Office Use Only

Project Code	
Project Type	

FINAL REPORT 2021

Applicants must read the *SAGIT Project Funding Guidelines 2021* prior to completing this form. These guidelines can be downloaded from www.sagit.com.au

Final reports must be emailed to admin@sagit.com.au as a Microsoft Word document in the format shown ***within 2 months*** after the completion of the Project Term.

PROJECT CODE :	**UA119**

PROJECT TITLE (10 words maximum)
Development of a probe for continuous measurement of soil nitrate

PROJECT DURATION

*These dates **must** be the same as those stated in the Funding Agreement*

Project Start date	1 July 2019
Project End date	20 June 2020

PROJECT SUPERVISOR CONTACT DETAILS

The project supervisor is the person responsible for the overall project

Title:	**First Name:**	**Surname:**
Dr	Glenn	McDonald
Organisation:		
School of Agriculture Food and Wine, University of Adelaide		

ADMINISTRATION CONTACT DETAILS

The Administration Contact is the person responsible for all administrative matters relating to the project

Title:	**First Name:**	**Surname:**
Ms	Anne	Wrzeszczynski
Organisation:		
Research Services, The University of Adelaide		

PROJECT REPORT

Provide clear description of the following:

Executive Summary (200 words maximum)

A few paragraphs covering what was discovered, written in a manner that is easily understood and relevant to SA growers. A number of key dot points should be included which can be used in SAGIT communication programs

The ability to continuously monitor the concentration of soil mineral N would be a valuable tool to help growers make informed decisions on N management. The purpose of this project was to provide funding for one year to investigate and provide proof of concept of photonic sensor technology to measure soil nitrate N continuously.

The project funded the development of a number of experimental probes. Each probe consisted of an optical fibre to which a molecular probe was embedded at one end. The fluorescence characteristics of the probe is sensitive to the concentration of specific chemicals in solution. Three types of molecular sensors were built and tested to examine their ability to respond to different concentrations of nitrate-N and ammonium-N.

The work in the project produced a portable demonstrator unit for the detection of N in solution. The sensors were able to detect changes in the presence of N in solution over relatively short times. However, the stability of the signal was variable and there was interference of unknown origin that affected the signals. The project demonstrated, that in principle it is possible to detect changes in N in solution using this technology but further work is required to understand the reasons for the differences in signal stability and causes of interference.

Project Objectives

A concise statement of the aims of the project in outcome terms should be provided.

Aim

To develop a probe for the continuous measurement of soil mineral nitrogen

Objectives:

Extend current research in nanoscale photonic probes to develop a method to measure soil mineral N in real time.

Overall Performance

A concise statement indicating the extent to which the Project objectives were achieved, a list of personnel who participated in the Research Project including co-operators, and any difficulties encountered and the reasons for these difficulties.

There was a delay of about 2 months in the commencement of the project because of negotiations relating to background IP and funding commenced in September 2019. A non-cost extension to the end date of the project was subsequently provided.

The work was conducted by Dr Roman Kostecki, Institute for Photonic and Advanced Sensing where all the work was conducted.

The project achieved is primary objective of developing experimental probes that were used to examine the responses to nitrate in solution, but failed to test the probes in a soil environment.

The work demonstrated the feasibility of using optical fibre technology as a non-destructive method of measuring nitrate in solution continuously.

Three different probes were assessed, but each encountered some technical problems associated with the stability of the signal and they showed different degrees of responsiveness. These problems meant that the probe could not be assessed in soils, which was an aim of the preliminary work. This can't be achieved until the problems can be overcome

Key Performance Indicators (KPI)

*Please indicate whether KPI's were achieved. The KPI's **must** be the same as those stated in the Application for Funding and a brief explanation provided as to how they were achieved or why they were not achieved.*

KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
Development of nitrate probe completed	Y	
Evaluation of nitrate probe in soil completed	N	The project was unable to develop a workable prototype to allow this KPI to be achieved. The reliability of the experimental probes was not great enough to allow the project to go to the next step of assessing the probes in soil
Final report on project submitted to SAGIT	Y	

Technical Information (Not to exceed <u>**three**</u> pages)

Provide sufficient data and short clear statements of outcomes.

Background to the concept

The rapid development of optics and photonic sensing technology in biological science, material science and food science opens up new opportunities for agriculture. This proposal used cutting-edge research being conducted by the ARC Centre for Nanoscale BioPhotonics (http://www.cnbp.org.au/sensing) to develop deployable optical fibre based photonic probes to measure soil mineral N. Current research has developed new devices for measurement and sensing of chemical concentrations within biological and mechanical systems using optical fibre photonic probes, and an aim of the work was to see if this technology could be extended to soil environments.

Construction of probe

The probe consists of an optical fibre which has the molecular probe embedded within a polymer base on the fibre surface. The molecular probe contains molecules of specific a chemical specific for a particular analyte, such as nitrate, which chemically binds to the probe. Light from a laser at a specific wavelength travels through the optical fibre to the molecular probe. When no nitrate molecules are bound to the probe, no signal is generated. When the nitrate molecules bind to the probe, the light is absorbed by the probe which then fluoresces, emitting a different wavelength of light that is transmitted back through the optical fibre and is detected by a spectrometer. The more nitrate that is bound by the probe the stronger the fluorescence and the greater the signal, resulting in a linear relationship between the concentration of nitrate and the signal in the probe. Being a light-based system, response times are instantaneous.

The system is shown in Fig 1. Briefly, it consisted of of a 488 nm laser light source and shutter to send a 0.1 sec pulse of 130 W excitation light to the tip sensor. The back-reflected signal was passed through a 488 nm long-pass edge and characterised using a spectrometer.

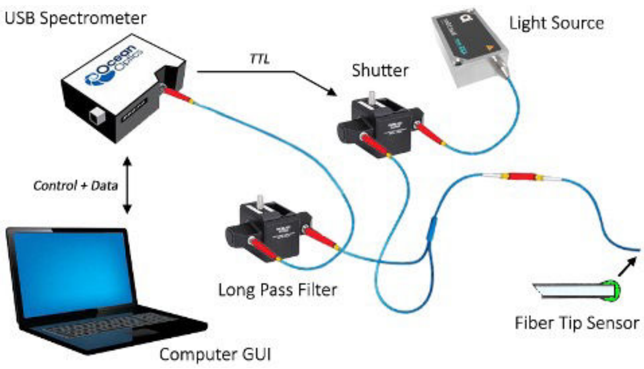

Figure 1. The experimental set up used to test different molecular probes

Experimental results

The project examined three types of probes based on different molecules that are sensitive to nitrate and/or ammonium.

(i) oxazine170 perchlorate
(ii) 5(6)carboxyl flurocein
(iii) fluorescein-5-thiosemicarbazide

In each case the probes were tested in solutions of sodium nitrate, ammonium nitrate and potassium nitrate at a concentration approximately 17 ppm (0.5 M). The aims of the experiments were to assess the responsiveness to N and the stability of the signal in different N solutions. If the sensor shows reliable results under controlled conditions then it can be assessed in soil. The results are presented as figures showing the outputs form the sensor over time when the supply of N around the sensor is changed.

An example of an output for the oxazine170 perchlorate probe is shown in Fig 2. The probe contains aluminium compounds which convert nitrate to ammonium which is detected by the membrane. Initially the sensor was placed in water. A response is recorded each time the solution is changed from sodium nitrate to potassium nitrate and ammonium nitrate, However, the signal from the sensor increase consistently over the 2-week period irrespective of the test solution. It is through the drift was due to the small increases in ammonia in solution over time.

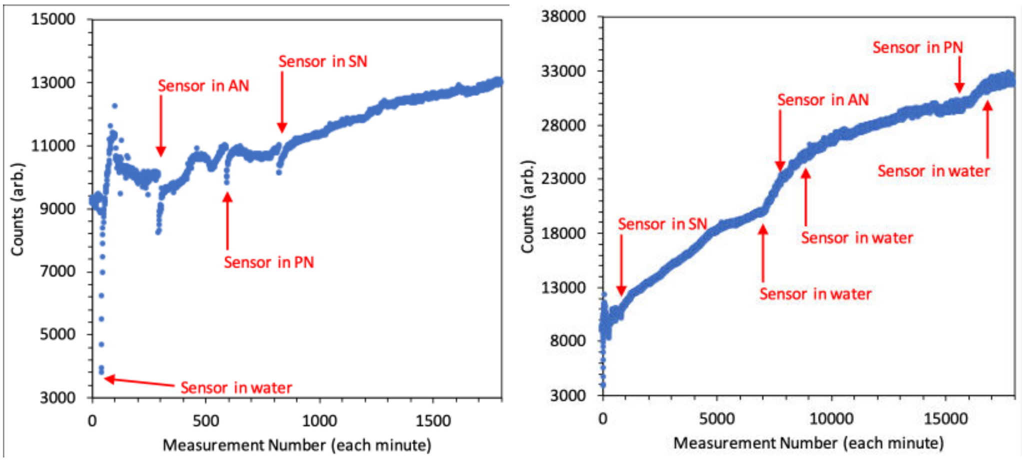

Figure 2. Output from the oxazine170 perchlorate probe over (a) the first 30 hours and (b) the two weeks of the experiment. The probe was tested with sodium nitrate (SN), potassium nitrate (PN) and ammonium nitrate (AN)

The results from the second probe (Fig 3) showed stabilisation in water over about a 1-hour period. There was a rapid response after it was placed in potassium nitrate (PN), but the magnitude of the response was smaller than expected with the concentration of nitrate in the test solution. Placing it in water again allowed the signal to return to the original value, but there was some drift over the following 30 mins. Replacing it in PN saw a rapid response but when the probe was left in the solution for a further 12 hours there was some oscillation in the response but the cause of this is not known. This probe showed good responsiveness to the presence of nitrate but the size of the response was low

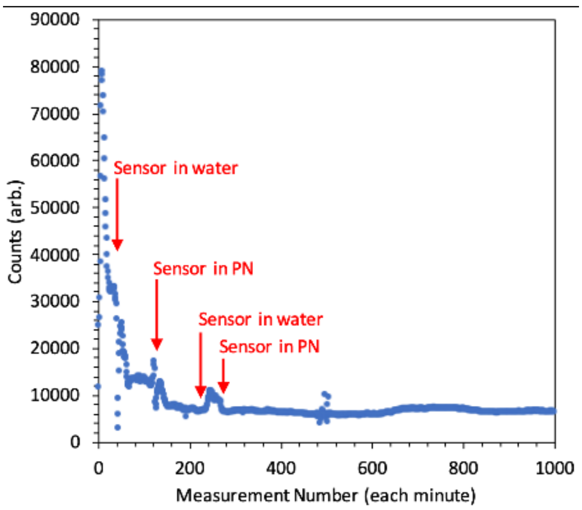

Figure 3. Response to potassium nitrate by the 5(6)carboxyl flurocein probe

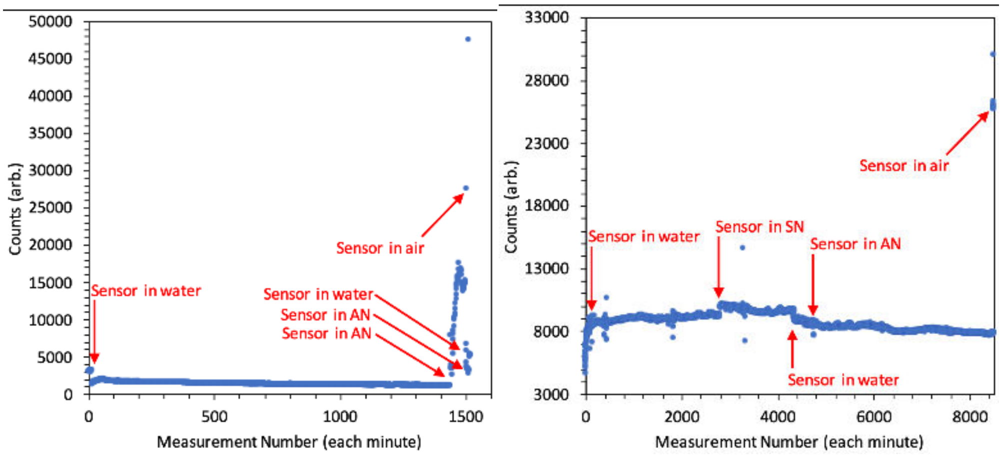

Figure 4. Output from the fluorescein-5-thiosemicarbazide sensor for (a) the first experiment with ammonium nitrate over 27 hours and (b) the second experiment with sodium nitrate and ammonium nitrate over 140 hours.

The third probe showed good stability in water over a 24-hour period and an instantaneous response when placed in ammonium nitrate (AN) but when placed in water again it failed to return to the original signal (Fig 4a) and putting it in AN again failed to stop the decline. Removing the sensor and leaving it air for one minute caused an increase in the signal.

The results suggest there may be slow diffusion of the ammonium in and out of the polymer environment of the sensor, but the initial stability and the rapid responsiveness of the sensor suggest it may be useful and worthy of further development. Another experiment was conducted using different sources of N (Fig 4b). There was reasonable stability over the initial 48 h (2880 min) in water and there was a small but immediate response when placed in sodium nitrate (SN) and the signal was reasonably stable when left in the solution for another 30 hours. When returned to water the signal returned to the initial water signal. However, there was no change in the signal after placing it in ammonium nitrate (AN) after 7 hours. The results indicate an instability in the sensor but the reason for it is unknown.

Conclusions Reached &/or Discoveries Made (Not to exceed one page)

Please provide concise statement of any conclusions reached &/or discoveries made.

Fibre optic technology has not previously been used to measure nitrate in solutions and in this regard the project has expanded the application of the technology. Notwithstanding some of the technical issues encountered, the preliminary results are encouraging. These experiments have provided evidence that there is potential for an optical fibre tip to be used to measure nitrate but further R&D is needed. Of the three sensors investigated, two showed a reasonable level of stability and responsiveness to N, but further work is needed to improve the performance. The immediate task is to improve the performance the sensors and assess their performance over a range of nitrate concentrations. The ability to measure N in the more complex environment of a soil solution also need to be investigated.

Intellectual Property

Please provide concise statement of any intellectual property generated and potential for commercialisation.

No IP was generated in the current project, but clearly in the long term the development of a soil probe to measure soil nitrate – either as a permanent *in situ* probe or a portable probe – may have considerable potential commercially.

Application / Communication of Results

A concise statement describing activities undertaken to communicate the results of the project to the grains industry. This should include:

- *Main findings of the project in a dot point form suitable for use in communications to farmers;*
- *A statement of potential industry impact*
- *Publications and extension articles delivered as part of the project; and,*
- *Suggested path to market for the results including barriers to adoption.*

Note that SAGIT may directly extend information from Final reports to growers. If applicable, attach a list of published material.

This project investigated the feasibility of using new technology to measure nitrate in soil solutions. Optical fibre sensor technology is being used increasingly in biological systems. The ability to use optical fibre molecular sensors to measure soil nitrate levels continuously would open up new opportunities to measure soil N and could have a significant impact in agriculture and in the management of N in environmentally-sensitive areas.

The work funded the development of a portable system to demonstrate the use of molecular sensors for the continuous measurement of nitrate. Three different sensors were evaluated and results from two of these provided evidence that there is potential to use this technology to measure nitrate N in solution. This is an important first step. The results of the project can be summarised as:

- Nitrate can be measure continuously and non-destructive using molecular sensors and optical fibre technology. To the best of our knowledge, this is the first demonstration of this application of optical fibre technology.
- There are a number of significant technical issues to be overcome to improve the performance of the sensors and to develop a robust sensor that can be sued in the field.
- The work has demonstrated an important first step in the development of soil nitrate probe, but considerable further work is required to make it a reality.

POSSIBLE FUTURE WORK

Provide possible future directions for the research arising from the project including potential for further work and partnerships.

This preliminary work had demonstrated the potential to measure nitrate in solution using optical fibre technology. However, there are a number of technical difficulties to be overcome to develop a workable prototype. In the short term, further development of the probe to remove the interference and improve responsiveness would be a suitable project for a PhD. This work would be best done at the Institute for Photonics and Advanced Sensing in the School of Physics.

In the longer term there is a need to develop a robust housing for the probes to make it suitable for field use. However, the SAGIT project was used to lever additional funds from the Waite Research Institute for Dr Kostecki to visit the Research Centre for Photonics & Instrumentation at City University of London. This research group are world leaders in packaging optical fibre sensors for industrial applications and their interest and possible future collaboration will assist in the development of a probe.