SRA Research Project
Final Report

Sugar Research Australia

Research Funding Unit

Cover page

SRA project number:	**NCA012**
SRA Project title:	**UV absorption as a tool for growers to benchmark humified organic carbon in soil**
Name(s) of the Research Organisation(s):	National Centre for Engineering in Agriculture (NCEA)
Principal Investigator's name(s), contact phone number, address and Email address:	Pam Pittaway Phone (w) 07 4631 2144 National Centre for Engineering in Agriculture University of Southern Queensland West St, Toowoomba Qld Aus 4350 Pam.Pittaway@usq.edu.au
A statement of confidentiality (if applicable):	n.a.

Body of the Report:

Executive Summary:

Growers do not have a simple, low-cost tool for benchmarking and monitoring soil health. Growers depend on experienced local agronomists to interpret conventional laboratory tests for Organic Carbon (OC), a key component of soil health. The commonly available laboratory methods for monitoring soil OC (wet oxidation and furnace induction) measure all of the different components of soil organic matter to varying degrees, and are insensitive to change in management practices over the shorter term (years to decades). Methods for selectively measuring different fractions of soil organic carbon have been developed (eg National Soil Carbon Analysis and Research Program), but measuring the more resilient soil humic substances costs $200 per sample, and requires sophisticated, expensive analytical equipment.

In this project, we investigated the feasibility of modifying well established methods for quantifying aquatic humic substances, for detecting soil humic substances. Soil humic substances are the dark coloured, organic compounds that stain the soil below the litter layer, distinguishing ‘topsoil’ from dirt. Soil humic substances are the by-products of organic matter repeatedly attacked by soil microbes, becoming more complex and resilient over time. Standard water quality methods for measuring dissolved organic carbon (DOC), aquatic humic substances (the fraction of DOC absorbing ultra violet light; $UV_{253.7}$), and specific UV absorbance ($UV_{253.7}$ divided by DOC) were adapted for measuring soil humic substances.

Different soil to water ratios, shaking times and the potential for other UV-absorbing elements to interfere with the test were investigated, to develop a reliable method for extracting DOC and measuring soil humic substances. Compost samples (the product of the accelerated microbial processing of organic matter), and soils with a history of repeat compost application were tested to determine if humus-like substances produced in weeks to months also absorbed $UV_{253.7}$, or if UV absorbance was specific for soil humic substances produced over years to decades.

Soil samples representing the major soil types located in the cane growing regions of Queensland were obtained from scientists with an extensive working knowledge of sugar cane soils (Dr Phil Moody and Dr Bernard Schroeder). The method developed for extracting and quantifying soil DOC and soil humic substances ($UV_{253.7}$ and SUVA) was validated as suitable for all soil textures and colours. The significant linear relationship between $UV_{253.7}$ and cation exchange capacity (CEC) suggests the UV Method can be used to monitor change in the nutrient holding capacity of soils (a key soil health property). Results also indicate the concentration of soil humic substances estimated using the UV Method may be associated with the capacity of some soils to resist soil acidification (pH buffering, another key soil health property).

A key outcome of our research was the development of a Laboratory Method for measuring $UV_{253.7}$, DOC and SUVA, and a Field Method measuring $UV_{253.7}$ absorbance only. The Field Method requires a level teaspoon of soil to be taken from bulked soil samples taken from within the dark-staining 'topsoil' zone of the vertical soil profile, for immediate shaking, filtration and UV testing using a portable spectrophotometer ($UV_{253.7}$). The purchase price of the field kit (about $3,000) should be recovered within one year if more than 300 tests at a unit cost of $10.00 are conducted. The estimated unit cost for the Laboratory UV Method is $50.

Growers attending the Mackay Soil Carbon workshop, and the Project Catalyst workshop were impressed with the simplicity and speed with which soil samples could be tested under field conditions. Increasingly, alternative management techniques and products claiming to improve soil health are being marketed to growers. Growers need soil health monitoring tools like the Field UV Method to test the merit of alternative products and management techniques for their farming system. Monitoring DOC and SUVA (Laboratory Method) also enables growers to distinguish between concentrations of rapidly recycled organic carbon, and the more recalcitrant soil humic substances that may attract carbon sequestration credits in the future.

The next steps to encourage adoption of the laboratory and Field UV Methods are:

1. Complete the NATA (technical note 17) reproducibility, repeatability and lowest order of detection specifications for an analytical test. Collaboration with other commercial testing laboratories will validate the utility of the UV Method as a commercial soil test.

2. Test sugar cane soil samples that have been fully characterized for yield, management history, soil health attributes and for carbon sequestration, to validate the utility of the UV Method as a soil health tool and for predicting carbon sequestration potential. Repeat sampling of field sites and stored samples that have been analysed in the past, will assist in detecting the rate of change in the concentration of soil humic substances.

3. Collaborate with managers of sugar cane industry yield databases to test whether a decline in the concentration of soil humic substances is associated with increasing soil acidification and yield decline.

Glossary of Terms and Abbreviations Used:

Aliphatic: linear chains of organic molecules with side chains, lacking the chemical ring structure of aromatic compounds.

Aromaticity (aromatic): chemical structure of organic compounds based on a six carbon ring, sharing carbon bonds. Aromatic compounds may contain one six carbon ring (eg ninhydrin), or many more complex ring combinations with side chains.

CEC: Cation Exchange Capacity, a measure of the ability of the soil to retain positively charged nutrients (cations) including calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na). In acidic soils, the positively charged ions hydrogen (H) and aluminium (Al) are also measured.

ECEC: Effective Cation Exchange Capacity is calculated by summing the cations removed with an extracting solution from soil particles, without fully testing cation retention. Soluble salts such as potassium in solution leads to an over-estimation of the CEC.

DOC: Dissolved Organic Carbon is defined as aggregates of organic carbon bound with other compounds which are small enough to pass through a 0.45 µm filter.

E2:E3 ratio: the amount of light at the specific wavelength of 253.7 nm that is absorbed by a soil solution that has been filtered through a 0.45 µm filter, divided by the amount of light absorbed at the specific wavelength of 365 nm.

E4:E6 ratio: the amount of light at the specific wavelength of 465 nm that is absorbed by a soil solution that has been filtered through a 0.45 µm filter, divided by the amount of light absorbed at the specific wavelength of 665 nm.

EC: Electrical Conductivity is a measure of the concentration of ions (positive and negative elements referred to as salts) in solution conducting an electric current.

Furnace Induction: Very high temperature combustion method for measuring OC.

Humic Substances: black to brown coloured complex organic molecules derived from leaf and bark litter, that stain the topsoil below the litter layer.

Humification: the microbially driven process of soil enzymes persistently attacking organic molecules, increasing the complexity and resilience of their chemical structure.

Labile: readily degraded, does not persist for long in soil.

NATA: National Australian Testing Authority overseeing the rigor and reliability of testing laboratories and testing methods.

nm: a nanometer is one millionth of a millimeter, and is the unit used to measure the wavelength of light.

NMR: Nuclear Magnetic Resonance is a sophisticated analytical technique that excites molecules in the test substance to differing degrees, enabling the composition of the substance to be determined. The equipment used for this method is too expensive for standard soil and water testing laboratories.

NCEA: National Centre for Engineering in Agriculture, a research institute which is part of the University of Southern Queensland, West St Toowoomba.

OC: Organic Carbon is the main building block and energy source used by aerobic organisms. In living and decaying tissue organic carbon is always bound to other elements, collectively referred to as Organic Matter.

rpm: revolutions per minute are the units used to describe how rapidly a rotating mixer or solid separator is turning.

SOM: Soil Organic Matter is the collective term for living and decaying organisms and substances in soil, made from organic carbon bound with other elements.

SHS (Soil Humic Substances): dark brown to black DOC produced by long-term microbial interaction with organic matter in soil. Soil humic substances are known to improve the CEC, water holding capacity and aggregate structure of soils.

Spectrophotometer: a scientific instrument generating light at specific wavelengths to test the ability of different compounds in solution to absorb or transmit light. Laboratory spectrophotometers produce wavelengths across the light spectrum from UV to infra-red. Field spectrophotometers most commonly generate only a very narrow spectrum or a specific light wavelength (eg $UV_{253.7}$ nm).

SUVA: Specific Ultra Violet Absorbance is the amount of light absorbed at the specific wavelength of 253.7 nm divided by the concentration of DOC in the sample.

TIC: Total Inorganic Carbon is measured as part of the procedure for measuring total organic carbon, using combustion of SOM to release the gas carbon dioxide (CO_2), which is quantified using infra red light. Inorganic (contains no organic carbon) substances including calcium carbonate (inorganic) also combust to produce CO_2, interfering with the quantification of TOC.

TOC: Total Organic Carbon is the measurement of organic carbon contained within an organic matter sample.

Transitional metal: metal elements such as iron (Fe) and copper (Cu) that exist in more than one charged form (eg ferric Fe^{3+}, or ferrous Fe^{2+}). Soils with a high concentration of iron are red in colour. Both iron and copper are elements required in trace amounts for plant nutrition.

µm: a micrometer or micron is one thousandth of a millimeter.

UV: Ultra violet light is the spectrum of light to the violet end, with a wavelength of less than about 350 nm (eg $UV_{253.7}$).

Wet Oxidation (WB): A method for measuring the concentration of organic carbon in soil, involving the addition of strong acid (wet oxidation). Walkley and Black (WB) developed one of the first methods commonly used in soil testing laboratories.

Background:

Issue 1: Organic carbon is a key component of soil health, but conventional test methods are generic and insensitive to change in the short term, or are very expensive.

Soil organic matter (SOM) is subdivided into animal or plant debris (particles > 2,000 µm), organic residues (particles > 53 µm and < 2,000 µm; particulate organic matter), soil biomass (0.1 – 2.0 µm; living and dead microbes), and humic substances (brown to black high molecular weight compounds < 53 µm; Rosell *et al.* 2001). Organic debris, residues and soil biomass may be recycled relatively rapidly, whereas the resilience of soil humic substances (SHS) increases over time, as microbial enzymes increase the chemical complexity of the compounds. Soil humic substances are associated with the soil health properties of water holding and nutrient holding capacity, pH buffering and aggregate stability (Krull *et al.* 2000).

The evaluation of SOM is important to primary producers, as the decline in organic C from historically cropped grain growing soils is associated with nutrient depletion, a decline in the nutrient and water holding capacity and aggregate stability of the soil, and an increase in bulk density (a decline in soil health; Dalal and Chan 2001). The association between sugarcane yield decline and SOM decline is less clear-cut (Skjemstad *et al.* 1999), presumably due to the very large seasonal fluctuation in rapidly recycled SOM associated with green cane harvesting and trash retention. The commonly available laboratory methods for monitoring soil organic C (wet oxidation and furnace induction; Rayment and Lyons 2011) measure all of the different components of SOM to varying degrees, and are therefore relatively insensitive to change in management practices over the shorter term (years to decades; Krull *et al.* 2000). More sophisticated laboratory methods based on photo-oxidation pre-treatment and Nuclear Magnetic Resonance (NMR) measure only extremely resilient SOM (Skjemstad *et al.* 1996), not the fraction that has declined from historically cropped grain growing soils (Dalal and Chan 2001).

Aim: To develop a detection method specific for soil humic substances that could be used as an index of soil health.

Issue 2: Growers do not have a simple, low-cost tool for benchmarking and monitoring soil health.

Increasingly, alternative farming methods and commercial products are being marketed to growers as improving soil health, often in the absence of an objective analysis of their merit (Quilty and Cattle 2011). What growers need is a low-cost soil test that will enable them to objectively monitor change in key soil indicators that are associated with nutrient retention, water holding capacity and aggregate stability. A test for quantifying soil humic substances (refractory dark-coloured heterogeneous organic compounds produced as a byproduct of microbial metabolism; Sutton and Sposito 2005) may have merit.

UV absorbance at the wavelength of 253.7 nm ($UV_{253.7}$) is an accepted method for quantifying aquatic humic substances (Pittaway and van den Ancker 2010), and could be adapted for measuring soil humic substances. Total Dissolved Organic Carbon (DOC) is also routinely measured as a water quality parameter (Clesceri *et al*. 2005), as is Specific UV Absorbance or SUVA ($UV_{253.7}$ divided by DOC; Weishaar *et al.* 2003). Dissolved organic carbon is defined as organic particles that pass through a 0.45µm filter (Sulzberger and

Durisch-Kaiser 2009), with concentrations in solution quantified using catalytic oxidation at 680 °C and near-infrared detection of CO_2 gas (Clesceri *et al.* 2005).

Aquatic, fresh water DOM includes coloured (chromophoric), hydrophobic, high molecular weight compounds that strongly absorb UV light (Sulzberger and Durisch-Kaiser 2009). Exposure to UV radiation at the water surface increases the chemical complexity and resilience of these yellow to black, high molecular weight compounds, referred to as humic substances (Hayes and Clapp 2001). The composition of aquatic humic substances includes carboxylic acids, alcohols, aromatic rings and aliphatic chains (Samios *et al.* 2007), with the benzenoid and electronic transfer bands in aromatic and carbonyl functional groups responsible for UV absorbance (Weishaar *et al.* 2003). The precursors to these compounds are lignins, tannins and quinones present in decaying plant residues in the litter layer of soil (Kalbitz and Kaiser 2008). The dark stain diffusing below the litter layer reflects the limited mobility of soil humic substances in the soil solution, defining the zone commonly referred to as ‘topsoil’.

Aim: To develop a low-cost method for monitoring soil humic substances based on their capacity to absorb ultraviolet light.

Issue 3: Is UV Absorbance Sufficiently Specific and Sensitive for Quantifying Resilient Humic Substances in Soil?

Methods for extracting DOC from soil have been published (eg Kalbitz *et al.* 2003, Akagi *et al.* 2007, Nkhili *et al.* 2012), but the ratio of soil to water, shaking time and filtration method vary substantially. Published interpretations of the composition of DOC also vary, with some authors considering it predominantly labile organic carbon (eg Akagi *et al.* 2007), with others reporting DOC half-lives of two to five days for the labile fraction to months to years for the humified fraction (Kalbitz *et al.* 2003). The formation of organo-mineral complexes may further increase the resilience of DOC from years to decades (Kalbitz and Kaiser 2008). The specificity of UV absorbance at 253.7 nm is also in doubt as other organic and inorganic compounds with covalent or transitional bonds also absorb strongly in the UV region (Weishaar *et al.* 2003), and other researchers use wavelengths of 280 nm and beyond as indicators of aromaticity (Yu-Ping Chin *et al.* 1994).

More recent research indicates UV absorbance at wavelengths above 350 nm may be associated with intramolecular charge-transfer reactions, not specific absorbance by discrete chromophores (Del Vecchio and Blough 2004). Thus absorbance at 253.7 nm, the wavelength specified for detecting aquatic humic substances, should indicate the concentration of humic substances in soil samples. In this project, a range of soil types varying in colour and texture were mixed with water at two different ratios for six different shaking times, to develop a laboratory-based protocol for quantifying the concentration of soil humic substances. Standard concentrations of the ions and functional groups most likely to interfere with absorbance at 253.7 nm (Crumpton *et al.* 1992) were also prepared to establish threshold concentrations for potential interference.

The specificity of UV absorbance for more resilient humified DOC was tested by comparing change in $UV_{253.7}$, DOC and $SUVA_{253.7}$, as well as the ratio of absorbance at 253.7 and 365 (E2:E3 ratio used as an index of the molecular weight of aquatic humic substances; Peuravuori and Pihlaja 1997) for the 17 different soil types. The stimulation of microbial metabolism associated with drying and re-wetting soil samples (Mikha *et al.* 2005) was used to validate the sensitivity of $UV_{253.7}$ as an index of soil humic substances. The feasibility of developing a field method for quantifying soil humic substances was also investigated, using the portable, single wavelength 253.7 nm spectrophotometers commercially developed for water quality testing. The utility of field method was tested on soil samples provided by attendees at a field demonstration and a workshop in Mackay.

Aim: To ensure the UV Method is suitable for all soil textures and colours, and is specific for detecting change in the concentration of soil humic substances associated with different management practices.

References Cited

Akagi J, Zsolnay A, Bastida F (2007) Quantity and spectroscopic properties of soil dissolved organic matter (DOM) as a function of soil sample treatments: Air-drying and pre-incubation. *Chemosphere* **69**, 1040-1046.

Clesceri L, Rice E, Greenberg A, Eaton A, Water Environment Federation US, American Water Works Association (2005) Standard methods for Examination of Water and Wastewater: 21st edition. American Public Health Association USA.

Crumpton W, Isenhart T, Mitchell P (1992) Nitrate organic nitrogen analyses with second-derivative spectroscopy. *Limnology and Oceanography* **37**, 907-913

Dalal R, Chan K (2001) Soil organic matter in rainfed cropping systems of the Australian cereal belt. *Australian Journal of Soil Research* **39**, 435-464.

Del Vecchio R, Blough N (2004) On the origin of the optical properties of humic substances. *Environmental Science and Technology* **38**, 3685-3891

Hayes M, Clapp C (2001) Humic substances: considerations of compositions, aspects of structure, and environmental influences. *Soil Science* **166**, 723-737.

Kalbitz K, Kaiser K (2008) Contribution of dissolved organic matter to carbon storage in forest mineral soils. *Journal of Plant Nutrition and Soil Science* **171**, 52-60.

Kalbitz K, Schmerwitz J, Schwesig D, Matzner E (2003) Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma* **113**, 273-291.

Krull E, Skjemstad J, Baldock J (2000) Functions of soil organic matter and the effect on soil properties. GRDC Project CSO 00029 Residue management, soil organic carbon and crop performance, Grains Research and Development Corporation.

Mikha M, Rice C, Milliken G (2005) Carbon and nitrogen mineralization as affected by drying and wetting cycles. *Soil Biology and Biochemistry* **37**, 339-347.

Nkhili E, Guyot G, Vassal N, Richard C (2012) Extractability of water-soluble soil organic matter as monitored by spectroscopic and chromatographic analyses. *Environmental Science and Pollution Research* **19**, 2400-2407.

Peuravuori J, Pihlaja K (1997) Molecular size distribution and spectroscopic properties of aquatic humic substances. *Analytica Chimica Acta* **337**, 133-149

Pittaway P, van den Ancker T (2010) Properties of natural microlayers on Australian freshwater storages and their potential to interact with artificial monolayers. *Marine and Freshwater Research*. **61**, 1083-1091.

Quilty J, Cattle S (2011) Use and understanding of organic amendments in Australian agriculture: a review. *Soil Research* **49**, 1-26.

Rayment G, Lyons D (2011) 'Soil Chemical Methods Australasia'. (CSIRO Publishing Collingwood: Australia).

Rosell R, Gasparoni J, Galantini J (2001) Soil Organic Matter Evaluation. In 'Assessment methods for soil carbon'. (Ed R Lal) pp 311-322. (Lewis Publishers Boca Raton: Florida).

Samios S, Lekkas T, Nikolaou A, Golfinopoulos S. (2007). Structural investigations of aquatic humic substances from different watersheds. *Desalination* **210**, 125-137.

Skjemstad J, Clarke P. Taylor J, Oades J, McClure S (1996) The chemistry and nature of protected carbon in soil. *Australian Journal of Soil Research* **34**, 251-271.

Skjemstad J, Taylor J, Janik L, Marvanek S (1999) Soil organic carbon dynamics under long-term sugar cane monoculture. *Australian Journal of Soil Research* **37,** 151-164.

Sulzberger B, Durisch-Kaiser E (2009) Chemical characterization of dissolved organic matter (DOM): A prerequisite for understanding UV-induced changes of DOM absorption properties and bioavailability. *Aquatic Science* **71**, 104-126.

Sutton R, Sposito G (2005) Molecular structure in soil humic substances: the new view. *Environmental Science and Technology* **39**, 9009-9015.

Weishaar J, Aiken G, Bergamaschi B, Fram M, Fujii R, Mopper K (2003) Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science and Technology* **37,** 4702-4708.

Yu-Ping Chin, Aiken G, O'Loughlin E (1994) Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environmental Science and Technology* **28**, 1853-1858

Objectives:

An outline of project objectives and the extent to which these objectives have been met is given below.

1. **Ensure different biological, physical and chemical properties of soil do not affect the accuracy, reliability and reproducibility of the test.**

   This objective was achieved. Pairs of grain growing soils categorized on their particle size distribution as clay, silt and sand, organic matter content and EC (refer Table 2 in Appendix 1, Milestone 2) were selected from the NCEA soil collection and prepared with either 5 g of soil in 25 mLs of distilled water or 2 g in 40 mLs (for more details refer to Milestone 2 in Appendix 1). Six replicate samples were shaken for 0, 2, 20, 60, 240 or 960 minutes on a clock-face shaker at 15 rpm prior to centrifugation at 2,000 rpm for 5 minutes and filtering the supernatant though a 0.45 µm glass fibre filter paper. Results indicate colloidal interference in the 5 g in 25 mL soil solutions interfered with the recovery of DOC (**Figure 1**), UV light absorbance (**Figure 2**), and E2:E3 ratio (**Figure 3**). All future extractions were conducted using the 2g in 40 mL soil solution, shaken for 60 minutes. This method was suitable for clay, silt and sand soil types, and was not affected by differences in the electrical conductivity (salt concentration) of the soil.

   The impact of differences in soil microbial activity and amending soil with humus-like organic carbon on the UV test was investigated by exposing two garden beds of the same soil type to wetting and drying conditions known to stimulate microbial activity. One of the garden beds was mulched with hay only, the other had repeat applications of compost applied over a period of 10 years (refer Figures 8 to 10 in Milestone 2 Appendix 1;and **Objective 4** below). Results indicate UV absorbance is specific for soil humic substances. Humus-like compost that resists microbial attack, and rapidly recycled (microbially degraded) organic carbon do not absorb light in the UV region. Results for 10 sugar cane soil samples varying in colour, organic carbon content and texture further established the UV method was suitable for all soil types (refer figures 1 to 3 in Milestone 3, Appendix 2).

2. **Establish if the UV method can be used as an index of key soil health properties such as aggregate stability, mineralisable nitrogen, yield decline, total water holding and nutrient holding capacity.**

   The soil health characterization of ten sugar cane soils provided by Drs Phil Moody and Bernard Schroeder for this project lacked the detail of the ‘Healthy Soils’ samples originally committed to the project., limiting our ability to correlate UV Method results with aggregate stability, mineralisable nitrogen, yield and total water holding capacity. As part of the project the ten soil samples were analysed for CEC. Pearson correlation and linear regression indicated UV absorbance was positively correlated with CEC (**Figure 4**), whereas DOC, SUVA, chloride, furnace induction OC and wet oxidation OC were not. CEC was also correlated with chloride, indicating soluble salts present in the solution may be affecting results. UV absorbance was not correlated with chloride, validating the specificity of UV absorbance for soil humic substances which are known to improve the cation exchange capacity of soils, improving soil health.

Figure 1: Effect of shaking time on the recovery of DOC from soil solutions. Samples 6a, 71a, 1a and 12 b were prepared as 5 g in 25 mL. Samples 7a and 7b were prepared as 2g in 40 mL. The recovery of DOC in the 2g in 40 mL solutions was less affected by shaking duration, and comparable with the 5 g in 25 mL solutions even though the amount of soil was less and the water dilution factor greater.

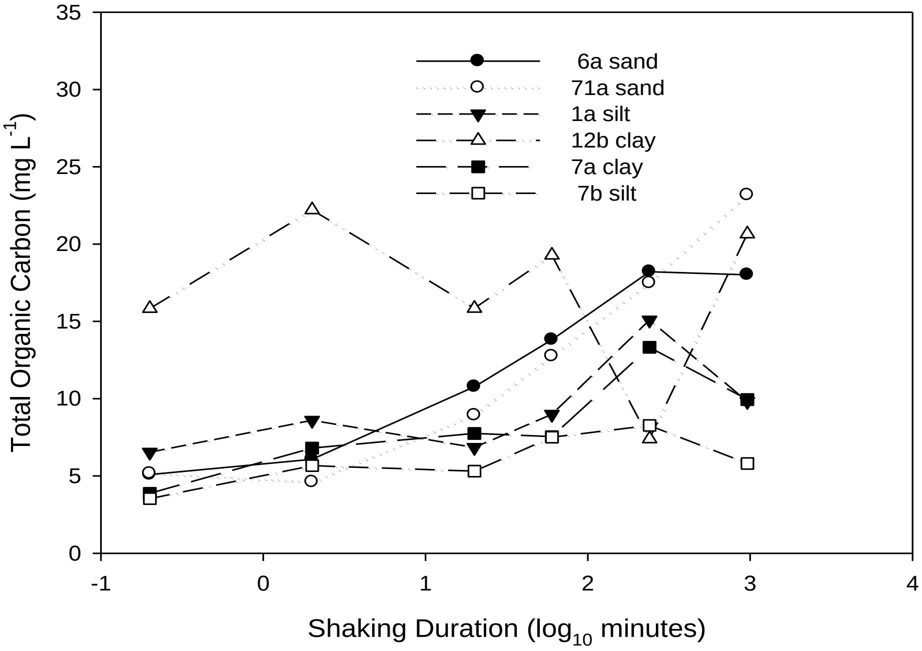


Figure 2: Effect of shaking time on UV absorbance of soil solutions varying in texture. The amount of light absorbed in the 2g in 40 mL samples (7a and 7b) increased linearly with shaking duration, whereas a greater duration of shaking was required to increase UV absorbance for the 5g in 25 mL samples.

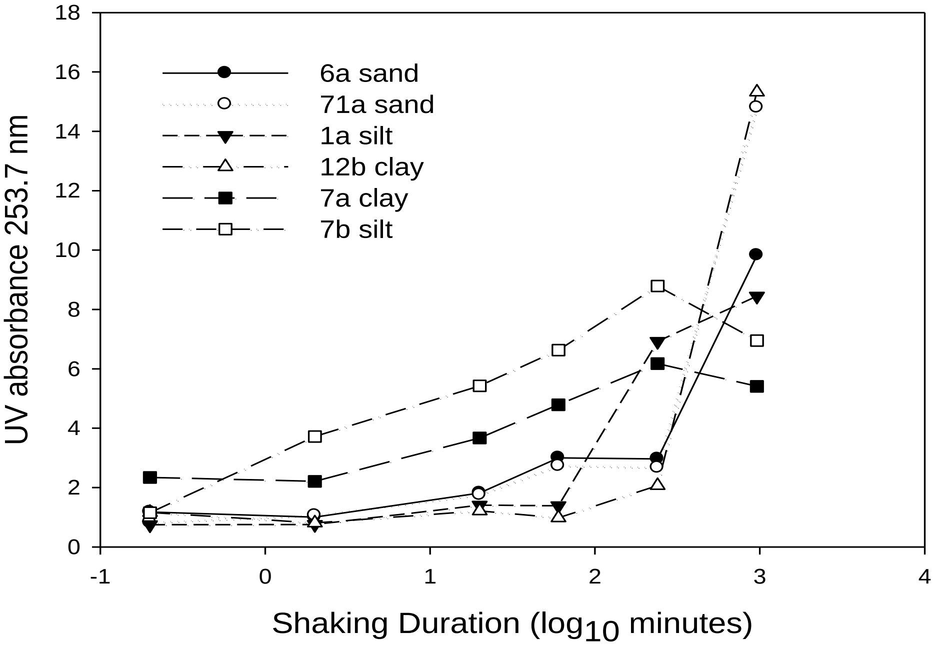
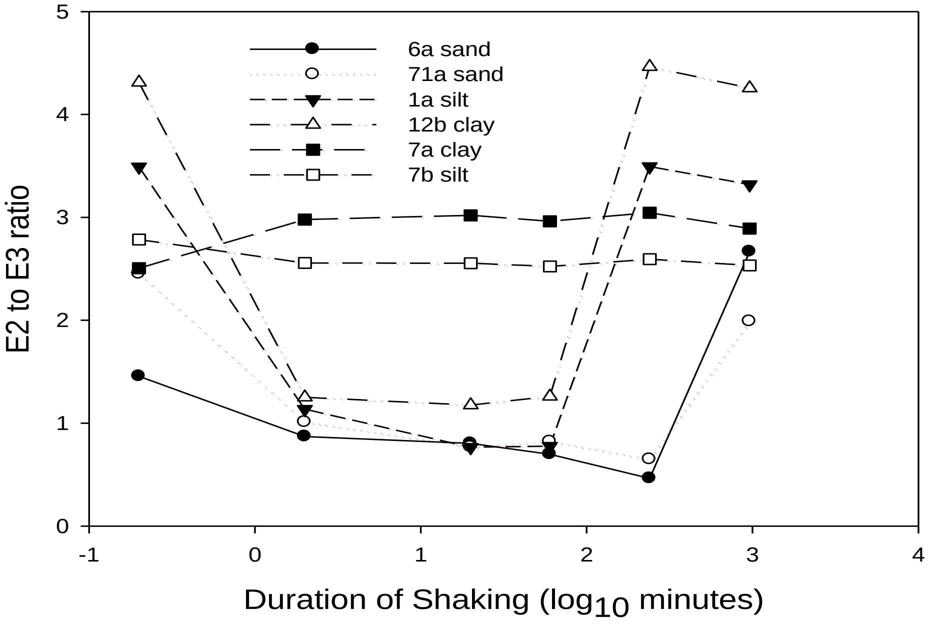


Figure 3: Effect of shaking time on the E2:E3 ratio of soil solutions prepared as 5g in 25 mL (6a, 71a, 1a, 12b) or 2 g in 40 mL (7a and 7b). Results for the more dilute 2g in 40 mL solutions are more consistent across all shaking times. The E2:E3 ratio is inversely proportional to molecular size, with results suggesting a longer shaking time is required to extract smaller molecules from the more concentrated 5g in 25 mL soil solution.



3. **Document the testing procedure, limits of quantitation and limits of uncertainty required for the method to be used by scientists and by commercial testing laboratories.**

The testing procedure has been documented (refer Output section 1 for complete details):

- 2g of air-dried soil sieved through a 2 mm diameter screen mixed with 40 mls of distilled water, shaken end over end at 15 rpm for 60 minutes prior to centrifugation at 2,000 rpm for 5 minutes.
- Filtration through a 0.45 m glass fibre filter paper .
- Record absorbance at $UV_{253.7}$ and at 365 nm using a spectrophotometer, DOC concentration using a combustion infrared analyser.
- calculate $SUVA_{253.7}$ and the E2:E3 ratio.

The procedure for specifying the limits of quantitation involves reporting $UV_{253.7}$, DOC, $SUVA_{253.7}$ and E2: E3 for a dilution series of the compound ninhydrin (**Figure 4**). Ninhydrin is an aromatic compound with a carbonyl functional group, with no nitrogen, carbonate or transitional metal functional groups likely to interfere with light absorbance at 253.7 nm. Results for dilution series of Na_2HPO_4, $FeCl_3$, $NaHCO_3$, and $CuSO_4$ (**Figures 5 and 6**, and Appendix 2 for further information) were used to establish threshold concentration limits above which interference at 253.7 nm may affect the accuracy of the method.

Figure 4: Chemical structure of the organic compound ninhydrin. The double bonds in the aromatic ring and pseudo-ring, and the double bonds in the carbonyl bonds strongly absorb light in the UV region.

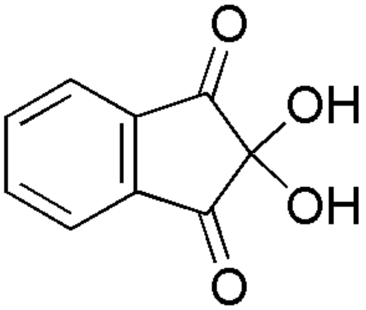


Figure 5: Absorbance spectra for standard concentrations of compounds of the transitional metals iron (Fe) and copper (Cu). Both compounds absorbed strongly at 253.7 nm. Elemental concentrations above 12.5% for Fe and above 7.5% for Cu in soil (air-dry mass basis) are likely to interfere with the UV Method.

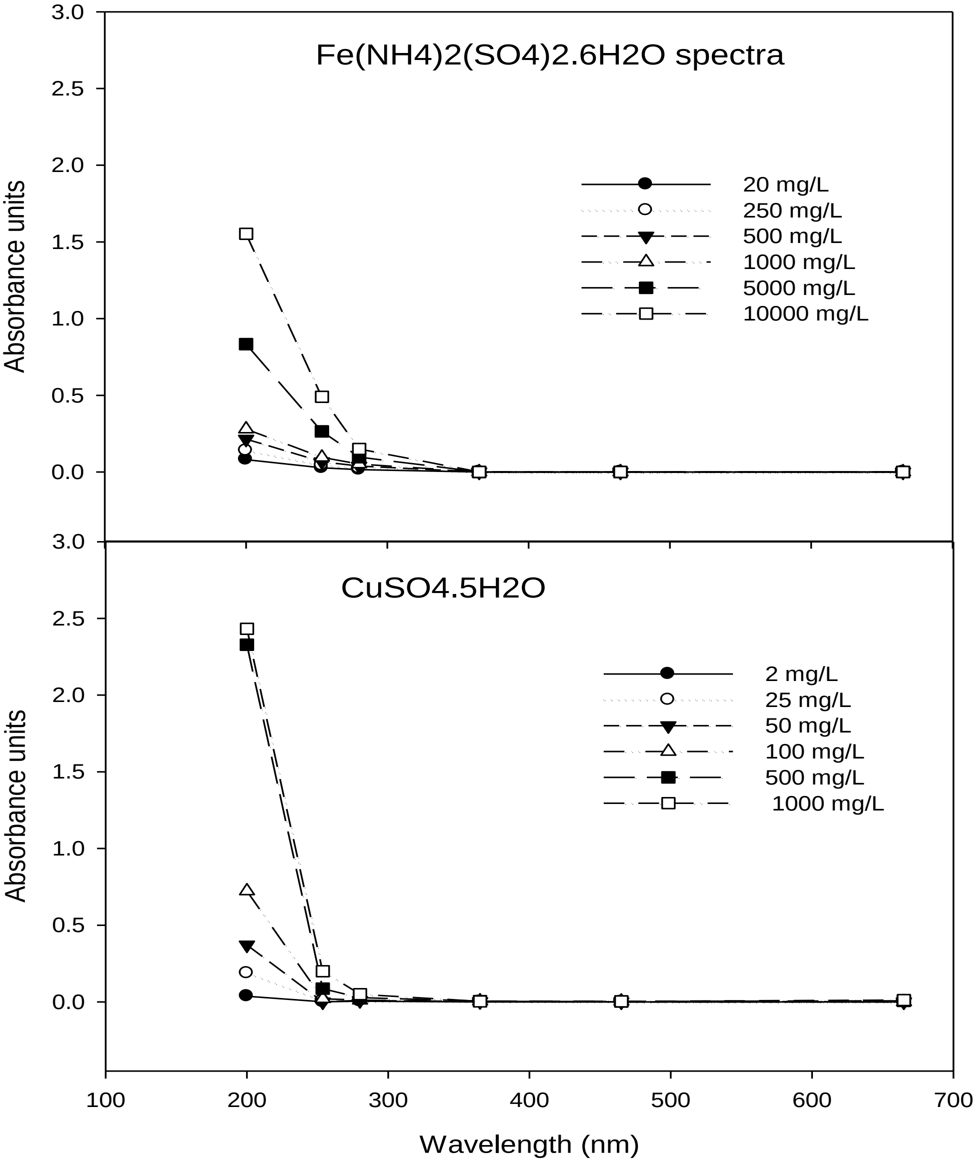
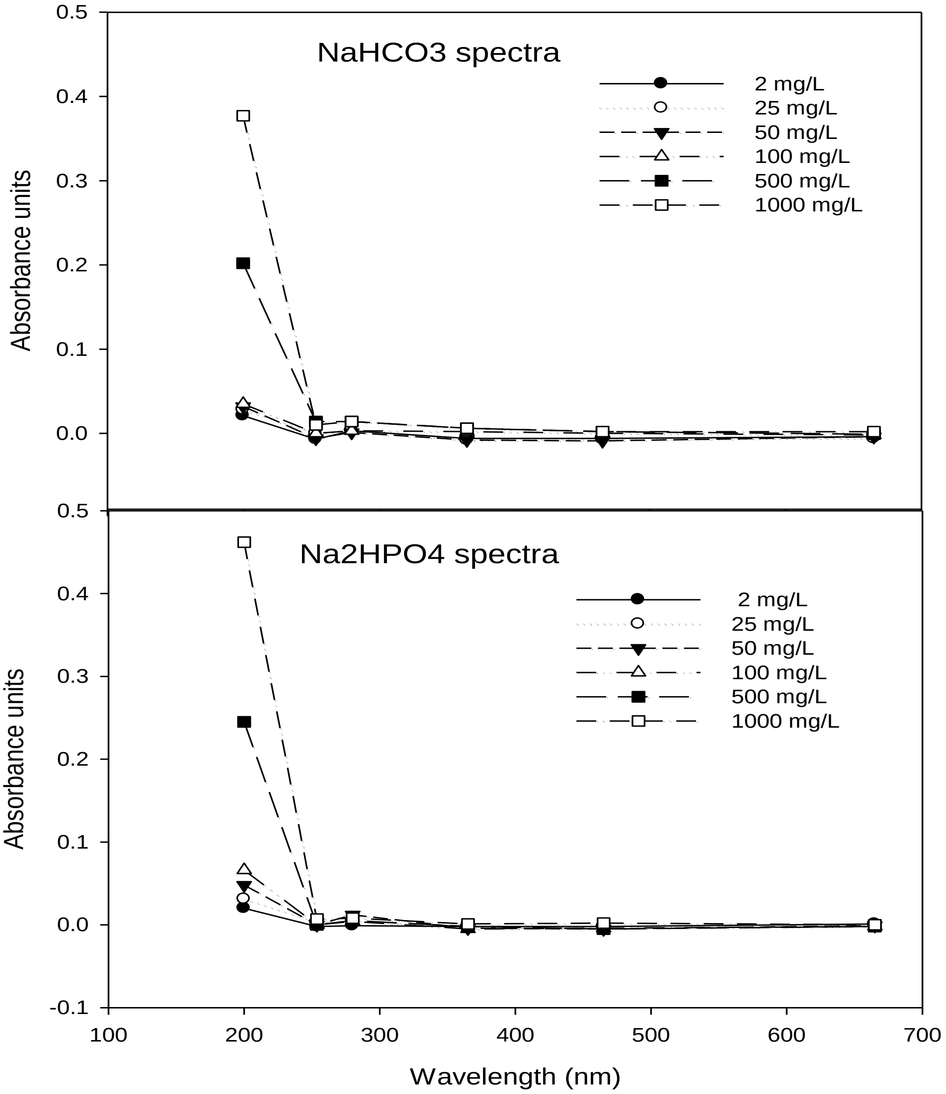


Figure 6: Absorbance spectra for standard concentrations of compounds containing carbonate ($NaHCO_3$) and phosphate (Na_2PO_4) bonds likely to absorb in the UV region. Neither compound absorbed very strongly at the wavelength of 253.7 nm. The presence of these compounds in soil even at high concentrations is unlikely to interfere with the UV Method.



4. **Work with Qld scientists and cane growers to document the value of this test in monitoring the impact of different management practices on soil health, and as a Method in the Carbon Farming Initiative.**

Ian Grant of Agricultural Chemistry Pty Ltd assisted in soil testing and in interpreting our results. Results for several compost samples and the paired, sodic garden soils with and without a history of compost amendment confirmed UV absorbance is a property of more resilient, humified soil organic carbon, and not humus-like compost. SUVA readings indicate change in UV absorbance of soils subjected to wetting and drying is due to the reduction in labile DOC depleted by enhanced microbial activity (**Figures 7 and 8**, and Appendix 2 for further information).

Figure 7: SUVA values for two garden beds on the same contour 100 m apart on a sodic soil type. Compost was applied every 3 to 5 years to one (D), with mulch only applied to the other (L). Four containers were used to capillary wet all samples for 48hr, with the water reservoir 15 mm below the soil. Two containers remained wet for the duration (wet), and two were dried for 12 days before re-wetting (w&d). The microbial flush induced by the rapid re-wetting reduced the concentration of labile organic carbon in the filtrate, increasing the proportion of DOC absorbing UV light (higher SUVA). The compost-amended D soil was less affected than the L soil.

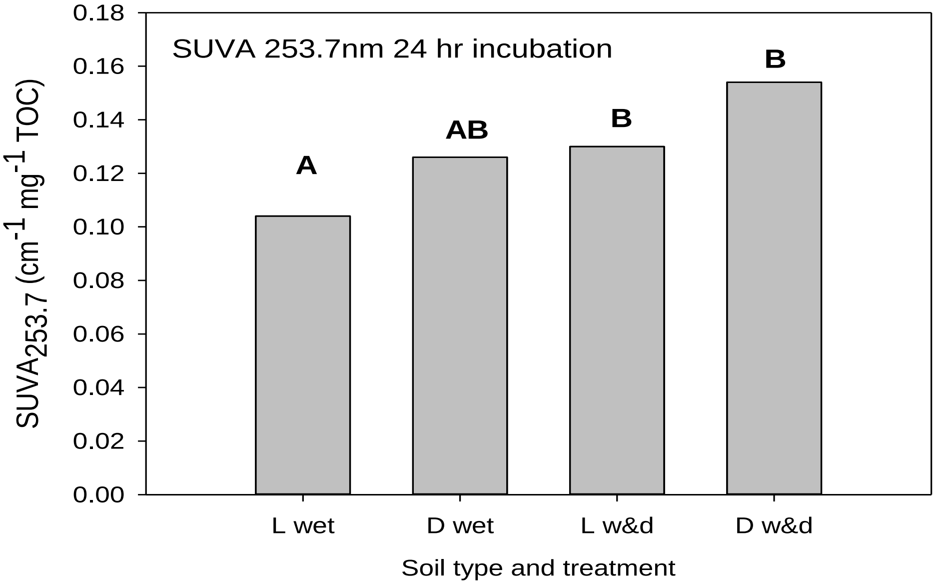
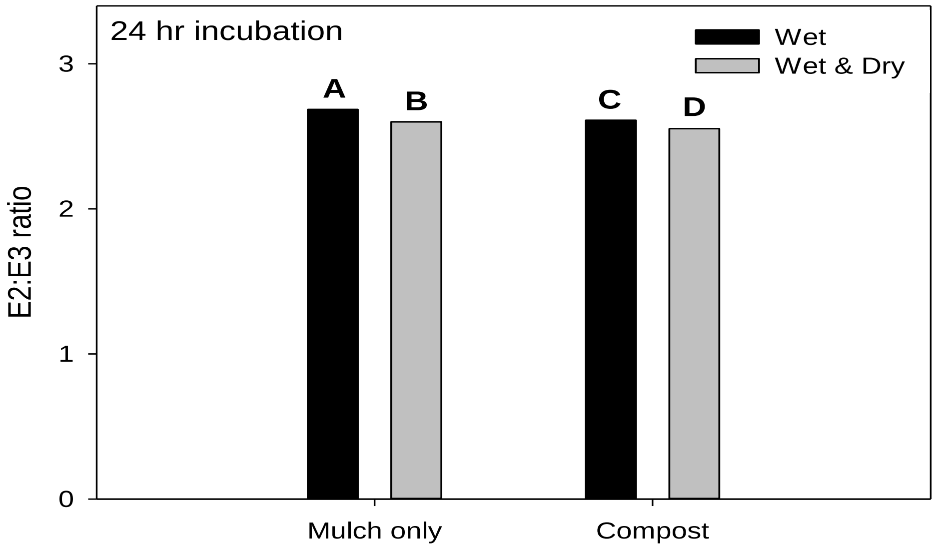


Figure 8: Change in the molecular size of soil humic substances in the garden bed soil samples continuously watered (wet), or exposed to a wet/dry cycle (Wet & Dry). The E2:E3 ratio is inversely proportional to molecular size, indicating for both the mulch only (L), and the compost-amended (D) soil, inducing a microbial flush increased the proportion of larger organic compounds in the DOC sample.



The ten sugar cane soils provided by Dr Phil Moody and Dr Bernard Schroeder were not as fully characterized as the ‘Healthy Soils’ soil samples originally committed to the project, so the utility of the UV test as a Method in the Carbon Farming Initiative could not be validated.

At the Mackay Soil Carbon field afternoon and workshop (25 & 26/11/2013) cane growers submitted soil samples for UV analysis. At the field event, three cane blocks soils treated with 37 t/ha compost only, inorganic fertilizer only or 50:50 for three years (SRDC project GGIP GGP034) were sampled. Results for the Laboratory UV Method for these soil treatments and the compost used for testing ion interference indicate compost samples do not absorb $UV_{253.7}$ as strongly as some soil types. The humus-like DOC produced during the curing phase of composting lacks the aromaticity of the much older, more complex soil humic substances (**Table 1**, and Appendix 3 for further information). Results for the sodic garden bed soil comparison (**Figures 7 and 8** and Table 1) suggest further humification after soil application is required before $UV_{253.7}$ absorbance increases in compost-amended soils.

Table 1: Summary of results for compost samples and compost-amended soils tested using the UV Lab Method. ProSoil compost is a cured compost produced from millmud and bagasse. D and L garden bed soils are those referred to in **Figures 7 and 8**. The D Hunter compost was submitted for testing at the Mackay Workshop.

The Walker trial was 37 t/ha of compost applied over 3 years (Walker plus compost), unamended cane soil treated with inorganic fertilizer only (Walker – compost), and a 50:50 compost and fertilizer treatment (Walker +50% compost). Compost $UV_{253.7}$ and SUVA values are lower than soil values, indicating the DOC is humus-like, lacking the aromatic bonds responsible for absorbing UV in soil humic substances. Compost application to the D garden soil (D soil + compost) diluted the SUVA value relative to the mulch only treatment (L soil mulch only). No increase in DOC was evident in the Walker trial, suggesting soil picked up during windrow turning and forming operations may have contaminated this compost.

Soil type	$UV_{253.7\ nm}$ abs. units	E2:E3 ratio	DOC mg/L	SUVA (mg/L/m)	% water in sample
ProSoil compost	0.43	3.86	10.2	5.0	56
D soil + compost	**0.99**	**2.63**	**11.03**	**8.98**	**20**
L soil mulch only	0.97	2.45	8.59	10.97	4
D Hunter compost	**1.92**	**2.13**	**8.85**	**21.7**	**13**
Hunter org bananas	3.08	2.1	6.4	48.4	7.36
Walker + compost	**2.65**	**2.42**	**7.00**	**37.93**	**29**
Walker – compost	2.53	2.39	6.68	37.99	29
Walker +50% compost	**1.73**	**2.09**	**5.71**	**30.33**	**35**

Uncultivated, slashed headland soil samples had the highest UV and SUVA readings ($UV_{253.7}$ of 4.2 and SUVA of 67.2, refer Appendix 3), suggesting they could be used for benchmarking concentrations of soil humic substances in specific cane growing soil types within local regions. Feedback from participants at the workshop, from Dr Bernard Schroeder (Principal Scientist SRA) and from Ian Grant (Principal of Agricultural Chemistry Pty. Ltd.) indicates the UV Method has merit as a soil health monitoring test.

5. **Develop a tool kit for using this procedure as a field based monitoring tool**.

A tool kit based on a volumetric soil sample taken under field conditions has been developed. Portable $UV_{253.7}$ spectrophotometers sold commercially for water quality testing are readily available for purchase by industry, grower or catchment groups interested in benchmarking soil humic substances as an index of soil health. The tool kit consists of :

- a portable spectrophotometer measuring at the wavelength of 253.7 nm (Real UVT portable spectrophotometer, developed for water quality testing, Australian supplier is ThermoFischer).
- medicine measuring cup for 40 mLs of distilled or reverse osmosis water.
- a standard kitchen measuring teaspoon.
- 50-60 mL cylinders with lids for shaking the x1 level teaspoon of soil with the 40 mLs of water.
- a 2 minute timer.
- heavy duty (suitable for Total Dissolved Solids testing) glass fibre filter papers with a nominal pore size of 0.45 µm (eg Advantec GB-140).
- a filter holder to support the filter paper and the syringe ‘pump’ (eg Advantec PP-47 polypropylene filter holder).
- a 60 mL hypodermic syringe pump (**Figure 9**).

All of these items are commercially available through pharmacies, supermarkets, or through scientific supply companies. The initial outlay for all of the equipment is about $3,000. For use in the field access to a power supply is required, and a flat surface or table to support the equipment during testing (**Figure 10**). Moist soils can be used as the sampling procedure is volumetric rather than mass based, and does not require pre-screening. Experience in the field with clay soils indicates the purchase of a second filter holder to screen out larger particles before filtering through 0.45 µm (eg Advantec GD 120 -047 nominal pore size 0.9 µm) may overcome the clogging associated with clay fines.

Figure 9: Components of the field UV method testing kit. From front left to right, 45 µm glass fibre filter paper discs, 40 mL measuring cup, filter disc holder, one teaspoon measure, one capped plastic container for shaking the solution, one 60 mL syringe for forcing the liquid through the filter, and a portable UV spectrophotometer is at the rear. Two capped vials for filtering the soil solution have not been included.

Figure 10: Field testing at a Project Catalyst Cane Grower Field Day on the property of Lou and Betty Raiteri, Proserpine on 24/2/2014.

Methodology:

1. **Determine how particle size, clay content and EC affect the reliability and reproducibility of DOC extraction from soil and UV absorbance using a spectrophotometer.**

The NCEA reference sample collection (characterised for pH, EC, particle size grades, WB OC, ECEC; refer Appendix 1) was used to select x 3 representative clay, silt and sand soils with low and high EC. Soil solutions were prepared using either 5 g of soil in 25 mLs of distilled water (soil to water ratio of 1:5) or 2 g in 40 mLs (ratio of 1:20). Six replicate samples were shaken for 0, 2, 20, 60, 240 or 960 minutes on a clock-face shaker at 15 rpm prior to centrifugation at 2,000 rpm for 5 minutes and filtering the supernatant though a 0.45 µm glass fibre filter paper.

Wide diameter, glass fibre filter paper was selected over smaller membrane filters as aquatic humic substances pass through glass fibre filter paper with minimal change to their chemical structure (Pittaway and van den Ancker 2010), and the glass fibre filters can be purchased in thicker, more robust filters that are designed to concentrate dissolved solids. Clay solids tend to blind the filters more readily, requiring the centrifugation step to minimize filter blinding.

UV absorbance and results for DOC, SUVA and E2 to E3 ratio were used to validate the utility of the test for soils of different texture and salinity, and to establish the most repeatable soil to water ratio and shaking duration. DOC was determined using a Shimadzu TOC analyser heated to 680 C calibrated with potassium hydrogen phthalate for TOC – C standards and sodium hydrogen carbonate and sodium carbonate TIC – C standards at concentrations ranging from 25 mg/L to 100 mg/L. UV absorbance was measured on a Jenway spectrophotometer with a 1 cm light path, reading absorbance at 200, 224, 253.7, 280, 365, 465, 500 and 665 nm wavelengths. The results were also used to establish if minimal, 2 minute shaking suitable for a field-based method, produced reproducible results.

The output was validating the UV Method is appropriate for clay, silt and sandy soils. Selecting the more dilute combination of 2g of sieved, air-dry soil added to 40 mLs of distilled or reverse osmosis water overcame colloidal interference that was evident in the 5g in 25 mL solutions (refer **Figures 1 to 3** and Appendix 1). Statistical analyses using median values and a one-way analysis of variance on ranks indicated there was no difference between a 2 minute, 20 minute and 60 minute shaking duration (refer Appendix 1). For the laboratory method, a duration of 1 hour was selected as this provides technical staff with a sufficient length of time to undertake other tasks whilst shaking occurs. For the field method, a duration of 2 minutes rapid hand shaking was selected (**Figure 11**).

Figure 11: rapid 2-minute hand shaking of the level teaspoon of soil in 40 mLs of distilled water, provided by a volunteer at the Project Catalyst field day held on the property of Lou and Betty Raiteri, Proserpine on 24/2/2014.

2. **Determine if soil colour and wet/dry cycles affect the reliability and reproducibility of the test.**

Drs Phil Moody and Bernard Schroeder provided ten sugar cane soils to validate if the UV Method is applicable to soils of different colour and texture. Standards of 2, 25, 50, 100 and 500 mg/L Na_2HPO_4, $NaHCO_3$, $Fe(NH_4)_2(SO4)_2.6H_2O$ and $CuSO_4.6H_2O$ were tested for $UV_{253.7}$ absorbance to establish threshold concentrations likely to interfere with the UV method (refer **Figures 5 and 6** and Appendix 2). The potential for these compounds to interfere with absorbance by soil humic substances was tested by adding standard concentrations of the compounds absorbing most strongly at 253.7 nm to 2g samples of air-dried, cured sugar milling compost. The Laboratory UV method was used to compare results for compost filtrates with and without the potentially interfering compounds.

The output from this research is that only red soils with iron (Fe) concentrations exceeding 12.5 % (mass basis) are likely to interfere with the UV Method. Phosphates, carbonates and copper ions are unlikely to be present in natural and fertilized soils at rates sufficiently high enough to interfere with the UV Method.

The paired sodic garden soils with and without compost amendment were used to establish if $UV_{253.7}$ was specific for soil humic substances that resist microbial attack. During the curing phase of composting humus-like compounds are produced, as microbial enzymes interact with recalcitrant organic compounds, increasing their chemical complexity. The composts added to the D garden soil shared the soil humus characteristic of improving soil water holding capacity and increasing the CEC of the amended soil (**Table 2**), but lacked the chemical complexity of aromatic compounds that are known to strongly absorb UV light.

Table 2: Humus-like properties (high water % and CEC) of a cured, sugar milling compost (ProSoil) and the change in properties of a sodic garden soil (D soil) amended with two different composts over a 10 year period. The unamended soil 100 m away on the same contour (L soil) had lower CEC, water %, wet oxidation OC % and DOC values than the D soil. However, the UV absorbance values are the same, as cured compost lacks the chemical complexity of soil humic substances and does not absorb UV light to the same extent. The SUVA values for the compost and the compost-amended D soil are therefore less than the unamended L soil.

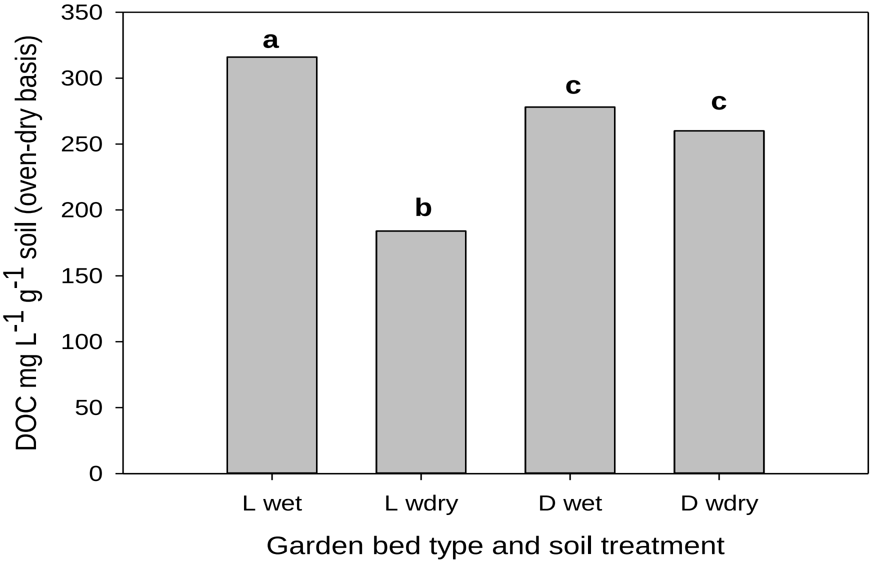
Soil type	Wet ox OC %	Water %	CEC mEq/100g	DOC mg/L	$UV_{253.7}$ abs. units	SUVA mg/L/m
ProSoil compost	4.7	56	34	10.2	0.43	5.0
D soil + compost	**2.8**	**20**	**14**	**11.0**	**0.99**	**8.98**
L soil mulch only	1.7	4	6	8.6	0.97	10.97

Humus-like organic compounds in cured compost are more linear in structure (aliphatic), lacking the aromatic ring structure characteristic of soil humic substances. This is reflected in the lower SUVA values for the ProSoil compost and the compost-amended D soil (**Table 2**). The apparent contradictory results for the D Hunter compost (**Table 1**), and the ProSoil compost and the compost-amended D soil can be explained by excess soil picked up during compost turning and windrowing operations contaminating the D Hunter compost. The UV absorbing capacity of most soils is higher than the absorbance recorded for cured composts known to contain minimal soil (ProSoil compost in **Tables 1 and 2**). These results highlight UV absorbance at the wavelength of 253.7 nm is selective for soil humic substances.

Humic substances have a residence time in soil of decades to centuries. Lignins, tannins, waxes and other complex organic compounds in leaf and bark litter degrade slowly over time, as soil animals and microbes utilize them as food. Soil enzymes continually attack these compounds, transforming them into humic substances. Composting is designed to speed up humification, with volume reductions of 30 to 50% in windrows indicating the concentration of labile organic carbon utilized by compost microbes. The organic carbon remaining in cured compost is humus-like, but is not as chemically complex as soil humic substances.

Microbial stimulation induced by the wet/dry treatment significantly reduced DOC concentrations in the L and D garden soils relative to the continuously capillary watered treatments (**Figure 12**, and Appendix 2). In the L filtrate, the loss of microbially labile DOC in the wet/dry treatment concentrated soil humic substances, increasing UV absorbance and SUVA. The change in DOC concentration, UV absorbance and SUVA values in the D soil exposed to drying and re-wetting was not as significant (refer **Figure 7**), suggesting the humus-like compost in it resisted microbial attack. Over time the addition of the humus-like compost to the D soil increased the proportion of microbially resilient DOC, reducing the proportion of DOC absorbing UV light (SUVA). These results validate UV absorbance is a property of soil humic substances produced over decades to centuries, and not humus-like compost processed over months to years.

Figure 12: The reduction in Dissolved Organic Carbon (DOC) associated with enhancing microbial activity by drying and re-wetting soil samples (wdry). The control samples (wet) were watered continuously by capillary action (refer Appendix 2). The drop in DOC concentration is statistically significant for the L soil that was not amended with compost. The drop in DOC for the compost-amended D soil was not statistically significant, as the humus-like DOC conferred by the compost resisted microbial degradation, but lacked the aromatic chemical structure known to absorb UV light.



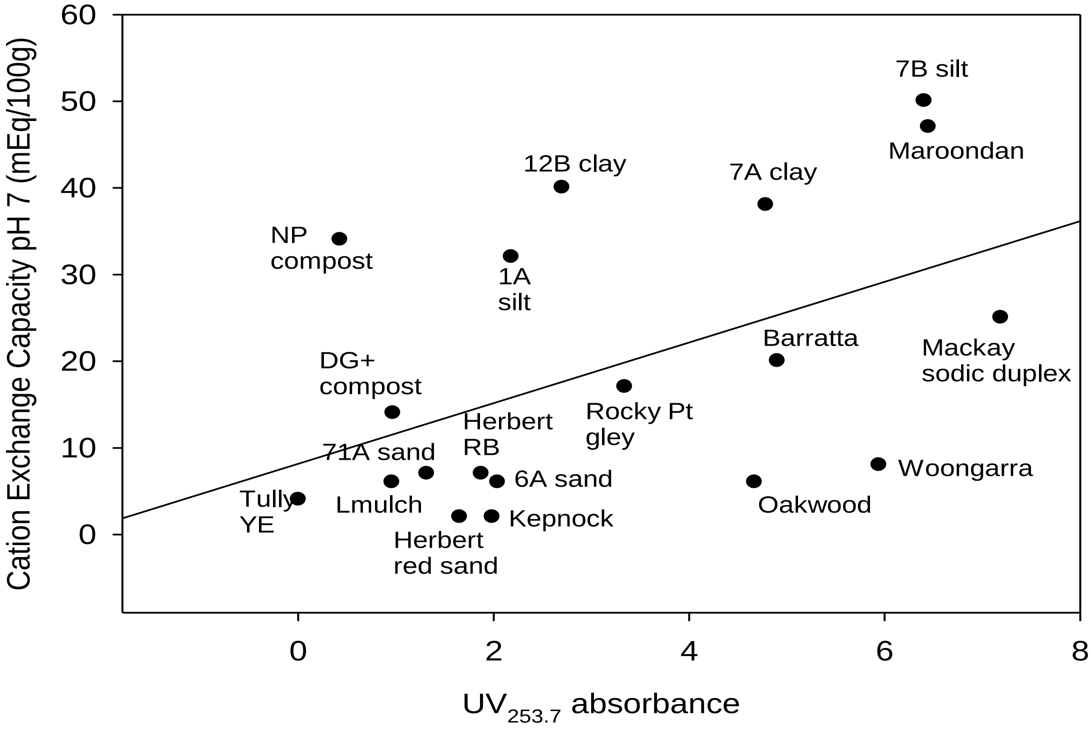
3. Determine the sensitivity of UV absorbance as an index of humified organic carbon in soil.

In lighter soils lacking the clay mineralogy of vertosols and ferrosols, cation exchange capacity CEC) is conferred by soil humic substances (variably charged CEC). If UV absorbance is associated exclusively with soil humic substances, in lighter soils UV absorbance should be positively correlated with CEC. Seventeen soils varying in texture and colour were tested for pH, electrical conductivity, Total Organic Carbon (furnace induction and wet oxidation), Cation Exchange Capacity at pH 7 and Cation Exchange Capacity at pH 8.6. Analysis of the heavy and light fractions of OC were not undertaken as published information indicated soil humic substances were predominantly in the heavy fraction.

Results for the 17 soils tested using the UV Laboratory Method were correlated with Total Organic Carbon (furnace induction and wet oxidation), pH, chloride, Electrical Conductivity and CEC. The CEC was tested at two pH levels, as the difference between the two results represents the variably charged contribution (P Moody personal communication). The output of this analysis was validating UV absorbance is the only soil property positively correlated with CEC and with no other soil properties (**Figure 13**). UV absorbance was not correlated with DOC or TOC, as soil humic substances are only one component of the organic matter present in soil (Rosell et al 2001). CEC was also correlated with chloride, as the most common anion paired with the cations on the exchange complex of clays is chloride. The anions on the humic substance exchange complex are organic acids. The difference in CEC at pH7 and pH 8 was not correlated with UV absorbance, as both decomposed clay minerals and humic substances confer variably charged CEC to soils.

Figure 13: Linear regression of $UV_{253.7}$ absorbance and cation exchange capacity (CEC) for the ten sugar cane soils, six NCEA soil collection samples (numbered sand, silt and clay samples), the sugar milling compost (NP compost) and the paired sodic garden soil samples mulched only or amended with mulch and compost (DG+ compost, L mulch). Outliers to the regression line include clay, silt and the Maroondan vertosol where clay type may confer more to the CEC than soil humic substances.

NP compost is an outlier as the humus-like compounds responsible for the higher CEC are aliphatic (long carbon chains) in nature and lack the aromaticity (carbon ring compounds) responsible for absorbing UV light. Iron oxides present in the Oakwood red kandosol and Woongarra red ferrosol may be responsible for the high UV absorbance associated with these soils, but would not contribute to the CEC.



As expected, results for the conventional wet oxidation and furnace induction OC analyses were positively correlated with DOC (Pearson correlation P value P = 0.017 and P = 0.037 respectively, n = 19). The E2:E3 ratio was also positively correlated with wet oxidation OC (P = 0.007), indicating organic compounds with a smaller molecular mass are readily oxidised under the conditions of this test (molecular mass is inversely proportional to the E2:E3 ratio). Chloride concentrations were also positively correlated with the CEC pH 7 (P = 0.007), but not with $UV_{253.7}$. The method for measuring CEC replaces cations held on exchange sites located either on clay mineral layers and/or on soil humic substances with ammonium ions (Rayment and Lyons 2011). The cations are detected and quantified, to calculate the CEC.

Chloride is the anion most commonly accompanying soil cations, so a correlation between chloride and CEC for soils is to be expected. Chloride was not correlated with $UV_{253.7}$, highlighting the specificity of UV absorbance for soil humic substances.

The sensitivity of UV absorbance for aromatic compounds was also assessed by testing a standard concentration series of the compound ninhydrin for $UV_{253.7}$ (**Figure 14**) and DOC (**Figure 15**). These results were used to establish the limit of detection of the Jenway spectrophotometer used for the laboratory method, and the portable $UV_{253.7}$ Real spectrophotometer used for the field method. The chemical structure of ninhydrin is an aromatic ring with a carbonyl functional group (refer **Figure 4**). The compound does not contain transitional metals, nitrate or carbonate functional groups that could interfere with UV absorbance.

Figure 14: Linear regression for ninhydrin concentration with $UV_{253.7}$ absorbance. Highly significant regression equations were established for the Jenway laboratory spectrophotometer (Lab spectro) and for the portable Real UV spectrophotometer (Field spectro). The aromatic and carbonyl bonds present in ninhydrin strongly absorb UV light at concentrations as low as 0.5 mg L^{-1}.

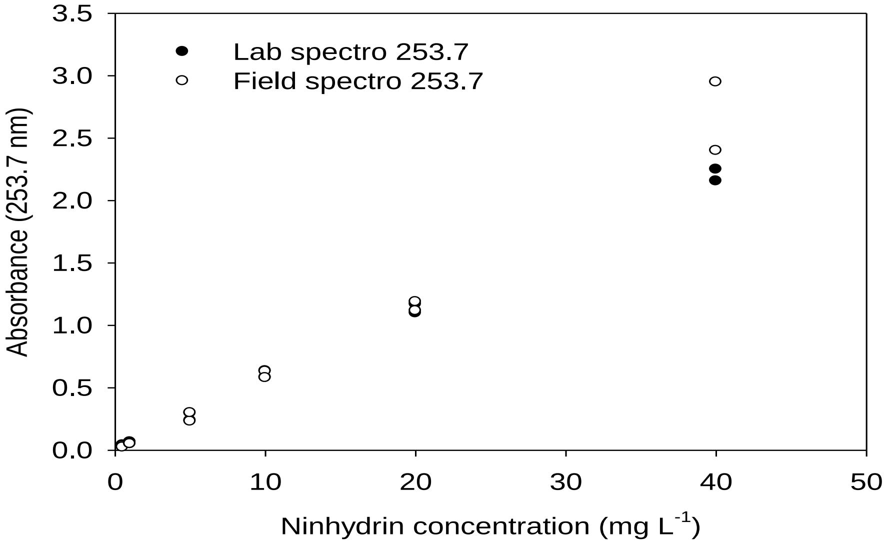
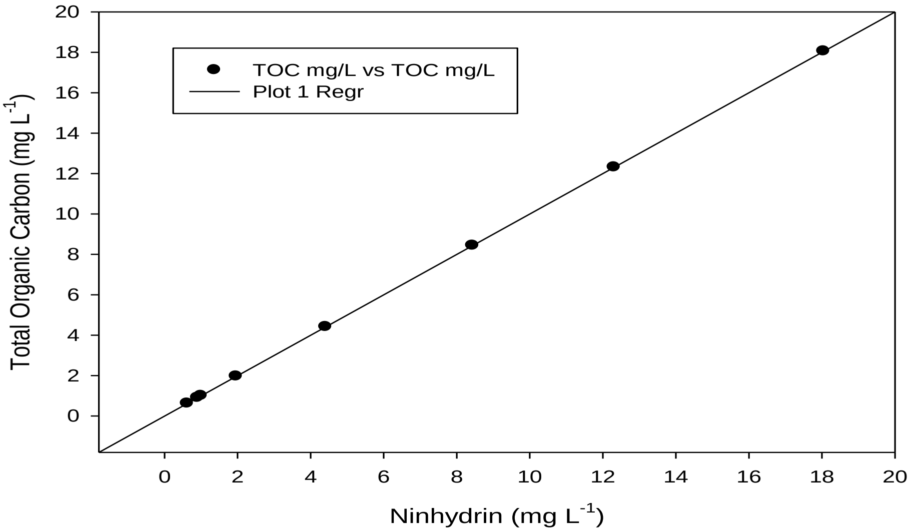


Figure 15: Linear regression for ninhydrin concentration with dissolved organic carbon (DOC). A Shimadzu combustion Infra-red analyser was used to measure DOC.



4. Determine if UV absorbance is a sensitive predictor of key soil health properties.

An indication of the range of values for $UV_{253.7}$, DOC, SUVA and the E2:E3 ratio in sugar cane soils was gained from analyzing the 10 soils provided by Drs Phil Moody and Bernard Schroeder, and the 10 soil samples submitted by growers attending the Mackay Field event and Workshop (Table 3; and Appendix 3). Descriptions of the management history and any organic amendments applied to the soil were filled in by participating growers. Soil management practices included mill mud application within the last 2 years, compost application, long-term uncultivated grassy headlands, and repeat applications of a fish, seaweed and molasses preparation.

Table 3: Range of values for UV absorbance, E2:E3 ratio, DOC and SUVA for the 20 sugar cane soil samples tested using the UV laboratory method during this project. No management information was available for the 10 cane reference soils within the black box. Grower soils in red could be used as regional soil health benchmarks.

Soil type	UV $_{253.7\ nm}$ abs. units	E2:E3 ratio	DOC mg/L	SUVA (mg/L/m)	% oven-dry moisture
Tully subsoil yellow earth	0.01	1.6	3.7	0.2	4
Kepnock yellow dermosol	1.66	0.7	5.9	27.3	14
Rocky Point gley	3.35	2.1	18.3	29.2	16
Oakwood red kandosol	4.68	2.2	9.4	50.1	10
Herbert red kandosol	2.00	2.5	12.2	16.8	1
Maroondan black vertosol	6.45	2.1	12.6	50.7	6
Woongarra red ferrosol	5.95	1.8	7.1	85.4	16
Herbert riverbank chernic tenosol	1.89	2.2	7.9	24.8	2
Mackay sodic duplex	7.19	2.3	14.1	52.3	7
Barratta clay	4.91	2.4	8.4	62.2	6
Baumann chicken manure	1.31	2.0	5.5	24.2	2
Hunter organic bananas	3.08	2.1	6.4	48.4	7
Hunter slashed headlands	**4.22**	**2.1**	**6.3**	**67.2**	**17**
River silt Burdekin	2.08	2.3	4.9	43.1	2
Ahern riverbank 50 yr cane, millmud 2011	2.48	2.4	6.3	38.6	4
Ahern org pumpkin millmud 2012	2.34	2.2	6.2	37.6	2
Attard Nth Eton 3151-2-1-3	1.51	2.5	7.6	20.0	1
Attard Eton 3151-2-1-2 **	2.10	2.5	7.3	28.8	3
Attard Nth grass 3079-7-2 **	**3.10**	**2.1**	**7.9**	**39.3**	**5**
Attard Nth Eton 3151-2-1-4	1.28	2.3	4.3	29.8	1

** Asterisks are soils treated for 5 years with fish, seaweed and molasses

Of the grower-supplied soil samples (10 samples below the black box in **Table 3**), the D Hunter slashed grassy headland could be used as a soil health benchmark for Mackay soils of the same soil type. This sample had the highest concentration of humified organic carbon (UV reading), a relatively high total dissolved organic carbon content (DOC), with the highest proportion of DOC-absorbing UV light (SUVA), and the highest moisture content (Oven-dry % moisture). The Attard soil samples were all below the D Hunter grassy headland benchmark, but again the grass fallow had the highest UV, DOC, SUVA and % moisture content. Assuming the samples are from similar soil types, Attard 3151-2-1-4 has the lowest DOC and UV absorbance values, which may indicate this soil has been cultivated for longer, with low inputs of organic carbon over that time.

Results for the two Ahern soils are similar to the Burdekin riverbank silt, with the exception of higher DOC values which may reflect the millmud applied in 2011 and 2012 to the cane and pumpkin blocks respectively. These results suggest millmud lacks the complexity of soil humic substances, which are responsible for UV absorbance. The riverbank silt soil had a higher SUVA value, due to the lower concentration of DOC in this soil. Soil samples from the 'Healthy Soils' Project soil types fully characterized for total water holding capacity, soil structural decline and other soil health parameters that were originally committed to this project, were not available during the project. Lack of access to fully characterized soils has limited our ability to validate UV absorbance as a soil health index. However, we were able to validate UV absorbance is a key indicator of CEC, which is a key soil health property.

5. **Based on the results of 4) above, we will identify the management practices that have the greatest impact on UV test results, and the time frame and scale in which change is detected.**

Results for imposing wet/dry cycles on the two garden soil samples indicated the time frame for detecting change in SUVA values can be as little as days to weeks (refer Appendix 2). For the UV laboratory Method, the DOC combustion infra-red method detects resilient aromatic soil humic substances, microbially recalcitrant aliphatic compost compounds, and simpler, microbially labile organic compounds. Change in DOC concentrations induced by stimulating microbial activity over a 12 to 20 day period will be reflected in the SUVA calculation, with the loss of labile DOC concentrating the more recalcitrant organic compounds in the sample. UV absorbance alone is insensitive to this change, as only the very resilient soil humic substances have the aromatic chemical complexity responsible for absorbing UV light.

Amending soil with cured, humus-like compost does increase SUVA values (evident as an increase in UV absorbance and the E2:E3 ratio for D compost-amended soil in **Table 1**), but soil microbial processing after application is required for the humus-like compounds to achieve the complexity of soil humic substances. The timeframe for this transformation is likely to be years. Results for the Ahern millmud amended soils (refer **Table 3**) suggest the DOC in millmud does not absorb UV light, and observed change in soil health properties such as effective CEC may be related more to the high concentration of soluble calcium in millmud and not to the variably charged CEC which is a property of soil humic substances. Lack of access to soil samples fully characterized for total water holding capacity, soil structural decline and cane yield decline limited our ability to identify the timeframe and scale required for soil health changes associated with different management practices to be detected using the UV Method.

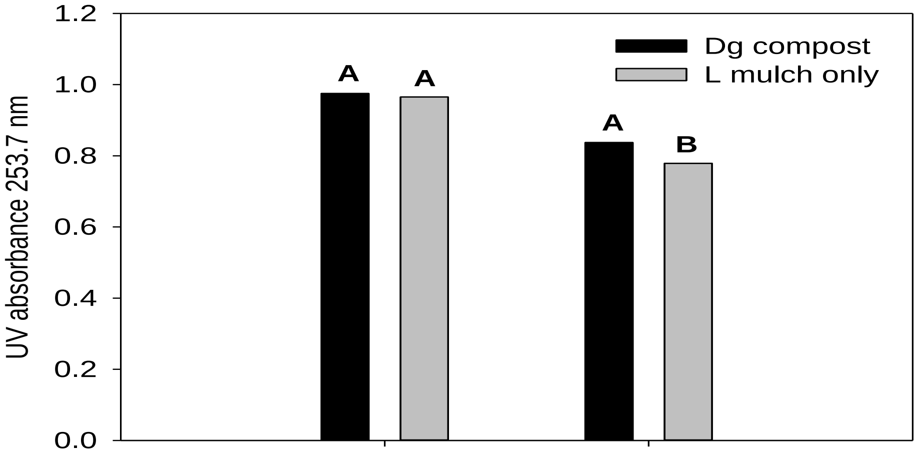
6. Development of a commercial laboratory testing protocol.

The accuracy, reliability and repeatability of the UV Laboratory Method, was tested according to NATA protocols (Guidelines for the validation and verification of quantitative and qualitative test methods, Technical Note 17 June 2012) using results from Methodologies 1 and 3 above. The accuracy of the method was tested by establishing the lowest concentration of the aromatic compound ninhydrin that could be detected using UV253.7 absorbance, the E2 to E3 ratio, combustion and near infra-red absorbance (DOC), and SUVA. Linear regression analysis was used to establish the relationship and sensitivity of detection of ninhydrin using the combustion near-infra-red absorbance method (DOC), and UV absorbance (refer **Figures 14 and 15**, and Appendix 4). Threshold concentrations established in Methodology 2 were used to specify concentrations of other compounds known to absorb in the UV region that could affect the accuracy of the method. The output from the NATA testing regime has been documented below (Output 1).

7. Develop a tool kit for using UV absorbance as a field-based soil health monitoring method utilising 'off the shelf' components currently used for unrelated applications.

Outcomes of Methodologies 1, 2 and 3 were used to develop a field-based UV method. A commercially available portable UV spectrophotometer set at 253.7 nm was purchased. A set of standard kitchen measuring spoons was used to select the volume of soil that could reproduce statistically significant differences between the L and D garden soils, exposed to the wet/dry and continuously wet treatments. The output of this research was volumetric sampling using a level teaspoon of in-situ field soil was not affected by differences in the gravimetric moisture content of soils, which dilutes the amount of soil tested using the mass based Laboratory Method sampling procedure (**Figure 16**).

Figure 16: UV results for air-dried samples of the Dg and L garden bed soils. Sampling was volumetric for the Field Method (one level teaspoon of soil), and gravimetric for the Laboratory Method (2g of soil). The difference in $UV_{253.7}$ was significant for the Field Method, as volumetric sampling was not compromised by the greater mass of water retained in the Dg compost-amended soil (**Table 3**).



The tool kit (refer **Figure 9** and Section 5 of the Objectives) was tested in collaboration with members of the cane grower group Advanced Nutrient Solutions (Barbara Walker Secretary), testing soil trial sites used during the grower-initiated project SRDC GGP034. From 2008 to 2011 compost was applied pre-planting to plant cane at a rate of 37 t/ha (70% dry matter content). Control strips of inorganic fertilizer only, and a 50:50 treatment of half the compost application with half the inorganic fertiliser were established and maintained into the first and second ratoon. During the field component of the Mackay Soil Carbon Workshop (November 25-26th 2013), samples from all three trial blocks were taken for analysis using the Field UV method (**Figure 17**). The testing protocol consisted of taking small teaspoon volumes of soil from 5 to 10 cm below the litter layer of soil, within the dark-staining topsoil zone. On the day of the field event the UV lamp and the compute function of the portable spectrophotometer would not function correctly. Soil samples were transported back to the NCEA laboratory where they were air-dried, and analysed using the laboratory UV Method (refer Appendix 3).

Figure 17: Sampling from the stained 'topsoil' zone under the litter layer of the cane blocks amended with compost as part of the SRDC GGIP GGP034 project. The soil profile was exposed by slicing away some of the soil with a spade. The dark-staining topsoil within a zone of 5 to 10 cm below the litter layer was sampled with a spoon. A composite sample of x6 heaped teaspoons of soil was mixed in a bag prior to returning to the shed for testing using the UV Field Method.

The portable spectrophotometer was returned under warranty to the supplier, who replaced the lamp. The UV Field tool kit was used successfully at a field event run as part of the 2014 Project Catalyst Cane Grower Forum (refer **Figures 10 and 11**,and Appendix 5). The two soil samples taken from very cane blocks recorded $UV_{253.7}$ values of 2.4 and 2.6, indicating the 'forgiving' nature of these soils may be related to these relatively high concentrations of soil humic substances. Eton topsoils have a relatively high CEC,, and the 15 to 20 years of green cane harvesting and trash blanketing that has been practiced on this property may have contributed to the relatively high concentration of humic substances the soils contain. Further research on a range of sugar cane soils characterized fully for soil health, sugar cane yield and quality, and standard soil physical and chemical attributes is required to validate the utility of the Field UV method as a soil health monitoring tool.

Outputs:

1. A low-cost commercial laboratory protocol for quantifying humified organic carbon in soil by researchers such as the Farming Systems group.

The protocol outlined below is based on NATA Technical Note 17 *Guidelines for the validation and verification of quantitative and qualitative test methods* 2012.

a) Purpose of measurement is to discriminate between rapidly recycled, labile Dissolved Organic Carbon (DOC) and more chemically complex, soil humic substances which are associated with soil health properties including pH buffering, water holding and cation exchange capacity, and soil crumb structure.

b) The sample matrices are dissolved organic matter extracted from air-dried soil passing through a 2 mm aperture mixed with water (1 to 20 mass to volume ratio) and filtered through a 0.45 µm glass fibre filter.

c) Interferences are likely from soils with high concentrations of the transitional metal ions of iron (Fe 111^+) and copper ($Cu11^+$). Organo-mineral complexes formed with aromatic soil humic substances are known to reduce light absorbance at 253.7 nm. Threshold soil concentrations of Cu and Fe above which ion interference is likely to occur are 12.5% and 7.5% (air-dry mass basis) respectively.

d) The range of concentrations recorded for ten sugar cane soil types are listed below. With further sampling the upper range is likely to increase.
DOC 3.69 mg/L to 18.33 mg/L
$UV_{253.7}$ 0.01 absorbance units to 6.45 absorbance units
SUVA 0.19 mg/L/m to 85.47 mg/L/m
E2:E3 ratio 1.43 to 4.39

e) There are no specific legislative or regulatory requirements associated with this test.

f) There are no specific equipment accommodation and environmental conditions that need to be considered for this test.

g) The equipment to be used is not specific to one instrument, but should apply to all instruments equivalent to:

- Advantec GB-140 filter paper held in an Advantec PP-47 filter holder with a port that fits a 60 mL syringe.
- DOC measured on a Shimadzu oxidative combustion-infrared detection TOC-V CSH Total Organic Carbon analyser heated to WW °C calibrated with potassium hydrogen phthalate TC, and sodium hydrogen carbonate and sodium carbonate IC standards at concentrations that range from 25 mg/L to 100 mg/L.
- UV absorbance measured on a Jenway 6705 UV/Vis spectrophotometer with a 1 cm light path reading absorbance at 253.7 and 365nm wavelengths ($UV_{253.7}$ and E2:E3 ratio respectively).

h) Method used for preparing the in-situ soil for sampling is to:

- remove the litter layer, and to use a sharp edged spade to cut a vertical profile to expose the black to brown staining ‘topsoil’ zone.
- A small trowel or spoon should be used to sample from within 5 to 10 cm below the litter layer, within the dark-staining zone.
- At least x6 sub-samples taken from x6 vertical profiles within the soil type of interest should be taken.
- The bulked soil sample should be air-dried under laboratory room conditions to facilitate screening thought a sieve aperture of 2 mm.
- The oven-dry mass of the screened soil should be determined to indicate the proportion of water contained in the sample on an oven-dry (105 °C) basis.

Comparative Validation of the UV Method:

Results for $UV_{253.7}$ need to be correlated with data derived from the NMR Carbon Farming Methodology to validate $UV_{253.7}$ is specific for recalcitrant organic carbon that has a residence time of years to decades. Obtaining a series of soils already characterised using the NMR Carbon Farming Methodology to validate the UV absorbance method is a high priority for the proposal submitted to SRA for the 2014-15 funding round.

Primary Validation of the Laboratory UV Method:

- The analyte to be measured is the concentration of dissolved humic substances in the test soil sample.
- Dissolved humic substances are defined as refractory, dark-coloured heterogeneous organic compounds produced as byproducts of microbial metabolism (Sutton and Sposito 2005), passing through a filter of nominal pore size 0.45 µm.
- The method to be used is absorbance of a 1 to 20 soil solution filtered through at 0.45 µm pore diameter glass fibre filter paper at the specific wavelength 253.7 nm.
- The spectrophotometer is to be blanked using distilled or reverse osmosis water.
- Seven calibration standards including the blank and six concentrations of ninhydrin within the measuring interval of 0.5 mg/L to 40 mg/L are to be used to assess precision.
- Linearity for UV absorbance and DOC should be established using linear regression and results for the ninhydrin standard concentrations. R^2 of the order of 0.998 and 0.994 for $UV_{253.7}$ and DOC respectively, should be reported.
- Samples with measuring intervals above this range will need to be diluted before reading $UV_{253.7}$ absorbance.
- Ninhydrin has been selected as the calibration standard as the chemical structure consists of one aromatic ring and one pseudo ring with carbonyl functional groups. The compound does not contain any of the other chemical functional groups known to strongly absorb in the UV region.

Analytical selectivity of the Laboratory UV Method:
Analytical selectivity of the UV Method depends on the concentration of potentially interfering transitional metal ions being below threshold values. Testing on standard concentrations of $Fe(NH_4)_2(SO4)_2.6H_2O$ and $CuSO_4.6H_2O$ added to a cured, sugar milling compost with a very low EC indicate the formation of organo-mineral complexes reduce absorbance at 253.7 nm. Threshold concentrations of $Fe(NH_4)_2(SO4)_2.6H_2O$ - Fe and $CuSO_4.5H_2O$ – Cu in the soil matrix likely to reduce the selectivity of the test are 150 mg/L and 250 mg/L respectively (or 75 mg/g and 125 mg/g respectively). Red ferrosol soils with iron oxide concentrations above 7.5% may interfere with the selectivity of the UV method.

Analytical sensitivity of the laboratory UV Method:
Analytical sensitivity of the UV Method depends on soil solutions of 1 to 20 (mass to volume) being shaken at 15 rpm for one hour on a clock-face or end-over-end shaker, and centrifuged for 5 minutes at 2500 rpm. Evidence of colloidal interference may reduce the sensitivity of this method if more concentrated soil solutions are used (eg 1 to 5 mass to volume).

Precision and repeatability of the Laboratory UV Method:
Over the duration of this project a total of 33 soil types and/or treatments were analysed for the Laboratory UV Method. Six replicates were used for statistical comparison for 18 of the samples, which were analysed on only one occasion. Precision and repeatability procedures recommend a minimum of x10 replicates, analysed on 3 or more separate occasions (NATA 2011). This was outside the scope of this limited ‘proof of concept’ research project.

Reproducibility of the Laboratory UV Method:
The reproducibility of the test for both ninhydrin standard concentrations and the x10 sugar cane samples needs to be determined by different operators using different analytical equipment (NATA 2011). The number of replicates and agreement from other laboratories required to undertake repeat analyses by technical staff working in other laboratories using the specified method was also outside the scope of our ‘proof of concept’ project.

Lowest Order of Detection (LOD) of the Laboratory UV Method:
The LOD of the Jenway 6705 UV/Vis spectrophotometer was 0.5 mg/L ninhydrin. Precision at this concentration was less for the TOC-V CSH Total Organic Carbon analyser.
At least x10 repeat analyses for UV253.7, DOC, SUVA and E2:E3 ratio for the lowest detectable concentration of 0.5 mg/L of ninhydrin are required to establish the standard deviation and confidence limits associated with the LOD (NATA 2011). Experience with the x6 replicates used during this study indicates the range of concentrations within soil samples is very high, and not normally distributed. Data reported in this project were often median values, with one-way analyses of variance and ad hoc T-testing undertaken on ranks. Larger replicate numbers may be required to establish normality for statistical purposes when calculating LOD values for all four testing procedures.

2 A field-based tool kit (utilising 'off the shelf' components in a novel application) for benchmarking and improving soil health. The outcomes of preliminary field testing, and comparison of results with conventional test results will be reported to growers and agronomists at a workshop in Mackay, and via industry publications (Cane Growers) and conferences (Aus Soc Sugar Cane Technologists).

Components of the field UV method testing kit include:

- One 47 mm diameter, 45 µm aperture glass fibre filter paper disc per soil solution sample (Figure 1). Heavy duty glass fibre filter papers suitable for the analysis of total dissolved solids are recommended.
- One filter paper disc holder to support a 47 mm diameter filter disc.
- One 60 mL syringe with a compatible tip that fits the filter holder aperture is required to pass the soil solution through the filter. The filtration equipment can be purchased through a retail laboratory supply company.
- One 40 mL measuring cup, one teaspoon measure, and at least x2 60 mL capped plastic containers per sample (unfiltered and filtered) for shaking the soil solution. This equipment can be purchased from retail pharmacies and/or kitchen and hardware shops.
- One portable UV spectrophotometer sold commercially for monitoring DOC concentrations in water samples. The Australian supplier of the Real UV spectrophotometer is Thermo Fisher Pty Ltd.

During the Mackay Soil Carbon field event the UV spectrophotometer malfunctioned. LED alerts indicated the lamp and/or the ‘compute’ button were faulty. Under warranty a new lamp was installed and no further malfunctions are anticipated. The soil samples submitted at the Soil Carbon events were analysed using the Laboratory UV Method (Appendix 3). Results and an interpretation of these tests were emailed to the Pioneer Catchment Landcare Group for dissemination to participants of the Soil Carbon Workshop.

Dr Pittaway demonstrated the Field UV Method to participants at the Project Catalyst field day held on 26/2/2014 at Proserpine. Dr Pittaway also participated in the Soil Nutrition Workshops held as part of the Forum on 27 and 28/2/2014 on Hamilton Island. Extracts from the report submitted to the Pioneer Catchment Landcare Group were included in the 2014 Project Catalyst Grower Forum publication. Dr Pittaway has also been invited to give a presentation on soil health and the UV Method to graziers at the Reef Catchments Grazier Forum to be held at the Mackay Entertainment and Convention Centre 9/4/2014.

Further collaboration with agribusiness and/or analytical equipment retailers is required to develop a commercially available Field UV Method kit. As part of this collaboration a Smart Phone app and/or web-based application needs to be developed to enable users of the Field UV Method kit to convert $UV_{253.7}$ absorbance readings to aromatic DOC mg/L readings. Direct conversion using the regression equations for ninhydrin and UV absorbance, and ninhydrin and DOC (**Figures 14 and 15**), is inappropriate. DOC estimates calculated using these equations exceed the DOC concentration determined by direct combustion infra-red analysis. Further work is required to develop an appropriate equation for converting $UV_{253.7}$ absorbance to mg/L aromatic DOC.

3 Knowledge of the relationship between humified organic carbon and key soil health properties including total water and nutrient holding capacity, aggregate stability, mineralisable nitrogen and resistance to soil acidification. Results will be shared with scientists providing the soil samples, sugar cane technologists, and one or two papers will be submitted to a research journal such as Australian Journal of Soil Research.

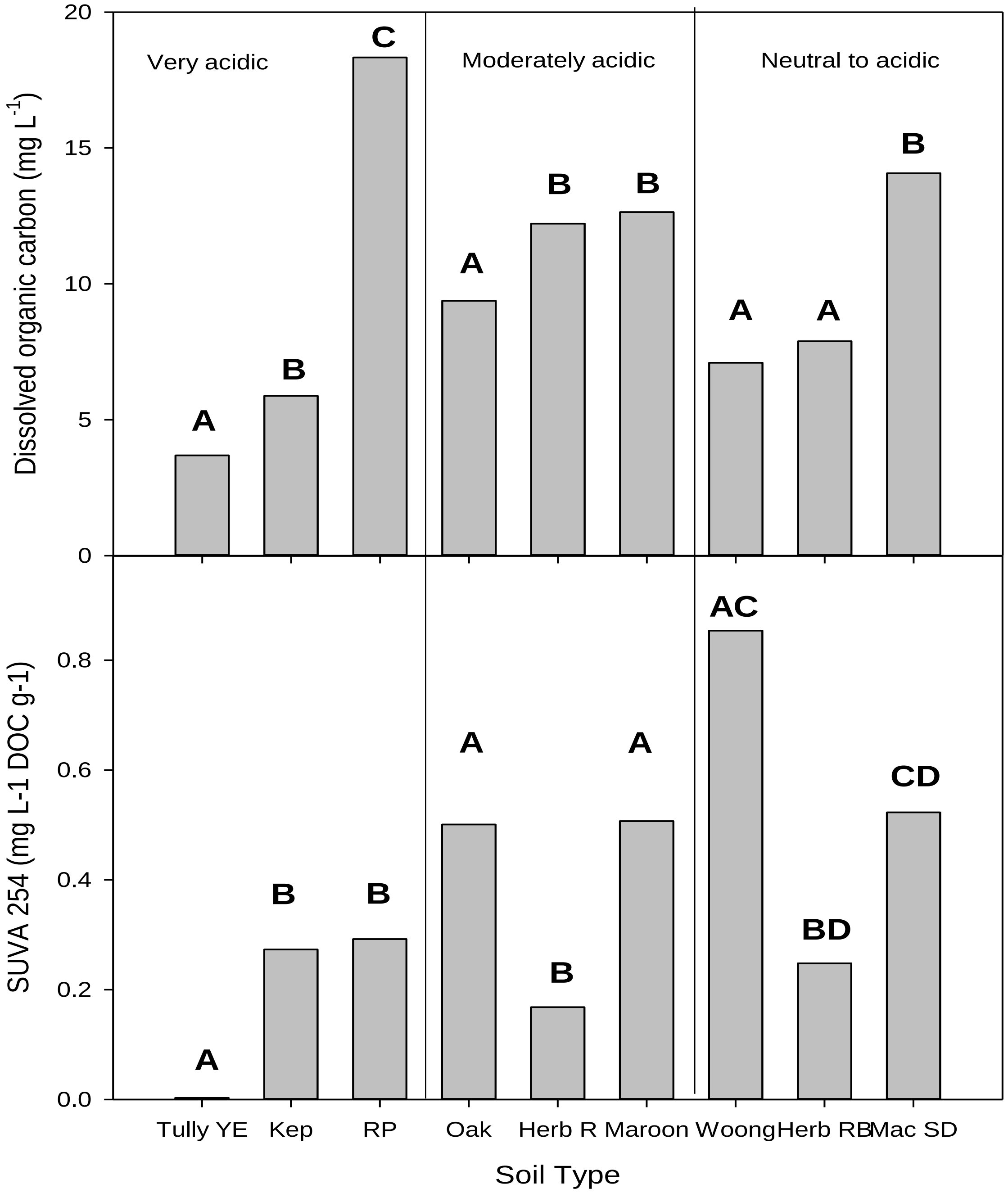
During the project the ten sugar cane soils, six NCEA reference collection soils and two sodic garden soils were analysed for wet oxidation organic carbon, furnace induction organic carbon, pH, conductivity and cation exchange capacity at pH 7 and at pH 8.6, to enable correlation and regression analyses to be applied to mean or median values of DOC, $UV_{253.7}$, SUVA and E2:E3 ratio. Results from these analyses confirmed DOC was significantly correlated ($P < 0.05$) with furnace induction ($P = 0.037$) and wet oxidation OC ($P = 0.017$), but not with $UV_{253.7}$, SUVA or E2:E3. Wet oxidation OC was positively correlated with E2:E3 ratio ($P = 0.007$), indicating wet oxidation preferentially oxidizes smaller molecular weight organic compounds.

$UV_{253.7}$ was the only analyte significantly correlated with cation exchange capacity at pH7 ($P = 0.035$). A high positive correlation was expected between $UV_{253.7}$ and the sandy soils as variably charged soil humic substances provide the only cation exchange capacity associated with these soils. Inclusion of the black vertosol (Maroondan in **Figure 13**) in the analysis reduced the correlation between $UV_{253.7}$ absorbance and CEC as inorganic clay particles are responsible for the very high CEC characteristic of these soils. The high absorbance but low CEC recorded for the red soils (Woongarra and Oakwood in **Figure 13**) is most likely due to high concentrations of iron oxides which also strongly absorb UV light. The sugar milling compost (NP compost) is also an outlier to the regression line as the humus-like compounds responsible for the high CEC lack the complexity of soil humic substances responsible for UV absorbance.

These results confirm UV absorbance can be used as an index for the soil health property cation exchange capacity. Results to-date will be presented and published in the Proceedings of the 2014 Australian Society of Sugar Cane Technologists to be held in April/May on the Gold Coast. The abstract has been included in the Publications List section. Additional papers on the Laboratory UV Method will be prepared and published in the Australian Journal of Soil Research. The extra work required to specify the precision, reproducibility and repeatability of the UV Method will need to be done before the Laboratory UV Method can be published.

The soils that were available to this project had not been characterized for aggregate stability, soil acidification or potentially mineralisable nitrogen, and no information was available on land use history or cropping management practices. However, when nine of the ten sugar cane soils were subdivided into very acidic, moderately acidic and neutral to acidic, soils with low SUVA values were more acidic (**Figure 17**). These results suggest the UV Method could also be used to monitor the susceptibility of soils to acidification. Further research on more completely characterized soils is required to conclusively establish a proportional relationship between humified organic carbon as measured by the UV Method, and aggregate stability, total water holding capacity and resistance to soil acidification. Repeat sampling of soils previously fully characterized a decade or so ago will validate the sensitivity of the UV Method in detecting change in these soil health parameters over time.

Figure 17: Dissolved organic carbon and $SUVA_{253.7nm}$ results for nine of the ten sugar cane reference soil samples provided by Dr Bernard Schroeder. The samples were grouped according to pH (water extract). Data for the Barrata clay have been omitted as it was the only alkaline soil. Within each pH category, bars with the same letter are the same (not significantly different using a one-way ANOVA). For further information refer to Appendix 4.



4 Recommendations will be made on the use of the UV method as a soil health index, including the potential use of the tool to measure the impact of management practices, and as a Method for the Carbon Farming Initiative (in collaboration with Phil Moody).

Results from this project validate the UV Method as an index of CEC, a key soil health property. The limited information available on the soil health properties of the soils we were able to access over the duration of this Proof of Concept project has limited our capacity to interpret the utility of the UV Method as an index of other soil health properties. Further work with soil types sampled from sites with a known cropping and management history is necessary to more conclusively establish the relationship between humified organic carbon and key soil health properties.

Repeat sampling from sites previously sampled five to ten years ago will also confirm the sensitivity of the UV Method for monitoring change in the more resilient, soil humic substance pool in soils over time, and the management processes associated with the change. The repeat sampling will only be of benefit if we have access to the original soil samples for testing with the Laboratory UV Method. Storage over that time should not affect $UV_{253.7}$ readings as results from this ‘proof of concept’ project confirm UV absorbance is associated with the aromatic structure of more resilient soil humic substances.

The utility of the UV Method as a candidate Method for the Carbon Farming Initiative still needs to be tested, as the soils we were able to access soils had not been characterized using the Carbon Farming NMR Methodology,. $UV_{253.7}$ absorbance and SUVA results for the compost used in the ion interference section of the project, and results from compost-amended soils (**Table 2**) indicate the UV method is selective for resilient, aromatic soil humic substances. The aliphatic nature of humus-like compounds in cured compost are known to increase soil health properties including cation exchange capacity and total water holding capacity, but they lack the aromatic bonds of humic substances responsible for absorbing light at 253.7 nm. These results indicate the UV method does have merit as a Methodology for the Carbon Farming Initiative, but more work is required to conclusively present the case for inclusion.

Intellectual Property and Confidentiality:

In the Project Proposal the methods specified for use in this study were known to be already in the public domain and were unlikely to have merit as intellectual property. However, use of the compound ninhydrin as a concentration standard for converting UV absorbance results to mg of aromatic DOC (soil humic substances) per litre of topsoil is novel (refer Appendix 5), and may merit consideration as Intellectual Property. Further work is required to develop the conversion factor, as estimates based directly on the linear regression equations (**Figures 14 and 15**) over-estimate aromatic DOC.

The development of a Smart Phone application or a web-based application would allow land holders and scientists using both the Laboratory and Field UV Methods to convert from absorbance units to mg of soil humic substances per L (or kg) of topsoil. Access to these internet applications could also be combined with an application for scientists and land holders to upload results for the Field UV Method and the Laboratory UV Method into a database that could be used for regional benchmarking purposes.

Environmental and Social Impacts:

During the relatively short duration of this 'Proof of Concept' project, the most evident social impact was the enthusiasm registered by attendees of the Mackay Soil Carbon Workshop (25-26/11/2013; **Figure 18** and Appendix 3) and at the 2014 Project Catalyst Grower Forum (24-26/2/2014; **Figure 19** and Appendix 4) for the UV Method as a soil carbon monitoring tool. Feedback from the Mackay attendees indicates growers are very receptive to the opportunities the UV Method offers for monitoring the concentration of soil humic substances in their cane blocks. Currently accepted methods for monitoring soil organic carbon measure all forms of OC to some extent, and are relatively insensitive to change in management practices over the short term. Results from this project validate the UV Method is selective for the more stable soil humic substances, and can be used to benchmark the impact of management practices.

Figure 18: growers attending the Mackay Field Event

Figure 19: Growers attending the Project Catalyst Proserpine Field Event

The most progressive growers routinely use soil testing for nitrogen, phosphorus and potassium to improve fertilizer use efficiency on their properties. However, with the exception of total organic carbon (wet oxidation or furnace induction methods), there are very few tests that can be used for monitoring soil health. The laboratory UV Method can be used to monitor change in the concentration of DOC, and UV absorbance and SUVA can be used to monitor change in labile DOC and in the proportion of soil humic substances. The relatively low cost of the field method will enable growers to monitor cane blocks more intensively, to select sites that merit a more detailed analysis.

The social benefit to growers is the immediacy of measuring field samples for UV absorbance, to compare the impact of different management practices on soil humic substances. Access to the Field UV Method kit will enable growers to undertake more on-farm monitoring, to identify which treatments, soil types or cane blocks merit more thorough testing. If the UV Method is proven to predict the concentration of recalcitrant OC as measured using the approved Carbon Farming NMR Methodology, the UV test could be used by growers to select paddocks that may qualify for carbon sequestration credits.

Further consultation with agribusinesses likely to market the Field UV kit and with local soil and/or water testing laboratories likely to adopt the Laboratory UV Method is required to fully realize the social benefits for land holders wishing to undertake their own soil monitoring programs. The low cost of both methods (refer next section) should further encourage uptake of these methods by land holders wishing to monitor the impact of amendments and management practices on the concentration of humic substances in topsoil. For the environmental benefits to be realized, further testing of soils with well documented histories of crop yield, soil health decline and associated management practices needs to be undertaken to establish the utility of the Laboratory and Field UV methods as an index of soil health.

Expected Outcomes:

This project has proven methods well established for monitoring aquatic humic substances have equal merit for monitoring soil humic substances. The Laboratory UV Method is sensitive for monitoring rapidly recycled soil organic carbon and for monitoring more resilient, soil humic substances (DOC, $UV_{253.7}$ and SUVA). This is very important for cane growers as the adoption of green cane harvesting and trash blanketing has increased total soil carbon concentrations, but previously no cost-effective method was available to distinguish between rapidly recycled soil carbon and soil humic substances. The cost of the Laboratory UV Method is estimated as $50 for $UV_{253.7}$, SUVA, DOC and E2:E3 ratio. This includes the cost of sieving 2 g samples through a 2mm mesh aperture sieve, shaking the 2g in 40 mL soil solution for 1 hour, centrifugation for 5 minutes and 0.45 µm filtration.

The impact on the cane industry will be greater if further research establishes a link between sugar cane yield decline and a decline in the concentration of soil humic substances in the topsoil. The low cost of the field UV method will enable agribusiness, grower and/or catchment and landcare groups to develop local benchmarks associated with sustainable sugar cane yields. The estimated cost per test using the Field method is $10. Individual land holders are not expected to purchase the Field UV Testing kit as the unit cost of the field spectrophotometer and the filter paper cartridge and glass fibre filter papers is around $3,000. The estimated payback period for groups purchasing the Field kit is 1 year, if more than 300 samples are done per year.

The impact for land holders generally is the potential to use the Laboratory and Field UV methods as a soil health tool. The soil humic substances detected by $UV_{253.7}$ absorbance are known to improve the aggregate structure and the nutrient and water holding capacity of the soil. Results from the Mackay Field event (Appendix 1) suggest UV testing of sites on the same soil type that have not been historically cultivated, may establish benchmarks for the concentration of soil humic substances in the topsoil. Results to-date suggest millmud application does not add to the pool of humified organic carbon, but over time, repeat applications of humus-like compost may add to the soil humus pool. Results to-date also highlight cured compost does not have the chemical complexity of soil humic substances that is associated with absorbance of light at 253.7 nm. It is humus-like, in increasing the nutrient and water holding capacity of soil, and over time further microbial processing will produce more resilient, soil humic substances. These learning outcomes on the properties of compost and its role in soil health were only possible with the development of the Laboratory UV Method.

The expected outcome that could not be realized within the timeframe of this project was submission of the UV Method for consideration as an approved Carbon Farming Methodology. Further research to document the repeatability, reliability and precision of the Laboratory and Field UV Methods, and validation of a significant correlation and linear regression between the currently accepted photo-oxidation NMR Methodology is required before a case for submission as a candidate Carbon Farming Methodology can be made. Establishing the UV Method as an approved Carbon Farming Methodology will be of major significance to all land holders, as the relatively inexpensive cost of both methods will allow growers to monitor which blocks of land may be worthy of testing using the more expensive photo-oxidation NMR method.

Future Research Needs:

The duration of this project and the budget was deliberately limited in scope and size, for the purpose of proving the concept that the UV Method could be used to monitor soil humic substances and could be adapted as a low-cost field monitoring method. This objective has been achieved, but more research is required to:

- Conclusively validate the utility of the UV Method as a soil health tool. Access to soils that have been fully characterised for key soil health properties and for sugar cane yield will be required, especially to establish if a decline in soil humic substances is associated with sugar cane yield decline.

- Document the repeatability, reproducibility and precision of the Laboratory and Field UV Methods to the standard specified in the NATA Technical Note 17 *Guidelines for the validation and verification of quantitative and qualitative test methods* 2012.

- Modify the equations for converting UV absorbance to ninhydrin equivalents, and ninhydrin equivalents to DOC. The concept of using ninhydrin as a concentration standard and for converting absorbance units to mg of soil humic substances per unit volume or unit mass of topsoil was discovered very late in the project. Estimates of aromatic DOC derived from the equations substantially over-estimate the concentration of soil humic substances in the original sample. Ninhydrin reacts readily with amino acids, and would not occur naturally in soils. UV absorbance associated with the reactive carbonyl bonds and the pseudo-ring may explain the over-estimation. If a valid calculation to account for this over-estimate cannot be developed, an alternative chemical standard (eg the international standard Suwannee River Normal Organic Matter) will need to be investigated.

- Develop the IP associated with the use of ninhydrin (or Suwannee River Normal Organic Matter) as a concentration standard for the Laboratory and Field UV Methods. More time is required to develop a Mobile Phone Smart App and a web-based App for converting results and for uploading results into a database for soil health benchmarking purposes.

- Establish if results for the Laboratory and Field UV Methods are significantly correlated with concentrations of resilient soil carbon tested using the approved Carbon Farming Methodology of photo-oxidation and NMR analysis. If this can be established, documentation to the standard of the NATA Technical Note 17 will assist in developing a case for adopting the UV Method as a Carbon Farming Methodology.

- Collaborate with commercial and government testing laboratories to meet the reproducibility requirement of NATA Technical Note 17 and to encourage the adoption of the Laboratory UV Method as a standard test for quantifying dissolved organic carbon and soil humic substances.

- Collaborate with agribusiness groups to encourage the marketing of the Field UV Method kit to grower, agribusiness, and/or Catchment Landcare or Reef Rescue groups.

Recommendations:

Further collaboration with sugar cane extension officers and with the SRA soil testing laboratory is required to encourage adoption of the UV Method as a soil health tool. Additional funding and time will enable the expansion of testing to soil blocks in different sugar cane growing regions, enabling the selection of sites that have been well characterized in the past, for repeat testing. Repeat testing should be on stored soil samples as well as on freshly taken samples from exactly the same site, to establish the merit of the UV Test for monitoring change in the concentration of soil humic substances over the short term (years to decades). Sampling of stored soil samples should not be a problem, as soil humic substances are chemically and microbially resilient, for years to decades.

Extending the project to enable the complete documentation of precision, repeatability and reproducibility of both the Laboratory and Field UV Methods to the standard specified in NATA Technical Note 17 is needed to validate the tests as laboratory and field analytical procedures. Once validated, the Method can be written up for publication in the journal Soil Research, and can be disseminated to government and commercial soil and water testing laboratories for adoption.

Collaboration with industry groups managing databases on sugar cane yield and management practices within the cane growing regions is also essential to select sampling sites with a known management and cane yield history. The information contained within these databases is sensitive, and collaboration with authorized users is essential for establishing if a decline in soil humic substances is associated with sugar cane yield decline. If this association can be established, soil blocks at risk of declining yields could be identified, as well as management practices associated with maintaining and/or building concentrations of soil humic substances. Historically maintained databases are required, as results to-date indicate soil humic substances require years of exposure to microbial activity to develop the chemical complexity associated with UV absorbance.

List of Publications:

Abstract of paper accepted for publication in the Proceedings of the Australian Association of Sugar Cane Technologists to be held on the Gold Coast in April/May 2014 :

Ag 10. A UV ABSORBANCE TEST FOR MEASURING HUMIFIED ORGANIC CARBON IN SOIL

By P PITTAWAY, F EBERHARD

National Centre for Engineering in Agriculture, University of Southern Qld.
Pam.Pittaway@usq.edu.au

KEYWORDS: Soil Carbon, Soil Health, Aquatic Humic Substances, Ultraviolet Light, Field Testing

Abstract

Standard methods used for measuring soil organic carbon are insensitive to short-term changes associated with soil management practices. The conventional wet oxidation and furnace induction methods measure all forms of soil organic carbon to some extent, not differentiating between rapidly recycled plant debris, organic residues, soil biomass or more resilient soil humic substances. The only accepted laboratory method for measuring resilient, sequestered carbon in soil (nuclear magnetic resonance or NMR), requires expensive laboratory equipment. Our new method uses off-the-shelf equipment developed for quantifying aquatic humic substances, measuring Ultraviolet (UV) light absorbance.

UV absorbance at 253.7 nm is strongly correlated with resilient aromatic compounds in aquatic humic substances quantified using NMR analysis. We have adapted water quality protocols for humified Dissolved Organic Carbon (DOC), for measuring humified organic carbon in soil. Under laboratory and field conditions, the UV method is sufficiently sensitive to detect changes in the concentration of humified DOC in soil amended with mulch only, or with mulch and compost. Results for total DOC and Specific UV Absorbance (SUVA; UV absorbance standardised per mg of DOC) for nine sugarcane soils, revealed large differences in total and humified DOC that were related to soil acidity. The soil health properties associated with humic substances include pH buffering, nutrient and water holding capacity, and improved soil structure. Further testing will validate the utility of the UV method for benchmarking and monitoring the impact of land management practices on soil health.

Appendix 1: SRDC Research Project Milestone Report

SRDC project number:	**NCA012**
Project title:	**UV absorption as a tool for growers to benchmark humified organic carbon in soil**
Research organisation(s):	National Centre for Engineering in Agriculture (NCEA)
Chief Investigator(s):	Pam Pittaway (NCEA)
Objectives:	Specific objectives include: • Determine how particle size, clay content and EC affect the reliability and reproducibility of DOC extraction from soil and UV absorbance using a spectrophotometer. • Select x 3 representative clay, silt and sand soils with low and high EC from NCEA reference sample collection (characterised for pH, EC, particle size grades, WB OC, ECEC). • Standardise UV absorbance results for total organic carbon content, and document how soil particle size distribution and salinity affects the methodology and results. • Determine if soil colour and wet/dry cycles affect the reliability and reproducibility of the test. Dr Mike Bell will provide representative red, black, yellow and grey top soils to determine if UV absorbance results are likely to be affected by soil colour and wet/dry cycles prior to analysis.
Milestone number:	2
Due date for milestone:	1-1-2013
Milestone Title:	**Proof of concept**
Achievement criteria:	• Three representative clay, silt and sand soils with low and high EC selected from NCEA reference collection. • UV method validated as not affected by soil particle size or salinity. • Topsoils selected from the "Healthy Soils' project (Mike Bell) • UV method validated as not affected by soil colour or wet/dry cycles. • Submission of Progress Report on suitability of UV Method for diverse soils

Milestone Achievement

With respect to the individual achievement criteria, we are able to report as follows:

Selection of 3 clay, silt and sand soil types with high and low EC

Preliminary reading of journal articles on extraction methods for water-extractable and hot water-extractable organic carbon indicated the duration of shaking the soil and water solution was highly variable. Instead of defining the selection criteria on soil texture and electrical conductivity alone, the project methodology had to be modified to allow for x6 different shaking times: 16 hrs, 4 hrs, 1 hr, 20 minutes, 2 minutes and 0 minutes (x5 rapid shakes only). We need to know how the concentration and type of organic carbon extracted from the soil samples varies with the duration of shaking, and if particle size affects the recovery. Our aim is to develop a field-based method, so we need to know how reducing the shaking time to minutes, affects the recovery of water-extractable, humified organic carbon.

In the samples selected below, pairs of high and low salinity (EC) were available in sand and clay textural classes (soils 1 to 4 in Table 1), but the availability of silt soils was limited to two only (soils 3 and 4). We were not able to select soils with high and low EC, whilst standardising for both soil texture and organic matter content. So a fourth pair of soils was selected, with a similar textural composition, the same organic matter concentration, but with different salt concentrations (soils 7 and 8 in Table 1).

Table 1: the particle size characteristics and chemical attributes of the eight soils selected from the NCEA soils collection. Sample pairs were matched on particle size (1 to 6), with high and low EC, or on organic matter content (7 and 8), with high and low EC

Sample	NCEA ID	Texture	Organic Matter %	EC dS/m	ECEC	Chloride mg/kg	Water %
1 Clay	**7A**	**clay 58%** silt 9% sand 33%	2.2	0.26	40.7	157	8.9
2 Clay	**12B**	**clay 55%** silt 20% sand 27%	0.8	0.76	45.4	835	7.1
3 Silt	**7B**	clay 11% **silt 30%** sand 55%	0.8	0.92	54	1351	7.2
4 Silt	**1A**	clay 5% **silt 34%** sand 62%	0.6	0.12	33.6	3	3.9
5 Sand	**6A**	clay 13% silt 5% **sand 81%**	0.9	0.03	5.5	4	1.5
6 Sand	**71A**	clay 14% silt 8% **sand 77%**	1.9	0.27	12.1	10	3.0
7 Organic matter	**3B**	clay 31% silt 6% sand 58%	**0.3**	0.64	24.6	869	5.5
8 Organic matter	**10B**	clay 35% silt 8% sand 56%	**0.3**	0.18	15.7	40	3.5

Ideally, our method must be suitable for field application, avoiding the need for laboratory equipment such as centrifuges. The initial experimental protocol of 5g of air-dried soil shaken for a specified time, pre-filtered through glass fibre filter paper to remove the soil particles prior to syringe cartridge filtration caused a lot of clogging, even after allowing the soil solution to settle for 30 minutes. The functional definition of dissolved organic matter (DOM) is organic matter in solution that passes through a 0.45 µm filter. Including 10 minutes centrifugation at 2,000 rpm to remove the soil particles reduced the number of filter cartridges used, and replacing the syringe filters with a much larger diameter glass fibre filter matrix of 0.45 µm further improved the method. Visually, shaking the soil solutions for 4 and 16 hours increased the concentration of DOM in the filtrate (**Figure 1**). Shaking times of this duration are impractical for field applications.

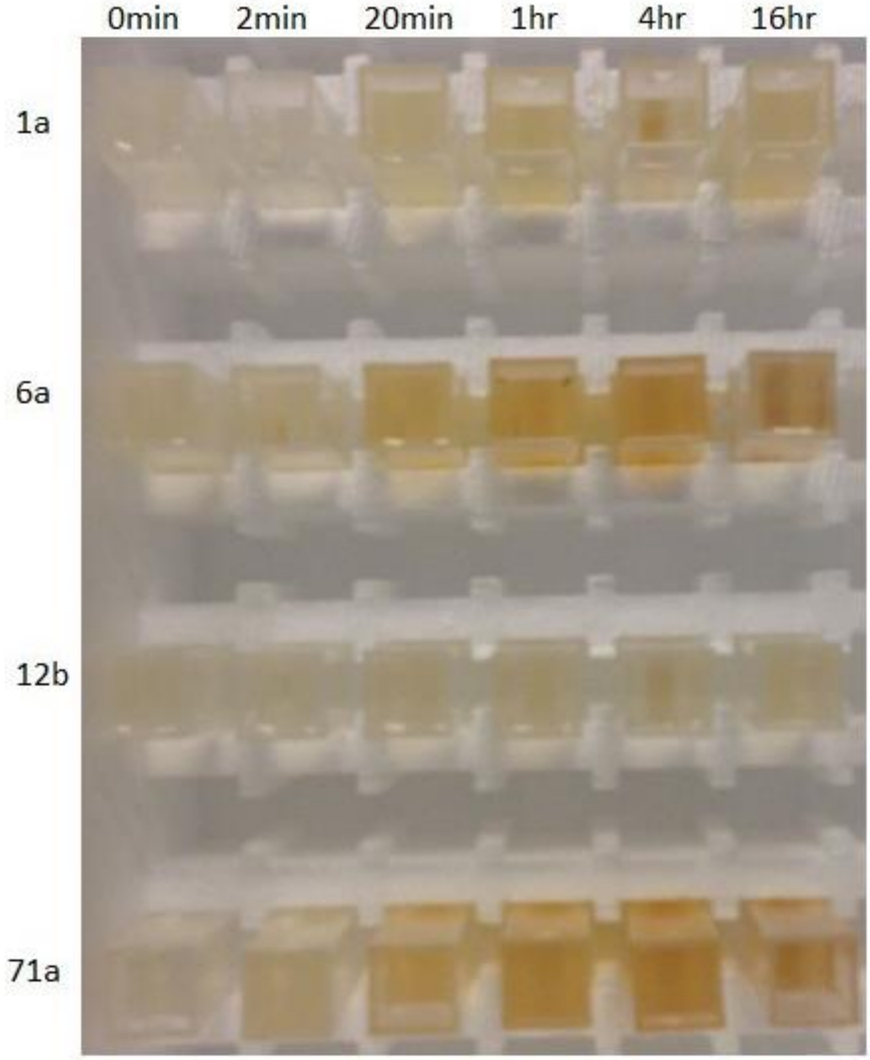


Figure 1: Gradations in the colour intensity of Dissolved Organic Matter (DOM) in the soil solution filtrate after shaking for 0, 2 minutes, 20 minutes, 2 hours, 4 hours and 16 hours (left to right). The lightest DOM (1a and 12b) are the silt and clay soils, whereas the darkest are sandy soils (6a, 71a). Samples 1a, 12b and 6a have similar organic matter concentrations (0.6, 0.8 and 0.9 respectively; Table 1), 71a is much higher (1.9%), but visually the clay and silt have lower DOM concentrations whilst the clays look similar.

Effect of soil texture and shaking on the quantity and quality of dissolved organic carbon extracted.

For all three soil types, the amount of total organic carbon extracted in the filtrate varied the most with minimal shaking (0 minutes), and least with 20 minutes or 1 hour (**Figure 2**). Results for the clay soil differ from the other soil types with recoveries highest at 0 minutes and 16 hours.

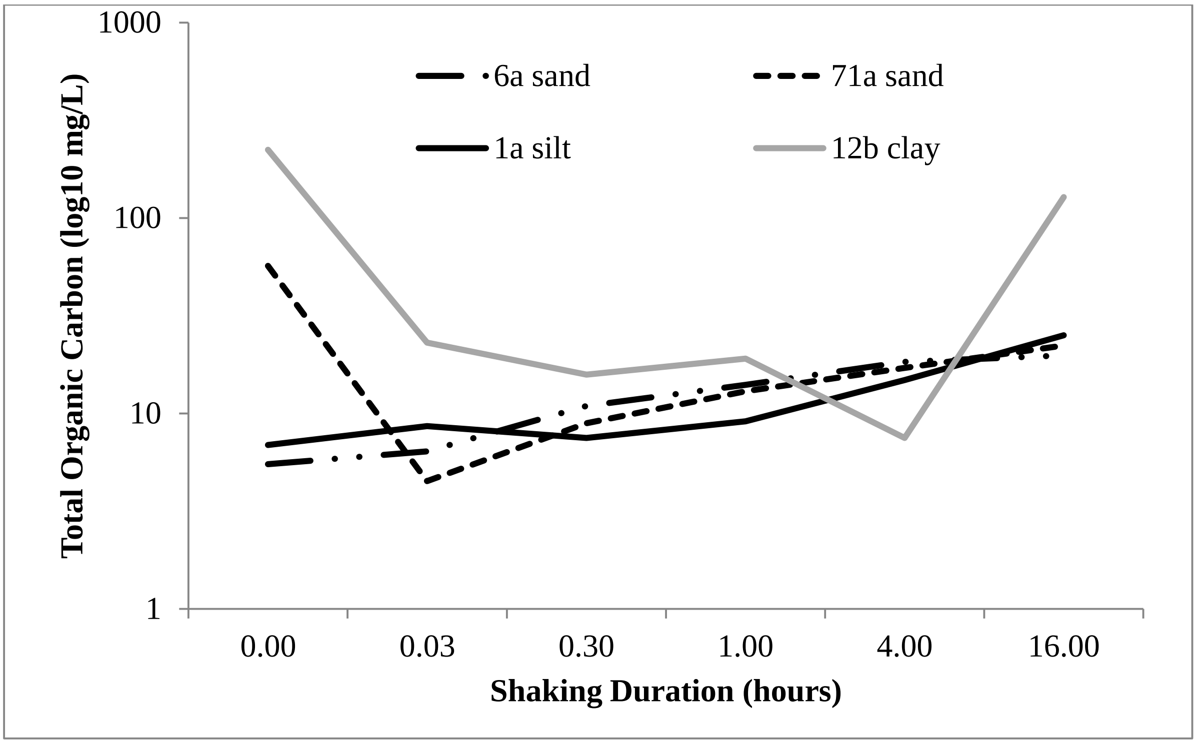


Figure 2: The effect of shaking duration on the concentration of total organic carbon recovered in the filtrate of the four soil samples. Six replicates were used for each sample. Total organic carbon was measured using a TOC auto analyser.

A minimum shaking time of 20 minutes extracted similar concentrations for all soil types. The very different peaks in recovery with no shaking and 16 hours of shaking for the clay (**Figure** 2) suggest different types of DOM were extracted. This hypothesis was tested using the light absorbing properties of different fractions of DOM. Aromatic (6 carbon ring configuration) and aliphatic (linear carbon chains) DOM containing conjugated double bonds absorb ultraviolet light. Humified DOM (large organic compounds that are structurally complex) absorbs light at wavelengths of 250 nm, whereas absorption at 240 nm is considered characteristic of simpler aromatic compounds.

Absorbance at 253.7 nm was highest for clay shaken for 16 hours, with very little difference between recoveries for 2 minutes and 2 hours (**Figure 3a**). Reducing the absorbance scale (omitting data for 16 hrs, **Figure 3b**) indicated the DOM in sand was extracted more readily than the silt or clay soils, with extending shaking from 4 to 16 hours having little additional benefit. Shaking the silt for 4 hours produced a higher absorbance than for 16 hours (not reflected in TOC results in **Figure 2**), indicating inorganic compounds that absorb in the UV region (eg nitrate and ferrous ions) may affect the results. Red soils containing high concentrations of ferrous ions have been selected as part of the experimental design, to determine if inorganic ions interfere with the UV method.

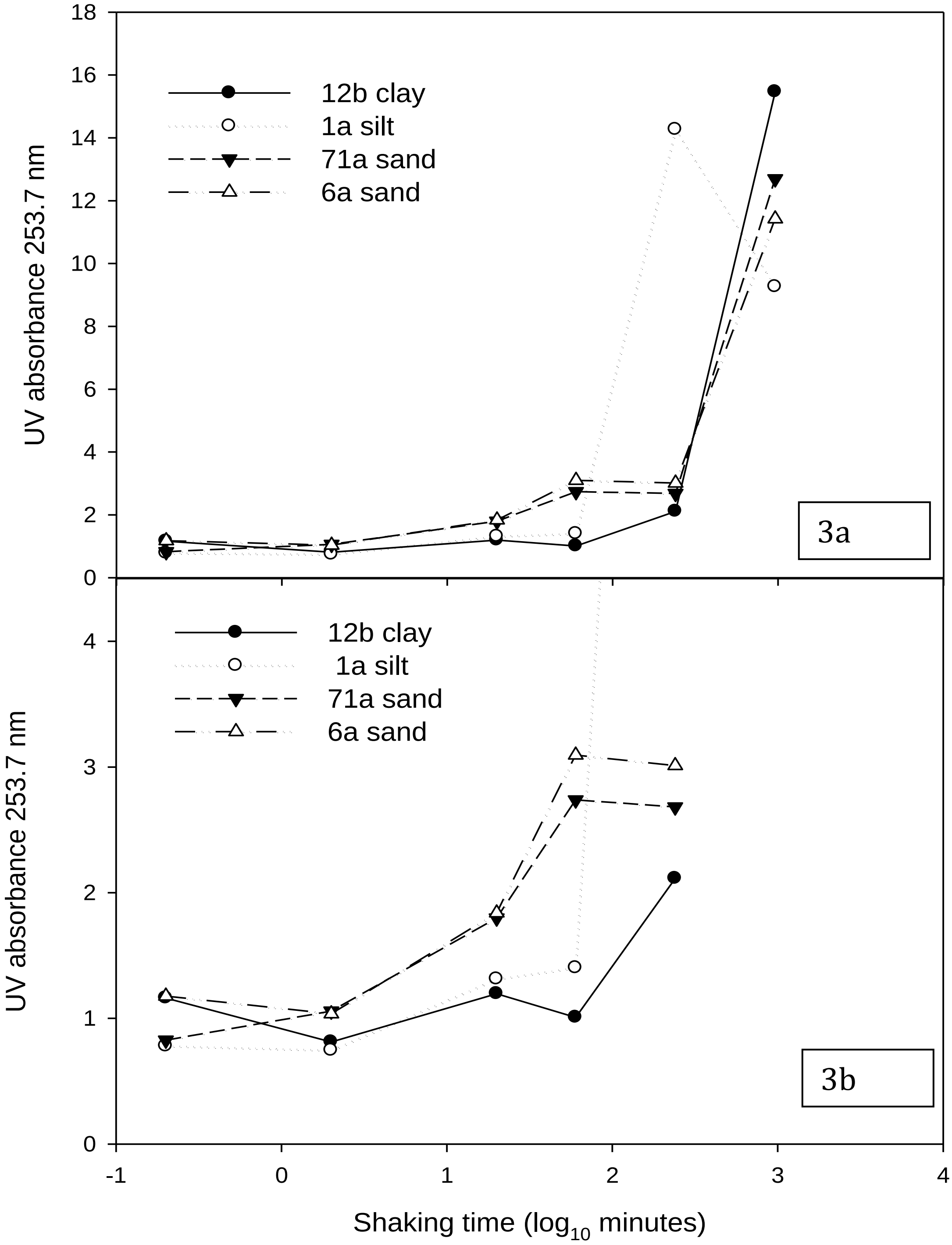


Figure 3: Impact of shaking duration on the concentration of humified dissolved organic matter (DOM) extracted from soils of different texture. Absorbance in 3b is presented with data for 16 hours omitted, to highlight the divergence between DOM recoveries the sand and silt/clay soils.

Standardising UV absorbance (SUVA) data for the total organic carbon extracted at each shaking time (**Figure 4**) highlights the difference in DOM recovery for the sand and silt soils, versus the clay soil. For most soils, shaking for 20 minutes extracted the highest concentration of humified (absorbance at 253.7 nm; **Figure 4a**) and aromatic (absorbance at 280 nm; **Figure 4b**) DOM. Standardising for the total organic carbon in solution also highlights the higher concentration of more resilient, aromatic DOM in the 71a sand sample (**Figures 4a** and **4b**). Further investigation is required to determine if the peak at 4 hours shaking for the clay soil is due to inorganic ions that absorb UV light, or humified DOM that has formed organo-mineral complexes with the clay particles, that require a longer duration of shaking for extraction.

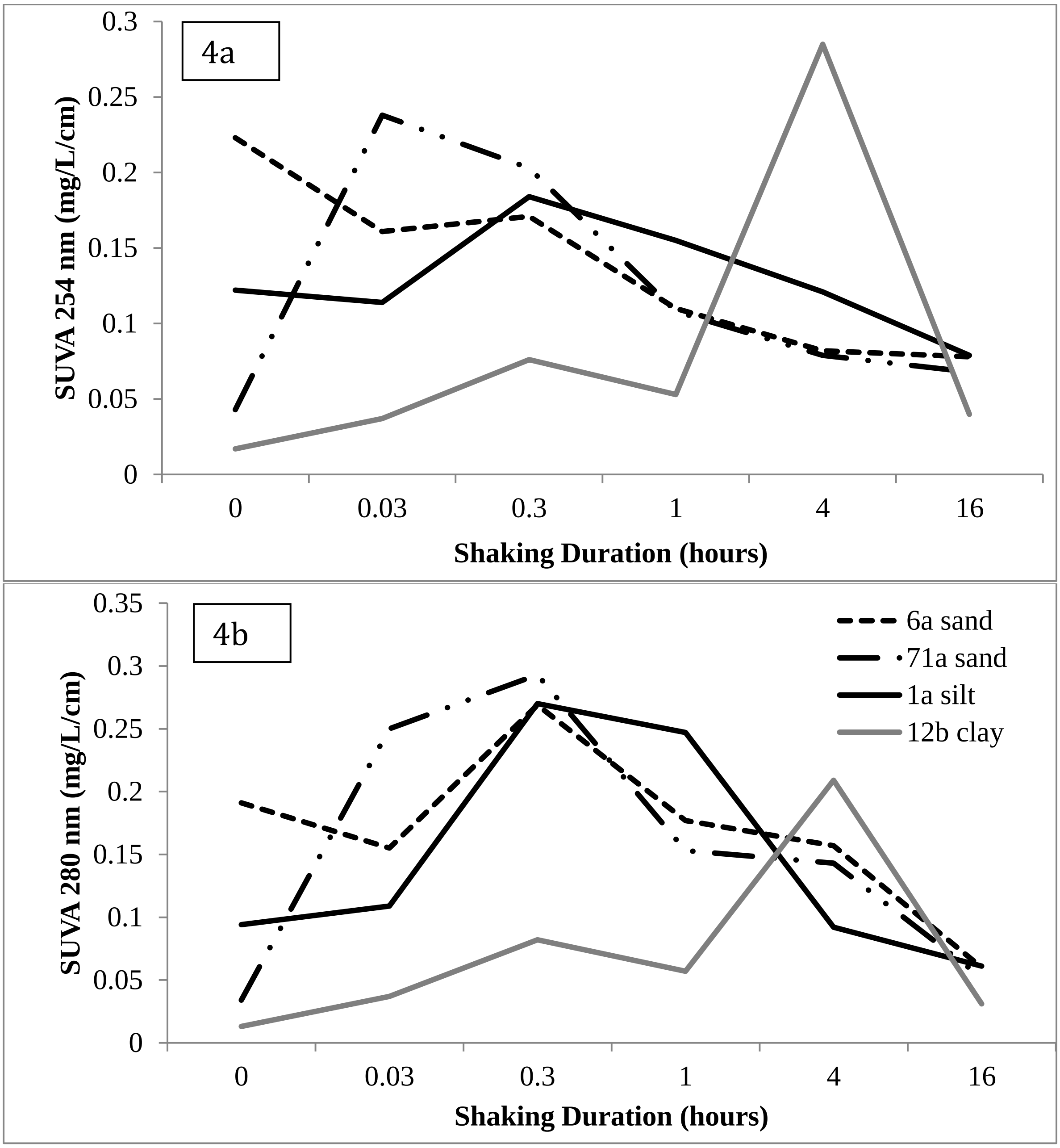


Figure 4: Absorbance at wavelengths of 253.7nm (humified DOM **4a**) and 280nm (aromatic DOM **4b**) standardised for the concentration of total organic carbon extracted at each shaking time. DOM refers to dissolved organic matter.

Effect of soil texture and shaking on the molecular size of DOM extracted in water

Large humified organic compounds bind at the secondary and tertiary structural level, to produce chemically resilient compounds with a longer residence time in soil. Smaller more biochemically reactive humified compounds react more readily with soil enzymes and microbes, reducing their residence time in soil. The ratio of absorbance at 250nm to absorbance at 350 nm (E2:E3) has been used by scientists studying aquatic humic substances, to indicate the molecular size of DOM. The E2 to E3 ratio is inversely proportional to molecular size.

Our results indicate the highest recovery of larger, humified DOM required a shaking duration of 20 minutes, with the exception of the 6a soil type (**Figure 5**). Shaking for a shorter or longer time increased the concentration of smaller humified organic compounds, especially for the clay and silt soil samples. These results suggest the peak in SUVA recoveries of DOM at both 253.7 and 280 nm for the clay soil (**Figure 4**) may be due to the extraction of smaller, physically protected organic compounds requiring a longer shaking time to break down the clay.

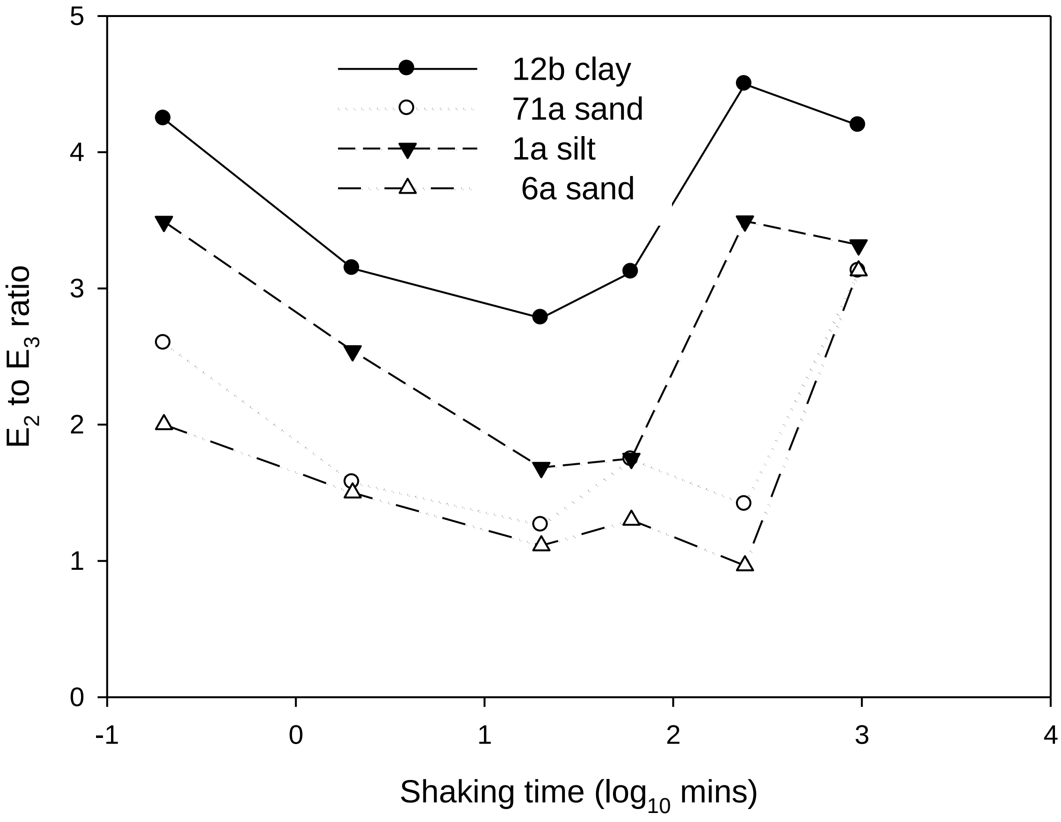


Figure 5: The influence of the duration of shaking on the molecular size of DOM extracted in a soil water solution for four soil samples. Molecular size is inversely proportional to the ratio of light absorbed at 250 and 350 nm (larger compounds have smaller E2:E3 ratios).

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Selection of topsoils from the 'Healthy Soils Project' in consultation with Dr Mike Bell

We have not started investigating the impact of wet/dry cycles or soil colour on the UV method due to the 3 month delay in commencing the project. Unfortunately, the soil samples from the 'Healthy Soils' Project that had been available for our use at the time of submission of our application (confirmed by Dr Mike Bell team leader), have subsequently been given to other researchers. We will be requesting access to soils from the Soil Carbon Research Project (SCaRP, co-ordinated by Dr Ram Dalal). Ram or the Soils Processes research scientist Kathryn Page will be contacted early in the New Year, to request access to the SCaRP soil collection.

We require less than one litre of each soil sample, preferably with different concentrations of iron and nitrate ions, known to absorb light in the UV spectrum. The SCaRP soils are preferred over coloured soils from the NCEA soil collection, as they are from cultivated, agricultural soils that have already been chemically characterised as part of a major Soil Carbon project. Reverting to the NCEA soil collection will not unduly impede our progress, but will require more detailed soil chemical analyses.

Suitability of the UV method for measuring the concentration of humified organic carbon in soils of different texture and salt content.

Results to-date, indicate the method is suitable for measuring the concentration of humified organic carbon in the main textural (particle size) soil classes, sand, silt and clay. Completing the analysis of the 8 soils listed in Table 1 will provide further information on the impact of the salt content (measured as the Electrical Conductivity or EC in **Table 1**) on DOM extraction, and on alternatives to centrifugation for removing soil particles prior to measuring UV absorbance. The optimal shaking time of 20 minutes indicated in **Figure 5** is feasible for a field-based test procedure.

The soil samples analysed so far have had relatively low organic matter contents (**Table 1**), but the humified DOM component has been relatively high (SUVA values in **Figures 4a** and **4b**). An alternative to centrifugation may be to reduce the soil to water ratio (2 g in 25 mL instead of the current 5 g in 25 mL), and to increase the diameter of the 0.45µ m glass fibre filter papers used to extract the DOM. These modifications will make the UV method more amenable for field use.

Environmental Impact

(detailing any new information on adverse or beneficial environmental impacts of conducting the project and/or implementing its findings)
The test is sensitive for the detection of large, humified organic compounds that improve the nutrient and water holding capacity, and the physical structure of soils. The carboxyl and phenolic structures that absorb UV light are primarily responsible for the cation exchange and buffering capacity of humified organic matter, of benefit both economically and environmentally where increases in the concentration of this fraction in cane soils can be demonstrated.

Intellectual Property & Confidentiality

(detailing any intellectual property considerations or discoveries made and if these are to be protected and how. Outlining any publications produced. Stating what information, if any, is to be treated as confidential, to whom and for how long.)
No change.

Communication and Adoption of Outputs

(indicating what messages have come from the project to date, how they have been communicated or what plans there are to communicate them, what activities have been conducted, and what information is available on adoption of project outputs. Include advice of any upcoming meetings, seminars, field days etc associated with the project, with dates, times, locations if known, and a brief description)
Once we have completed the first series of tests we will discuss results with Dr Phil Moody and Drs Schroeder, Dalal and Page if available. Recent discussions with Lawrence Di Bello (Herbert Cane Productivity Board) indicate he is interested in using our test to survey soils in the Herbert region suffering cane yield decline. Lawrence will be included in the Communication and Adoption phase of our project.

Proposed variations

(if any, to the project objectives, timing, costs, subsequent milestones or methodology. A request for a variation detailing the proposed changes should be included)
Only variation is substituting soil samples from the 'Healthy Soils' project with soils from the SCaRP project, and investigating the impact the duration of shaking has on the quantity and quality of DOM extracted from sand, silt and clay soil samples.

Personnel Changes

(advising any changes to the location or time input of project investigators or other related personnel. Note that SRDC must be advised if any Chief Investigator, or other Investigator who is paid half or more of a full time salary from SRDC funds, becomes unavailable, and alternative arrangements must be determined in consultation with SRDC)
No proposed change.

Other Matters

(relevant to the conduct or outcomes of the project that the investigators wish to bring to the attention of SRDC)
Losing access to samples from the 'Healthy Soils' project has reduced our capacity to utilise knowledge gained from related, completed research projects. However, access to soils from the SCaRP project may provide equivalent data for soils that represent the major agricultural soils of Queensland. Linking with this Australia-wide project may also improve communication of outputs.

Summary

Progress at this early stage (2 months into an 18 month project) is very encouraging and we anticipate no impediments to meeting future milestones. Analytical methods developed for investigating aquatic humic substances appear to be equally applicable for investigating dissolved organic matter in soils. The 20-minute shaking time for extracting large, humified organic compounds from soils of different texture is amenable for field testing, provided an alternative to centrifugation can be developed.

The loss of this more resilient organic carbon fraction from cropping soils on the Darling Downs over 20 to 50 years of cultivation is coincident with a decline in soil structure and fertility. Our test may enable growers to monitor the impact of organic inputs and other management practices on the concentration of humified organic matter in soil, with the potential to improve the resilience and sustainability of cropping systems.

Appendix 2: SRDC Research Project Milestone Report

SRDC project number:	**NCA012**
Project title:	**UV absorption as a tool for growers to benchmark humified organic carbon in soil**
Research organisation(s):	National Centre for Engineering in Agriculture (NCEA)
Chief Investigator(s):	Pam Pittaway (NCEA)
Objectives:	Specific objectives include: • Standardise $UV_{253.7}$ absorbance results for dissolved organic carbon content (SUVA) for a set of x10 sugar cane reference soils provided by Dr Bernard Schroeder • Compare SUVA results for a range of soils with other soil health criteria (eg pH, EC, CEC, wet oxidation SOC, DTPA extractable trace elements) • Determine if other elements known to absorb in the UV region interfere with absorbance at 253.7 nm • Determine if wet/dry cycles affect the reliability and reproducibility of the test. • Compare SUVA results for one soil type with a history of compost addition, with the same soil type amended with hay mulch only
Milestone number:	3
Due date for milestone:	1-8-2013
Milestone Title:	**Validation of UV method as a soil health index**
Achievement criteria	• Cane reference soils selected (Phil Moody, Bernard Schroeder), with varying concentrations of TOC and CEC. • UV method validated as a sensitive indicator of soil health, and management practices that are best suited for UV monitoring identified. • Documentation of the accuracy, reliability and repeatability of the method. • Submission of Progress Report on UV Method as a Soil Health Index

Milestone Achievement
(giving evidence to confirm the achievement of the milestone or reasons it was not achieved, including an overview of data and/or other relevant results)

A key outcome of Milestone 1 (MS1) was the validation of the suitability of the UV method for measuring the concentration of humified organic carbon in soils of different texture and salt content. The laboratory protocol developed for preparing soil solutions for testing, specifies adding 2 g of air-dried soil to 40 mL of distilled water, shaking at 15 rpm for 1 hour prior to centrifuging at 2,000 rpm for 5 minutes, then passing the supernatant through a glass fibre filter with a pore size equivalent to 0.45 µm. This protocol has been used in all subsequent research.

Results from Nick Stuckey's PhD project using UV absorbance and the hot permanganate test to characterise the chemistry and photochemistry of aquatic humic substances, confirmed UV absorbance is sensitive to the concentration of total organic carbon, and the hot permanganate test is a sensitive indicator of the photo-reactivity of the water. Nick's research also indicated SUVA and the hot permanganate index (also standardised for total organic carbon) measure very different properties of organic matter.

On the basis of these results, all UV absorbance (UVA) data obtained in our SRDC project were standardised for the concentration of dissolved organic carbon (SUVA).

With respect to the individual achievement criteria for MS2, we are able to report as follows:

Sensitivity of UV absorbance as an index of humified organic carbon in x10 sugar cane soils

The ten sugar cane reference soils were prepared for analysis using the UV protocol described above, with the concentration of dissolved organic carbon and dissolved nitrogen determined using an infra-red auto-analyser (**Table 1**). Dissolved organic matter was selected for analysis, as the dark brown staining visually used to indicte the depth of topsoil is due to the limited leaching of these compounds down the soil profile.

Literature on aquatic humic substances indicates absorbance at 253.7 nm is due to the light-absorbing properties of plant-derived aromatic compounds, and some aliphatic compounds containing unsaturated bonds. In the litter layer, chemically resilient aromatic compounds including lignins (plant structural compounds), phenols and tannins (dark coloured anti-microbial and anti-insect compounds), are slowly transformed into humus by microbial enzymes. Less chemically resilient organic matter is more rapidly used as a microbial energy source, with the organic carbon transformed into the gas carbon dioxide. What remains becomes increasingly complex, a process described as humification. In light soils including sands, the concentration of humus-like organic matter determines the nutrient exchange (cation exchange capacity) and water retention properties of the soil. This more slowly degraded organic matter also provides the 'maintenance' supply for soil microbes, once the more available (also termed labile) organic matter sources have been depleted.

In the ten sugar cane reference soils analysed, the concentration of dissolved organic carbon covers a much greater range than the concentration of total organic carbon (3.69 – 18.32 mg L^{-1}, and 0.85 to 3.52% respectively; **Table 1**). The Rocky Point humic gley as the name suggests, has the highest concentration of total organic carbon, and of dissolved organic carbon. However, the concentration of dissolved, humified organic carbon is much lower (**Figure 1**), indicating the anaerobic conditions under which this soil type developed limited microbial decomposition.

Table 1: Sugar cane reference soils provided by Dr Bernard Schroeder for analysis. Bulk density was tested to determine how field volumetric measures of soil for UV absorbance testing may affect results. Iron (Fe 3^{+}) was measured to indicate the potential for interference with absorbance in the UV spectrum. Dissolved organic carbon (DOC) and dissolved nitrogen (DN) were determined on a filtered soil solution of 2g in 40 mL.

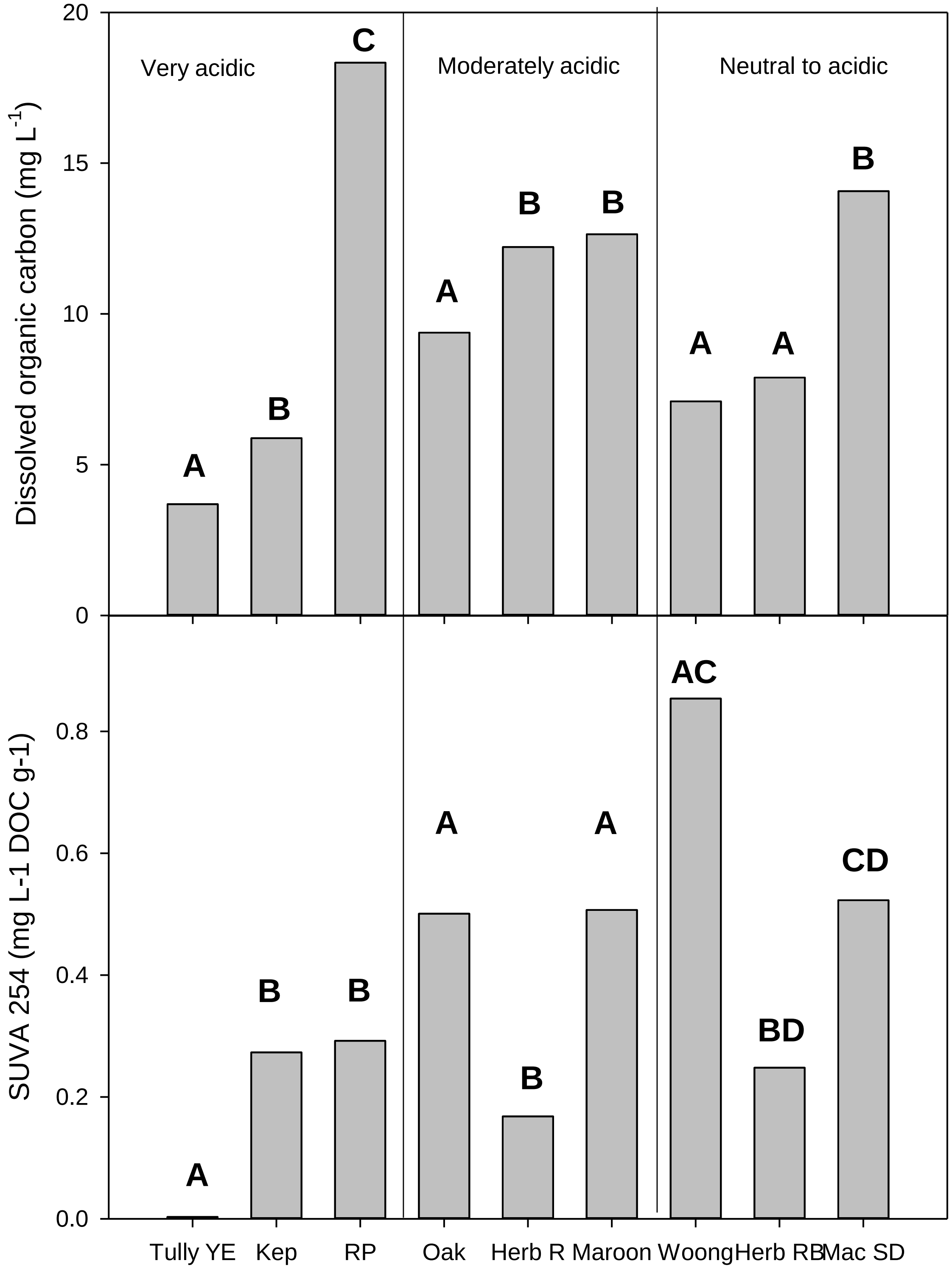
Reference soil	Bulk density g L^{-1}	pH	EC µS	Fur Ind Org C %	<0.45µm DOC mg L^{-1}	<0.45µm DN mg L^{-1}	Fe mg kg^{-1}
Woongarra Bundaberg	1188	6.17	86.7	1.58	7.10	2.60	645
Yellow Earth Tully	903	4.43	170.1	1.17	3.69	6.20	2
Kepnock Bundaberg	1214	4.89	170.1	0.96	5.88	4.58	80
Red Sand Herbert	1225	5.12	32.22	1.49	13.09	0.87	71
Sodic Duplex Mackay	1183	6.7	188.8	1.64	14.07	5.06	294
Riverbank lee Herbert	1139	6.35	34.66	0.79	7.89	0.96	136
Oakwood Bundaberg	1104	5.29	179.1	1.66	9.32	6.32	331
Humic gley Rocky Point	989	4.52	463	3.52	18.32	11.00	203
Barratta clay Burdekin	1158	8.07	69.8	0.85	8.35	1.26	262
Maroondan Bundaberg	1005	5.62	441	2.31	12.78	13.45	405

For most of the cane reference soils, the concentration of dissolved organic carbon (DOC in **Table 1**) is proportional to the concentration of total organic carbon (furnace induction OC in **Table 1**). Results for the $\text{SUVA}_{253.7\text{nm}}$ (SUVA) absorbance test are very different, as would be expected if the test is sensitive for humified organic carbon only (**Figure 1**). In **Figure 1**, nine of the ten cane reference soils were grouped according to their acidity. The Barratta clay soil has not been included, as this is the only soil that is alkaline (pH 8.07).

Very acidic soils lack the buffering conferred by specific clay minerals such as smectite, which also determine the cation exchange capacity of the soil. In very acidic soils, the presence of humified organic acids may improve soil health by providing an alternative form of pH buffering and cation exchange capacity. Of the three very acidic soils, the Tully Yellow Earth (Tully YE in **Figure 1**) would suffer most from lack of buffering, whereas the higher concentration of humified DOC (high SUVA) in the Kepnock (Kep) and Rocky Point (RP) soils should improve soil health.

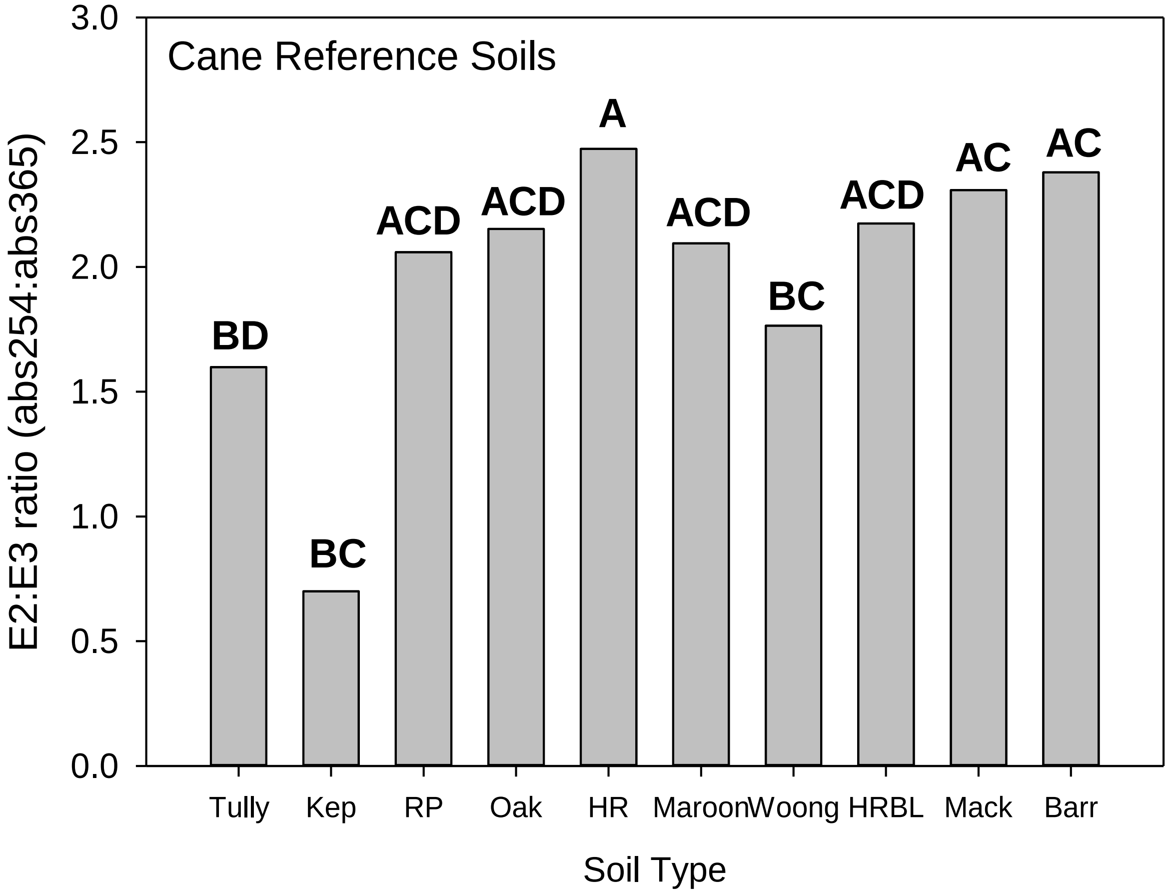
Of the moderately acidic soils, the Herbert Red Sand has a very low SUVA relative to a high DOC value, suggesting fertiliser use efficiency and soil health may be lower than the Oakwood and Maroondan soils which have higher SUVA values. Of the neutral to acidic soils, SUVA is highest for the Woongarra soil, conferring the soil health benefits of additional buffering and cation exchange capacity, potentially improving phosphorus fertiliser use efficiency in P-sorbing soils.

Figure 1: Dissolved organic carbon and $SUVA_{253.7nm}$ results for nine of the ten sugar cane reference soil samples provided by Dr Bernard Schroeder. The samples were grouped according to pH (water extract). Data for the Barrata clay have been omitted as it was the only alkaline soil. Within each pH category, bars with the same letter are the same (not significantly different using a one-way ANOVA). Soil types are specified in **Table 1**.



The ratio of absorbance at 253.7 nm with absorbance at 365 nm has been used by aquatic scientists to indicate molecular size, which is inversely proportional to the ratio. The data for the ten cane soils indicates soils with a higher concentration of dissolved organic carbon (**Figure 1**), tend to have a higher proportion of smaller molecular weight compounds (**Figure 2**). As with the $SUVA_{253.7}$ data (**Figure 1**), the rank order of the ten sugar cane reference soils for E2:E3 ratio is different to $SUVA_{253.7}$ and to DOC, indicating these tests provide different information on the properties of the DOC in the soil solutions.

Figure 2: Molecular size of humified organic matter as indicated by the inverse of the ratio of absorbance at 253.7 nm with absorbance at 365 nm. Bars with the same letter are the same (not significantly different).



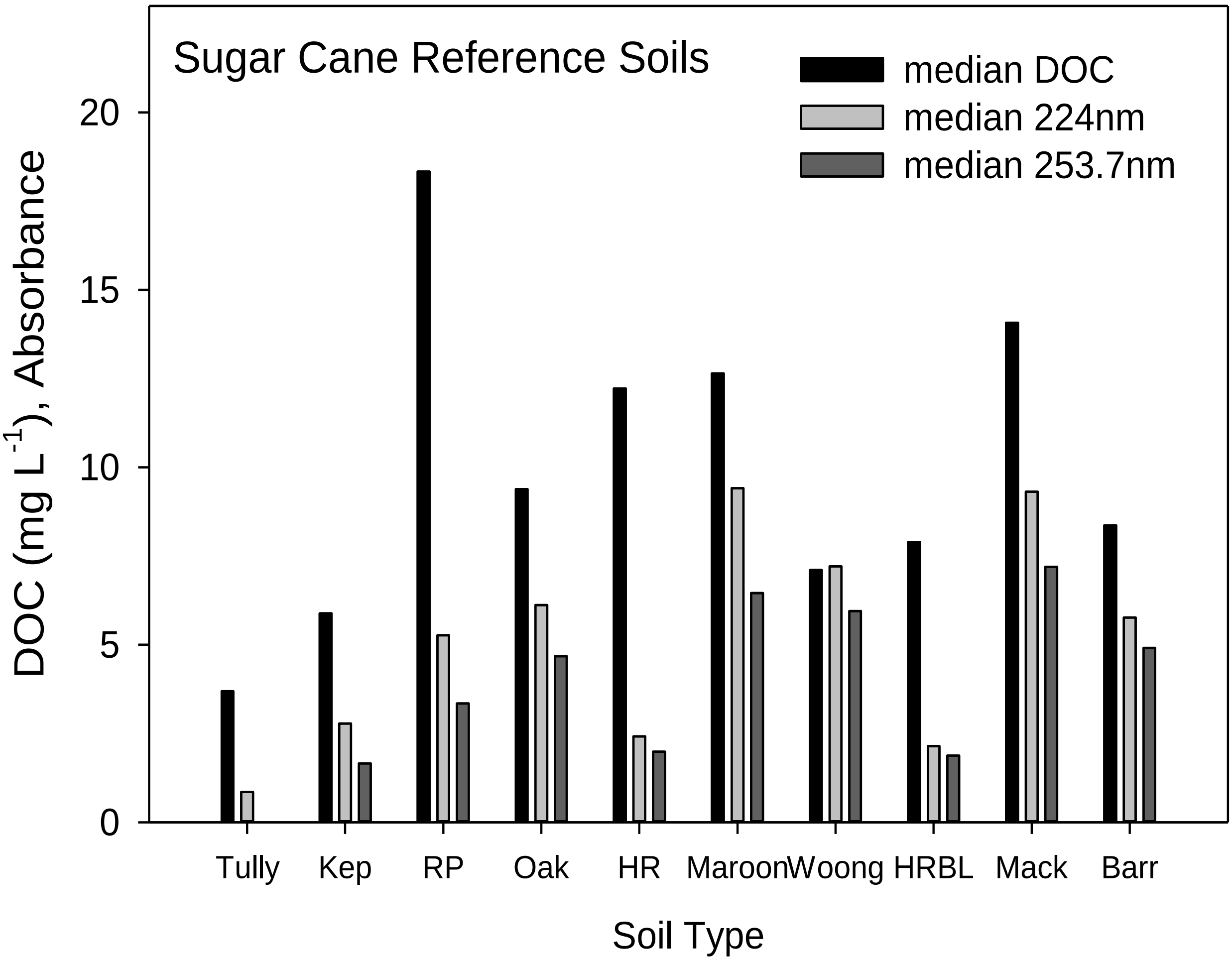
Absorbance at 253.7 nm for aquatic humic substances are correlated with DOC concentrations (Nick Stuckey pers. comm.). However, DOC was not linearly related to absorbance at 253.7nm for the ten sugar cane reference soils ($R^2 = 0.441$, $P = 0.202$, $N = 10$; the median value of the x6 replicates for each soil type were used). Dissolved nitrogen concentrations were relatively high in some of the soils (**Table 1**), as indicated by very high values for absorbance at 224 nm. Absorbance at 224 nm is considered to be specific for the covalent bonds in nitrate.

It is possible that absorbance at 224 nm may interfere with absorbance at 253.7 nm. Absorbance at 224 nm is linearly related to absorbance at 253.7 nm ($R^2 = 0.980$, $P < 0.001$, $N = 10$; the median value of the x6 replicates for each soil type were used), with absorbance in the nitrate region (224 nm) reducing absorbance in the aromatic organic carbon region (253.7 nm). The relationship is:

$$\textbf{Absorbance at 253.7 nm} = -0.179 + (0.777 \times \textbf{absorbance at 224 nm})$$

This result suggests that light energy absorbed within the nitrate wavelength (224nm), reduces the amount of light absorbed within the aromatic organic carbon wavelength (253.7nm). The bar graph in Figure 3 highlights the close relationship between absorbance at 224 nm and absorbance at 253.7 nm, but lack of an association with the concentration of dissolved organic carbon (DOC). Median values have been used, as the individual data sets are not normally distributed. This means the average value of each data set does not accurately describe the range of values.

Figure 3: Concentration of dissolved organic carbon (infra-red auto analyser), humified organic carbon (absorbance at 253.7 nm) and nitrate (absorbance at 224 nm). Median values are given as data is not normally distributed.



Sensitivity of SUVA as an index of soil health

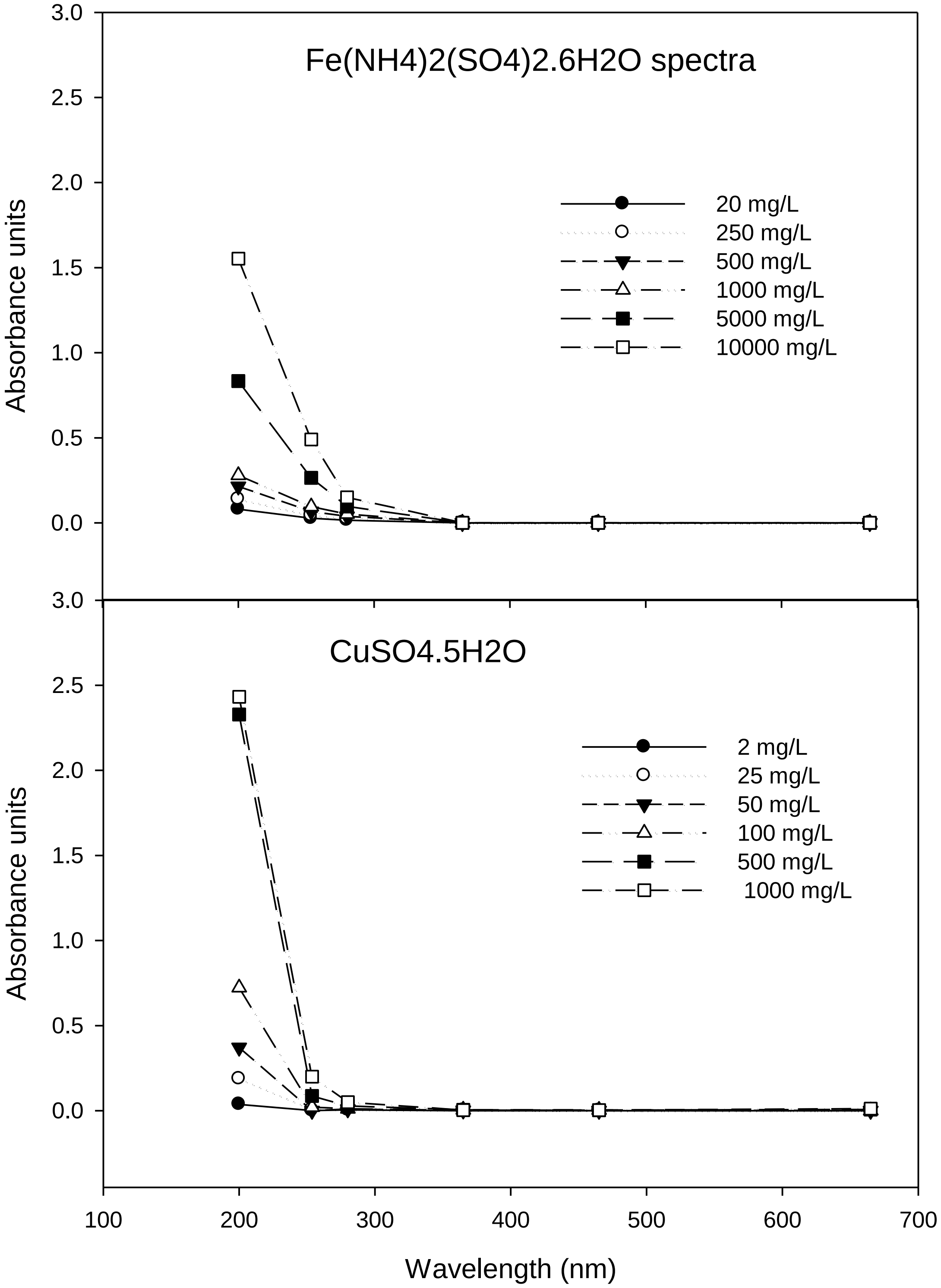
The preliminary analysis of the soil health status of the sugar cane reference soils will be expanded once we receive further information on the properties of these soils from Drs Phil Moody and Bernard Schroeder. Further testing of the NCEA soils analysed as part of Milestone 1 were undertaken to determine if the SUVA values correlate with the proportion of the cation exchange capacity that is conferred by humified organic matter (variably charged cation exchange capacity). The humified organic matter contribution to the CEC was derived by subtracting the CEC measured using 1M NH_4Cl alcoholic solution adjusted to pH 8.6, from the effective CEC measured using 1M NH_4Cl alcoholic solution adjusted to pH 7.0 (P Moody personal communication). The percentage contribution was calculated by dividing by the effective CEC and multiplying by 100 (**Table 2**).

SUVA results for the NCEA soils in **Table 2** need to be repeated using the 2g in 40 mL protocol, as results presented in MS1 indicate colloidal interference reduces SUVA when higher soil to solution ratios are used. Of the data available, SUVA results appear to be correlated with the percentage of the ECEC attributed to humified organic matter. The % variably charged ECEC contribution is highest for the lighter silty soil (1A in **Table 2**), but the low value for the 6A sand suggests the SUVA values will also be very low. The high SUVA value for the 7a clay but low % variably charged ECEC indicates the clay is predominantly responsible for the ECEC. As expected, the proportion of the variably charged CEC in the ECEC for the cured, sugar milling compost (NP compost) and the compost-amended soil is relatively high (59% and 22% respectively, **Table 2**).

Table 2: Updated data for the NCEA soils used in MS1, and for the sugar milling compost and the organically amended soils used for investigating the potential of wet and dry cycles, and transitional metals and unsaturated bonds, to interfere with absorbance at 253.7 nm. Variably charged ECEC% refers to the proportion of the CEC attributed to humified organic matter (P Moody pers. comm.). NA indicates data on the 2g in 40 mL solutions are still to be done. Asterisk* indicates suspected interference with $SUVA_{253.7}$ value.

Reference soil	pH	EC mS	Org matter %	SUVA cm^{-1} mg^{-1}	ECEC mEq $100g^{-1}$	% ECEC variably charged
7a clay	9.0	0.271	2.2	0.680	38	15.8
12B clay	8.9	0.716	0.8	NA	40	32.5
7B silt	9.1	1.107	0.8	0.963	50	40
1A silt	8.7	0.106	0.6	NA	32	68.8
6A sand	8.0	0.028	0.9	NA	6	0
71A sand	7.8	0.034	1.9	NA	7	14.3
NP compost	6.8	2.660	10.2	**0.0462***	34	58.8
DG mulch + compost	7.3	0.130	6.2	0.259	14	21.4
LA mulch only	5.5	0.530	3.8	0.184	6	0

Figure 4: Absorbance spectrum of standard concentrations of ferric ammonium sulphate and copper sulphate in distilled water. The gradual reduction in absorbance from 253.7 nm into the visible and infra-red spectrum is characteristic of most light-absorbing (chromophoric) compounds.



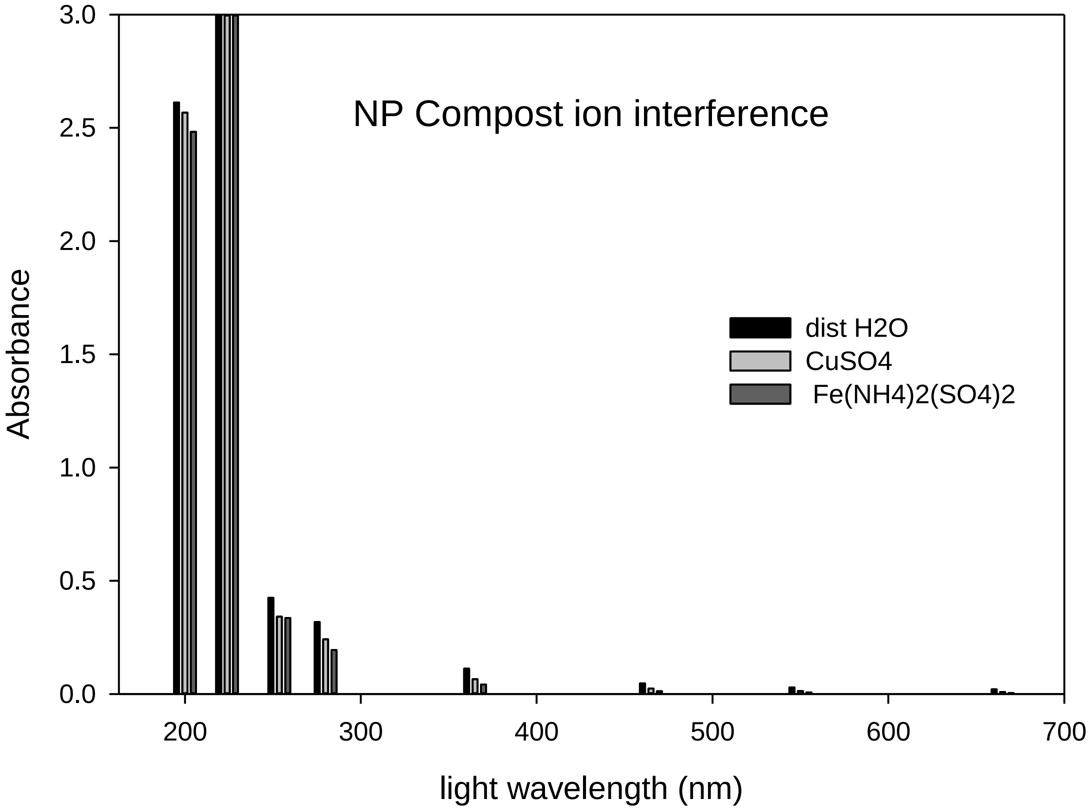
Potential for other UV absorbing elements to interfere with UV absorbance results

Transitional metals including iron (Fe), copper (Cu) and manganese (Mn) also contain unsaturated bonds that absorb in the UV region, as does nitrate, phosphates and carbonates. Nitrate is known to absorb wavelengths around 224 nm most strongly, with the potential to interfere with absorbance at 253.7 nm. To test for possible interference, a dilution series (2, 25, 50, 100 and 500 mg/L) from stock solutions of 50 mg/50 mL (1,000 mg/L) of Na_2HPO_4, $Fe(NH_4)_2(SO_4)_2.6H_2O$, $NaHCO_3$, $CuCl_2$ and $CuSO_4.5H_2O$ were prepared to determine at which wavelengths the chemicals absorb most strongly. Of the solutions tested, only $Fe(NH_4)_2(SO_4)_2.6H_2O$ and $CuSO_4.5H_2O$ absorbed strongly at 253.7 nm, and only then at the highest concentrations tested (**Figure 4**)

In some published papers the contribution of interfering transitional metals to absorbance at 253.7 nm has assumed to be additive. Typically, scientists use standard concentrations as indicated in **Figure 4**, to determine the absorbance associated with a given concentration of the transitional metal in solution. They then subtract this value from that of the solution being tested, to indicate absorbance due to humified organic matter only.

Evidence from our results (**Figure 5**) indicates the interference is not additive, but conversely, reduces the intensity of UV light absorbed by humified organic matter. High concentrations of $Fe(NH_4)_2(SO_4)_2.6H_2O$ and $CuSO_4.5H_2O$ were used instead of distilled water to prepare a 2.5g in 40 mL solution of the humified NP sugar milling compost characterised in **Table 2**. 2.5 g was used instead of 2.0 g as this compost was known to have a high water content (56.3%). The control solution was the standard protocol of 2.5 g in 40 mL of distilled water.

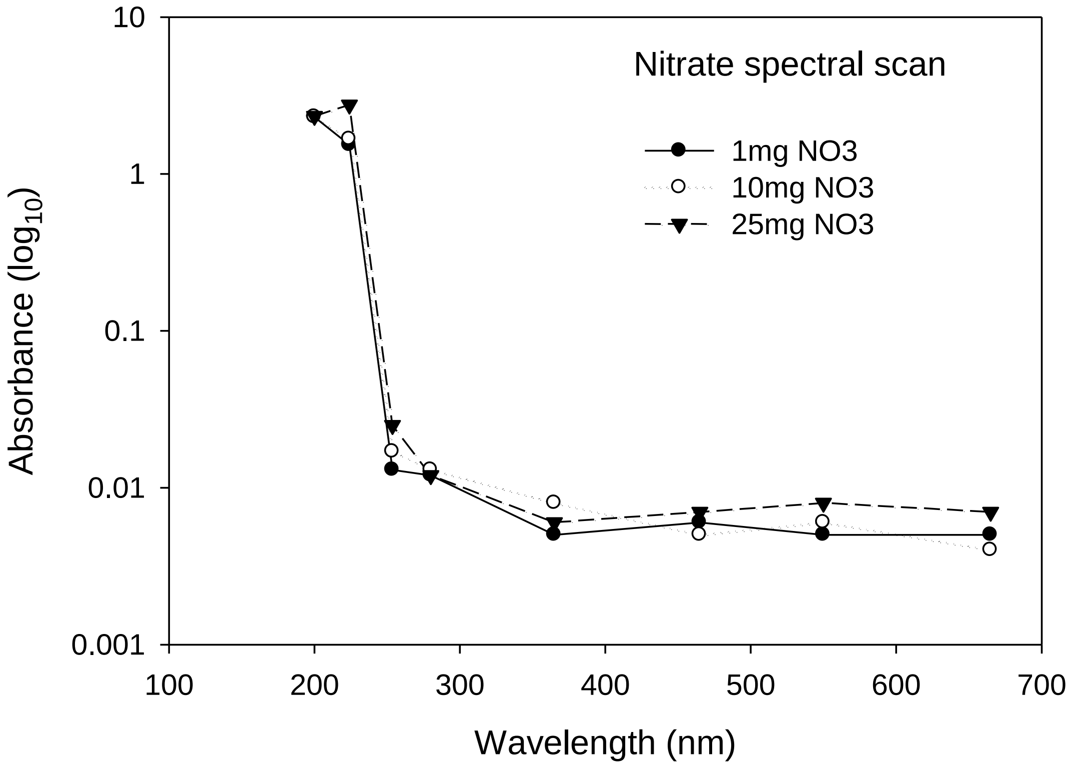
Figure 5: The reduction in UV absorbance of dissolved organic matter extracted from a cured sugar milling compost (NP) containing high concentrations of copper (Cu) and iron (Fe) ions. The off the scale peak at 224 nm indicates the high concentration of nitrate in the compost.



The **reduction** in UV absorbance for the NP compost in Cu and Fe solutions (**Figure 5**) indicates organo-metallo complexes formed between the humified organic compounds in the compost and the transitional metal ions may reduce the concentration of chromophores absorbing light. Recent research into the photochemistry of UV light absorption indicates why this is the case. Highly accurate lasers were used to destroy the compounds in aquatic humic substances responsible for light absorption (chromophores) at specific wavelengths. They found that destroying specific chromophores absorbing above a wavelength of about 255 nm reduced absorbance at that wavelength, and also at successive wavelengths below 255 nm. The scientists concluded absorbance above 255 nm was associated with different classes of chromophores, whereas absorbance below 255 nm was due to successive electron donor and acceptor reactions.

This property of donor -acceptor transfer explains why absorbance of transitional metals around 253.7 nm reduces the capacity of humified organic matter to absorb light. The electrons donated by the transitional metals saturate the shared bonds that would otherwise absorb light. The very low absorbance at 253.7 nm observed for the NP compost (**Table 2**) may also be explained by this hypothesis. In **Figure 5**, UV absorbance at 253.7 nm is extremely low, for a humified compost known to have a high concentration of dissolved organic matter (9.6 mg L^{-1}, or 17.1 mg L^{-1}on a dry mass basis). The off-scale peak at 224 nm is due to the high nitrate concentration mineralised by this cured compost during 5 years of storage (Total N 5.0 g kg^{-1}, or 8.9 g kg^{-1}dry mass basis, nitrate N is 1.65 g kg^{-1}), which may donate sufficient electrons to saturate the aromatic bonds that otherwise absorb light at 253.7 nm.

Figure 6: Spectral scan for standard nitrate concentrations (mg L^{-1}). Absorbance values have been log10 transformed to highlight absorbance at 253.7nm.

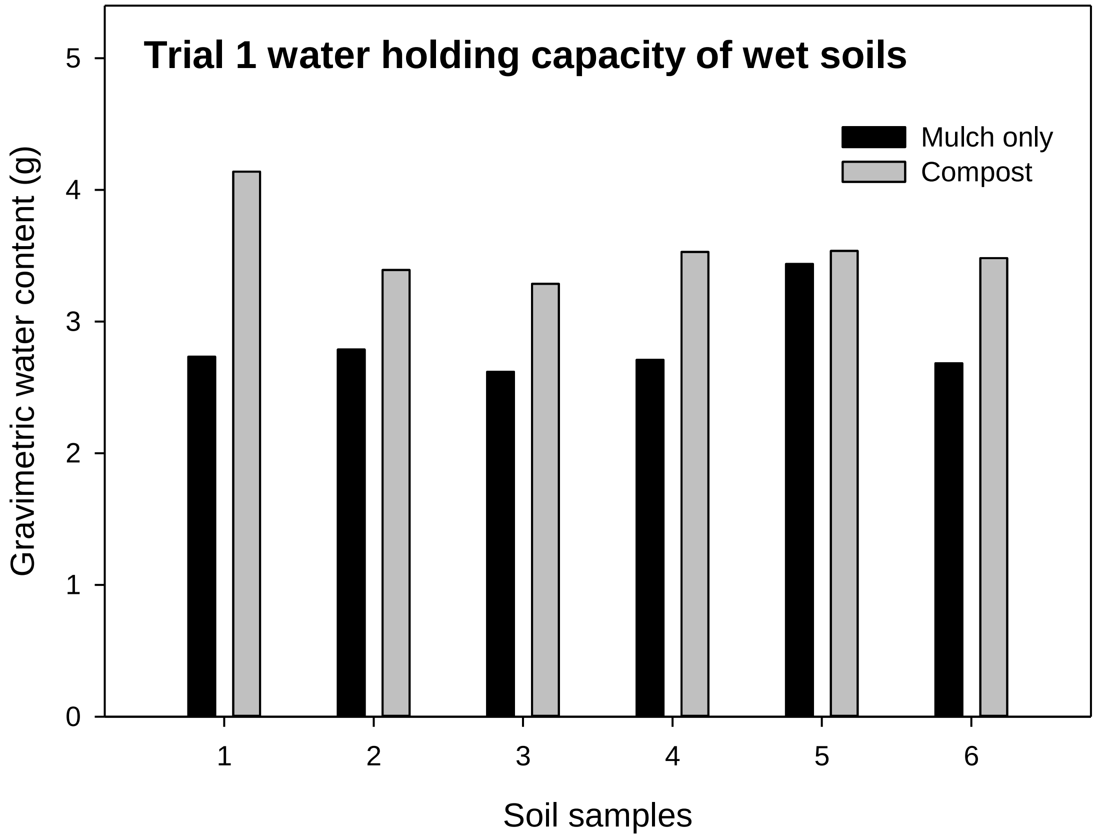


During the final stage of this project, benchmarks for the transitional metals Fe and Cu, and for nitrate will be calculated to indicate the maximum concentration of these chemicals in solution that can be tested using the UV method. Above these concentrations, the likelihood of interference compromises the sensitivity of the UV method as an index of humified organic carbon.

Management practices that affect UV absorbance test results

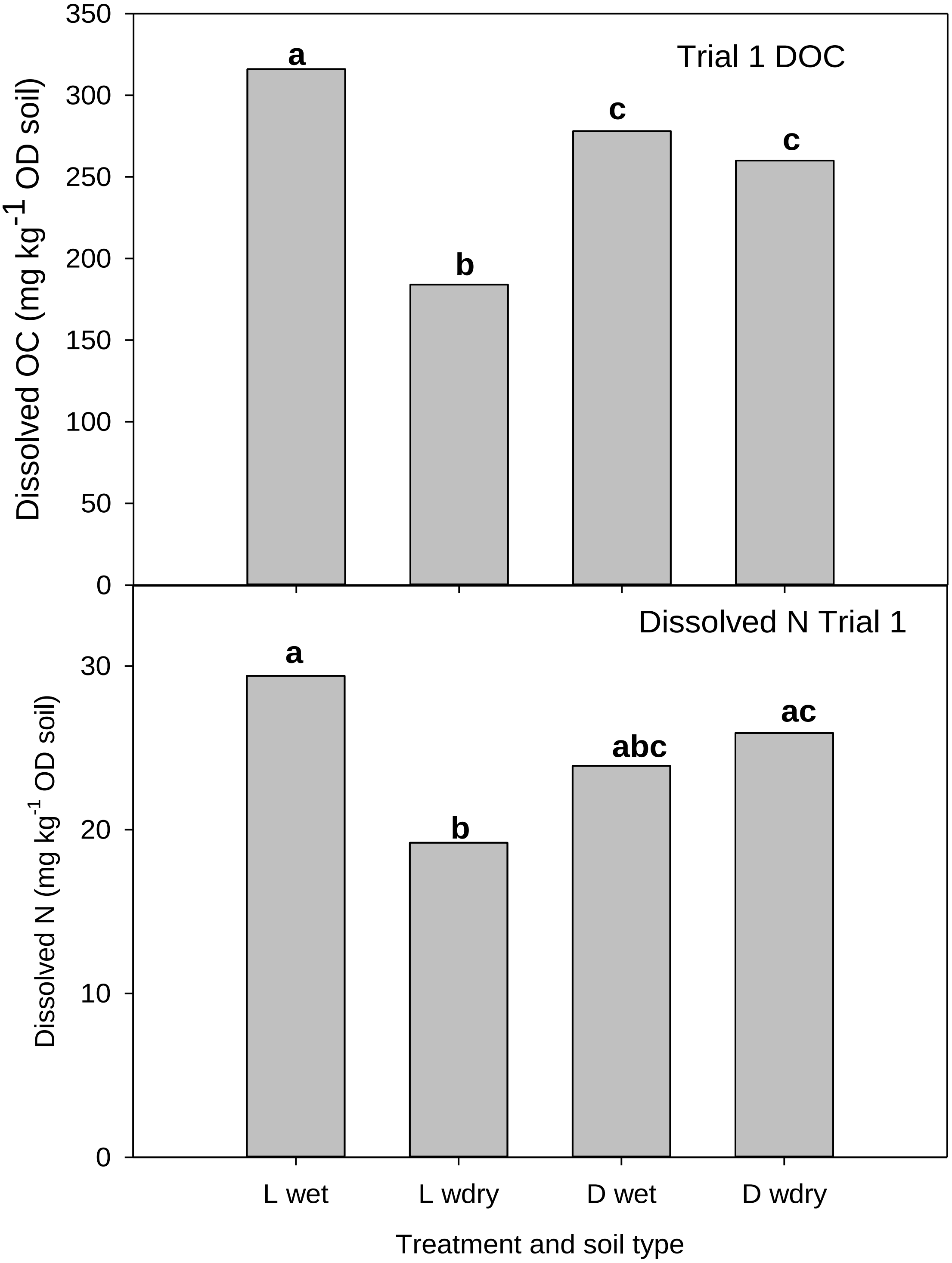
In the absence of the ‘Healthy Soils’ samples originally offered to the project by Dr Mike Bell, a sodic soil type in the Laidley area was used to test whether the UV test was sufficiently sensitive to detect changes in humified organic carbon associated with the application of organic amendments. Two adjacent plots separated by about 100 m have a history of hay mulch being applied annually to the soil surface, but only one plot (DG) has a history of repeat applications of cured compost over a 10 year time span. A comparison of the gravimetric water content of the two soils wetted up in containers described in **Figure 11** confirmed that the addition of four or five applications of compost at a rate of approximately 5 t ha^{-1}over the 10 year period has increased the water holding capacity of the soil relative to the mulch only plot (**Figure 7**).

Figure 7: The amount of water held in the capillary wetted soil plots treated with mulch only or with repeat applications of compost over 15 years. Compost addition has increased the water holding capacity of the soil.



The mulch only soil had a higher concentration of dissolved organic carbon (L in **Figure 8**), which may reflect the soil sampling method. The compost and mulch-amended soil was sampled at a depth of 20 cm, whereas the mulch only soil was sampled immediately below the litter layer. About a third of the dissolved organic carbon originally in the mulch only soil was readily available for microbial activity, as exposing soil samples to a sequence of wetting followed by 20 days of drying, reduced the concentration of DOC (**Figure 8**) and of dissolved nitrogen (DN, **Figure 8**). Some of the readily available DOC compounds must absorb UV light, as the drying cycle (w&d in **Figure 9**) increased the SUVA values of the soil samples relative to the continuously wet samples (microbially produced light-absorbing compounds).

Figure 8: The effect of mulch only and mulch and compost on the concentration of dissolved organic carbon (DOC) in a sodic soil. DOC values were expressed as mg per kg of soil on an oven-dry basis, to account for the different water holding capacity of the two soil treatments (**Figure 7**). Columns with the same letter have the same SUVA values (are not significantly different).



The DOC concentration in the mulch only plot was higher than the compost-amended plot for the continuously wet samples (L wet versus D wet in **Figure 8**). The microbial stimulation induced by drying and rapidly re-wetting the samples reduced the mulch only concentration below concentration of the compost-amended samples. Within one hour of rapidly rewetting the dried mulch only samples, microbial stimulation had reduced the concentration of dissolved nitrogen. There was no corresponding reduction in the compost-amended soils sampled below the litter layer, in the absence of fresh, more readily available organic carbon.

$SUVA_{253.7}$ values indicate the concentration of humified organic carbon was higher in the compost-amended soil samples (D wet and D w&d in **Figure 9**), with the microbial stimulation induced by rapidly rewetting dried soils having no impact. $Suva_{253.7}$ results for the mulch only soil samples indicate the proportion of DOC absorbing UV light was smaller in the continuously wet samples, increasing as microbial activity reduced the concentration of readily available organic compounds (L wet and L w&d in **Figure 9**).

These results indicate management practices including amending soils with mulch and with compost, and exposing sol samples to wet/dry cycles, can be detected and interpreted using the UV method. Fine, microbially available particles immediately under the mulch contributed to a significantly higher concentration of dissolved organic carbon, with these simpler, more microbially available organic compounds diluting the $SUVA_{253.7}$ values for the mulch only samples (L wet in **Figure 10**). The mulch and compost amended plot was sampled well below the mulch layer, and contained a higher proportion of humified organic carbon (SUVA253.7 values for D wet and D w&d in **Figure 10**), that did not significantly change under drying and rapid re-wetting. These results were repeatable when a shorter duration of drying was applied, with a longer (24 hour) incubation period after rapid re-wetting (**Figure 11**). However, the longer incubation period reduced the SUVA values by about 30%.

Figure 9: SUVA values for the adjacent soil plots treated with mulch only (L) and with mulch and compost (D). Wet refers to soil samples that remained capillary wet for the duration of the experiment, w&d refers to wet samples dried over 20 days (**Figure 11)**. Rapidly re-wetted soils were incubated for one hour before sampling. Columns with the same letters have the same SUVA values (are not significantly different).

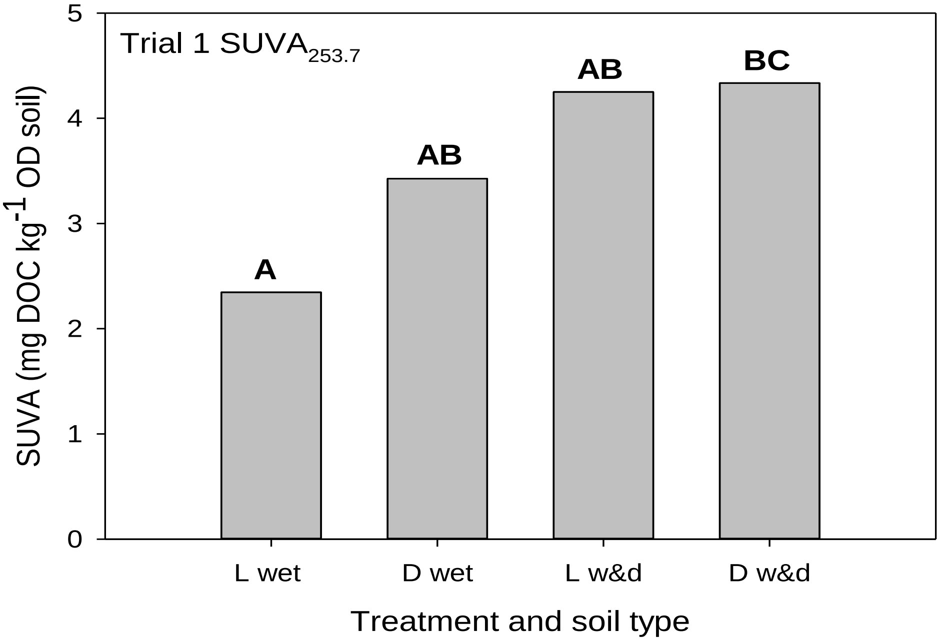
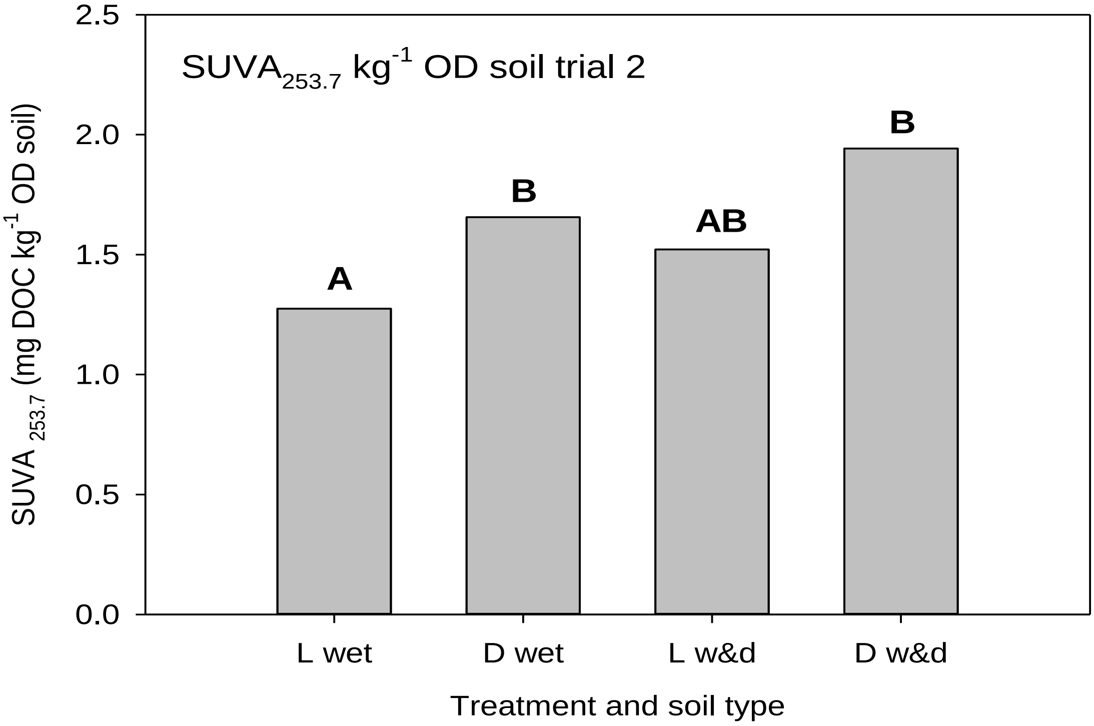


Figure 10: SUVA values for the second wet/dry trial established as described for the first trial (Figure 9), but with the soils incubated for 24 hours before sampling. Separate containers were used to capillary wet all soil samples for 48hr, establishing the water reservoir 15 mm below the soil samples



Impact of wet/dry cycles on validity of UV method as an index of humified organic carbon

The main objective of investigating the sensitivity of SUVA values to differences associated with exposing soil samples to continuously wet versus wet/ dry cycles, was to establish how much of the absorbance at 253.7 nm was due to chromophores present in less resilient DOM. Garden soil from a vegetable patch historically amended with humified compost and annually covered with grass mulch was compared with an adjacent plot receiving annual mulch applications only. Both soils were covered with mulch at the time of sampling, but the garden soil sample was taken from well below the litter layer.

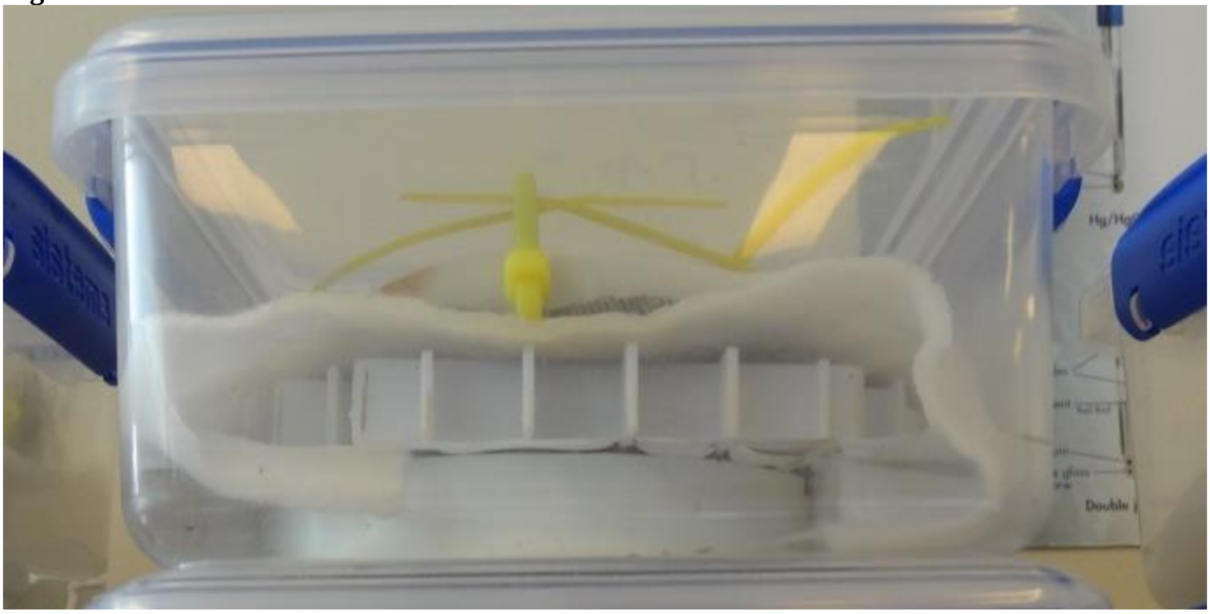
Separate containers were used to capillary wet all soil samples for 48hr, establishing the water reservoir 15 mm below the soil samples (**Figure 11**). Half of the samples were replaced in taller drying chambers, with silica gel added to yellow screw top medical specimen containers glued to the base of the lid. In Trial 1 the samples were slowly dried over a 20 day period. In Trial 2, a mesh support replaced the hydrophilic geofabric in the drying chambers, reducing the drying period to 12 days. The dried geofabric soil samples were removed from the dessication containers, and water was added using a syringe to rapidly rewet the soil to the equivalent weight of the original capillary watered samples (DW, dry wet treatment).

In the literature, applying one wet/dry cycle and rapidly re-wetting is known to stimulate microbial activity. Over the drying cycle stressed microbes release organic matter, which is available for microbes once the soils are re-wetted. This is evident as a reduction in the concentration of dissolved organic carbon and nitrogen in the L wd treatment relative to the L wet treatment in Trial 1 (**Figure 8**). In Trial 1 the compost and mulch soil samples were not affected by wet/dry cycles.

Figure 11: Plastic chambers used to capillary wet soil samples (short containers on right of Fig 11a, enlarged in Fig 11b), and to dry pre-wetted samples with silica gel used as the dehydrating agent (tall containers on left). Silica gel was added to perforated screw-capped vials fixed to the base of the lids. In the wetting chambers water was added to the top of the PVC ring, maintaining a distance of 15 mm between the soil samples and the water. The hydrophilic white fabric was removed from under the soil samples for the drying cycle. The soil samples were placed on a filter paper, supported by a wire mesh (detailed in Fig 11b). During the drying cycle, the PVC ring raised the wire mesh above the base of the chamber.

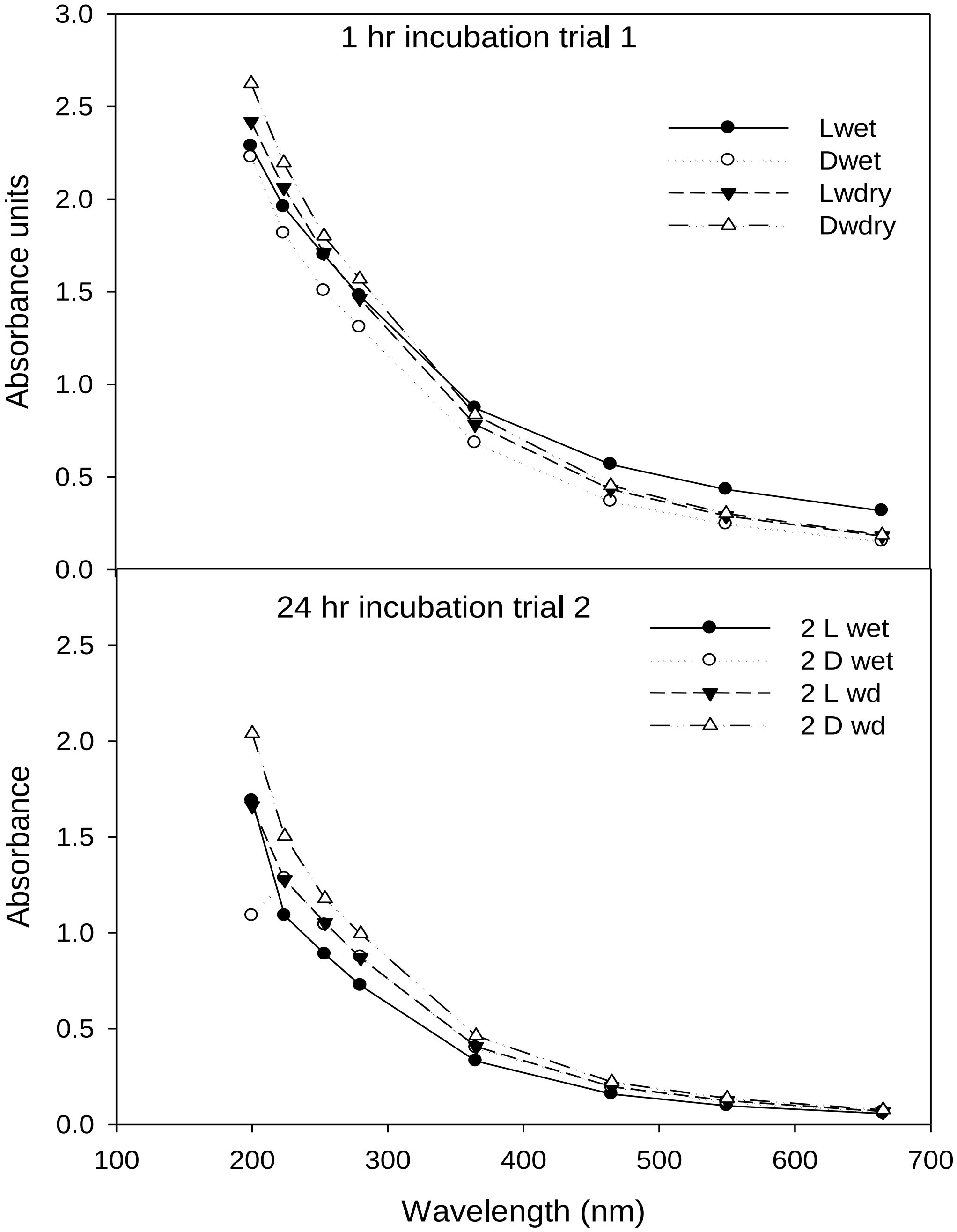
Figure 11a

Figure 11b



$SUVA_{253.7}$ values indicated the compost-augmented plots had a higher concentration of more resilient humified organic carbon than the mulch only plots (**Figures 9 and 10**). However, the SUVA values for Trial 2, where wet/dry/wet soil samples were incubated for 24 hours prior to analysis, were about 60% of the values recorded for Trial 1. This difference is also evident in the spectral scan data (**Figure 12**).

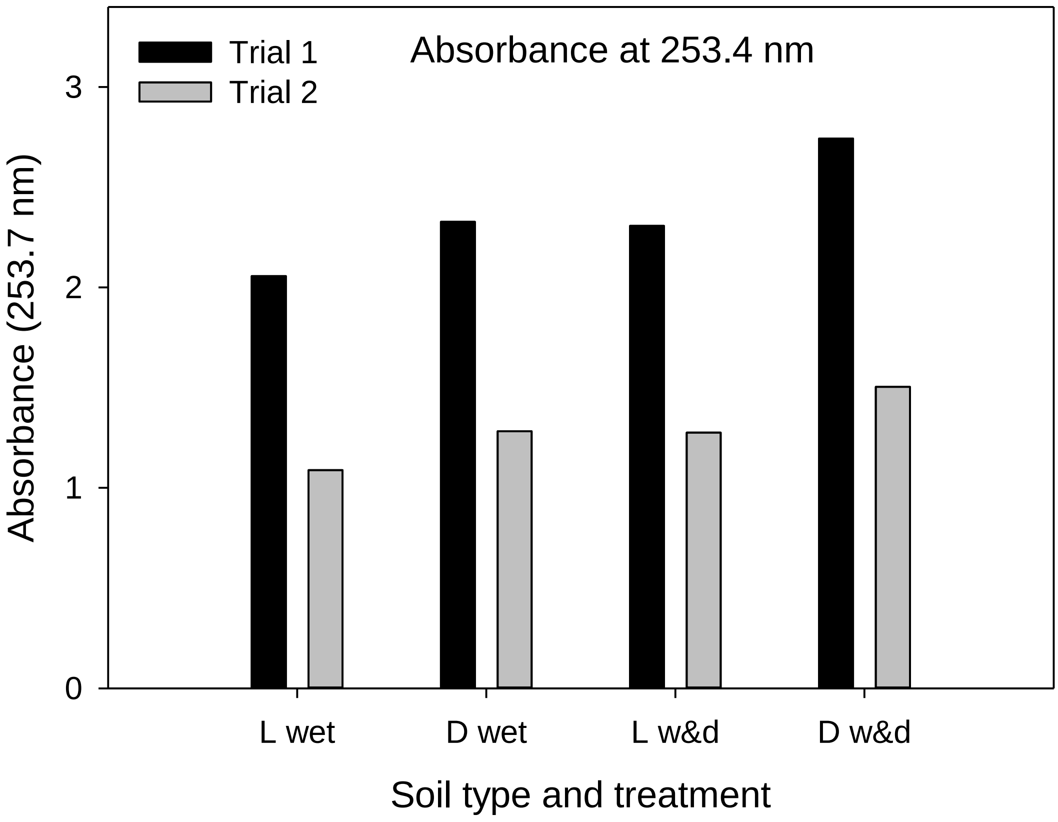
Figure 12: Spectral scan (average of 6 replicates) for soil samples from adjacent plots treated with mulch alone (L), or compost and mulch (D). Soil samples were either capillary-wetted for the duration of the trial (wet) or wetted for 48 hr, dried for 20 d (12 d for trial 2) then rapidly re-wet (wd). Trial 1 soils were prepared for UV testing after 1 hour of incubation, whereas Trial 2 soils were prepared after 24 hours of incubation.



A closer analysis of absorbance at 253.7 nm (humified organic carbon) suggests incubating the rapidly re-wet soil samples for 24 hours before testing them reduced the concentration of humified organic carbon (**Figure 13**). These results indicate microbially labile compounds also absorb in the UV region, indicating the UV method is not specific for humified, microbially resilient DOC.

Interpretations of the results from the SUVA test will need to consider this aspect, as the data in **Figure 13** suggest humified, microbially labile compounds may account for about 30 to 40% of the absorbance at 253.7 nm. Under field conditions, most soil sampling will occur after soils have dried, although some chromophoric compounds released by microbes during the preparation of the soil solution (equivalent to rapid re-wetting) may be present. Sampling from below the litter layer (still within the dark, organic stained zone of the soil profile) will also reduce the impact of rapid re-wetting on the concentration of labile chromophoric compounds in soil solutions.

Figure 13: Average values for absorbance at 253.7 nm (humified organic carbon) for Trial 1 (1 hour incubation before processing), and Trial 2(24 hrs incubation before processing). L and D refer to mulch only and compost and mulch treatments, and wet and w&d refer to continuously wet and wet/dry/rapidly rewet treatments.



Environmental Impact

(detailing any new information on adverse or beneficial environmental impacts of conducting the project and/or implementing its findings)

The test is sensitive for the detection of large, humified organic compounds that improve the nutrient and water holding capacity, and the physical structure of soils. The carboxyl and phenolic structures that absorb UV light are primarily responsible for the cation exchange and buffering capacity of humified organic matter, of benefit both economically and environmentally where increases in the concentration of this fraction in cane soils can be demonstrated.

A portable spectrophotometer specifically measuring light absorbance at 253.7 nm has been purchased, and will be used to detect differences in the concentration of humified organic carbon in sugar cane soils on the property of Barbara and Alan Walker. The Walkers participated in two SRDC producer-initiated projects (GGP034, GGP062), comparing cane productivity with conventional inorganic fertiliser and with the addition of compost. Use of the UV test should validate if repeat applications of the compost over several years, has benefited soil health by increasing the concentration of humified organic carbon in the soil.

Intellectual Property & Confidentiality

(detailing any intellectual property considerations or discoveries made and if these are to be protected and how. Outlining any publications produced. Stating what information, if any, is to be treated as confidential, to whom and for how long.)

No change.

Communication and Adoption of Outputs

(indicating what messages have come from the project to date, how they have been communicated or what plans there are to communicate them, what activities have been conducted, and what information is available on adoption of project outputs. Include advice of any upcoming meetings, seminars, field days etc associated with the project, with dates, times, locations if known, and a brief description)

The outcomes of an updated Milestone 1 were discussed with Dr Phil Moody and with Ian Grant earlier in the year. Both expressed their interest in the UV test, and its potential to provide insights into soil health. Communication with Lawrence Di Bello (Herbert Cane Productivity Board) has been limited, due to the additional research effort required to investigate the cane yellowing syndrome affecting the Herbert region.

The workshop to be held in Mackay in conjunction with testing the Walker compost trial soils will be planned for September/October. Dave Hunter (Pioneer Catchment Management Co-ordinator) will be organising the workshop, to assist in communicating the outcomes of this project to local growers.

Results from our laboratory and field studies will be prepared for the submission of a complete paper for presentation at the Gold Coast Meeting of the Australian Society of Sugar Cane Technologists commencing April 29th 2014. The synopsis of the paper must be submitted by 11th October 2013.

Proposed variations

(if any, to the project objectives, timing, costs, subsequent milestones or methodology. A request for a variation detailing the proposed changes should be included)

The only variation is substituting soil samples from the ‘Healthy Soils’ project with sugar cane reference soils provided by Dr Bernard Schroeder. Communication with Dr Ram Dalal indicated the SCaRP soils he had access to were restricted to vertosols only, with the other Queensland samples in the collection not available for analysis. Dr Phil Moody has undertaken to provide us with information on the physical, chemical and biological properties of the sugar cane reference soils, as characterised in previous research projects.

Personnel Changes

(advising any changes to the location or time input of project investigators or other related personnel. Note that SRDC must be advised if any Chief Investigator, or other Investigator who is paid half or more of a full time salary from SRDC funds, becomes unavailable, and alternative arrangements must be determined in consultation with SRDC)

Nick Stuckey accepted full time employment in February 2013 and was no longer available to the project. Dr Friederike Eberhard was employed in his place, to assist with the laboratory work.

Other Matters

(relevant to the conduct or outcomes of the project that the investigators wish to bring to the attention of SRDC)

Losing access to samples from the ‘Healthy Soils’ project has reduced our capacity to utilise knowledge gained from related, completed research projects. Access to the sugar cane reference soils will indicate the sensitivity of the UV method as an index of humified organic carbon, but published data on the soil health status of these soils may be limiting. Dr Phil Moody has undertaken to provide us with the information that is publically available on the x10 soils.

Summary

(of progress towards the stated objectives, outputs and outcomes of the project. Highlight adoption of project outputs to date. Indicate any circumstances that could potentially prevent future milestones being met as agreed)

We have successfully met the achievement criteria for Milestone 3:

- x10 sugar cane reference soils provided by Dr Bernard Schroeder have been characterised for $SUVA_{253.7}$, E2:E3 ratio, dissolved nitrogen and organic carbon, and their spectral scans in the UV region. The data provided from these tests provides qualitative information on soil properties that is different to standard organic carbon testing. Results from these soils indicates a high absorbance in the nitrate peak at 224 nm may reduce absorbance at 253.7 nm.

- The UV method has been validated as a sensitive indicator of soil health, as the humified organic matter that absorbs the 253.7 nm wavelength is also responsible for increasing the water holding and nutrient holding capacity of lighter soils.

- High concentrations of nitrate, copper and iron reduce absorbance of the 253.7 nm wavelength, and may affect the accuracy of the UV test. Concentrations of microbially labile, UV light-absorbing compounds immediately beneath the litter layer may also affect the accuracy, reliability and repeatability of this test. We will calculate the maximum concentrations of N, Fe and Cu known to affect $UV_{253.7}$ absorbance, and will specify sampling protocols that take dry soil from below the litter layer, but within the visual organic stain of the topsoil. Documentation of the Lowest Order of Reporting will be undertaken in the last phase of the project, with the guidance of Ian Grant (Agricultural Chemistry Pty Ltd).

- **We have submitted the Progress Report as required for Milestone 3.**

Appendix 3:

OUTCOME OF TESTING GROWER SAMPLES PROVIDED FOR THE MACKAY SOIL HEALTH WORKSHOP NOVEMBER 26th 2014

All soil samples either taken on the day of the field component of the workshop, or submitted during the workshop presentations were allowed to air-dry for at least 7 days before analysis. The tests undertaken on all samples were:-

1. Amount of $UV_{253.7}$ light absorbed by the sample solution (humified organic carbon absorbs UV light).

2. Amount of dissolved (particles less than half a thousandth of a mm) organic carbon in the sample (2 g of air-dried soil shaken in 40 mLs of water before 0.45 µm filtration)

3. Proportion of the dissolved organic carbon (DOC) that absorbs UV light (Specific UV absorbance or SUVA, calculated by dividing 1. by 2.)

4. The amount of light absorbed at $UV_{253.7}$ divided by the amount of light absorbed at UV_{365} nm; the E2 to E3 ratio (a high ratio indicates a higher proportion of smaller, less complex organic carbon in solution)

5. The amount of moisture retained in the original samples used to produce the solution after air-drying (higher % moisture is a property of clays with very fine particles or humified organic carbon, both of which hold onto water)

Results listed in the first table are for the sugar cane reference soils provided by Drs Bernard Schroeder and Phil Moody (list of x10 soils within the box), and the x10 soil samples provided by attendees at the Mackay workshop (below the boxed x10 cane reference soils). No information on the land use and cropping history of the x10 reference soils was available. However, they do provide a reference for UV, DOC, E2:E3 and SUVA values for Qld sugar cane soils.

Of the grower-supplied soil samples (compost samples and treatments are in the second table), the D Hunter slashed grassy headland can be used to benchmark Mackay soils. This sample had the highest concentration of humified organic carbon (UV reading), a relatively high total dissolved organic carbon content (DOC), with the highest proportion of DOC-absorbing UV light (SUVA), and the highest moisture content (Oven-dry % moisture). The E2:E3 ratio was the same as the average for the cane reference soils (2.1), indicating the soil humic substances are a mix of more complex soil humic substances, and more recent, less complex organic carbon. The E2:E3 and DOC values of the organic banana soil are very similar, but the lower UV and SUVA values and lower moisture content may indicate a history of cultivation with low organic carbon inputs.

The Attard soil samples are all below the D Hunter grassy headland benchmark for UV absorbance, SUVA and % moisture, but again the grass fallow had the highest UV, DOC, SUVA and % moisture content. Assuming the samples are from similar soil types, Attard 3151-2-1-4 has the lowest DOC and UV absorbance values, which may indicate this soil has been cultivated for longer, with low organic carbon inputs over that time.

Results for the two Ahern soils are similar to the Burdekin riverbank silt, with the exception of higher DOC values which may reflect the millmud applied in 2011 and 2012 to the cane and pumpkin blocks respectively. These results suggest millmud lacks the complexity of soil humic substances, which are responsible for UV absorbance. The riverbank silt soil has a higher SUVA value, but this is due to the lower concentration of DOC in this soil.

The Baumann soil has low UV absorbance and low DOC, suggesting historically this block was cultivated with low inputs of organic carbon. In the short-term, chicken manure inputs are not expected to change these parameters as the nitrogen in the manure will feed microbial activity, but the proportion of complex organic carbon in the manure capable of resisting microbial activity may be too low to be detected by the Laboratory UV Method tests.

Summary table of cane reference soils for UV lab method

Soil type	UV $_{253.7\ nm}$ abs. units	E2:E3 ratio	DOC mg/L	SUVA (mg/L/m)	% oven-dry moisture
Tully subsoil yellow earth	0.01	1.6	3.7	0.2	4
Kepnock yellow dermosol	1.66	0.7	5.9	27.3	14
Rocky Point gley	3.35	2.1	18.3	29.2	16
Oakwood red kandosol	4.68	2.2	9.4	50.1	10
Herbert red kandosol	2.00	2.5	12.2	16.8	1
Maroondan black vertosol	6.45	2.1	12.6	50.7	6
Woongarra red ferrosol	5.95	1.8	7.1	85.4	16
Herbert riverbank chernic tenosol	1.89	2.2	7.9	24.8	2
Mackay sodic duplex	7.19	2.3	14.1	52.3	7
Barratta clay	4.91	2.4	8.4	62.2	6
Baumann chicken manure	1.31	2.0	5.5	24.2	2
Hunter organic bananas	3.08	2.1	6.4	48.4	7
Hunter slashed headlands	**4.22**	**2.1**	**6.3**	**67.2**	**17**
River silt Burdekin	2.08	2.3	4.9	43.1	2
Ahern riverbank 50 yr cane, millmud 2011	2.48	2.4	6.3	38.6	4
Ahern org pumpkin millmud 2012	2.34	2.2	6.2	37.6	2
Attard Nth Eton 3151-2-1-3	1.51	2.5	7.6	20.0	1
Attard Eton 3151-2-1-2 **	2.10	2.5	7.3	28.8	3
Attard Nth grass 3079-7-2 **	**3.10**	**2.1**	**7.9**	**39.3**	**5**
Attard Nth Eton 3151-2-1-4	1.28	2.3	4.3	29.8	1

** Asterisks are soils treated for 5 years with fish, seaweed and molasses

INTERPRETATION OF COMPOST AND COMPOST TO SOIL SAMPLES

A set of reference results has also been provided for the compost and compost-treated soil samples submitted by workshop participants. The ProSoil compost is from a fully cured millmud and bagasse compost formerly produced at the Proserpine mill. The moisture retained by this sample was very high (56%). Water weighs a lot (1 mL weighs 1 g), and where samples are prepared on a mass basis high moisture concentrations will effectively dilute UV and DOC values. However, even allowing for a dilution of about 50%, the ability of the humus-like organic carbon in this sample to absorb UV light was low (0.43 x2 = 0.86), below the capacity of all of the sugar cane topsoils listed in both tables (the Tully yellow earth is a subsoil, sampled well below the topsoil layer). The concentration of DOC is higher than all other samples tested (about 20.4 mg/L if corrected for the 56% water dilution factor), and the E2:E3 ratio was the highest for both tables (3.86). These results highlight the high concentration of organic carbon in this cured compost is humus-like with respect to water and nutrient holding capacities (this compost has a high cation exchange capacity), but the molecules are still comparatively simple (a high E2:E3 ratio), and require further microbial processing in the soil (years to decades) before they achieve the complexity of soil humic substances (high UV absorbance, low E2:E3 ratio, high SUVA).

The ‘Pam’ soils indicate how cumulative additions of cured compost over a timeframe of 10 to 15 years, can transform humus-like compost into soil humic substances. In the absence of compost (‘Pam soil – compost’ received grassy mulch only), the E2 to E3 ratio and the water retained in the soil was much lower than the corresponding values for the ‘Pam soil + compost’ (4% and 20% respectively). Over the 10 – 15 years repeat, relatively low compost applications (about 10 t/ha every 3 to 5 years only), the proportion of simple humus-like compounds in the soil has increased (a higher E2 to E3 ratio) and the DOC has increased, reducing the SUVA value. The UV absorbance values remain very similar, as the compost is humus-like, still lacking the chemical complexity of soil humic substances responsible for absorbing UV light.

The D Hunter compost had absorbance and SUVA values that were lower than the soil treated with the compost (Hunter org bananas), but the E2 to E3 ratios are very similar. These results suggest the compost may be contaminated with soil from under the windrow, mixed with the compost during turning or other windrow management operations. Soil contamination may also explain why the moisture content of the air-dried compost was so low (13%, less than twice that of the receiving soil, and less than half that of the ProSoil compost).

The addition of compost in the Walker trial did not change the properties of the receiving soils that were tested using the UV Laboratory Method. In the Walker trial compost was applied at a rate of 10 t/ha per year (compost only treatment, 5 t/ha with half conventional fertiliser (Walker + 50% compost), or no compost with full inorganic fertiliser (Walker – compost) annually over three years. Lack of evidence of an increase in the DOC and % water content suggests either this rate of application over 3 years was too low to be detected, and/or soil contamination in the compost was high enough to mask any treatment effects. Soil contamination is likely, as the compost windrows are relatively low in height (refer to the photo), and soil from under the windrows may be mixed in with the compost during turning and other windrow management operations. We need to test a compost sample to confirm this. The crumb structure of the 10 t/ha and 5 t/ha compost treatments during compost sampling appeared more friable than the inorganic fertiliser only treatment, but more objective testing is needed to confirm these observations. Results over a longer timeframe of regular 10 t/ha compost application may be required to confirm the soil health benefits expected with repeat applications of a cured compost.

Summary table of compost samples for UV lab method, compost samples and treatments

Soil type	UV $_{253.7\ nm}$ Abs. units	E2:E3 ratio	DOC mg/L	SUVA (mg/L/m)	% water in sample
ProSoil compost	0.43	3.86	10.2	5.0	56
Pam soil + compost	**0.99**	**2.63**	**11.03**	**8.98**	**20**
Pam soil –compost	0.97	2.45	8.59	10.97	4
D Hunter compost	**1.92**	**2.13**	**8.85**	**21.7**	**13**
Hunter org bananas	3.08	2.1	6.4	48.4	7.36
Walker + compost	**2.65**	**2.42**	**7.00**	**37.93**	**29**
Walker – compost	2.53	2.39	6.68	37.99	29
Walker +50% compost	**1.73**	**2.09**	**5.71**	**30.33**	**35**

Photo: Compost windrows on the property of John and Barbara Walker.

Many thanks to Barbara and John Walker and John Ross for hosting the field event, and to Dave Hunter, Kate Steele and Belinda Billings for organising the field and workshop events.

Appendix 4: MEDIA RELEASE 24-Feb-2014

Project Catalyst 2014 connects global experts, farmers and the Great Barrier Reef in Mackay and Whitsundays

Farmers and the Great Barrier Reef might seem to have little in common – but for 70 cane producers along Queensland's coast,taking care of one of Australia's most iconic natural assets has become a top priority.
This week, growers will join leading experts in innovation and sustainability for the Project Catalyst Growers Forum 2014 to discuss a future for sugarcane production that continues to reduce the environmental footprint of agriculture on the Great Barrier Reef.

The event is set to attract more than 150 participants, including cane farmers from Mackay and the Whitsundays, the Wet Tropics and across the Burdekin; as well as leading global and Australian researchers, industry partners, economists and sustainability experts.

The forum will be held from Mon 24 – Wed 26 February on Hamilton Island, beginning with a farm Field Day in the Proserpine region.
Now in its milestone 5th year, Project Catalyst is a pioneering partnership between cane farmers, the Coca Cola Foundation, WWF, the Australian Government and Natural Resource Management Groups: Reef Catchments, Terrain Natural Resource Management and NQ Dry Tropics. The forum is being coordinated locally in Mackay and the Whitsundays by Reef Catchments. The three-day event will link those involved in regional sugar production with leading global innovators.

Reef Catchments CEO Robert Cocco said Project Catalyst recognised the significant efforts of sugar producers who were taking responsibility for their own environmental footprint.
"This major event celebrates the work of forward-thinking Australian cane farmers who are at the fore of truly innovative practicethat also protects our region's natural assets, including the iconic Great Barrier Reef," Mr Cocco said.
"The actions to date of the farmers involved in Project Catalyst have directly improved water quality to the Great Barrier Reef, while also helping increase farm productivity and profitability.
"These farmers are leading the way in the adoption of cutting-edge farm management that promotes better business and a better environment – essentially, learning to do more with less.
"We are proud to be involved in this unique partnership that connects our sugar producers with a leading think-tank of organisations nationally and globally to break new ground in sustainable agriculture."

Michelle Allen, from Coca-Cola South Pacific, said the Coca-Cola Foundation funded Project Catalyst because it provides the holistic resources farmers need to pioneer new and promising sustainable agriculture advances.
"We provide financial support to enable growers to speed the adoption of innovative sugar cane practices which can lead to tangible improvements in water quality. Integral to the Project's success, it is the growers who are the ones generating the ideas, they are the ones leading Project Catalyst's success," she said.

WWF Sustainable Agriculture Program Manager, Rob Cairns, said Project Catalyst brought together a diverse group of people with the ability to effect real change.
"The challenges facing agriculture and the Great Barrier Reef are immense and all stakeholders need to work together like never before," Mr Cairns said.

"Project Catalyst brings a diverse group of people and organisations together to trial and validate practices that are good for farmers, good for the community and good for the reef. We can all be very proud of this collaboration, as well as the outcomes achieved so far."
Project Catalyst farmer Lou Raiteri will be hosting the 2014 Field Day on his property, north of Proserpine. Mr Raiteri said the Project Catalyst fitted in with what growers were trying to do.
"I was one of the first 20 growers involved in Project Catalyst right from its inception. To me, it just fits in with what we're trying to do, to improve our sustainability and productivity and be more conscious of the environment," Mr Raiteri said.
"I'm a third generation grower, my family has been on the same property for over 85 years and my son is coming on to take care of the farm. This land is still taking care of us after all that time and I think it is our responsibility to look after what we have.
"What Project Catalyst does is bring together likeminded growers with enthusiasm who are all ready to try something new. It gives us the support we need to step out and raise the bar and challenge ourselves – we can look up and say 'ok, there's the benchmark' and we find new ways to reach it. It's absolutely remarkable what comes out of it."

Over the event, growers will have the opportunity to hear from keynote guests including
Dave McLaughlin - WWF Vice
President of Agriculture (United States) and
Dr David Cropley - University of South Australia Associate Professor (Engineering Innovation) and scientific consultant for the ABC TV Documentary, *Redesign My Brain*.
ENDS

Background Information – Project Catalyst

Now in its milestone 5th year, Project Catalyst is a pioneering partnership between more than 70 innovative Queensland cane growers and major program partners - Reef Catchments, NQ Dry Tropics, Terrain Natural Resource Management, the Australian Government, WWF and The Coca-Cola Foundation.

The program aims to promote the rapid adoption of innovative farm practices that improve water quality from cane farms impacting the Great Barrier Reef. Project Catalyst supports a network of farmers from the Mackay Whitsunday, Burdekin and Wet Tropics region who are leading the way in the use of cutting-edge management practices for a more sustainable and effective farming future.

To date, Project Catalyst growers have provided major water quality benefits to the Great Barrier Reef through significantly improved farm practices. Project Catalyst growers have reduced total pesticide and nutrient runoff by more than 60 tonnes annually. Over the five years of the project (2008 – 2014) the following reduction of chemical, nutrient and sediment runoff from farms into freshwater estuaries which connect to the Great Barrier Reef catchment have been recorded:

- Reduction of particulate nitrogen by 25 tonnes per annum

- Reduction of particulate phosphorus by 12 tonnes per annum

- Reduction of dissolved inorganic nitrogen by 22 tonnes per annum

- Reduction of filterable reactive phosphorus by 4 tonnes per annum

- Reduction of pesticides leaving the farm by 190 kg per annum

Appendix 5: SRDC Research Project Milestone Report

SRDC project number:	**NCA012**
Project title:	**UV absorption as a tool for growers to benchmark humified organic carbon in soil**
Research organisation(s):	National Centre for Engineering in Agriculture (NCEA)
Chief Investigator(s):	Pam Pittaway (NCEA)
Objectives:	Specific objectives include: • Development of a commercial laboratory testing protocol, with any pre-treatment equilibration steps evaluated by Ian Grant (Agricultural Chemistry Pty. Ltd) • Develop and test a tool kit for using UV absorbance as a field-based soil health monitoring method. Advanced Nutrient Solutions Mackay (Barbara Walker Secretary), will assist in field-testing the tool kit. Conduct a workshop in the Mackay region (in-kind support from Dave Hunter, Pioneer Catchment Management Group) to communicate the merits of the test and to encourage local adoption.
Milestone number:	4
Due date for milestone:	1-12-2013
Milestone Title:	**Field testing and validation of toolkit**
Achievement criteria	• Consultation with Ian Grant, ANS cane growers and SRDC Farming Systems researchers on relevance of UV method results for interpreting change in soil health in response to changed management practices, and • Outcome of survey results reported. • Documentation of the feasibility of using the UV method as a field test, and assessment of the potential use of the procedure as a Method in the Carbon Farming Initiative. • Submission of Progress Report on UV Method as a Soil Health Index

Milestone Achievement

(giving evidence to confirm the achievement of the milestone or reasons it was not achieved, including an overview of data and/or other relevant results)

A key outcome of achieving Milestone 4 is validation of the sensitivity of UV absorbance for quantifying aromatic compounds. Standard dilutions of the simple aromatic compound ninhydrin (molecular formula $C_9H_6O_4$) established the lowest order of detection at 0.5 mg/L. The highly significant linear regressions for UV absorbance and ninhydrin, and ninhydrin and total dissolved organic carbon can also be used to express field test results for UV absorbance as mg of dissolved aromatic carbon per L of soil.

The simplicity of the UV field method for quantifying humified soil organic carbon impressed many of the Mackay Soil Carbon Workshop attendees and field event participants. The two landscape-scale methods for soil carbon described by the two other presenters at the workshop (Terry McCosker and Rob Slugget) required substantially more expensive equipment, and results interpretation depended on off-site data analysis. In contrast, the UV method requires a portable UV spectrophotometer, a measuring teaspoon, a measuring cylinder (40 mLs), capped vials for shaking the soil solution, a container for decanting the soil solution leaving the settled solids, a 60 mL syringe for forcing the solution through a 0.45 µm glass fibre filter, and a holder to support the filter. UV absorbance is read directly off the spectrophotometer.

Moreover, in contrast to these two landscape-scale methods, results from the UV absorption method is more likely to correlate with results for more resilient soil organic carbon analysed using the accepted Carbon Farming Initiative Methodology of Nuclear Magnetic Resonance (NMR). Access to soil samples analysed under the completed “Healthy Soils” Project would have facilitated comparisons between the NMR method and the UV method. Replacement samples provided by Drs Phil Moody and Bernard Schroeder have not been analysed using the NMR Methodology. During the final months of this project, researchers in Queensland (Drs Ram Dalal and Kathryn Page) and/or in Adelaide (Dr Jeff Baldock) will be approached for access to reference soils already characterised for soil organic carbon as part of the SCaRP (National Soil Carbon Research Program) program.

Relevance of UV method results for interpreting change in soil health in response to changed management practices

Outcomes arising from discussions with Drs Bernard Schroeder and Lawrence diBella

Data presented in MS 3 have been discussed with both Dr Bernard Schroeder (SRA) and Ian Gant (Agricultural Chemistry Pty Ltd.). Dr Schroeder has agreed to collaborate on a proposed project to further validate the UV method as a soil health index, by re-visiting sugarcane soil sampling sites analysed 10 to 15 years ago. Results from the laboratory UV method tested on recent soil samples will be compared with results from stored samples taken 10 to 15 years ago, to analyse how management changes over time have affected key soil health properties including the concentration of aromatic organic carbon, specific UV absorbance (SUVA; $UV_{253.7nm}$ absorbance divided by total dissolved organic carbon), E2:E3 ratio ($UV_{253.7\ nm}$ divided by $UV_{365\ nm}$; an index of molecular size) and total dissolved organic carbon.

Dr Lawrence di Bella has also expressed interest in assessing the utility of the UV method for evaluating the difference in the health of soils in the Herbert region that have been affected by sugarcane yield decline, with paired equivalent soil types where yield has been maintained. Staff from the Herbert Cane Productivity Board will also collaborate on the project, using their database on yield to further asses the utility of the UV method as a soil health index. A joint application will be developed for submission to the SRA to fund the soil sampling and data analyses that will be required in the Ingham and Bundaberg regions, and the laboratory analyses to be undertaken at the NCEA.

Validation and verification of the UV method as a Quantitative test

In consultation with Ian Grant (Agricultural Chemistry Pty Ltd.), the organic compound ninhydrin was selected to test the lowest order of detection possible with the UV method. The chemical structure of ninhydrin is comprised of an aromatic ring and two carbonyl bonds, which absorb UV light (**Figure 1**). This compound was used instead of the humified compost sample originally specified in the Research Contract, due to the simplicity and known chemical composition of Ninhydrin.

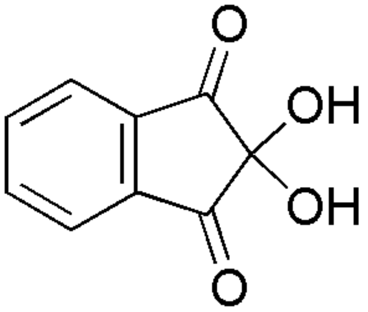


Figure 1: Chemical structure of the organic compound ninhydrin. The double bonds in the aromatic ring and pseudo-ring, and the double bonds in the carbonyl bonds strongly absorb light in the UV region.

A dilution series from 0.5 to 40 mg L^{-1} ninhydrin in distilled water was prepared, with samples from each dilution analysed for absorbance at 253.7 nm, E2:E3 ratio, and total dissolved organic carbon. The simple regression equations for ninhydrin concentration and absorbance were highly significant (R^2 of 0.998 and 0.98 for laboratory and field spectrophotometers respectively), with results for both the laboratory and field spectrophotometers very similar (**Figure 2**). Plotting ninhydrin concentration against SUVA values for the laboratory and field methods indicated that below a concentration of about 5 mg/L, the constant relationship between SUVA and ninhydrin concentration did not hold (**Figure 3**).

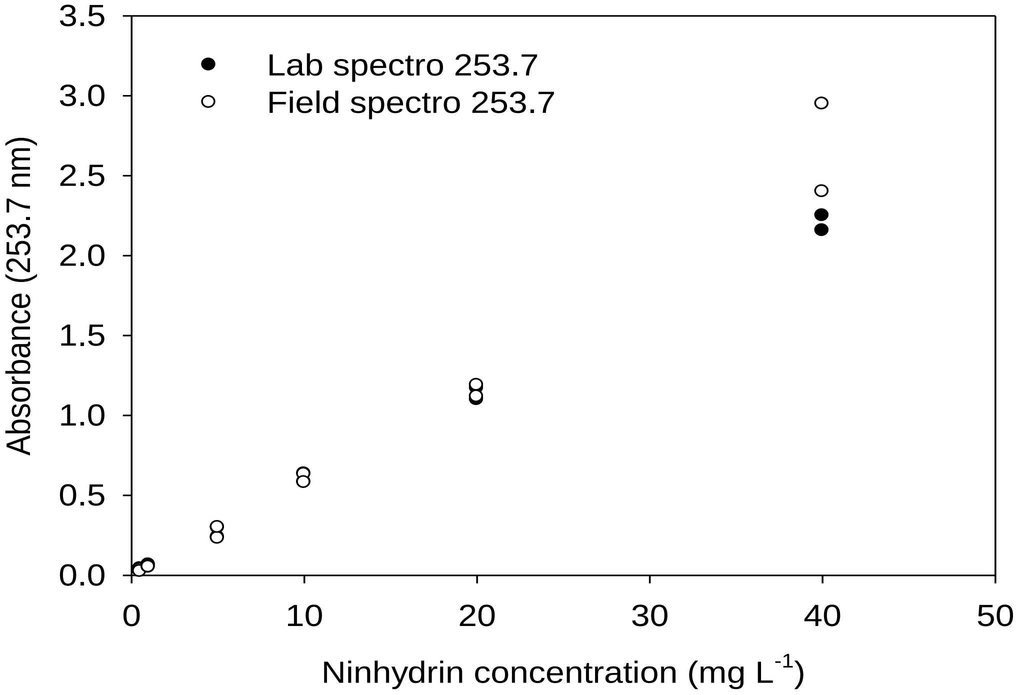


Figure 2: Linear regression of ninhydrin concentration with UV absorbance at 253.7 nm. Results for the laboratory and field spectrophotometers were highly significant ($P < 0.001$).

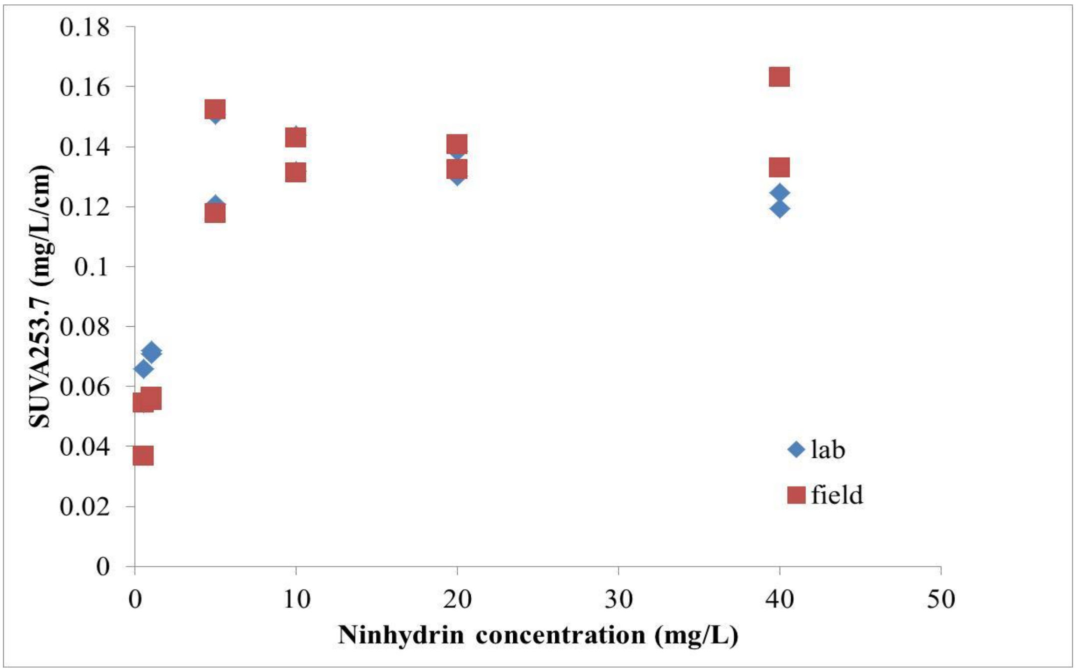


Figure 3: Ninhydrin concentration plotted against SUVA values derived using the laboratory and field spectrophotometers. The expected constant relationship between SUVA and ninhydrin is valid above a concentration of 5 mg/L ninhydrin.

These results indicate the UV method detects concentrations of ninhydrin as low as 0.5 mg/L. Relative to ninhydrin, the chemical structure of humic substances is much more complex (**Figure 4**), and would be expected to absorb much more strongly than 0.5 mg/L of ninhydrin. These results indicate UV absorbance is a very sensitive method for quantifying the concentration of humic substances in solution. The humic substances extracted from the soil solution are responsible for the dark organic stain observed in a soil profile immediately below the litter layer (visually referred to as ‘topsoil’), and contribute to key soil health properties including pH buffering, water holding capacity and cation exchange capacity, and soil aggregation.

SUVA is less sensitive, with the expected constant relationship between ninhydrin and SUVA only appliccable above a ninhydrin concentration of about 5.0 mg/L (Figure 3). One explanation for the lower sensitivity of the TOC instrument for detecting ninhydrin is the amount of organic carbon present in the compound. The chemical formula for ninhydrin is $C_9H_6O_4$, with carbon comprising only 61% by mass of the compound. Comparing the two linear regression equations for ninhydrin and UV absorbance (**Equation 1**) and ninhydrin and TOC (**Equation 2**), the x-intercept for UV absorbance is closer to zero, and the slope is much steeper (**Figure 5**).

Equation 1: Ninhydrin mg/L = -0.295 + (18.149 * lab spec 253.7)

Equation 2: Ninhydrin mg/L = 7.363 + (1.427 * TOC mg/L)

(2a: Aromatic TOC mg/L = -5.099 + (0.700 * ninhydrin mg/L)

Equation 3: Ninhydrin mg/L = 1.020 + (14.742 * field spec 253.7)

Using equations 3 and 2a, field absorbance readings for level teaspoon samples of soil can be expressed as mg of dissolved aromatic carbon per L of soil sample. The standard of one level teaspoon equalling 4.9 mL will be used for the calculation. For the laboratory method, equations 1 and 2a and results from oven-dry weights can be used to express UV absorbance readings as mg of dissolved aromatic carbon per kg of soil.

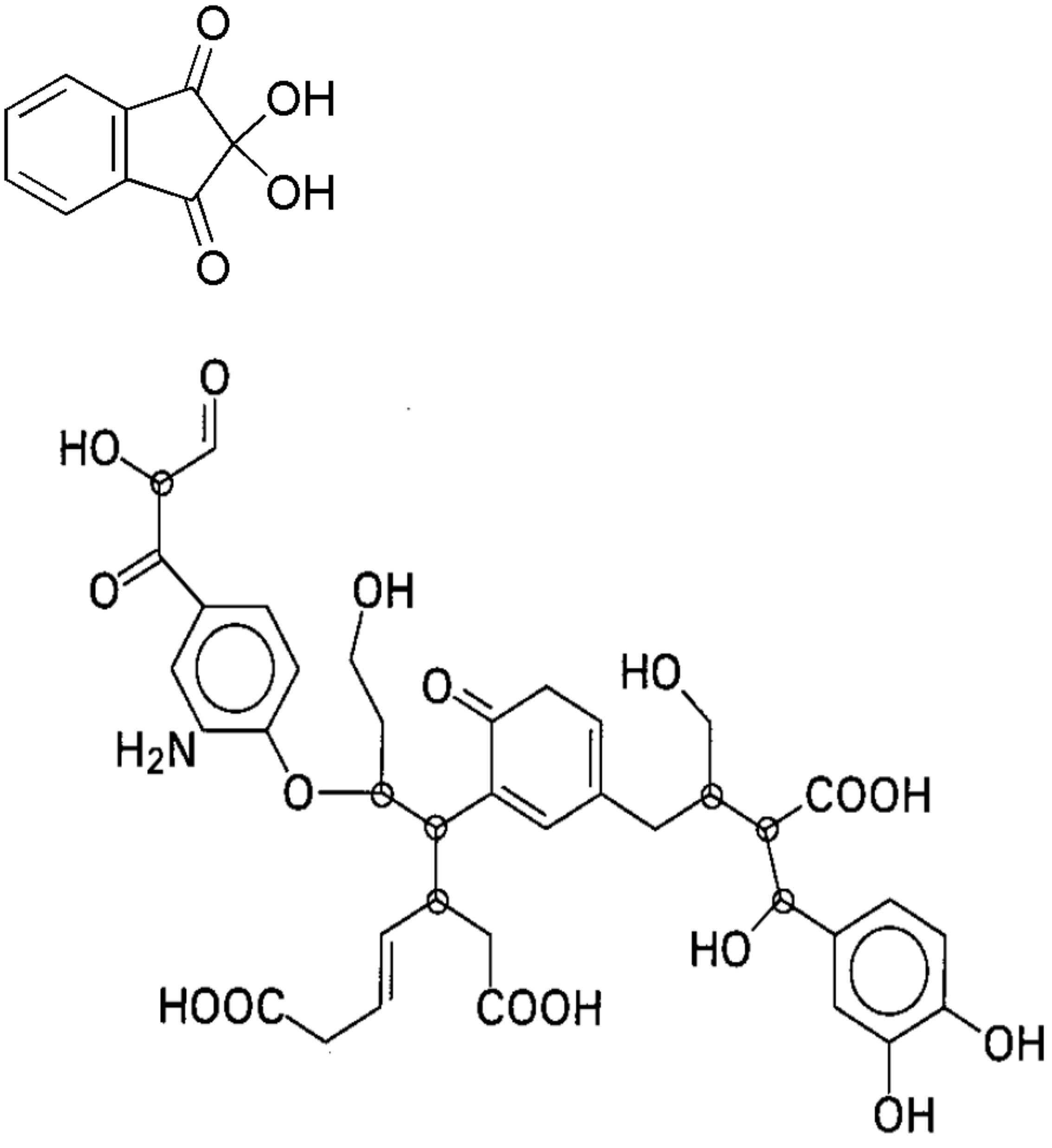


Figure 4: Comparison of the simple aromatic compound ninhydrin and a proposed structure for a humic acid compound. This structure contains multiple aromatic rings, and carbonyl bonds responsible for absorbing UV light. The open circles at bonds on the aliphatic portion of the molecule indicate chiral centres (Sein et al 1999).

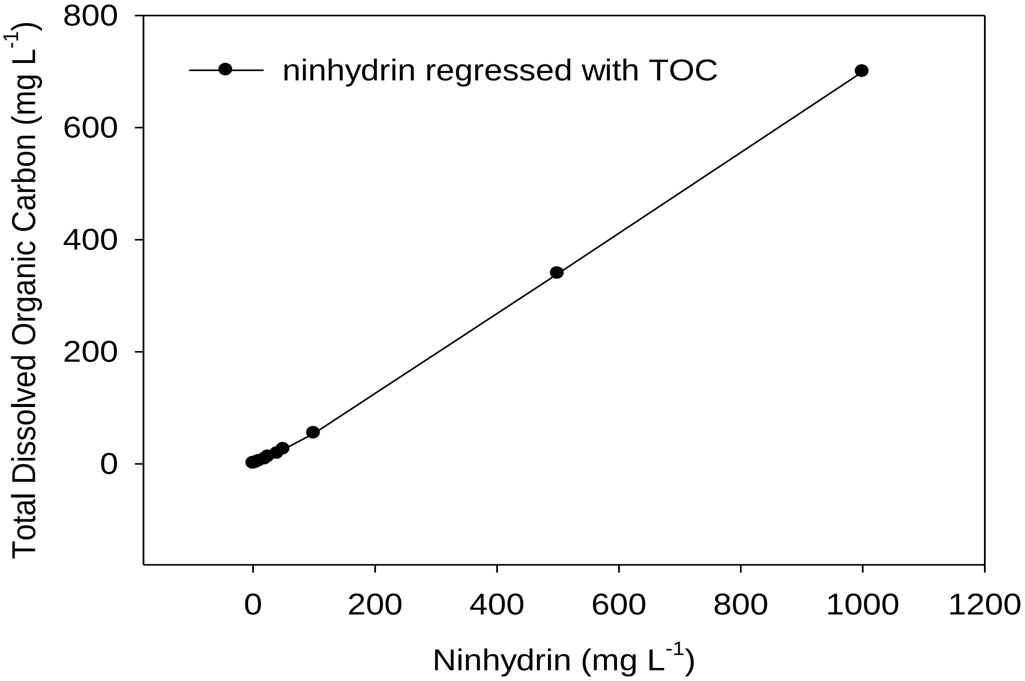


Figure 5: Regression of ninhydrin concentration with total dissolved organic carbon determined in the laboratory with a TOC infrared analyser. The R^2 value for the linear regression equation was P = 0.9999.

Outcome of Survey Results

The survey will be conducted after the field testing event and Mackay workshop. These events had to be delayed until after the cane crushing season had finished in the Mackay region, to enable the participation of the host family (Barbara and John Walker, local harvesting contractors) and other sugarcane growers. The field testing and workshop were held on 25th and 26th November respectively, and outcomes from these events are still being analysed. Examples of some of the comments provided on my presentation at the workshop are listed in the *Report on the Mackay UV Method Field Trial Event* section below.

Surveying selected agronomists on their perception of the UV method may be compromised by lack of soil reference samples that have been tested using the approved Carbon Farming Initiative Methodology (NMR analysis). The soil set that had provisionally been allocated to the project at the time of project submission had been tested for NMR results. However, by the time the project had started, these samples were no longer available. Substitute samples provided by Drs Moody and Schroeder have only a limited dataset with them (refer samples 1.1 to 1.10 in **Table 1**), which does not include NMR testing. Further discussions with Dr Phil Moody who has agreed to evaluate the UV method as a CFI Method, will continue over the final 3 months of the project.

Documentation of the feasibility of using the UV method as a field test

Laboratory comparisons of the sensitivity of the off-the-shelf portable UV spectrophotometer (Real Tech Inc. Real UVT) with a multiple wavelength laboratory spectrophotometer (Jenway 6705 UV/Vis spectrophotometer) indicated the accuracy of both instruments was comparable (**Figures 2 and 3**).

The field method requires no pre-treatment, other than manually breaking soil aggregates into smaller particles. The limitations of the field method are differences in the moisture content and bulk density of soil samples may affect comparisons between samples. Under laboratory conditions, air-drying standardises the moisture content, and weighing 2.0 g samples standardises sample bulk density. Under field conditions, SUVA calculations cannot be done, as accurate dissolved organic carbon analyses can only be conducted using laboratory analytical instruments (eg a Shimadzu oxidative combustion-infrared detection TOC-V CSH Total Organic Carbon analyser).

However, despite these limitations, results for laboratory mass based testing and field volumetric testing of the DG compost-amended soil and L mulch only soil (soil samples used for testing the sensitivity of the UV method to short-term changes; refer Milestone 3) were comparable (**Figure 6**). The volume of x1 level teaspoon (equivalent to 4.93 mLs) is of the order of twice the 2.0 g sample used for the laboratory test, and at this rate a statistically significant difference in UV absorbance for the compost-amended soil was achieved.

Using equations 3 and 2a, the average amount of aromatic dissolved organic carbon in the two soil samples was 355 and 75 mg per litre of soil for the compost-amended DG and mulch only L soils respectively. The higher value for the DG soil reflects the management practice of applying low rates (<10 t/ha) of a fully cured compost to this soil over the last 10 years. The statistically significant results validate the sensitivity of the UV method in detecting differences in humified organic carbon in soil, associated with different management practices.

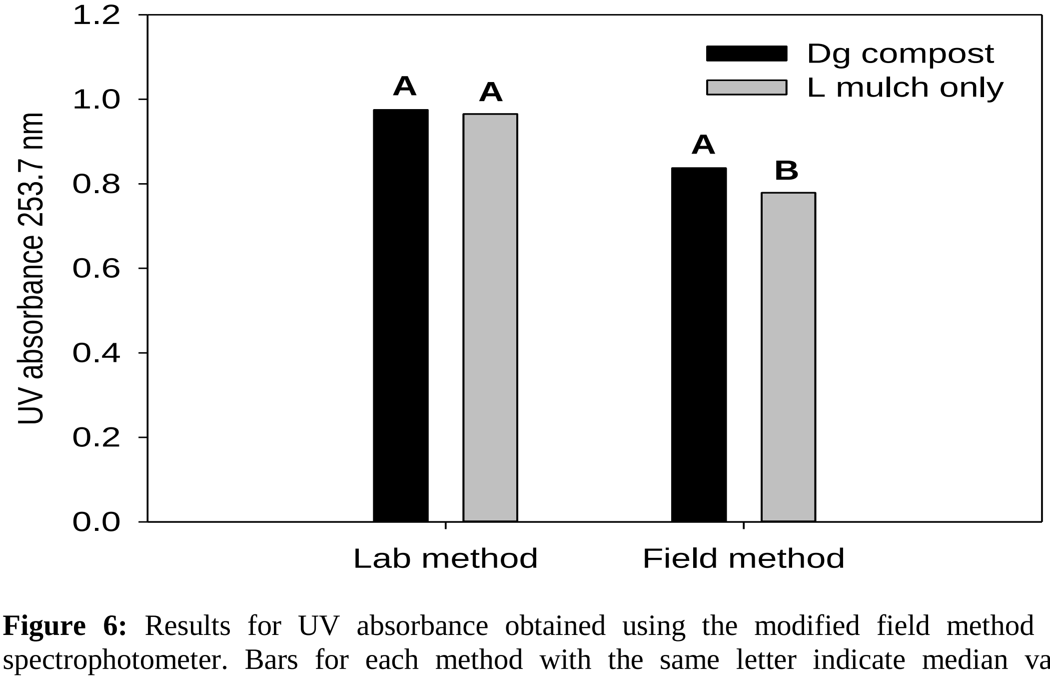


Figure 6: Results for UV absorbance obtained using the modified field method and portable spectrophotometer. Bars for each method with the same letter indicate median values are not significantly different at the 95% confidence level (Tukey post hoc test).

The field method developed to approximate the accuracy of the laboratory method consists of:

- Using a spade to expose the upper 20 cm of a vertical soil profile
- Using a metal teaspoon or rigid but relatively small volume implement to extract a sample of soil from the dark-staining zone 5.0-10.0 cm below the litter layer
- Repeating the procedures above to obtain a composite sample of about 1.0 L that can be manually crushed and/or pulverised within a plastic bag into smaller particles.
- Placing x1 level teaspoon of soil particles into a capped 60 – 100 mL container with 40 Ml of distilled water added.
- Shaking the capped container vigorously by hand for 2 minutes.
- Setting the shaken container to one side for a few minutes to allow the larger particles to settle.
- Using a 60 mL syringe take at least 30 mLs of the filtrate well above the settled sediment layer, for filtration through the 0.45 µm glass fibre filter paper. A 47 mm diameter filter paper holder is required as the larger surface area is less susceptible to blinding with soil particles in solution.
- Placing a sample of the filtered solution into the cuvette of a portable UV253.7 nm spectrophotometer and recording the absorbance value.
- Using equations 1 and 2a to calculate the concentration of aromatic dissolved organic carbon in the soil sample (units are mg per L of soil).

The equation to calculate mg of aromatic dissolved organic carbon per L of soil consists of three steps:

1. converting the UV absorbance results to ninhydrin equivalents (Equation 3),
2. converting ninhydrin equivalents to mg aromatic dissolved organic carbon per L of solution (Equation 2a)
3. dividing the value in 2 above by the volume of soil used (one level teaspoon = 4.9 mLs), and multiplying by 1,000 to express the result as mg per L.

The field testing kit consists of a volumetric teaspoon, a 40 mL measuring cup, 47mm diameter glass fibre filter papers filtering solutions to 0.45 µm, one 47 mm diameter filter paper cartridge, one 60 mL syringe, distilled water and a capped > 50 mL vial for shaking the soil solution in (**Figure 6**). The total cost of the kit should come to under $3,000. The spectrophotometer can be purchased from scientific supply companies providing portable water quality testing equipment. The portable single wavelength (253.7 nm) spectrophotometer was developed for monitoring the concentration of aquatic humic substances in water. Aquatic humic substances have the potential to react with chlorine during potable water treatment, producing disinfection by-products that may adversely affect the health of the public.

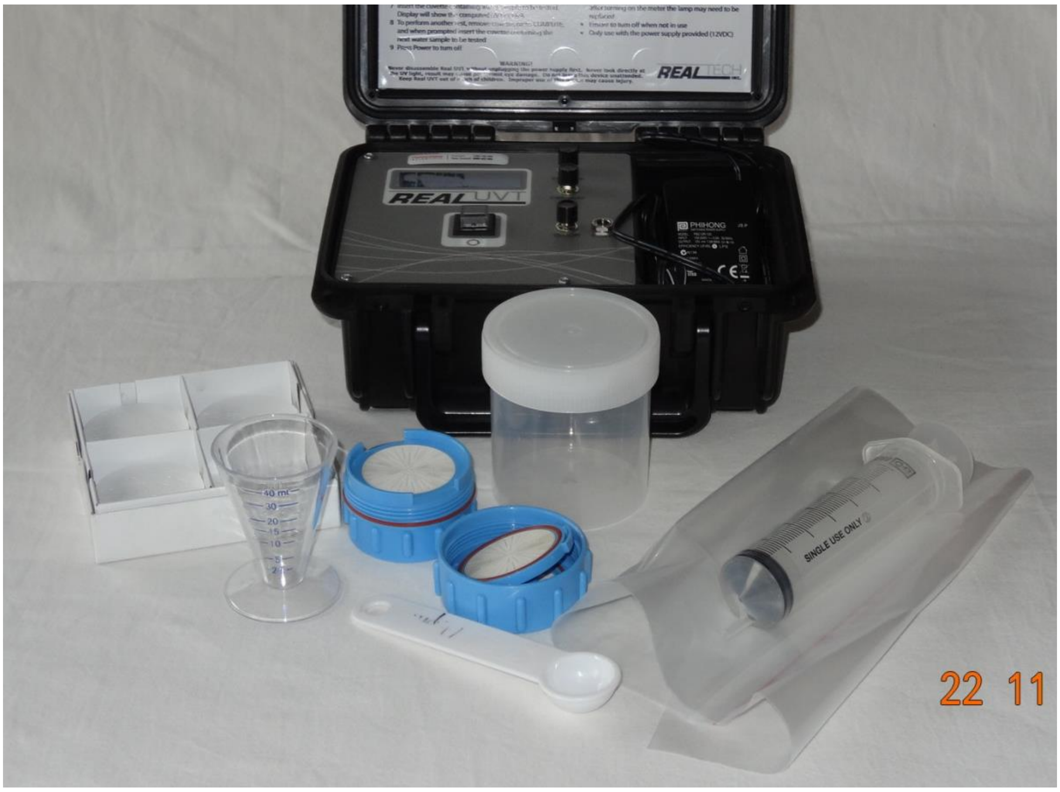


Figure 6: Components of the field UV method testing kit. From front left to right, 47 mm diameter, 45 µm glass fibre filter paper discs, 40 mL measuring cup, 47 mm filter disc holder, one teaspoon measure, one capped plastic container for shaking the solution, one 60 mL syringe for forcing the liquid through the filter, and a sealed plastic bag for pulverising the soil sample. Portable UV spectrophotometer is at the rear.

Report on the Mackay UV Method Field Trial Event

Attendees at the workshop were very attentive and interested in both the background to the trial site, and in the logic behind developing the UV method to measure humified organic carbon in soil. The simplicity of soil sampling, the small amount of soil required for testing, and the relatively simple shaking and filtering procedure appealed to the participants. The immediacy of results on the day, also appealed to participants.

Questions arising from my presentation at the Workshop the following day were also very supportive, indicating strong interest in the potential of both the laboratory and field testing methods.

Responses to my presentation included on the feedback sheets handed out at the workshop included:

- Spot on research – develop & get approved – farmers will need schooling to understand.
- New innovative idea
- Good, but I'm concerned that she has not yet tested if charcoal affects UV absorption
- Good scientific perspective. Hope the instrument works & the field test she's developing is reliable and robust
- Will work with Pam in further projects
- Testing the soil carbon test very interesting

Laurent Verpeaux, field officer for sugar and horticulture for the Dry Tropics was very interested, and expressed interest in purchasing a portable spectrophotometer to use for benchmarking and testing purposes in his region.

One key limitation of the field half-day was the malfunction of the portable Real UV spectrophotometer. Designed as a robust, single wavelength portable instrument for testing water samples, the operation of the instrument should not have been affected by airport scanning of hand luggage and minor knocks during flight. The UV lamp continued to operate and air and zero calibration procedures functioned, but the 'compute' function could not be activated. Participants at the field event were very interested in the simplicity and direct read-out of the UV method, and were very supportive of the potential of the method as a soil health field test.

The day of the workshop the field method was again demonstrated after the three speakers had given their presentations. Initially the compute function of the instrument again failed to operate, but persistent operation of the knob appeared to initiate contact and the prompts for operating this function appeared. Complete testing of the soil samples contributed by attendees was postponed until my return to the laboratory due to the unreliability of the instrument. Results for the soil samples collected during the field event and contributed by workshop attendees will be emailed to participants once completed, by Kate Steel Project Manager of the Pioneer Catchment group. Results will be expressed as mg aromatic dissolved OC per kg air-dried soil, and as SUVA.

Figure 8:

View of half the attendees at the field half-day. Host John Walker is seated on right hand side of photo. SRA staff in orange had to leave prior to field sampling.

Figure 9: Participants discussing compost production with John Walker by the compost windrows.

Figure 10:
Sampling of cane block to one side of planting row where fertiliser treatments were banded. A spade was used to expose at least 20 cm of the soil profile. The soil sample was taken with a kitchen spoon, at a depth of 5 – 10 cm below the litter layer in the dark-staining region of the soil. A composite sample of at least x3 teaspoons from x3 soil profiles per treatment was taken.

Detecting the humus stain in this soil was difficult as the soil was wet, and black in colour.

Figure 11: Vigorous shaking one level teaspoon of each of the soil treatments for 2 minutes.

Potential use of the UV Method as a Method in the Carbon Farming Initiative

The sensitivity of UV absorbance for the simple aromatic compound ninhydrin and the highly significant regression equation derived (**Equations 1 and 3**), can be combined with the known TOC concentrations of ninhydrin standard solutions to calculate the concentration of aromatic dissolved organic carbon per unit volume (field method) or per unit mass (laboratory method) of soil sample. Not all aromatic compounds have the chemical and biological resilience required for Carbon Sequestration purposes.

However, once a set of sugarcane reference soils that have been analysed for NMR organic carbon are available, results for SUVA and aromatic DOC will be regressed against NMR organic C data to determine if the UV laboratory and field methods meet the requirements for a Carbon Farming Initiative Methodology. The laboratory method used to determine the TOC result used for calculating SUVA and aromatic DOC should meet the requirements, as Mid-Infrared Spectroscopy is currently accepted ‘as a quick and effective tool for measuring numerous soil attributes’ (Sanderman et al 2011). The Shimadzu oxidative combustion-infrared detection TOC-V CSH Total Organic Carbon analyser used in the University of Southern Queensland Water Quality Laboratory works on a similar principle to mid-infrared spectroscopy, and is an accepted method for measuring dissolved organic carbon in water samples.

If SCaRP reference soil samples characterised for NMR organic C are not locally available, Dr Jeff Baldock (CSIRO Land and Water Adelaide) will be contacted. If a statistically significant regression can be established between SUVA and aromatic DOC and the NMR organic C, a case can be made for the inclusion of the laboratory UV method as a Carbon Farming Initiative Method. Further discussions with Dr Phil Moody will be undertaken on this topic during the final months of this project.

Potential use of the UV Method as a Soil Health field test

The alternative sugarcane soil samples provided by Drs Phil Moody and Bernard Schroeder (samples 1.1 to 1.10 in **Table 1**) had not been characterised using the Soil Carbon Method, and the additional testing required to characterise all ten samples for wet oxidation organic carbon, furnace induction organic carbon, effective cation exchange capacity and an index of variably charged cation exchange capacity have yet to be completed. Once completed, the index of variably charged cation exchange capacity will be regressed with UV absorbance, SUVA and aromatic DOC to determine if the UV method is sufficiently sensitive to predict the contribution variably charged cation exchange capacity contributes to the total cation exchange capacity.

Variably charged cation exchange capacity is a key soil health parameter in light textured soils, and in some clay soils. A field-based method testing for this capacity in soil samples will be an attractive addition to existing soil health tools. During the Mackay Field Event soils were sampled from the three treatments used in the SRDC GGIP GGP034 project conducted by Advanced Nutrient Solutions Mackay. Over the duration of this project (2008-2011), the three treatments of: compost only at 37 t/ha, 50:50 compost and conventional inorganic fertiliser, and inorganic fertiliser only (DAP at 259 kg/ha) were compared for their efficiency in producing sugarcane.

The compost produced on the Walker property and applied during the GGP034 project was processed into the curing phase, when the process of humification occurs. The humus-like organic carbon within the compost should have a soil residence time of years to decades, and should improve soil health properties including pH buffering, cation exchange capacity, soil structure and soil water holding capacity. The UV field test should detect differences in the concentration of aromatic organic carbon in the soil, arising from repeat compost applications.

As part of the Mackay Workshop, attendees were invited to bring soil samples from their properties. Due to the malfunction of the portable spectrophotometer, the nine soils submitted at the workshop and the three Walker GGP034 soils will be analysed in the laboratory for UV absorbance, aromatic DOC, total DOC, SUVA and the E2 to E3 ratio. Data provided with the samples on recent management history will be used to evaluate the potential use of the UV method for benchmarking humified organic carbon in soil. In future research, local databases with information on sugarcane yield, bulk density, total water holding capacity, furnace induction and wet oxidation organic carbon will be used for selecting well characterised soil samples to be analysed with the UV method, to further validate the utility of the UV method as a soil health field test.

Table 1: Soil sample information base to be completed during last 3 months of this project.

Analyses that cannot be undertaken at the NCEA will be commercially analysed by Agricultural Chemistry Pty Ltd.

	soil type	Wet Oxidn organic matter %	wet ox OC %	Furnace Ind OC%	ave E2:E3	ave E4:E6	ave TOC	ave SUVA 253.7	water %	CEC pH buffered	CEC up 1pH unit	% CEC assoc with org acids
7A	clay	2.2	1.00		2.96	3.20	7.41	0.34	8.8	38	32	15.78947
12B	clay	0.8	0.36		2.88	2.50	8.42	0.33	7.3	40	27	32.5
7B	silt	0.8	0.36		2.52	3.39	7.50	0.96	7.1	50	30	40
1A	silt	0.6	0.27		2.61	2.85	4.25	0.53	3	32	10	68.75
6A	sand	0.9	0.41		1.96	2.84	4.90	0.43	1.1	6	6	0
71A	sand	1.9	0.86		2.31	3.06	4.24	0.32	2.1	7	6	14.28571
NP	compost	10.4	4.73	6.96	3.86	2.31	10.20	0.05	56.3	34	14	58.82353
DG	soil+comp	6.2	2.82						20.1	14	11	21.42857
Linda	soil mulch	3.8	1.73						4.7	6	8	-33.3333
1.1	Woongarra		1.2	1.58	1.43	3.74	7.10	0.78		9.4		
2.1	Tully YE			1.17	3.40	0.00	3.69	0.00				
3.1	Kepnock		1.1	0.96	2.08	2.75	5.88	0.28		2.07		
4.1	Herbert Red		0.54	1.49	2.16	2.64	13.09	0.16		0.98		
5.1	Mackay SD			1.64	2.49	2.31	14.07	0.52				
6.1	Herbert riverb		0.81	0.79	2.07	2.42	7.89	0.25		3.15		
7.1	Oakwood		0.9	1.66	1.78	2.90	9.32	0.50		2.33		
8.1	Rocky Pt gley			3.52	4.39	4.89	18.33	0.29				
9.1	Barratta			0.85	2.32	2.46	8.35	0.62				
10.1	Maroondan		1.2	2.31	2.41	2.57	12.78	0.49		37.37		

Communication and Implementation Strategies

Linking the selection of soils and analysis of results to past soil health projects was not possible during this project. The soil health and sugarcane yield data available with the alternative soil samples provided by Drs Bernard Schroeder and Phil Moody was very limited.

Collaboration with the ANS grower group (Mackay) has been very productive, culminating in the half-day field testing of the cane blocks used during the project SRDC GGIP GGP034 on the Walker property. The support of Mrs Barbara Walker and Mr John Ross of ANS, and the assistance of Mr Dave Hunter and Kate Steel from the Pioneer Catchment Management Group has been fundamental in promoting and advertising the half-day field testing and one-day workshop events held on Monday 25th and Tuesday 26th November.

A paper on the UV Method (*A UV absorbance test for measuring humified organic carbon in sugar cane soil* authors P Pittaway, F Eberhard; abstract is provided below) has been accepted for presentation at the Australian Society of Sugar Cane Technologists meeting to be held at the Gold Coast in April/May 2014. The final 3.500 word paper will be submitted not later than 6th December 2013. A second paper on the UV Method as a Soil Health Test will be prepared for submission to the Australian Journal of Soil Science in 2014.

Further discussions with Drs Lawrence Dibella, Bernard Schroeder and Greg Shannon will be used to develop a collaborative research proposal to be submitted for consideration of funding in the 2014/2015 SRA research funding round. Datasets on sugarcane yield and from soils that have been chemically characterised in the past, will be used to identify a range of soils to be re-sampled using the UV Method protocols, to further develop the UV method as a soil health testing method. A selection of these soil samples will also be analysed using the NMR method, to further test the merit of the UV Method as a candidate Method under the Carbon Farming Initiative.

Abstract of the paper accepted for presentation at the Australian Society for Sugar Cane Technologists annual conference at the Gold Coast, April/May 2014.

A UV ABSORBANCE TEST FOR MEASURING HUMIFIED ORGANIC CARBON IN SUGAR CANE SOIL

By

P Pittaway, F Eberhard

National Centre for Engineering in Agriculture, University of Southern Qld.
Pam.Pittaway@usq.edu.au

KEYWORDS: soil carbon, soil health, aquatic humic substances, ultraviolet light, field testing

Abstract

Standard methods used for measuring soil organic carbon are insensitive to short-term changes associated with soil management practices. The conventional wet oxidation and furnace induction methods measure all forms of soil organic carbon to some extent, not differentiating between rapidly recycled plant debris, organic residues, soil biomass or more resilient humic substances. The only accepted laboratory method for measuring resilient, sequestered carbon in soil (nuclear magnetic resonance or NMR), requires expensive laboratory equipment. Our new method uses off-the-shelf equipment developed for quantifying aquatic humic substances, measuring Ultraviolet (UV) light absorbance.

UV absorbance at 253.7 nm is strongly correlated with resilient aromatic compounds in aquatic humic substances quantified with NMR analysis. We have adapted water quality protocols, for measuring humified Dissolved Organic Carbon (DOC) in soils. Under laboratory and field conditions, the UV method is sufficiently sensitive to detect changes in the concentration of humified DOC in soil samples exposed to wet/dry cycles, known to stimulate microbial growth flushes. Results for total DOC and Specific UV Absorbance (SUVA; UV absorbance standardised per mg of DOC) for nine sugarcane soils revealed large differences in total and humified DOC that were related to variation in soil acidity. The soil health properties associated with humic substances include pH buffering, nutrient and water holding capacity, and improved soil structure. Further testing will validate the utility of the UV method for benchmarking and monitoring the impact of land management practices on soil health.

End of Milestone 4 for NCA012