spatial heterogeneity in DGT measured NO_3-N are illustrated (Figure 3). In the bulk of the soil sampled, NO_3-N fluxes were low at 20 pg cm^{-2} s^{-1},but the 3 microniche zones with maxima ca. 4-fold higher, were also observed. It is inconclusive, whether these geochemical features arose due to plant root influence, microbial activity or abiotic factors. With nitrogen remaining the most important limiting nutrient for plant growth[7] there is great potential in applying the 2D DGT chemical imaging methods to characterise NO_3-N availability simultaneously with other elements (nutrients/toxins) for further understanding of uptake efficiency, which would assist plant breeding programmes and improve the selection of cultivar's with optimised ionomes[25, 26]. Combing the presented NO_3-N DGT with new developments in DGT for NH_4-N affords the opportunity to further develop *in situ* nitrogen speciation measurement/mapping[27,38]..

We have demonstrated that NO_3-N in soil porewaters can be measured in a quantative manner using standard DGT devices fitted with a SIP-100-HP/agarose binding layer. One-hundred percent elution efficiencies can be obtained without the need for hazardous mineral acids in a simple procedure using only NaCl solution, while the analysis can be completed easily with a standard spectrophotometer. The cost effectiveness of the technique allows deployment in sufficient numbers to obtain a good spatial coverage, while simultaneously providing a time-integrated measurement. The adsorption kinetics, selectivity and capacity of the SIP-100-HP binding layer met the prequisites for use in DGT and have been shown to be sufficient for deployment in normal soil conditions, and validated in three different soils (humic rendzina, brown sand, and rice paddy soil). Preliminary results for 2D measurements of NO_3-N in soil clearly show the potential for the DGT technique to be used in chemical imaging applications

for further understanding of bioavailability and biogeochemical processes of NO_3-N in soils[26]

Acknowledgements

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Supporting Information

Additional information as noted in the text. This material is available free of charge via the internet at http://pubs.acs.org

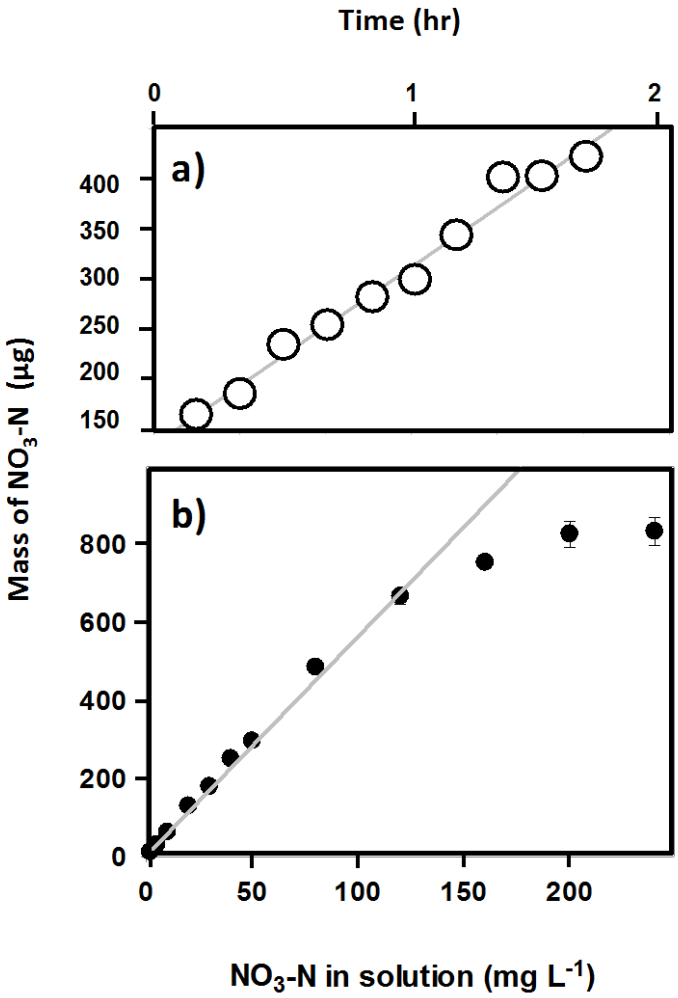

Figure 1. a) Kinetics of NO_3-N adsorption by SIP-100-HP binding gel. Error bars are calculated from the standard deviation of replicates (n=3). b) Mass of NO_3-N accumulated by DGT devices placed in solutions containing 50 mg L^{-1} NO_3-N. Error bars are calculated from the standard deviation of triplicates.

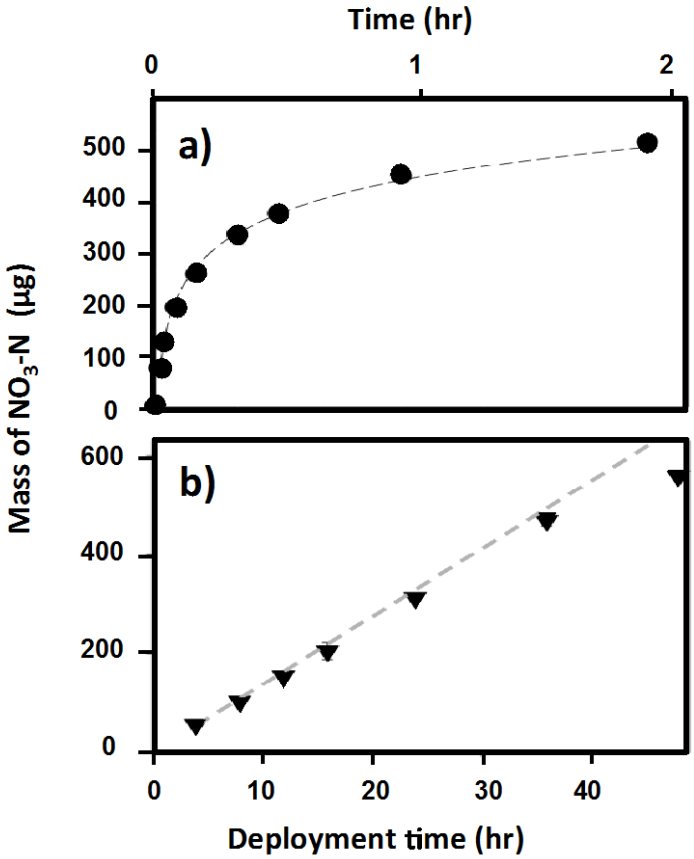

Figure 2. a) Plot of mass of NO_3-N sampled from compartment B vs time in the diffusion cell experiment at 25°C. The correlation coefficient between mass and time was 0.98. b) Dependence of mass of nitrate accumulated by DGT for the binding gel on solution concentration. The line is the theoretical slope calculated from known concentrations in solution. The grey solid line was calculated from independently measured solution concentrations according to the equation $C = Mg/DAt$.

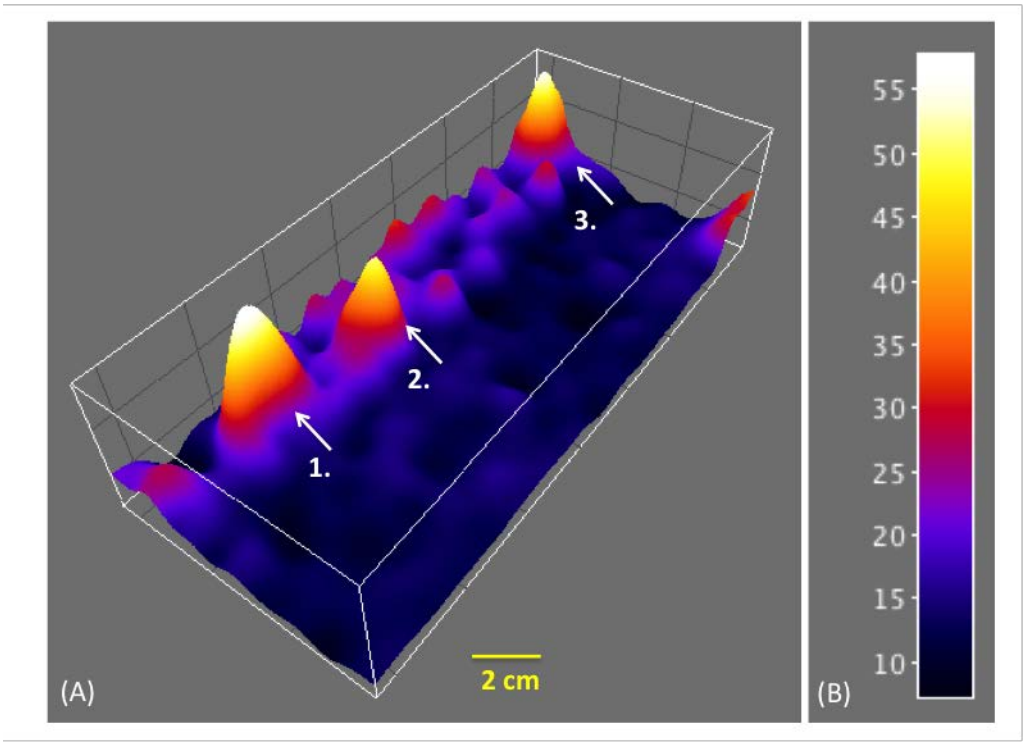

Figure 3. 2D mapping of NO_3-N fluxes in a paddy soil. (A) Profile view of flux measurements, points 1-3 denote microniches/hot spots of enhanced NO_3-N mobilisation. (B) The dark-blue to white colour scale represents a sequential increase in NO_3-N fluxes (pg cm^{-2} s^{-1}).

Table 1. **Optimisation of nitrate elution from SIR-100-HP DGT**. Two-factor factorial design, NaCl solution concentrations (% m/v) with different elution times (hr).

Elution Time	1% NaCl	2% NaCl	3% NaCl	5% NaCl
4 hr	0.78 ±0.009	0.86 ±0.007	0.91 ±0.007	0.93 ±0.005
8 hr	0.87 ±0.014	0.96 ±0.018	0.97 ±0.013	1.02 ±0.019
20 hr	0.87 ±0.006	0.96 ±0.009	1.01 ±0.006	1.04 ±0.002

Table 2. **Competition Effects and Applications in Soil**. *A)* Effect of pH on the ratio of concentrations of NO_3-N measured by DGT, C_{DGT}, to deployment solution concentrations, C_{soln}. *B*) Effect of concentration of supporting electrolyte, Na_2SO_4, on the ratio of C_{DGT} / C_{soln}. *C)* different anions; Cl^-, NO_2-N, $CaCO_3$, HPO_4^{2-}, D) C_{DGT}, to soil solution concentrations.

		4 hr Deployment **CDGT / C Soln**		24 hr Deployment **CDGT / C Soln**	
		Average	*Stdev.*	*Average*	*Stdev.*
A)		***pH***			
	3.1	1.06	± 0.02	1.01	± 0.06
	4.0	1.09	± 0.02	1.03	± 0.04
	5.0	1.09	± 0.03	0.98	± 0.04
	6.2	1.07	± 0.03	1.05	± 0.01
	7.1	1.10	± 0.03	0.98	± 0.06
	8.1	1.09	± 0.02	1.03	± 0.07
B)		***I ONIC STRENGTH (mM)***			
	0	1.01	± 0.03	0.93	± 0.03
	0.3	1.05	± 0.01	0.91	± 0.05
	3	1.06	± 0.01	0.97	± 0.02
	9	1.02	± 0.03	0.95	± 0.08
	18	0.92	± 0.02	0.96	± 0.02
	36	0.78	± 0.02	~	± ~
C)		***ANION COMPETITION***			
	Control	1.01	± 0.04	0.89	± 0.04
	Cl^- (250 mg L^{-1})	0.99	± 0.02	0.86	± 0.02
	NO_2-N (1 mg L^{-1})	1.01	± 0.02	0.95	± 0.09
	$CaCO_3$ (13 mg L^{-1})	1.04	± 0.02	0.88	± 0.03
	HCO_3^- (50 mg L^{-1})	1.04	± 0.07	0.90	± 0.02
	HPO_4^{2-} (10 mg L^{-1})	0.95	± 0.03	0.86	± 0.04
D)		***S OIL DEPLOYMENT***			
		Brown Sand		0.92	± 0.09
		Rendzina		0.95	± 0.08
		Paddy soil		0.93	± 0.05

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