SOIL CRC

Performance through collaboration

FINAL REPORT

SMELLING SOIL

PROJECT 2.1.004

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The Soil CRC recognises the value of knowledge exchange and the importance of objective peer review. It is committed to encouraging and supporting its research teams in this regard.

The author(s) confirm(s) that this document has been reviewed and approved by the project's steering committee and by its program leader. These reviewers evaluated its:

- originality
- methodology
- rigour
- compliance with ethical guidelines
- conclusions against results
- conformity with the principles of the Australian Code for the Responsible Conduct of Research (NHMRC 2018), and provided constructive feedback which was considered and addressed by the author(s).

PROJECT PARTICIPANTS

CONTENTS

1. EXECUTIVE SUMMARY

The objective of this project was to determine if a low-cost electronic nose (eNose) could be built to detect volatile organic compounds (VOCs) in soil that might provide an indication of soil biological activity and soil health. The project intended to seek grower involvement from an early stage to ensure that any device produced was useful and usable. This project has generated a significant amount of national interest from a wide range of potential users both within, and external to, the Soil CRC.

Before travel restrictions in 2020, we were able to meet with several grower groups. The responses we received suggest that growers want to know more about soil performance, that they are prepared to adopt new technology if it provides this information and that ideally, the eNose would provide both an easy-to-understand output as well as the ability to access more detailed data relating to soil VOCs and performance.

Low cost eNoses were built using a range of off-the-shelf components and a housing for the eNose was produced using a 3D-printer. The iterations of the eNose were tested in laboratory pot trials and a field trial. We demonstrated that an eNose built using low-cost, off-the-shelf components can detect changes in soil VOCs. Further work is required to optimise the production of the circuit board and to establish power and communication systems that will allow it to operate remotely. The data generated in the pot trials suggests that the eNose can detect signals from biological activity, and changes in signals due to variations in the soil environment. Further work is needed in a range of soils to fully understand the correlation between soil performance and eNose signals.

2. OBJECTIVES

The overarching purpose of this project was to provide 'proof of concept' for an eNose that can measure, monitor and evaluate soil microbial health via existing sensor technology. The specific aims were to:

1. gain a clear understanding of what end and next users require from a soil eNose, and in what ways would they both understand and use the information
2. develop and produce a prototype eNose that uses a low-cost sensor array capable of detecting aromas emitted from soils
3. correlate eNose response with specific gas emissions and microbial activity
4. demonstrate the prototype eNose in the field.

3. RESULTS

Users are willing to adopt a rapid low-cost sensing technology such as the eNose, particularly if it gives real-time actionable information about the biological activity in their soil that is not available through other existing technologies.

An eNose was developed using low-cost, off-the-shelf gas sensors that can detect different signals in different soils under different conditions.

Changes were observed in microbial community activity and in the production of VOCs measured by gas chromatography-mass spectroscopy (GC-MS) when saturated soil was dried.

Differences in eNose signals were also observed in soils containing specific legume plants when rhizobial nodulation was successful. The eNose was able to continue recording data in the field for several weeks when connected to mains power supply.	
4. NEXT STEPS	**5. TIMING**
1. Determine the relationship between eNose signal and soil health, or particular functions of the soil ecosystem in multiple soil types and systems.	1. This will require several multi-year field trials. Complete within three to five years.
2. Modify eNose design to improve device robustness.	2. Improvements to design complete within 12 months.
3. Develop analytics or user interface to deliver usable outputs from the eNose.	3. Algorithm and user interface developed with growers over one to two years.
4. Identify commercialisation opportunities and pathways.	4. Identify commercialisation opportunities and develop plan within 12 months.

INTRODUCTION

The overall aim of this project was to determine if it was possible to build a low-cost electronic nose (eNose), using existing sensors, to monitor and evaluate soil health. The eNose is intended to measure multiple, soil derived non-specific biogenic volatile organic compounds (BVOC) rapidly over time. After further analysis, the BVOC profile (which is like a fingerprint) can potentially provide an overall indication of how the soil is functioning.

The specific aims of this initial project were to:

- gain a clear understanding of what end and next users require from a soil eNose, and in what ways would they both understand and use the information
- develop and produce a prototype eNose that uses a low-cost sensor array capable of detecting aromas emitted from soils
- correlate eNose response with specific gas emissions and microbial activity
- demonstrate the prototype eNose in the field.

BACKGROUND

THE SOIL VOLATILOME

The importance of the production of VOCs by biological activity in soil has been known for some time. BVOCs are likely to have an important role in affecting ecosystem function (Leff and Fierer, 2008). These gases are produced in soil through the metabolic activity of plant roots, soil fauna, fungi and bacteria and are collectively referred to as the soil 'volatilome'. Over one thousand different microbial volatiles have been characterised from soil, but it is likely that there are many more (Schenkel et al. 2015). Importantly for agricultural production, interactions among plants, invertebrates and microorganisms in the soil are mediated by BVOC signals (Peñuelas et al. 2014). In addition, studies have shown that BVOC have a role in influencing the nitrification process (Mohanty et al. 2019) and that BVOC production varies in response to soil amendments with different types of organic matter (Potard et al. 2017).

As a marker of biological activity, measuring soil BVOC may offer a way to improve our understanding of the complex biogeochemical and biological processes in the soil. Currently, BVOC are difficult to measure due to their low concentrations, high diversity and the complexities of the soil matrix. The most common analytical methods of detecting and measuring soil BVOC are either based on mass spectrometry techniques (e.g. proton-transfer mass spectrometry or membrane inlet mass spectrometry) or on gas chromatography (Peñuelas et al. 2014). Rapid, field-based methods do not yet exist although small portable gas chromatography–mass spectroscopy (GC-MS) machines are available that can be used on site.

ELECTRONIC NOSES

Electronic noses (eNoses) for environmental monitoring have been in use for some time. In 2000, the first validated 'eNose' was revealed for environmental detection of pollutants (Staples, 2000). Essentially this device uses a gas chromatography type analysis and is now

commercially available albeit at a high cost (>$US20 000 purchase with ongoing maintenance costs (https://www.cbrnetechindex.com/Print/4362/electronic-sensor-technology-inc/znose-series). In agriculture, various eNoses have been proposed to detect pests and diseases in plants (for example Cui et al. 2019; Gębicki and Szulczyński 2018), for the detection of soil moisture levels (Bieganowski et al. 2016), respiration (Pineda and Perez 2017) and organic matter (Huang et al. 2021). Bieganowski et al. (2016) found that their eNose could distinguish between different moisture levels in several different soil types, but they also found that the signal was different from different soils at the same moisture levels. The SENose (Pineda and Pirez 2017) was developed as a low-cost (less than $US 50) device to measure soil respiration although it included sensors for methane and hydrogen as well. Most recently, an eNose system to measure soil organic matter was developed (Huang et al. 2021) using a metal-oxide-semiconductor sensor array. The device was able to predict organic matter content based on the VOCs measured. Specific components of organic matter, humic and fulvic acids, have been previously quantified using an E-nose with a specific array of metal-oxide sensors (Lavanya et al. 2017).

The use of BVOCs as a measure of microbial activity was first proposed in 2007 (Bastos and Magan 2007). Despite this very little work on using BVOC to measure microbial activity has appeared in the literature in the intervening time. De Cesare et al. (2011) tested an eNose to measure microbial activity successfully in a semi-artificial system in which irradiated soil inoculated with a single bacterial species was used. A recent review (Nahirniak et al., 2020) of the remote monitoring of ‘soil air’ and ‘soil breathing’ focussed on the main gases that are found in the soil: oxygen, carbon dioxide (CO_2), nitrogen oxides, methane and hydrogen. These gases are all important and linked to specific soil biogeochemical processes but are likely to have less of a role in the below-ground interactions between plants and other soil organisms.

GAPS IN CURRENT KNOWLEDGE: ENOSES AND SOIL HEALTH

None of the devices developed so far have been built or tested with the aim of specifically linking soil biological activity to ‘long-term sustainable agricultural productivity’ (the definition of soil health provided by Arias et al. (2005)). Soil health is usually defined as the ‘capacity of soil to function as a vital living system’ (Doran and Zeiss 2000) but there is no agreement on the best measure of soil health (see for example Powell et al. 2020). The link between biological activity and soil health is complex and often misunderstood (Fierer et al. 2021). There is a need for an indicator or measure that clearly links biological activity to the overall performance of the soil without requiring specific identification of the thousands of different organisms that live in a single sample of soil. Electronic nose technology may be able to fill this gap by measuring the products of biological activity without specifically identifying them.

METHODOLOGY

END USER INPUT

As part of the eNose development we wanted to understand grower requirements for on-farm soil sensing. The aim was to collect this data at an early stage of the project to inform eNose development. Our approach was to ask growers very open questions to encourage as many ideas as possible. At later stages of the eNose development (in future projects) further feedback will be sought on more specific ideas. At this stage, the aim was to gather as much information as possible on what information growers want to have about their soils and how an eNose might be used to obtain this information.

The workshops were held in Ross, Tasmania and Young, New South Wales under the University of Tasmania Social Sciences Ethics approval H0018461.

Participants were welcomed and given a general introduction to the day. As part of the Smelling Soils section of the day participants were asked to read information sheets and sign consent forms which were then collected and stored securely.

A short presentation on the goals of the Smelling Soils project was presented culminating in a request for answers to two major questions:

1. Imagine that this technology can tell you anything about your soils. What would you like this technology to tell you about your soil?
2. What would you be prepared to do to get this information? What time, effort or other investment would you make to get some value from the technology?

Participants were given a pile of sticky notes and asked to jot down their thoughts and place them on the relevant card (see Figure 1 as an example from one workshop). Participants were free to talk to others and to ask questions of the presenters but were not required to discuss their ideas. A card for ‘any other comments’ was also provided.

Before the workshops, the project team developed a series of themed questions to explore what information growers wanted and how they might use the eNose. Ideas from workshop participants were transcribed from the sticky notes into written documents. Ideas were grouped together, initially where the same or very similar comments were made, and then into groups of similar ideas. The next stage grouped the notes into themes under each of the previously developed questions.

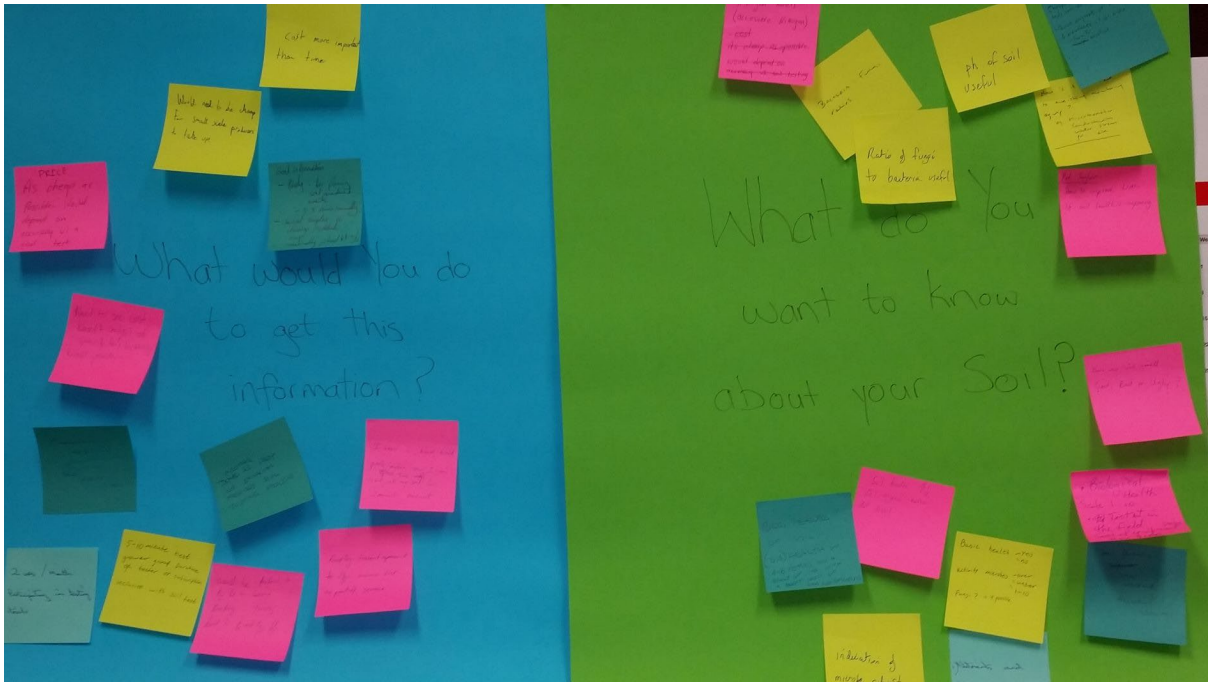

Figure 1: Workshop outputs - grower responses to questions about information and soil

ENOSE TESTING: WATER STRESS POT TRIAL

A pot trial was set up to determine whether the eNose was able to produce different signals in soil that went through different stages of drying to simulate a 'high performing' and 'stressed' soil. Various moisture levels were chosen as stress points as soil moisture affects both plant growth and microbial activity. These stages of soil moisture – saturation (SAT), readily available water (RAW), field capacity (FC), permanent wilting point (PWP) and very, very, dry (VVD) – are well understood by growers. Alongside the continual recording of eNose data, tests of microbial activity and analysis of soil VOC by GC-MS were carried out.

Soil was collected from two sites (Campbell Town and Cambridge) at a depth of 10-15 cm. Soil was homogenised, sieved through a 20 mm^2 mesh followed by a further sieve through 5 mm^2 mesh then placed into 6 x 400 mm pots per soil type (total of 12 pots). The Campbell Town soil was referred to as the 'ferrosol' and the Cambridge sample was referred to as the 'sandy loam' throughout the study.

Pots were placed in large tubs which were then filled with water. This allowed the pots to soak up the water rather than disturbing the soil by pouring water directly onto it. Water was topped up each day and moisture measurements were taken. Once moisture level appeared stable using both 10H-S probes and TDR probes, pots were deemed 'saturated', and were taken out and placed in a temperature-controlled room set at 25°C.

Pots were placed in a complete randomised design. Tensiometers were installed to help gauge the relevant stages of soil moisture. eNoses were placed in pots with the aim of constantly measuring gas output.

Both 10H-S and TDR probes were used to determine soil moisture content. Soil moisture was then determined gravimetrically. Samples were collected at approximately SAT (0 kPa), FC (10 kPa), RAW (60 kPa), PWP (1500 kPa), VVD (~75,000-170,000kPa). Subsamples were either frozen immediately or air-dried and stored.

VOLATILE ANALYSIS BY GCMS

A 50/30 µm DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane) StableFlex fibre was selected. The fibre was pre-conditioned by exposing it within the GC injector at 270 °C for 10 min. A blank (background) analysis was undertaken by exposing the fibre to the headspace of a screwed cap vial as per conditions below.

Samples were contained in 20 mL headspace vials. An internal standard mixture (20 µL in methanol) containing Cyclohexanol (50 µg), Undecanone (0.48 µg), Undecanoate (0.48 µg), Pentadecanal (0.48 µg) and Pentadecanoic acid (50 µg) was spiked into each sample prior to analysis. SPME sample processing was undertaken using a Gerstel Multi-Purpose Autosampler equipped with SPME and heated agitator modules. Samples were heated at 80 °C for 30 minutes with an agitator program of five seconds on, one second off at a speed of 400 rpm. Thereafter, the pre-conditioned fibre described above was inserted into the sample vial and exposed for 20 minutes to capture headspace volatiles. The fibre was then inserted into the GC injector for thermal desorption of volatiles for four minutes.

GC-MS analysis was performed using a Varian CP3800 GC coupled to a Bruker 300MS triple quadrupole MS with a split/splitless injector in splitless mode. Compound separation was carried out using Agilent DB-5MS capillary column 30 m x 0.25 mm x 0.25 mm film thickness (Agilent Scientific, USA) with the following conditions: carrier gas (helium) with a flow rate of 1 mL/min, injector temperature 270 °C. Oven initial temperature 40 °C, held for four minutes, to 80 °C at 6 °C/min, to 250 °C at 8 °C/min and to 290 °C at 25 °C/min. The MS was operated in full scan analysis (m/z) 35-350. Source temperature was 220 °C and electron impact ionisation (EI) at 70 eV was used.

MS data review was within MS Workstation Software Version 7 (Bruker) used to plot and process chromatograms and spectra obtained. The mass spectrum of each compound was matched with the National Institute of Standard and Technology (NIST, USA) 2017 library of compounds using > 90 relative match as standard. The Kovats retention index was also calculated for each compound and compared to the library reference value.

COMMUNITY LEVEL PHYSIOLOGICAL PROFILING

The functional diversity of the soil microbial communities was tested using EcoPlates™ (Biolog) which provide a range of 31 carbon substrates in individual wells. The ability of the soil community to use each carbon source is measured by spectrophotometrically measuring the growth in each well. One sample from each pot at each sampling time point was tested following the manufacturer's suggested protocol. 1 g of soil was diluted in 19 mL of sterile water and 140 µL of this dilution was added to each of the substrate wells in the EcoPlates. 140 µL of sterile water was added to the blank control plates. Plates were incubated at 25 °C. Absorbance measurements were taken with a Spectrostar-Nano Spectrophotometer (BMG Labtech) at 750 nm and 590 nm immediately and after 48, 96 and 168 hours. To correct for background turbidity the absorbance at 750 nm was subtracted from the absorbance at 590 nm (ie A_{590} - A_{750}). The colour development due to substrate usage in each well was then calculated as the difference between the initial reading and the reading after 48, 96 and 168 hours.

ENZYME ASSAYS

Air-dried soil samples were used for the enzyme assays. For each sample point, each pot (replicate) had two soil solutions prepared. These solutions were then assayed using the fluorimetric microplate assay method described by Dick et al. (2018). Four different enzymes were assayed using fluorescent methylumbelliferyl substrates:

1. B-Glucosidase; representative of carbon cycling.

2. B-N-Acetyl-Glucosaminidase; representative of nitrogen cycling.
3. Acid Phosphomonoesterase; representative of phosphorus cycling.
4. Arysulfatase; representative of sulphur cycling.

Relative fluorescence was measured at 360 nm excitation and 460 nm emission through a FLUOstar Omega spectrophotometer (BMG LABTECH).

ENOSE TESTING: RHIZOBIAL INOCULANTS

A pot trial was carried out to determine if the eNose was able to detect VOCs associated with root-rhizobia signalling. This trial compared signals from pots containing plants inoculated with rhizobia and pots containing plants that were not inoculated. This system had the benefit of including plants with a limited diversity of microorganisms in a system highly relevant to many growers where differences in biological activity between treatments were expected.

A 2 x 2 factorial design was used on two different legume species (lucerne *Medicago sativa* var. Stamina ® GT5 and subterranean clover *Trifolium subterraneum* ssp. Yanninicum). Two inoculation treatments were used (inoculated or non-inoculated) and two applications of N were used (either N–containing or a N–free solution).

One hundred and twenty seeds of *Medicago sativa* and 192 seeds of *Trifolium subterraneum* were germinated in a growth cabinet at 20 °C for 6 days until the cotyledons emerged. The seedlings were transplanted into disinfected 1.5 L plastic pots containing a mix of 70 % sand and 30 % perlite that had been sterilised by steam at 70 °C for 40 minutes.

Five seedlings per replicate were planted near the edge of the pots. The seedlings were inoculated immediately after transplanting with their respective rhizobium strain (*Sinorrhizobium meliloti*, strain RRI128 (group AL) for lucerne and *Rhizobium leguminosarum* bv. *trifoli*, strain WSM1325 (group C) for sub clover (Drew et al. 2012)). Pots were kept in a glasshouse and seedlings were watered every 48 hours with either half-strength Hoagland's solution (N+) or half-strength N-free Hoagland's solution (N-).

The above and below-ground biomass were collected four weeks after planting. Roots were carefully removed trying to recover most of the root system of three specimens per replicate. The roots were assessed for nodulation according to the nodulation score chart developed by Howieson and Dilworth (2016).

The six eNoses were rotated across replicates, with a minimum data collection of two time points per replicate. A tube with a cone-shaped end was inserted in the soil in the centre of each pot at the time of the eNose placement. The eNose was placed on top of an inverted cone-shape structure that allows the collection of gases from the soil, with the sensing material facing towards the exit of the tube. Sensor gas readings were regularly collected from the SD cards of each eNose and checked to confirm that the gas readings, date, and time had been recorded properly before being rotated to another replicate. Due to the limitation in the number of eNoses, and to maintain consistency in the data collection, three eNoses were rotated through the different treatment combinations,

ENose readings were collected every two days from the SD cards. The data was classified by treatment groups and species and processed in the SPYDER 4 (Scientific Python Development Environment) running Python 3.7. A parser was written to convert raw eNose data into a useable data frame, permitting initial exploratory analysis. Graphs and statistical parameters were obtained from each replicate, allowing graphical comparison among treatments and legume species. Due to the large data set – approximately one million data points per replicate – the data was reduced from seconds to quarter-hours by linear

interpolation. The data was processed using a mixed-effects model to assess the effect of inoculation, N and legume species on the gas signals in the software SAS version 9.4. Random factors such as date of data collection and repetitive measures were also included. The results from pairwise comparisons were adjusted using the Tukey-Kramer method.

PRELIMINARY FIELD TESTING

Four eNoses were deployed on the University of Tasmania Farm at Cambridge in December 2020. The eNoses were connected to a power box which supplied mains power via weather-proof leads. The eNoses were placed with the sampler in the soil with the main eNose housing sitting on top of the soil. Plastic pots were used to provide additional weatherproofing and protection (see Figure 2).

Figure 2: Field deployment of eNoses

RESULTS

END USER REQUIREMENTS

Some common responses were observed across both groups. Both groups wanted to know about:

- biological diversity
- nutrient levels (including organic matter, carbon, nitrogen specifically and ‘nutrients’ in general)

- soil health (although this may mean different things to different people)
- whether soil health is improving over time.

Somewhat surprisingly, only one group had responses that fitted under the theme ‘what happens when you change management practices?’ despite both sessions being held after talks about different management practices.

Neither group had any responses relating to the financial value of their land.

Both groups said that they would use an eNose to monitor their soil several times a year, this frequency ranging from three to five times annually to monthly and ‘frequently’. An output of a simple scale was put forward by people in both groups although other comments suggest participants would also like detailed data to be available as an output..

Both groups were willing to invest time into learning how to use the device and in the actual monitoring. However, one person suggested a data logger, which takes measurements automatically, would be better. Both groups identified cost as important although an exact cost was difficult to calculate as the cost will change with product accuracy and cost-benefit analysis.

An extensive ‘wish list’ of functions for the eNose was provided by one group and these are listed in Appendix A. Although this list does not answer the main questions that we were seeking to address in the workshops, it is a source of ideas.

CURRENT ENOSE DESIGN

ENOSE DESIGN

The eNose is, in essence, a mini-computer. It was built as an Arduino compatible device so that it could be easily used and adapted using existing Arduino tools and devices. The Arduino ecosystem was used as a starting point as this is a mature platform which many are familiar with. Arduino is an open source, series of single board easy to use microcontrollers which are ideally suited to operation and integration of sensors. Much like the heart of a computer is the central processing unit (CPU) (i.e. Intel i7), the eNose used a modern low cost SAMD21G18 M0 cortex 32-bit microcontroller, which is much faster and more powerful than most existing 8-bit microcontrollers. The program to operate the eNose is easily modified through the Arduino open-source software (Arduino.ide) and can be stored on the eNose so that it can be operated without being connected to a computer. The board consists of many surface mounted components which largely manage power and regulate voltage to improve data quality. The board also contains two megabytes of flash memory for storing the operating program (SPI interface), an SD card for logging data (SPI interface), DS1307 real time clock (I2C) for time stamping data, and two ADS1015 Analogue to digital converters. All of these devices can be controlled using the Arduino.ide software. The board has been designed to allow for flexible configuration with different sensors to meet different use cases.

The Arduino bootloader was flashed on the microcontroller which allows for the use of the Arduino IDE to write and upload code, although there is the possibility to use micro-python as the programming language if desired. Most of the components on the circuit board were surface mounted allowing for automated production of the basic board. Several of the sensor and connectors used are through hole mounted and must therefore be added later. This allows for a number of base circuit boards to be produced and the end user can then add sensors

which are most relevant to their situation. The board is 100% Arduino compatible meaning functionality for other Arduino boards that use the same microcontroller can be added to the eNose. The I2C bus is broken out, which allows for any number of I2C compatible devices to be added.

While the board operates at 3.3 volts to minimise power consumption, both 3.3-volt and 5-volt sensors can be used. A separate 5-volt input is used to heat the sensors, and the second highly regulated 5-volt input used to operate the sensors to minimise variations in voltage to reduce signal noise. Passive noise cancelling is also conducted by the ADC's (Analogue to Digital Converters) which have Programmable Gain amplifiers (PGA), that allow the user to amplify the input signal before it is converted to a digital signal. The eNose can be fitted with up to 10 sensors. Up to three of the low cost six pin format MOSS (mobile operations unattended sensor system) sensors, two sensors of the four pin format sensors, and up to two sensors with the final pin pattern are also able to be installed (Figure 3).

Additionally, there are three surface mounted devices (SMD) that act as sensors. Air temperature, pressure and humidity are measured on the underside of the board using a BME 280 chip, and eVOC and eCO_2 measurements are measured using a CSS811 chip to estimate total VOC load. Finally, there is a MISC 4514 chip that has two analogue sensors; one oxides gases and the other reduces gases.

The device has been designed to be as easy as possible to fabricate by hand. Most of the components are surface mounted using 0805 size parts which makes it possible to place the parts without the need for a microscope. Fabricating the boards involves:

1. applying solder paste using an electropolished stencil
2. populating components by hand placement
3. baking in a toaster oven to melt the solder
4. inspecting the boards under a microscope for errors
5. testing each component (i.e. SD card, RTC, etc.) separately by flashing the Arduino bootloader onto the microcontroller via a modified JTAG/SWD. This bootloader allows for code to be uploaded using the Arduino IDE or Circuit python as these are very common entry level ways of programming microcontrollers.
6. adding the through hole components (large sensors, wires, etc.) by hand soldering. These include power wires, inputs for other devices and sensors.

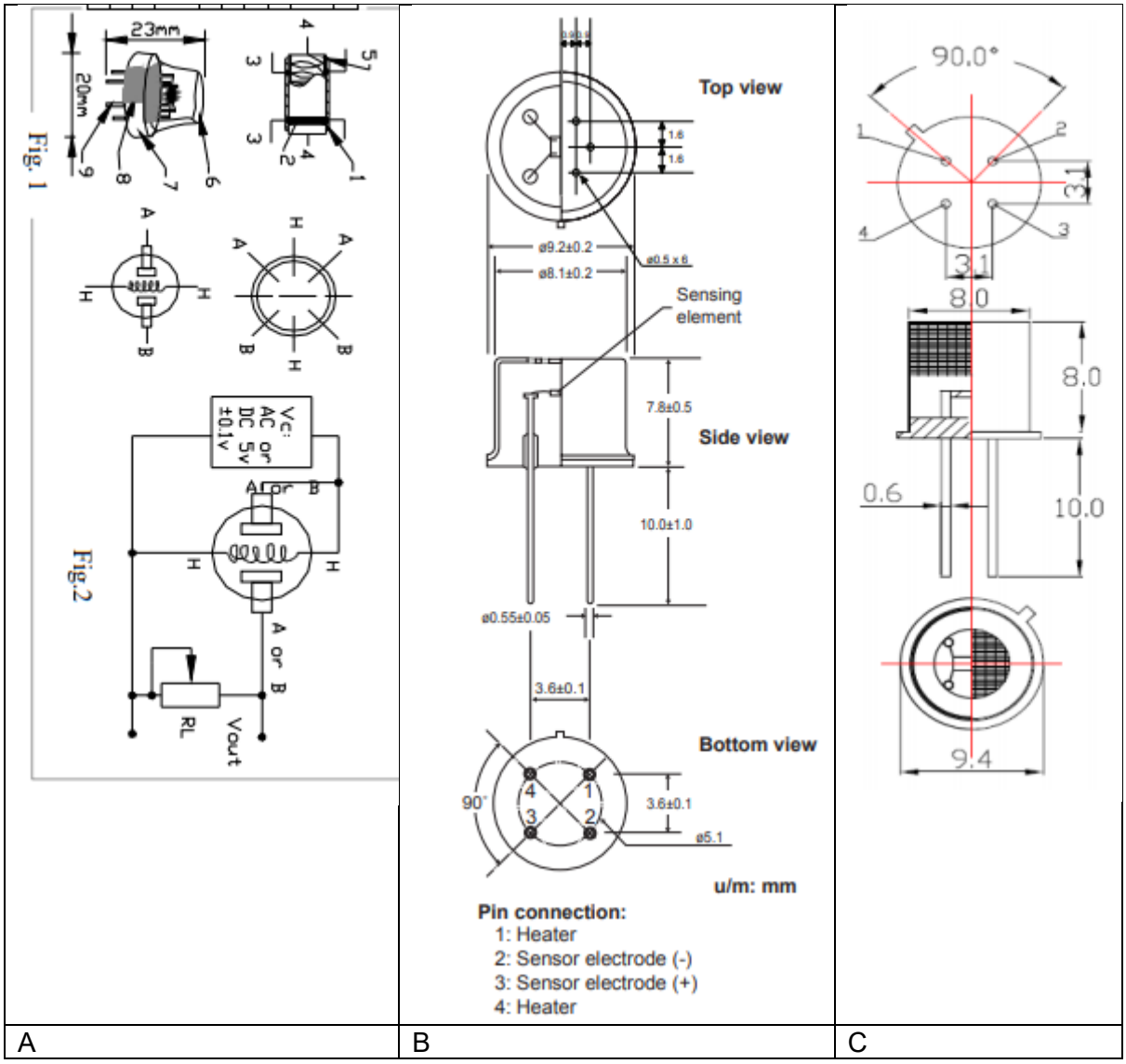

Figure 3: Different MOSS sensor configurations

PRELIMINARY FIELD PERFORMANCE

After six weeks in the field, two of the four eNoses were still running and data was downloaded from SD cards in all eNoses.

Two of the eNoses collected data for the entire deployment period. However, one of these eNoses had a faulty sensor that did not provide a signal. Of the two eNoses that did not run, one ceased operation after approximately 15 minutes and the other after three weeks. It is not clear why they stopped working although the most likely explanation is a problem with the power cables. One eNose had two broken wires that required repair.

Six weeks of data from all sensors were downloaded from one eNose. The data was resampled to include every 500th data point and then plotted (Figure 4). In this plot, diurnal temperature variations are observed, as expected. This indicates that the sensors are detecting environmental change. Other variations can also be observed over time that do not correspond to diurnal variation. This means that these sensors are detecting changes other than those caused by temperature.

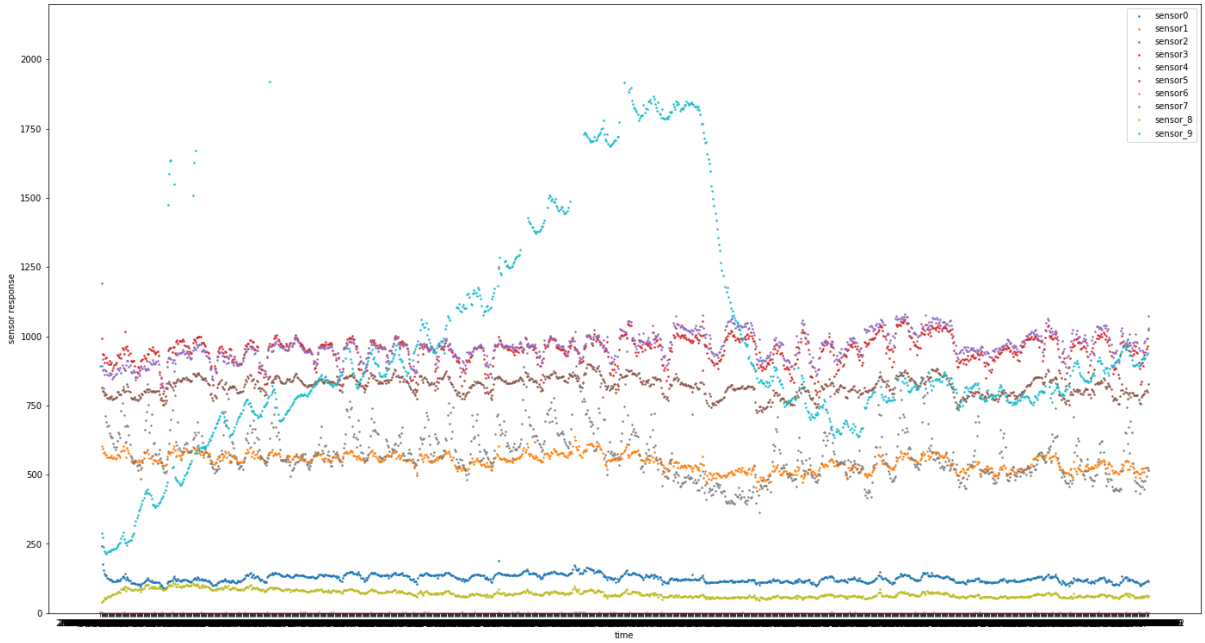

Figure 4: Resampled data from field deployed eNose showing diurnal and other variations.

WATER STRESS POT TRIAL

ENOSE SIGNALS

The eNoses in this trial included up to nine different VOC sensors plus sensors for circuit board temperature, soil temperature and humidity and in some cases CO_2 and total VOC. In some eNoses, some individual sensors failed to record a signal, but most sensors recorded a signal for the duration of the pot trial. Figure 5 shows the average signals recorded each day for each sensor in one of the pots. These plots do not show changes occurring on a smaller timescale (for example diurnal changes) but do show changes on a longer timescale which are more likely to be associated with drying.

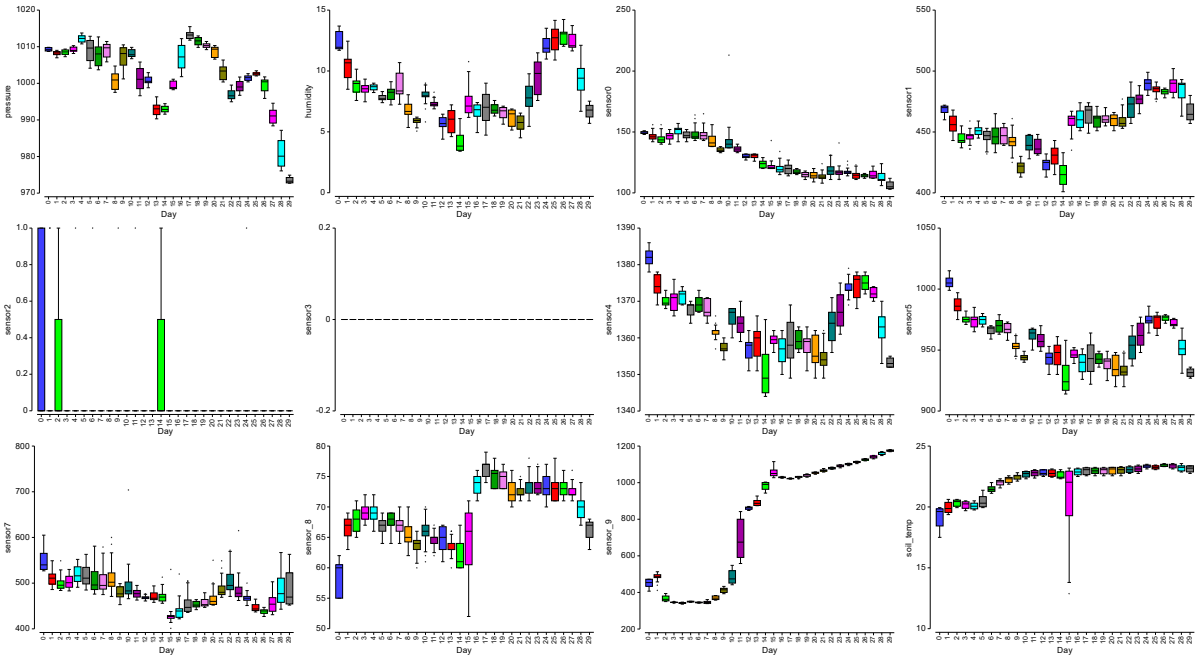

Figure 5: Box plots from pot C containing a ferrosol subject to drying

In this study, classical multivariate techniques were used to examine the patterns in the combined signals from all gas sensors (excluding carbon CO_2 and total VOCs) to determine if there was any correlation with soil moisture at SAT, FC, RAW and PWP). Figure 6 presents a nonmetric multidimensional scaling (MDS) plot of a typical response from a pot containing ferrosol (A) and a pot containing a sandy loam (B). Changes over time can be clearly distinguished in both soil types, however it is also clear that both soil types behave differently.

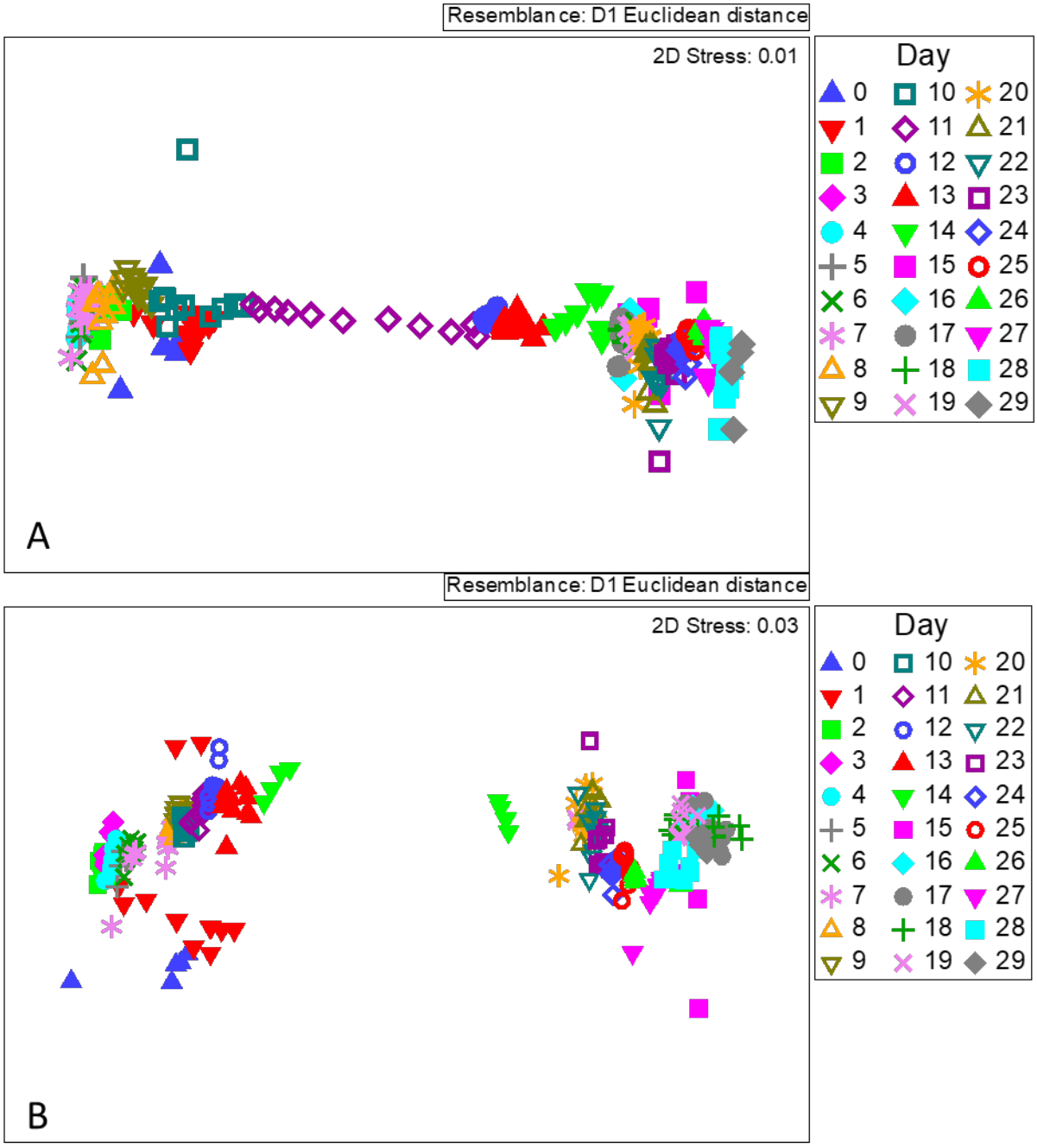

Figure 6: nMDS based on Euclidian dissimilarity matrices for a ferrosol (A) and sandy loam (B).

MICROBIAL ACTIVITY

The activity of four major classes of enzymes (representing four major nutrient cycles) were measured. There was a clear difference in enzyme activity between the two soil types, however, differences among the different soil moisture levels were not significant (Figure 7).

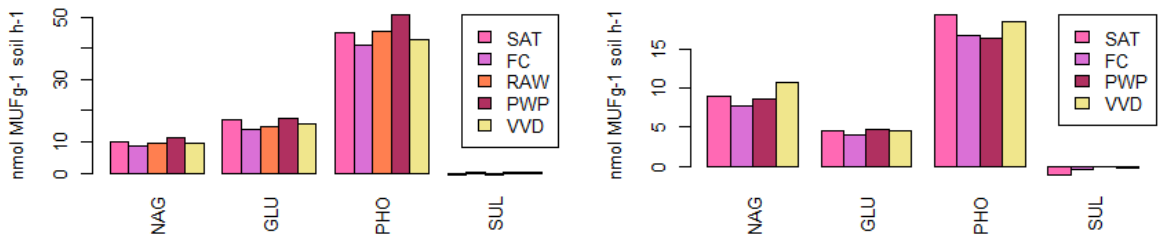

Figure 7: Activity (nmol MUF g-1 soil h-1) of N-Acetyl-Glucosaminidase (NAG), Glucosidase (GLU), Acid Phosphomonoesterase (PHO) and Arylsulfatase (SUL) in Ferrosol (left) and sandy loam (right) soils at saturation (SAT), field capacity (FC), readily available water (RAW), permanent wilting point (PWP) and very, very dry (VVD).

Community level physiological profiling measures the overall use of a range of carbon substrates by a microbial community. Changes in the profile indicate changes in carbon use and hence potential changes in the functioning of the community. Multivariate ordination (MDS) shows a clear difference in the communities in the two soil types (Figure 8). More importantly, there are differences in the function of the microbial communities among the different sampling points (moisture levels) in both soils (Figure 8). ANOSIM tests were significant ($P<0.01$) for all pairwise comparisons within the sandy loam soil. Within the Ferrosol, the PWP sampling point was different to the other sampling points (SAT, FC, RAW) which were not significantly different to each other. This suggests that the activity of the microbial communities varies both between the two soils and among the different soil moisture level states within the same soil type. The sandy loam (Figure 8B) had much greater differences between the sampling points than the Ferrosol (Figure 8A).

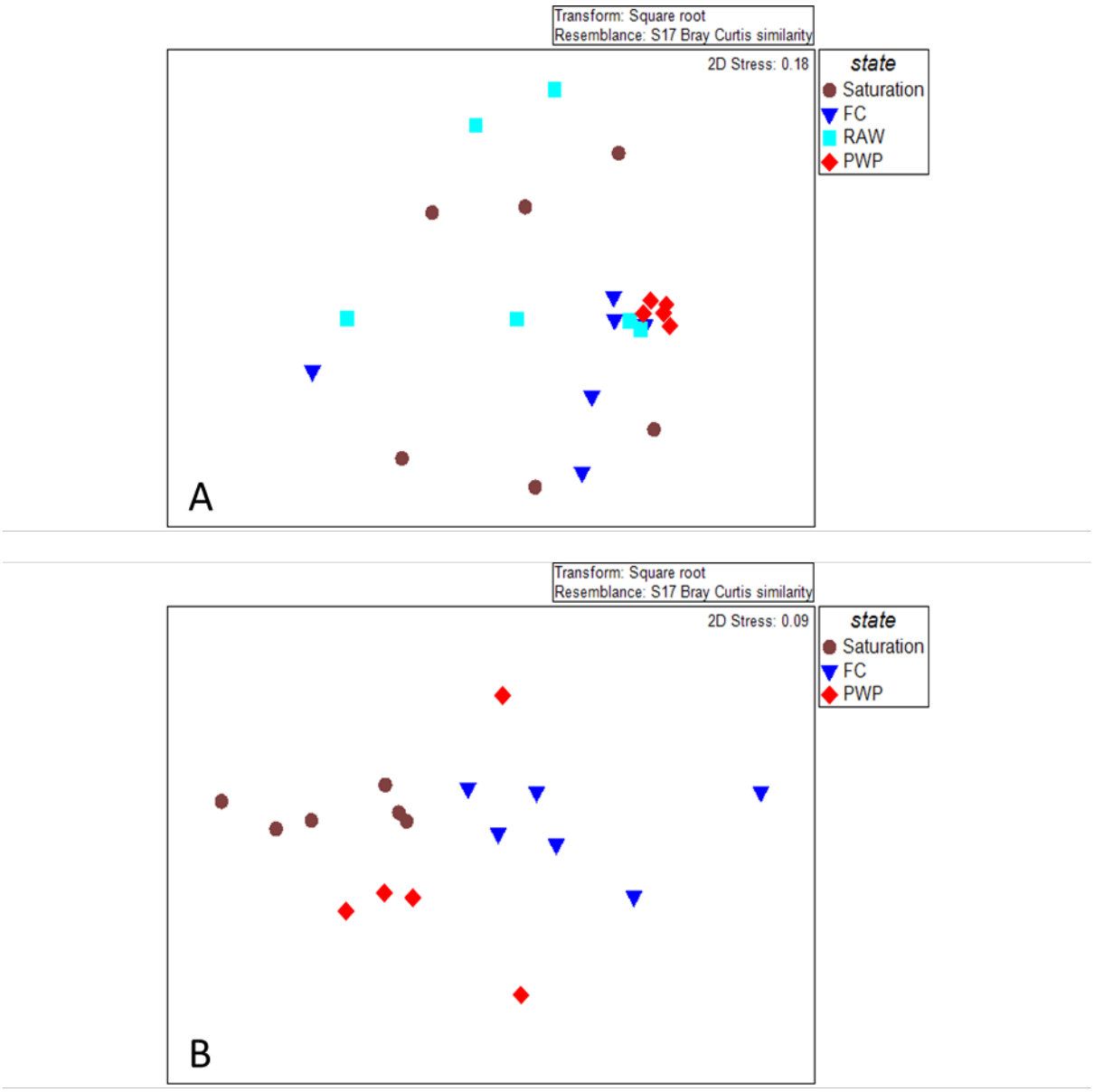

Figure 8: nMDS showing the difference in community activity based on CLPP at different sampling times (saturation (SAT), field capacity (FC), readily available water (RAW) and permanent wilting point (PWP) in Ferrosol (A) and sandy loam (B) soils. The different behaviour of the two soils can also be observed in these figures.

SOIL VOC

Gas chromatography-mass spectrometry was used to detect and identify VOCs in soil samples collected periodically from the pots. The chromatograms contained a total of 91 different compounds. Some of these were identified using existing libraries; others could not be identified. Multivariate ordination of this data revealed that the overall mix of volatiles detected varied with sampling point for both the sandy loam and Ferrosol soils. ANOSIM tests confirmed that these differences were statistically significant ($P < 0.01$).

While outside the scope of this study, it is interesting to note that some VOCs were only detected in one soil or the other (Figure 9). More surprisingly, some were only detected in dry soil (PWP) or in saturated (SAT) soil. This is likely due to differences in biological activity although it could also be due to differences in the chemical activity in the soil and the interaction between water, soil particles and the VOCs.

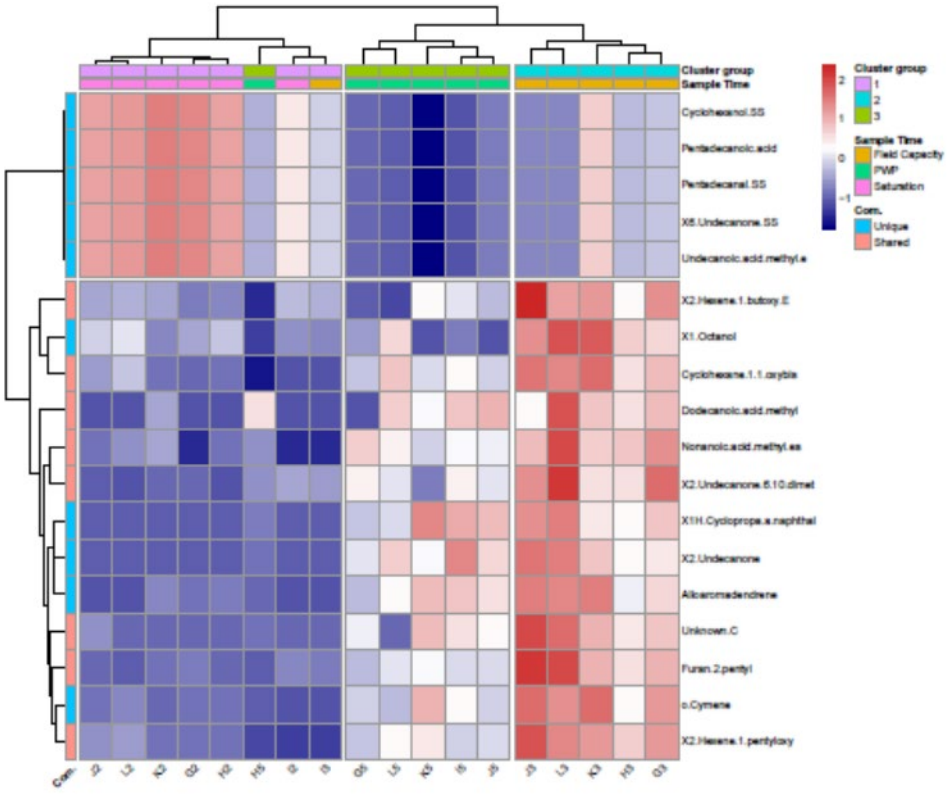

Figure 9: Heatmap of a selection of VOCs present in the sandy loam soil where red colours represent a higher relative abundance and blue colours a lower relative abundance. The first row across the top indicated the sampling point as either field capacity (orange), permanent wilting point (green) or saturation (pink).

RHIZOBIAL INOCULATION TRIAL

The ability of the eNose to detect successful nodule formation in legumes inoculated with appropriate strains of rhizobia was tested in pot trials in a growing medium with or without the application of nitrogen (N+ or N-).

Nodule formation in each of the pots was assessed (Table 1). Inoculated plants grown without N application (N-) developed nodules whereas inoculated plants that received N (N+) did not develop a good nodulation system. Some nodules were observed on N- non-inoculated plants.

Table 1: Mean nodulation score (standard deviation)

Species	Treatment	Nitrogen addition	Mean nodule score
Sub clover	Inoculated	-	4.7 (0.58)
	Inoculated	+	0.5 (0.05)
	Non-inoculated	-	0.5 (0.05)
	Non-inoculated	+	0
Lucerne	Inoculated	-	3.3 (0.58)
	Inoculated	+	0.7 (0.58)
	Non-inoculated	-	1.2 (1.61)
	Non-inoculated	+	0

The eNose detected changes in gases and VOCs, shown as variations in the sensor responses across most treatments. Specifically, seven of the nine sensors in the eNose detected changes in gases and volatiles and two of the sensors did not record readings. Gas readings varied over time in all treatments except N+ inoculated.

The eNose was able to distinguish between gas mixtures emitted from sub clover plants that had root-rhizobia associations and those that did not have root-rhizobia associations (Figure 10). However, the eNose did not detect significant differences between gas mixtures emitted from lucerne plants with and without root nodules.

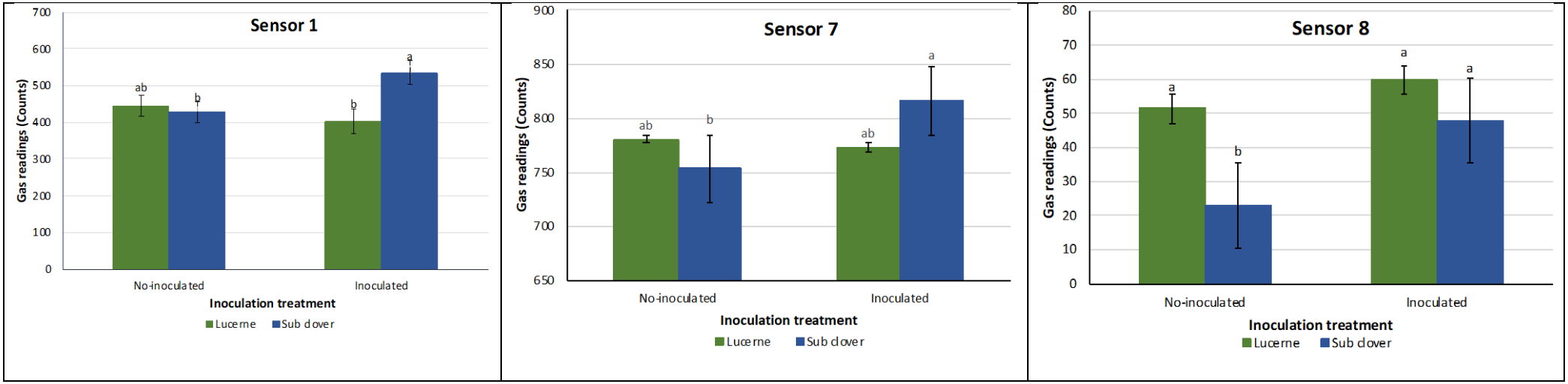

Figure 10: Mean gas readings of lucerne and sub clover plants under two inoculation treatments (Inoculated and Non-Inoculated) from sensor 1, sensor 7, and sensor 8. Error bars indicate +/- 1SEM, different letter represent significant different predictive means (Tukey-Kramer; $p<0.05$).

Using a mixed-effects-model including inoculation, N and legume species, sub clover plants that developed root nodules recorded significantly higher (Tukey-Kramer, $p<0.05$) gas readings from sensors one, seven and eight compared to sub clover without nodules (Table 2). In contrast, no significant difference between gas mixtures from lucerne plants with and without root-rhizobia associations were found from any of the sensors.

Table 2: Summary of the results from the mixed-effects model on the effect of inoculation, nitrogen and legume species on gas readings from seven sensors.

Variable	Sensor 0	Sensor 1	Sensor 3	Sensor 4	Sensor 5	Sensor 7	Sensor 8
Inoculation	0.457	0.012*	0.094	0.729	0.741	0.056	<.0001*
Species	0.174	0.069	0.982	0.688	0.810	0.818	0.008*
Inoculation*species	0.773	<.0001*	0.055	0.064	0.274	0.012*	0.007*
Nitrogen	0.124	0.0003*	0.446	0.491	0.836	0.003*	0.154
Nitrogen*species	0.681	<.0001*	0.021*	0.925	0.271	0.086	<.0001*

DISCUSSION

GROWER INTEREST IN SENSORS AND SOIL INFORMATION

IMPLICATIONS FOR ENOSE RESEARCH

This project aimed to provide evidence that the eNose can detect changes in the soil VOCs that are correlated with changes in the soil and to initiate the co-development process with growers. Understanding grower needs is essential to develop a useful product.

Grower understanding and definitions of soil health vary and different aspects of the long-term productivity of soil (a common definition of soil health e.g. Arias et al. 2005) are important to different growers at different times. Our interactions with growers show that many want to ‘measure’ their soil health and that the eNose could be a pathway to providing measurements, particularly on the status of biological activity.

Some things that individual growers would like to know, and they associate with indicators of soil health, can already be determined by well-known measurements e.g. worm density, N levels, organic matter or bacteria:fungi ratio. There are existing tests that can provide this information and as noted by a grower, a new test ‘must cost less than existing tests’. Our goal is to provide more information that they cannot currently obtain easily and a measure of overall soil health status.

Some growers indicated that they would like to use the eNose to monitor soil health over time and in response to different soil management practices. There were many comments and questions relating to aspects of soil management that are well outside the scope of this project. Growers obviously want to understand more about their soil, soil processes, productivity, and long-term sustainability and how they can use such information to move towards improved soil health.

Some growers indicated that they would be willing to pay for an eNose service while others wanted to use the device themselves. This opens several different pathways for the development and adoption of this technology. In future, development of this technology, the user interface with the output (signal) of the eNose will be particularly important. A meaningful

linear scale (good to poor soil health) will be difficult, however, we can aim for an easily understandable output that is calibrated for different soils and practices. The eNose may have to be available in different formats i.e. for ‘beginner users’, ‘intermediate users’ and ‘advanced users’.

Due to COVID-19 and the scope of the project, interactions with growers were limited. However, the workshops provided the Smelling Soils team with grower perspectives that generally aligned with our assumptions about what they might want to know and the diversity of targeted potential applications for the eNose. It will be essential to seek input from a wider range of groups in the co-development of the eNose as we start to develop algorithms targeting specific applications and contexts e.g. detecting nodulation.

SUMMARY

Growers have a strong desire to know more about their soil. One group was very interested in how their management practices affected the soil. The other group did not provide any comments that related to this theme but were clearly interested in having information to help them decide what they should do. Our goal of providing low cost, potentially real-time information about soil health to growers in an actionable form fulfils a need in the sector.

USING THE ENOSE TO DETECTION NODULATION

In the rhizobia inoculation trial, the growing medium was disinfected before inoculating with the rhizobia. This resulted in a low abundance, low diversity microbial community in the growing medium so that the rhizobia inoculants created a major difference in biological activity for the different treatments. The differences detected in the eNose signals are therefore very likely to be the result of different biological activity. However, we cannot be certain whether the VOC are produced by the rhizobia, the plant roots or as a signalling mechanism between the two. The results are also confounded by the difference in nodulation between the two legume species with poorer nodulation in the lucerne.

Further work on this system is required to refine the choice of sensors used, to test in a greater variety of legume-rhizobia pairs and to test in real soil rather than a growing medium. Despite this, non-destructive detection of nodulation is a potential use of this technology.

RELATIONSHIP BETWEEN SOIL CHARACTERISTICS, MICROBIAL ACTIVITY, VOC PRODUCTION AND ENOSE SIGNAL

The second trial using water stress to change the function of two field soils generated very large data sets which are still being explored. Preliminary analysis revealed that there are differences in the biological activity in the soils at different moisture contents and that the two soils behave differently when subjected to saturation and drying. We have observed differences in the eNose signals and VOCs present when measured by GC-MS. This suggests that the eNose can detect differences in soils under different stress conditions and that some of the differences are likely associated with changes in biological activity. Not only could differences between the soils be detected but different trends were observed in the two soil types. This phenomenon has been reported previously (Bastos and Magan, 2007) and suggests that what constitutes a ‘good’ (or equally a ‘bad’) eNose signal will be different in different soils. The eNose will need to be calibrated for different soils and different farming systems.

Past work has used eNoses to measure soil moisture and soil organic matter (Huang et al. 2021; Lavanya et al. 2017). These are parameters for which other means of measurement currently exist. Our goal is somewhat different – to link the eNose signal to soil health. Building the underlying models and algorithms to do this will require much larger data sets in different systems over long periods of time. Machine learning, neural network and other big data approaches are required to develop models that relate the eNose signal, or fluctuations in the signal, to parameters of interest. This will require working with other large field trials to generate this data.

ENOSE PERFORMANCE

LABORATORY TRIALS

The two trials performed in this project are laboratory-scale tests of a set of hand-built eNoses. Each eNose underwent preliminary testing to check that they functioned prior to use in the pot and inoculation trials. The main problem encountered with the initial eNoses was power stability. The eNoses recorded signals continuously for eight weeks in the laboratory trials. The trials were then stopped to analyse the data (not because of technical problems with the eNoses). Some of the sensors failed to record on some of the eNoses. Standardisation and simplification of the circuit boards will allow production of the eNoses by automated machine-picking methods. This will decrease the fail rate of circuit board production.

FIELD TRIAL

Field deployment was a more complex test of the eNoses as there are a range of plant species present and large variations in environmental conditions. Previously we tested the eNose in bare soil or with one individual plant species under more controlled conditions. Despite the range of plants, the signals detected remained in range. The external temperature fluctuations were much larger than those previously experienced by the eNose but again the sensors operated as expected.

This experiment successfully demonstrated the ability of these eNoses to collect VOC data in the field over six weeks. Further development is needed to improve power supply and the ability to access data remotely.

CONCLUSION

This project had four very clear objectives:

1. To gain a clear understanding of what end and next users require from a soil eNose.

We have evidence that growers want to know about their soil, particularly biological diversity and activity, and some growers specifically want to know how their management practices affect the soil. We know that they want easily interpreted information but also the ability to access and analyse data in more detail. Growers are also prepared to dedicate time to learning how to use new technology when it will provide them with the information that they need. We have established a good basis for the further co-development of the eNose.

2. To develop and produce a prototype eNose using low-cost sensors that was able to detect gases emitted from soils.

We have produced a low-cost eNose device that can detect soil VOCs along with a housing for soil gas sampling.

3. To correlate the eNose response with gas emissions and microbial activity.

In laboratory trials we were able to show that the eNose signal varied under different conditions. We were also able to measure differences in microbial activity and VOC emissions as measured by GC-MS. This provides us with confidence that the eNose signal is measuring soil VOCs and it can detect differences in biological activity. The eNose was also able to detect whether legumes were nodulated or not in a simplified laboratory pot system.

4. To demonstrate the prototype eNose in the field.

The eNose was able to function in the field for at least six weeks and record data that varied in the way that we expected it to.

In conclusion, the eNose technology shows promise as a way to monitor soil biological activity and it may be possible to develop this further for specific contexts.

RECOMMENDATIONS

This project has shown that electronic nose technology has the potential to provide growers with the information that they want about their soil. There is, however, more work that needs to be done. The most important priorities for further investment are:

- greater understanding of the link between soil VOCs and soil health as measured by other parameters
- further engineering of the eNose to build a robust prototype. This should focus on power, communication and reducing the number of unique components
- testing of a greater range of sensors. Many more sensors are available over a range of costs (from <$5 to >$500) and some of them may be useful for soil
- market analysis to determine the best initial focus for commercialisation of the eNose. Given that different soil types produce different signals, it may be prudent to work in one system first before expanding into other systems
- the non-destructive detection of successful nodulation. This may be an easy first use of this technology, however, further work on the eNose is required to determine the parameters under which it can detect nodulation.

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APPENDIX A FURTHER IDEAS FROM GROWERS

Table A1: Other comments and ideas provided by growers that are currently out of scope

Device	Data collection
Mobile, broad spectrum (measures lot of things)	Send data back to computer
Multifunctional	Needs to relay data to mobile phone
Farmer friendly	Bluetooth compatible
Hand-held size or similar	App on phone to submit data
Can it go on a header?	Ability to print report as well as view online
Take photos at the same time	Record conditions at time of test
GPS data for mapping and repeat measurements over time	Collect date and GPS
Other ideas	
Should suggest how frequently to monitor	
Application add-ons or different subscription levels	
Support line	
Have permanent monitoring sites as a way to see seasonal changes	
Suggest test locations	