

Monitoring the effectiveness of biological products

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Mark Farrell, CSIRO talking to a group of farmers at SFS regarding biological product trials







Contents

1. Introduct	ion	4
2. Implemer	nting on-farm paddock trials	4
2.1 Trial c	design and analysis	4
2.2 Samp	ling	5
2.2.1	Sampling time	5
2.2.2	Sampling method	5
2.2.3	Sample collection	6
2.2.4	Monitoring points	6
3. Measurer	ments to indicate changes	6
3.1 Popul	ation Analysis – who is present and diversity	7
3.1.1	Worm Numbers & Eco-engineer counts	8
3.1.2	Fungal to Bacteria ratios	8
3.1.3	Mycorrhizal fungi	9
3.1.4	Nematodes	9
3.1.5	PreDicta B	
3.2 Biolog	gical activity	
3.2.1	Microbial activity CO_2 respiration test	
3.2.2	Potentially Mineralisable N	
3.2.3	Total Soil Microbial biomass	
3.2.4	Microbial Biomass Organic Carbon	
3.2.5	FDA enzyme assay test	12
3.3 Habita	at measures of soil biological change and health	
3.3.1	Soil organic carbon	
3.3.2	Penetrometer to measure compaction	14
3.3.3	Bulk density	14
3.3.4	Cation Exchange Capacity	15
3.3.5	Moisture infiltration	15
3.3.6	Soil pH	15
3.3.7	Albrecht soil testing	15
3.3.8	Sap analysis using Brix test	16
3.3.9	Effective root depth	
3.4 Testin	ng quality of farm manures, composts and effluents	16

4. What biological product work has been done in the past	17
5. Types of biological products used in CCMA trials	18
5.1 Composts	18
5.2 Biological stimulants	18
5.3 Humates	18
5.4 Animal Manures	19
5.5 Liquid alternative fertilisers	19
6. Recommendations for monitoring within trials	20
7. Acknowledgements	21
8. References	21
Appendix 1	23
Appendix 2	27

1. Introduction

There is great interest by farmers in improving their soil condition and health and it is likely that biological products can play a part in helping them achieve their aims. Farmers test biological products but can't tell if they have worked or not. There is limited independent scientific evidence available that farmers can tap into to help them make decisions on where and when to use them, when they start to improve soil condition or increase plant production and what products can reliably be used. One of the main issues producers and extension officers face is trying to establish the benefits and if they are cost effective.

Corangamite Catchment Management Authority through the support of the Australian Government funded numerous projects to evaluate biological products. Some of these include replicated trials and others include paddock trials. All require monitoring of potential changes in soil properties and understanding what is driving responses.

This paper focuses on reviewing what measurements can be taken to help evaluate biological products in research and on farm trials. It also briefly discusses trial design and lists other relevant biological product work locally and in southern Australia.

2. Implementing on-farm paddock trials

Farmers use paddock scale trialling as part of their decision making process to determine the usefulness of biological products but it is generally ineffective. Even though they apply the product they do not always include a control treatment to compare to, their monitoring is often limited to visual assessment and there is no data analysis. If farmer testing is to be encouraged then it should be made easy for them to carry out and analyse the results objectively. There will be claims made about what the product will do. For example increase yield and improve soil structure. These claims should drive what is measured within the trial and test if the product does what it says it will.

2.1 Trial design and analysis

It's often suggested that when setting up your own field trials it is a good idea to replicate and randomise the treatments and a minimum of three replicates of each treatment is needed to determine statistical significance. Roger Lawes, 2010 identifies that randomised block designs with three replicates within paddocks are not very practical to implement by farmers who want to use their own equipment. He suggests side by side strip treatments, with 10-12 monitoring points located in each treatment which are in close proximity and can be statistically analysed using paired t tests. Farmers generally apply treatments in strips or to one half of the paddock but they need assistance through case studies or calculators to show them how to analyse the results to enable confidence in comparing products. This information which shows farmers how to conduct analysis is currently not available.

Useful on farm trial design resources which are available:

• Designing your own experiments GRDC, 2006

- Doing successful on farm research, David Lawrence, Nick Christodouolou and Jeremy Whish, QLD gov.
- Setting up PA experiments, 2008. Sam Trengrove
- Understanding trial results Soil Health Knowledge bank, Australian Government
- Protocol for 'Paired Paddocks' in the DAFF project: 'Farmers leading and learning about the soil carbon frontier' Harm Van Rees, Dec 2003
- Trial design and analysis using precision agriculture and farmer's equipment, 2010. Roger Lawes, CSIRO Sustainable Ecosystems, Centre for Environment and Life Sciences, Floreat, WA
- Chapter 6. "A practical guide to improving soil health and reducing losses from soil borne disease', In Soil health, soil biology, soilborne diseases and sustainable agriculture, 2016. By Graeme Stirling, Helen Hayden, Tony Pattison, Marcelle Stirling, CSIRO publishing.

2.2 Sampling

2.2.1 Sampling time

If trying to monitor and identify changes in soil condition biological products have made then it is important to sample and compare at the same times and under the same seasonal conditions each year. Results can provide guides rather than absolute figures and be used to look at trends over time.

Different tests will have optimum times as microbial populations vary greatly during the year and will be affected by the presence of a crop and its management. Generally measurements should always be taken at times when soil biology is likely to be relatively stable. Therefore sampling should not be sampled in the height of summer, depth of winter or immediately after rain (Stirling et al, 2016).

2.2.2 Sampling method

For researchers, the sampling method used can vary depending on what is being measured or the research question. How many samples to take to get a fair representative sample of the paddock's biology or chemistry within treatments is different from researchers who want to know how much spatial variation there is across a paddock.

Dr Helen Hayden, is a soil microbial ecologist with AgVic, Bundoora and part of Professor Pauline Mele's Department of Applied System Biology Research Scientist with AgVic. Hayden is generally looking at responses to a treatment and will be comparing treatments across several paddocks. The approach used is to minimise field variation due to spatial heterogeneity. For her biological testing she collects 6 to 10 cores or more by walking in a W across a field and then bulks them into one sample, then repeats by walking the W in the other direction to collect a second sample. Hayden will ensure that her 6 to 10 samples from a paddock are made of homogenised cores to give a representative sample of that paddock's biology and matching soil chemistry. Collecting two bulk samples for the same soil will give her "biological replicates" for the paddock but they are not true technical replicates as every spoonful of soil may have a slightly different soil biology population. Helen Hayden's soil biology group uses approaches for some projects that are outlined in the journal paper "Environmental and spatial characterisation of bacterial community composition in soil to inform sampling strategies," by Baker et al, 2009. This paper shows why their sampling approach is effective given the spatial variability in biology that occurs within soils.

At other times the group have used a strict grid system to sample at set distances. The SCaRP project also used a grid approach. They used 10 sampling points within a 25 by 25 metre area as a representative sample of a given agriculture management on a particular soil type. By sampling from a small area of each paddock, errors due to encountering a soil type that is not representative of the targeted soil type were minimised (Sanderman et al, 2011).

2.2.3 Sample collection

There are specific soil collection and storage protocols to be followed for biological analyses, which are critical to sort out before collection. This is likely to involve keeping samples cool and send samples by courier with ice-bricks in foam boxes.

2.2.4 Monitoring points

One of the issues with on farm trials is losing the monitoring points or trial edge corners. Stock or equipment knock out markers. Taking GPS points can get you in the vicinity of one metre two but are generally not accurate enough. Having trials located within 5 or 10 metres of a fence provides a good landmark to measure from but may be unrepresentative of the rest of the paddock. Using metal detectors to find buried corner markers (nothing sharp that could penetrate tyres) has been used in crop lime trials eg metal rods and jar lids.

3. Measurements to indicate changes

Trials will generally involve monitoring of crop or pasture yield and quality and measurements within soil or products to help understand the responses. There is a huge array of soil biology type tests available and it is quite confusing to farmers and extension officers which ones are worthwhile using. This is partly because soil biology as a term encompasses all living organisms in the soil, the bacteria, archaea, fungi, micro-, macro- and meso-fauna. Not all measures cover all of the organisms present within the soil.

The soil biology testing factsheet by NSW Agriculture points out there are three main types of soil biology testing; population testing, biological activity and indirect indicators and describes tests which can be used to measure them. There are also functional tests for soil microbes that are generally only available to scientists. It's noted that much of the available extension material recommends tests but provides no details about where to get the tests done or potential costs which also extends to soil chemistry. Lyn Abbott and Dan Murphy, University of WA also provide an "overview of soil biology tests," in Soil biology in Agriculture, 2004. DPI 2009 has a review of tools and systems for assessing soil health which includes soil biology, chemistry and physical conditions. This report is available from the CCMA Soil Health Knowledgebase or via VRO.

Kibblewhite *et al* 2008 points out that there are practical difficulties with assessment because soil biology performance is multifunctional, variable and interactive with environmental factors, and does not respond instantaneously to altered conditions. They propose that assessment should be achieved using non living conditions or things that are indicative of their habitat (i.e. physical and chemical conditions such as bulk density, aggregate stability, pH, cation exchange capacity), and the levels of key energy and nutrient reservoirs (e.g. ratios of organic matter fractions and nutrient balances); as well as measures, which describe the community composition and populations of key functional groups of organisms (earthworms, N fixers, pest-control populations).

The Soil Quality website is no longer being updated but does contain soil biology benchmark tests for different regions which included three sites within the Corangamite catchment. It contains measures of microbial activity (mg CO2-C/kg, soil/day), total organic carbon, carbon stock (0-30 cm), microbial biomass carbon, soil nitrogen content via potentially mineralisable N (PMN) and numberous fungal and nematode pathogens from DNA testing. Dr Helen Hayden, suggested there is an opportunity to add to these benchmarks via Prof Daniel Murphy runs the site at UWA. Most new measures are undertaken by research groups who require financial support to be involved in analyses.

3.1 Population Analysis – who is present and diversity

Up to 6 billion microbes per cup of soil have been quoted which contain millions of different species. To complicate this, populations can change throughout the seasons and with stage of the farming practice.

Cam Nicholson, Nicon Consultant recalls testing soil biology in Woady Yaloak catchment trials. He first sampled different treatments and the results back were vastly different. Cam then sampled soil cores from within the same treatments side by side and sent them off for analysis. The lab tests back showed again completely different results. He concluded that testing for soil biology species was not helpful to determine product effects.

Dr Helen Hayden suggests Cam experienced the spatial variation that exists with soil biology which is evident in Baker et al 2009 sampling strategies paper. Variability of bacterial community composition was reduced within small 1 m² sampling plots but not significantly different compared with random pooled samples or between sampling plots. This paper also showed a dominant effect of variable pH on bacterial community composition which had more effect than moisture content and carbon.

Counting the numbers and types of protozoans, bacteria, nematode types, fungi and Actinomycetes using microscopes or growing them on nutrient media is measured by some laboratories. NSW DPI 2005 stated the values of recommendations from such analysis at that time were yet to be proven.

This sentiment has been echoed by Professor Pauline Mele, microbiologist with Agriculture Victoria and by NSW DPI (2002) who says that the absolute numbers of individual organisms are not important but the variety of different soil organisms is. A healthy functioning soil contains a wide

diversity of soil organisms. Dr Helen Hayden further explains that for the counts of bacteria or fungi they are based on the number of colonies that grow on an agar plate of culture medium. For bacteria alone it is estimated that between 95-99% of bacterial species may not be culturable so these counts are capturing only the very small number of species that can be grown in the laboratory.

Specific tests are discussed below.

3.1.1 Worm Numbers & Eco-engineer counts

Ecosystem engineers are worms, dung beetles, springtails and are essential for breakdown of organic matter. The best part about these assays/counts is anyone can do them and get results quickly and for free. It's noted that in many studies on the tillage and liming that worm counts are often included as a measure of the impact on soil structure as they improve macroporosity and are considered a general indicator of soil health, particularly soil structure and carbon levels.

Bacteria provide lots of soil functions and if one type of bacteria drops out its function can be replaced by another species of bacteria but the role of ecosystem engineers cannot be replaced.

Visual soil assessment guides often say 6 worms per shovelful of soil are an indicator of good activity. Helen Hayden advises the number of worms is a good baseline indicator, but to also consider change over time in monitoring provided the measure is done at the same time each year.

The VRO site under biology tests says worm counts are not useful in areas where rainfall is below 600 mm as very little variation in count numbers observed between samples. Also, seasonality is a factor as earthworms tend to be dormant (deep underground) in hot dry months and therefore it is best to sample in wetter periods.

In Soil Health Guide, North Central Victoria, chp "Evidence of soil biological activity", it recommends count numbers of six different species evident within the topsoil is a good measure of biological activity because it shows diversity within the soil biology.

3.1.2 Fungal to Bacteria ratios

Dr Helen Hayden suggested the ratio of fungi to bacteria more important than absolute numbers. Fungi are important because they contribute to decomposition of plant residues, and provide additional soil functions in terms of structural stability and aeration by binding soil particles into aggregates.

Some management practices can change the relative abundance of fungi and bacteria in the soil so this ratio can be used as an indicator to assess the effects of management strategies and gives a general indication of soil health. For example tillage induces shifts in the fungi:bacteria ratio which influences the rate of organic matter decomposition and nutrient availability. Reduced tillage systems support a fungal based system whereas conventional tillage systems support a bacterial based system (NSW DPI, 2004). Reduced tillage systems could however be hostile to plant growth and soil biology if compacted.

The VRO site on biology tests reports several ways to measure fungal:bacterial ratios:

- 1) direct count method Fungi and bacteria can be directly assessed by plate counts and the ratio of their abundance calculated.
- 2) Phospholipid fatty acid analysis (PLFA) -this method uses biochemical tests of fungi and bacteria (fatty acid analysis) as a basis for estimating the proportion of fungi and bacteria in soil.
- 3) substrate induced respiration (SIR) this method assesses the ratio of fungi and bacteria in soil based on response to addition of carbon substrates. It is based on inhibition of fungi and bacteria in separate assays and inhibition of all biological activity as a control which is difficult to achieve across different soils.

The VRO comments that Plate count method is straightforward which technicians could carry out but the PLFA and SIR are more complex and all are time consuming to do but there are no benchmarks which makes data quite difficult to interpret. Therefore the validity of this measure is questionable.

Some commercial laboratories which may offer this test include Microbiology Laboratories Australia, SWEP and AgVita analytical.

3.1.3 Mycorrhizal fungi

Mycorrhizal fungi form mutualistic associations with plant roots that aid nutrient uptake particularly for phosphorus (P) and zinc (Zn). Arbuscular mycorrhizal (AM) fungi plays an important role in providing glues such as glomalin which help maintain aggregate stability. Tillage and fungicides can reduce AM colonisation of crops and so may be an indicator of the effects of these management practices.

Helen Hayden commented nearly all crop plants can form AM associations. The exceptions are lupins and brassicas such as canola. Crop sequences which involve mainly lupin or canola or have long fallows which have extended periods without AM plants can reduce nutrient uptake and yield of following host crops (personal communication Helen Hayden).

With mycorrhizae naturally occurring in soil with cereal crops there may be little need to apply it via biological products. You can't also always visually see it on plant roots without the use of a microscope. The fact sheet for the Soil Quality website contains good information on arbuscular mycorrhiza. http://www.soilquality.org.au/factsheets/arbuscular-mycorrhizas-s-a

3.1.4 Nematodes

Dr Helen Hayden suggested nematodes were important to assess because as well as pathogenic nematodes there are also beneficial nematodes that regulate the soil food web. Some nematodes eat bacteria, some trap fungi, some eat other nematodes and thus they are important in regulation of other organisms and the transfer of nutrients and energy within the soil food web.

The Soilquality organisation website contains a factsheet titled "A practical test to assess the biological status of Australian grain-growing soils" that discusses using nematodes as a biological

indicator. It also discusses PreDicta B tests by SARDI for measuring Free Living Nematodes and also parasitic nematodes. The costs of the tests are \$95 each and are quantitative tests.

3.1.5 PreDicta B

SARDI offer DNA tests for identifying specific microbes. These are namely ones that are pathogens or pests which cause soil borne diseases.

Predicta B is important because it primarily measures soil plant pathogens and nematode pests for grain production. It will give farmers a guide to the disease risk for forthcoming season. Samples need to be collected by trained agronomists of which there are some in the Corangamite region. Helen Hayden recommends it especially for farmers who may not be aware of diseases or those who are and wish to monitor them before selecting the next season's crops.

3.2 Biological activity

Biological activity tests measure what soil organisms <u>are doing</u> at a point in time and so are useful in comparing the effectiveness of soil amendment treatments. For example biological assays such as CO_2 respiration measure CO_2 released into soil by soil biology and capture "activity" at that point. These tests are different to the population studies which show "who is there" at a point in time.

3.2.1 Microbial activity CO₂ respiration test

Microbial activity can be determined by soil respiration test. Carbon dioxide is respired by soil microbes as they decompose organic matter and the rate of soil respiration is determined by measuring the CO_2 efflux over 7 days at 25°C. This is a basic test of soil biology and is offered by some soil biology labs (e.g. Microbiology Laboratories Australia (www.microbelabs.com.au) and Nutrient Advantage is exploring including this measure in their tests. CSBP does not offer it. Helen Hayden describes this test as a useful baseline measure of soil biology. It is one of the measures used in the Soil Quality website measuring microbial activity (mg CO2-C/kg, soil/day). Their website contains a good description of soil respiration at http://soilquality.org/indicators/respiration.html#

3.2.2 Potentially Mineralisable N

As the active microbial biomass decomposes soil organic matter and plant residues, nitrogen can be released into plant available mineral forms. Potentially mineralisable nitrogen (PMN) is an indicator of the capacity of the soil microbial community to convert (mineralise) nitrogen tied up in complex organic residues into the plant available form of ammonium.

Soil samples are incubated for 7 days and the amount of ammonium produced in that period reflects the capacity for nitrogen mineralisation. The higher the number the more likely that soil will release nitrogen. Soils with high levels of nitrogen-rich organic matter (e.g. soils where legumes are in rotation) tend to have the highest populations of microbes involved in nitrogen mineralisation and the highest PMN rates.

Nutrient Advantage can provide this test upon request and the UWA group led by Prof Daniel Murphy's group. It was used in the soil biology benchmarking program and is featured within the Soil Quality website as a test for Soil Nitrogen Supply.

Both Pauline Mele (AgVic) and Mark Farrell, CSIRO who leads the GRDC funded project, "Understanding Biological input farming," suggested this test as a good reliable measure of soil biology. The advantage of this test is that it can be performed by commercial soil chemical testing laboratories unlike microbial biomass which is explained below.

3.2.3 Total Soil Microbial biomass

Microbial biomass is important for many reasons:

- with fauna it's part of the living component of soil organic matter which consists of 2 to 7% of the organic carbon in soils.
- acts as the engine for organic matter turnover and nutrient release.
- a storehouse of plant essential nutrients. For example nitrogen levels in microbial biomass range from 15 kg to 150 kg N/ha.
- nutrients held in microbial biomass are not prone to leaching and are released for plant uptake as a result of their death through predation or soil drying.
- Its interaction with organic matter largely determines the fertility and overall soil quality.

Microbial biomass is a measure of the total living microbial weight (mass) of a soil). It measures all bacteria and fungi present in the soil, including those which decompose crop residues and organic matter in soil. It is measured by using a soil chloroform to kill microbes and the the soil biomass carbon content is extracted and calculated based on the difference between the carbon content of the treated versus untreated soil.

Microbial biomass is not equal to CO₂ respiration through some laboratories try to make out they are the same. Pauline Mele, AgVic, also suggests this is a good measure of soil biology which has done in the past by the UWA group led by Prof Daniel Murphy's group as fee for service. It was used as part of the soil biology benchmarking within the Soil Quality website, microbial biomass carbon.

However, it is not measure in most commercial labs because it uses chloroform which is dangerous, labour intensive, and is not so easily made into a high throughput assay for them to use commercially. Therefore it is a measure only scientific laboratories might be able to do and not readily available to farmers.

The Soil Quality website states the best time to sample for microbial biomass is during the dry summer months when soil is collected for chemical analysis by commercial laboratories. Microbial biomass varies greatly during the year, however during summer it is more stable because both organic carbon inputs and soil water are low. Single measurements of microbial biomass can be difficult to interpret, but trends over time are a relatively simple way of assessing the effect of management on soil microorganisms.

3.2.4 Microbial Biomass Organic Carbon

Microbial biomass organic carbon (MBOC) uses a relatively simple microwave treatment to obtain a measure of the carbon bound in microbes. This test is an indirect measure of microbial activity which expresses mg Biomass Carbon/gram dry weight equivalent soil. Lisa Warn, formerly a consultant with Mackinnon Project used this test performed by the NSW DPI, Woollongbar laboratory as part of her study of poultry litter amendments 2012.

3.2.5 FDA enzyme assay test

Specific microbial enzyme activities can be measured to give an indication of microbial processes carried out in the soil. Various biochemical assays exist for a range of enzymes. Such assays can be used as general indicators of soil microbial health or more likely may be used to address more specific questions about microbial processes.

FDA assay (fluorescein diacetate) is a specific enzyme assay which includes phosphatase and urease, enzymes involved in phosphorus and nitrogen transformations. It expresses values as ug sodium fluorescein/gram dry soil/minute and relatively simple and is widely used to estimate general microbial activity (Reid and Cox, 2005). This assay was also used by Lisa Warn, 2012.

3.3 Habitat measures of soil biological change and health

Monitoring the soil environment which encourages favourable conditions for microbiology is an indirect way of measuring soil biology or to help explain yield responses. Some biological products may improve soil condition or lay claims to and so require assessment. Indirect ways soil condition can impact on soil biology are:

- Soil organic carbon as a food source
- Porosity for air and water movement which can be measured through bulk density testing.
- Compaction the use of penetrometers for detection of compaction
- Soil temperature which can be measured directly or indirectly by groundcover percentage for soil protection.
- Cation Exchange Capacity measured by laboratory testing reflects the nutrient holding capacity of the soil and is influenced by carbon levels.
- Soil pH as a measure of soil acidity can affect microbiology composition, survival and performance. Ideal pH for microbial activity is pH(Ca) 6.0 to 7.5 (Stirling et al, 2016).

Monitoring plant yield is an obvious measurement for evaluating biological products. Sap analysis can be claimed by some laboratories to provide a useful indicator of biological performance.

Further measurements in soil condition and other indirect test options are discussed.

3.3.1 Soil organic carbon

Organic carbon provides a broad measure of biology because soil organisms represent a portion of the carbon in soil, and the more soil carbon there is the more biological activity and diversity there will be.

There are concerns that it takes a long time to detect changes in organic carbon and that with soil variability measuring soil carbon may not be a worthwhile measurement. However, in a crop residue trial, differences in carbon (Leco measured) between treatments were identified in the 0-2.5 cm and 2.5 to 5cm (Chowdury *et al*, 2015). Most carbon sources are surface applied and so it makes sense that they are more likely to affect the top few centimetres of soil. These depths would not usually be examined in soil samples taken for chemistry analysis which may cover 0-10 cm and 10-20 cm in the profile.

The VRO biology test site also states that research has shown that active carbon is a good "leading indicator" of soil health response to changes in crop and soil management, usually responding to management much sooner (often, years sooner) than total organic matter percent.

Measurement of soil organic carbon is also affected by variation caused by contamination of plant material, dung and organic debris and so as much as possible should be avoided at sampling before analysis (Conyers et al, 2011).

There are four commonly available organic carbon tests which include:

1. Labile Carbon or Active Carbon

Labile carbon is an indicator of the fraction of soil organic matter that is a readily available food source (carbon) which all soil microbes depend on and thereby provides an indicator of biological activity.

In the past labile carbon field test kits have been used by the Agriculture Victoria, soil health team which use colour indicators of levels and may be a good tool for extension.

The soil is mixed with potassium permanganate (deep purple in colour) and as it oxidises the active carbon the colour changes (becomes less purple), which can be observed visually, but is very accurately measured with a spectrophotometer. Active carbon is positively correlated with percent organic matter, aggregate stability, and with measures of biological activity such as soil respiration rate.

Monitoring the changes in active carbon can be particularly useful to farmers who are changing practices to try to build up soil organic matter (e.g., reducing tillage, using new cover crops, adding new composts or manures).

Labile carbon test is offered by Nutrient Advantage.

2. Walkley- Black Wet Oxidation method

This method measures readily oxidisable Carbon. It's reported by most laboratories and often measuring total organic carbon (TOC) but underestimates soil organic carbon, detecting only approximately 76% (Convers et al, 2011).

3. Leco dry combustion method

It directly measures carbon by burning of soil and plant material and measuring carbon dioxide. It was expensive but has come down in price (about \$17 per sample).

Leco test measures total carbon which includes organic carbon but also inorganic carbon such as calcium carbonate which can be common in calcarosol soils but is an accurate measure of the total organic C in other soils. The Walkley Black test does not measure carbonates. The Leco test can also be used to measure Nitrogen and gives cropping farmers an accurate reading on the amount of nitrogen contained within their soil.

4. Mid-Infrared (MIR) spectroscopy

Mid Infrared spectroscopy measures light reflectance of soils and carbon amounts are then calculated using accurate calibration curves developed from actual carbon data collected using LECO tests. It is also used to measure the carbon fractions, which provides better information on carbon stability. It is a cheaper measurement tool that has been under development. It is not known if it is commercially available but it can be done through CSIRO, SA or AgVic, McCleod laboratories.

3.3.2 Penetrometer to measure compaction

The penetrometer gives an indication of penetration resistance/soil strength and is highly dependent on soil moisture content. This can make comparisons between sites and days a bit difficult to interpret without having some detailed measurement and recording of soil moisture content. This can be complicated by different treatments using moisture differently.

The penetrometer is useful for field demos and demonstrating differences in compacted versus uncompacted zones at the same site on the same day. According to Tim Johnson, former Agriculture Victoria Soil Extension Officer Scientist they offer less value for research trials. However, its attractiveness is its ease of measurements and the number of tests that can be taken. A penetrometer costs approximately \$500.

A cone penetrometer is the most suitable type of penetrometer for this type of use. The basic penetrometers have a "speedo" type dial which you have to manually read and this can be tricky to push the probe in and read at the same time, especially if you break through a less resistant layer. Graeme Ward, former Agriculture Victoria, researcher with the dairy team says it takes a little bit of time to use to using them. Graeme suggests a better but more expensive type is a recording penetrometer that records the soil strength/resistance down the soil profile in increments and you then download the data to a computer for analysis which overcomes the reading difficulty.

3.3.3 Bulk density

Bulk density is an indication of how compacted the soils are. It's a measure of the weight of the soil in a known volume. Light soils contain lots of air, but heavy soils have usually had the air squashed out of them and pore spaces collapsed and so compacted soils will contain more carbon and nutrients. Bulk densities above 1.5 g/cm3 are usually considered compacted.

Bulk density tests are less dependent on soil moisture but are difficult to take with each bulk density test requiring cores of soils to be cut out without compacting them. For a 0-10cm bulk density core its estimated to take about 10 minutes per core for sampling. However hydraulic soil corers can be used to sample large soil cores (75 cm diameter) provided they don't compact the soil at sampling and are often used in field sampling. They do however need to be taken when soils contain moisture

so that the cores remain intact so that the exact soil volume is collected. Soil moisture content can also be calculated using samples.

Graham Ward commented that bulk density remains a sound experimental technique, but given the spatial variability amongst soils, that you have to take lots of cores. Bulk density can also be related to porosity.

3.3.4 Cation Exchange Capacity

Cation exchange capacity (CEC) is a measure of the soil's ability to hold positively charged ions. It is a very important soil property influencing soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilisers and other ameliorants. Bill Grant, Blue Environment consultant who spoke at the SW Dairy Compost March 2016 seminars was confident that composts will increase cation exchange of the soil.

3.3.5 Moisture infiltration

McEwan *et al* (2009) describes two moisture related measurement tools that are relatively simple tools that may be useful for determining changes in soil condition by amendments. Determination of volumetric soil water content which involves measuring soil bulk density once for the horizon and then measurements of soil water content.

Infiltration tests can be used as an indicator of compaction where a 200 mm PVC tube in inserted into the ground and a known amount of water is applied to pre-wet soil or soil near field capacity. The time taken for the water to infiltrate is recorded and the infiltration rate is calculated. DPI reports the method as inexpensive and fairly rapid (30 minutes to 4 hours).

Soil moisture probes could also be useful. Web sites with a range of soil measuring devices are shown in Appendix 2.

3.3.6 Soil pH

Most biological products won't claim to increase soil pH. However, soil acidity levels are important to measure as they can affect potential responses to products. For example the rate of organic matter breakdown of composts can be slowed if acidity restricts microbial activity (pHCa less than 5.0). Composts can also slightly increase soil pH by providing alkalinity.

3.3.7 Albrecht soil testing

Some soil testing laboratories offer "Albrecht" concept soil testing and it can be a preferred soil testing method by sellers of biological products. Its concept is based on a balanced soil concept where optimal plant growth will only occur in soils with ideal cation ratios. However many researchers and research organisations have disputed this concept in favour of using a sufficiency level concept, where there are definable levels of individual nutrients in the soil below which crops will respond to added fertilisers and above which they will probably not respond (Kopittke and Menzies, 2007 and NSW Agriculture 2002). Examples of soil testing laboratories that offer this testing are EAL and SWEP.

3.3.8 Sap analysis using Brix test

Some biological product sellers can recommend plant sap testing using "Brix". "Brix" measures primarily sugars and minerals in plant sap that cause refraction. A refractometer is the instrument used to obtain a brix reading. Refractometers can be hand-held instruments or more sophisticated instruments costing up to \$600. EAL laboratory offers Brix sap testing.

It has mainly been used in wine making and the fruit and vegetable industry to measure quality and has been adapted for use for farmers. The idea behind it is to test the leaf of the growing plant to detect potential deficiencies before the crop matures. Supporters of the Brix method claim crops with a leaf Brix of 12 or better leaf Brix will be of high nutritional value and disease and pest resistant. A low rating means that your crop will not grow to its potential due to some external limiting factor, such as: a dilution of its nutrients due to high nitrate content, a mineral imbalance in the soil allowing weeds to flourish and take from your harvest, a low calcium content in the soil or a low/steady boron reading indicating an issue with the translocation of sugars within the plant. A Brix chart allows interpretation of results based on refractive index of different crop species.

3.3.9 Effective root depth

Effective root depth methodology is described in Soil Analysis: An interpretation manual, Chp 4 pg 66. It involves visual observation and estimation of the presence of roots. It requires a soil pit face and placing a wire frame of $100m^2$ and estimating the amount of visible roots which are < 2 mm in diameter within the frame and classify as few < 10; common 10-200 and abundant > 200.

The method could possibly be adapted by using a hydraulic corer and extracting large soil cores and splitting them lengthways to create a vertical face and estimating roots within 10 cm depth increments whilst also visually recording horizons and compaction areas. More cores could be taken to account for spatial variation.

3.4 Testing quality of farm manures, composts and effluents

Bill Grant, Blue Environment consultant provided good independent advice on the purchase of composts and what specifications they should meet at the SW Dairy Compost March 2016 seminars. Bill's specific advice was to ask composters for specifications for the product you are getting from them which includes:

- Australian Standard 4454 2012 test results which sets minimum quality management and product quality thresholds and not just for contamination,
- documented compost management systems and records showing pasteurisation, monitoring and product testing.
- sampling and product analysis by a reputable laboratory.
- Analysis which contains information on carbon to nitrogen levels which can pose issues for nitrogen draw down on crops or pastures.

Many biological products like composts or manures contain nutrients but it's important for farmers and extension officers to realise that these may not be readily available to the plant and have to be first converted into plant useable nutrients by soil biology. The amount of nutrients readily available differs for different product types and will vary from year to year. For example only 5% of nitrogen might be available in the first year from composts but 80% could be available in poultry litter. In poultry litter 25% of nitrogen is in the ammonia form which could be lost to the atmosphere if broadcasting the product two weeks before rainfall. Most of the nitrogen in litter becomes urea within a short time after spreading (Warn, 2014)

Bill Grant discussed how composts are primarily applied to improve soil condition and are generally not regarded as fertilisers. Therefore it is unfair to value nutrients \$/kg of nutrient in composts to inorganic fertilisers.

Product	Nitrogen (kg/ha)	Phosphorus (kg/ha)	Potassium (kg/ha)	Sulphur (kg/ha)	Source
Compost	5%	50%	70%	50%	Bill Grant & Graham Ward
Poultry Litter	75%	13%	100%		DAF QLD

Table 1 Availability of nutrients in different products in the first year of application

Many commercial laboratories do not seem to offer effluent testing and it's not known where or if dairy farmers have dairy effluent tested. There is information available on the difficulties of where to test within effluent ponds to get good representation of the likely nutrients applied on farm. Rachel Campbell, DEDJTR dairy officer reported that Deakin University laboratories have previously been used to do testing for effluent testing projects in the past.

4. What biological product work has been done in the past

There are a number of trials which have been done in the past and are currently occurring that can provide useful information for the CCMA biological testing trials. A list of these trials is given below.

These trial reports are yet to be comprehensively reviewed but many seem to indicate that they have not been able to identify changes in yield or soil condition. This could be because there are no changes but there is some concern about the reliability of tests used to detect changes or that the trials have not gone on long enough to result in changes in soil condition.

- CSIRO –GRDC project Understanding Biological input farming 2014-2018- 8 sites across Australia including one at Inverleigh SFS site all using wheat. Contact Mark Farrell
- Dairy Australia, Biological fertilisers for dairy farmers 2014 By Serve- Ag
- Dairy Australia 2009-2012: Harnessing soil biological fertility to optimise dairy production efficiencies.
- Woady Yaloak catchment Group 2009-2011 A range of biological products used on 3 pastures, 3 lucerne and 3 crops. Contact Cam Nicholson

- Holbrook Landcare Network 2010-2012 phalaris pasture on one high fertility site
- Binalong Landcare Group 2009-2011 native pastures on three low fertility sites
- Mackinnon Project 2009-2012 Use of poultry litter and humic acid applied to inorganic fertiliser on two improved pastures on two high fertility soils. Contact Lisa Warn.

5. Types of biological products used in CCMA trials

Biological amendments can include microbes, composts, manures, biochars, biosolids and biostimulants, as well as their value-added products. There are a wide range of products available in each of these categories, and products may come in bulk, pelletised or liquid forms. Some biological products remain within the soil for a short time (e.g. bio-stimulants, amino acids) and may require multiple applications per season to be effective. Other products have slower rates of turnover, remain in the soil for multiple seasons, and can impact soil function over a longer timeframe (e.g. manures, biochars). While many of these biological farming inputs have been available for a long time, their effectiveness and return on investment has not been consistently independently assessed. Products used in the CCMA landhealth trials are discussed below.

5.1 Composts

There were two different types of commercial compost used, one based on animal manures and one made from plant green waste. Analysis testing of these different composts has been done through Nutrient Advantage.

Compost claims may include that they increase soil carbon by adding slower-degrading organic carbon/humus to the soil, increase cation exchange, add nutrient, improve soil structure, improve water and nutrient holding, boost soil biology and suppress diseases.

5.2 Biological stimulants

The biological stimulant used in the trials (TM) claims it allows for a reduction in the use of inorganic fertilisers, creates disease resistance in plants and improves soil condition by promoting loose aerated soil.

It requires application several times a year on pastures and on bare soil with crops. Manufacturers say that it mimics plant exudates, which stimulates soil biology. There was some thought that it was citric acid based which tapped into usually unavailable phosphorus, however manufacturers deny this. The Woady Yaloak Catchment Group followed up testing of TM in 2012 after success in one of three trial sites in their testing of alternative fertilisers in 2009 to 2010 which included Cape Clear (crop) and Mannibadar (pasture). TM appeared to show responses on sites which contain nutrient deficiencies.

5.3 Humates

Humates were applied at three soil biology trial sites and have been used in a SCIPN case study on stubble digestion. They have also been trialled by Lisa Warn, Mackinnon Project to compare poultry litter versus inorganic fertilisers plus humic acid.

Sellers of humates claim they can contain up to 60% humic acid. Humus is a relatively stable form of organic carbon which is appealing. However, Roget and Gupta (2004)reports that humate products are generally extracts from leonardite or lignite which are minerals similar to brown coal, and differ from those extracted as humic acid from soil organic matter. They also say that improving soil organic matter through application is unlikely given the amount needed to be applied. However, they report there are many claims and some reports that adding humic acid products may stimulate plant growth and increase yield, possibly due to mechanisms such as delaying precipitation of phosphorus from mineral fertilisers in certain soil types. Humic acid type products are also used in stubble digesters which are claimed to hasten stubble breakdown.

Humates are generally regarded to be a soil conditioner that increases cation exchange capacity and the ability of the soil to retain nutrients, promote good soil structure and improve water holding capacity. They are also claimed to stimulate beneficial microbial activity and root growth.

5.4 Animal Manures

Animal manures are frequently claimed to be a natural alternative fertiliser and soil conditioner which improve soil structure and organic carbon content. Examples are poultry litter, pig manure and dairy effluent. Lisa Warn, 2014 has done a comprehensive report on poultry litter.

5.5 Liquid alternative fertilisers

Liquid type products used in the Corangamite catchment trials have included Seasol, Powerfeed, Nutrisol, Worm casting liquid and MCal.

Seasol is a seaweed plant conditioner that claims to promote vigorous plant growth by boosting plant metabolism, stimulates root growth and enhances flowering, increases tolerance to heat, drought and frost and stimulates beneficial soil microbial activity.

Powerfeed is a fish fertiliser (NPK: 14:1.4:8) that is blended with liquid humus to provide essential nutrients which promotes vigorous growth, conditions soil, stimulates beneficial soil microbial activity and increases plant nutrient uptake and reduces leaching.

Nutrisol is a concentrated liquid plant food produced from a vermiculture (worm recycling system). It claims to enhance the natural growing mechanisms of the plant and soil through increasing photosynthesis, feeding microbial communities and growing larger root systems. This allows plants to cope better with changes in stress such as extreme climatic conditions and chemical use.

MCal is a calcium based product which claims to increase calcium in the plant and increases cell strength.

Worm castings have been claimed to stimulate plant growth, enhance the ability of the soil to retain water and inhibit root diseases such as root rot and provide humus.

6. Recommendations for monitoring within trials

Many of the treatments or biological products used within the Corangamite catchment landhealth biological product trials claim to achieve greater microbial activity and improved physical properties. Tests should be chosen that can evaluate those claims and are practical and cost effective to implement. Some suggestions for testing are recommended in table 1. Ideally soil samples taken are sent to the one laboratory where a range of tests can be undertaken rather than having to split samples and send to multiple laboratories.

Table 1. Recommended test methods for evaluating soil biological treatments.

Claims	Monitoring tools
Boosts or stimulates soil microbial activity	Potentially Mineralisable Nitrogen test OR Microbial Carbon dioxide respiration test 0-10 cm
Increase soil carbon	Leco 0-5 cm, 5-10 cm OR Labile carbon 0-5, 5-10 cm
Increase CEC or nutrient holding capacity	CEC
Add some nutrients, unlocks nutrients	Common soil nutrient analysis rather than Albretch. Compost or effluent testing to establish nutrients applied.
Increasing calcium in the plant, increasing cell strength	Plant tissue test in spring
Improves pest and disease resistance and plant vigour	Brix

For CCMA landhealth cover cropping projects the claims are around that growing vegetation over summer versus fallow creates a more stable microbial environment by providing a food source and by keeping soil temperatures more cooler and thereby increases microbial activity. Also cover crops can improve soil health by adding more soil carbon. It's unlikely that any measured changes in soil physical condition between treatments could be easily determined given spatial variability within soils.

Table 2 Recommended test methods for cover cropping land health project

Claims	Monitoring tools
Groundcover keeps ground cooler during summer.	Measure soil temperature
Increased microbial activity	Potentially Mineralisable Nitrogen test OR Microbial Carbon dioxide respiration test 0-10 cm
Adds soil organic matter and so increases carbon	Leco 0-5 cm, 5-10 cm OR Labile carbon 0-5, 5-10 cm test.

7. Acknowledgements

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8. References

Abbot, L & Murphy, D (2004), Overview of Soil biology tests, In 'Soil Biology in Agriculture'. *Proceedings of a workshop on current research into soil biology in agriculture*. Ed Lines-Kelly R, Department of Primary Industries Tamworth, NSW.

Baker K, Langenheder S, Nicol, G, Ricketts D, Killham K, Campbell CD and prosser J (2009) Environmental and spatial characterisation of bacterial community composition in soil to inform soil sampling strategies. *Soil biology and biochemistry* **41**, 2292-2298.

Chowdhury S, Farrell M, Butler G and Nolan N (2015), Assessing the effect of crop residue removal on soil organic carbon storage and microbial activity in a no-till cropping system. *Soil use and management* **31**, 450-460.

Convers MK, Poile G J, Oates AA, Waters D and Chan KY (2011) Comparison of three carbon determination methods on naturally occurring substrates and the implication for the quantification of 'soil carbon'. *Soil Research*, **49**, 27-33.

DAF QLD (2012) Chicken Litter Factsheet. (Available on-line at http://www.daf.qld.gov.au/animal-industries/dairy/feed-and-nutrition/chicken-litter (verified 3 March 2017).

Gupta VVSR and Ryder MH (2003) Soil biology in pasture systems: knowledge and opportunity audit. Final Report BSC.007. Meat and Livestock Australia, North Sydney

Hannam R (2013) Soil biology review and project prioritisation for the feedbase investment plan. Meat and Livestock Australia Final Report

Kibblewhite MG, Ritz Kand Swift MJ, 2008. Soil health in agricultural systems. *Philosophical Transactions of the Royal Society B*, **363**, 865-701

Kopittke PM, Menzies NW (2007) A Review of the Use of the Basic Cation Saturation Ratio and the "Ideal" Soil, *Soil Science Society Australia Journal*, **71**: Number 2, 259-265

MacEwan R, Bougoure D, Cann M, Crawford D, Heemskerk G, Imhof M, Johnston T, Noonan B, Pearl D, Rab A. (2009) Tools and systems for assessing health, Department of Primary Industries, Bendigo, Available on-line at

http://www.ccmaknowledgebase.vic.gov.au/soilhealth/soils_resource_details.php?resource_id=3310 (Verified April 6 2017)

Roget D and Gupta VVSR (2004) Impact of management practices on soil microbial functions in alkaline Mallee soils. In 'Soil Biology in Agriculture'. *Proceedings of a workshop on current research into soil biology in agriculture*. Ed Lines-Kelly R, Department of Primary Industries Tamworth, NSW. P 37

Lawes R (2012) Trial design and analysis using precision agriculture and farmer's equipment, Grains Research and Development Corporation, Available on-line at https://grdc.com.au/uploads/documents/Designing%20farm%20trials%20with%20PA%20equipment .pdf (verified April 6, 2017)

Rural Directions (2013) Chicken Litter as fertiliser for broadacre grain crops: a user's guide. Rural Industries Research Development Corporation. Available on-line at

http://www.ruraldirections.com/media/Documents%202013/Chicken%20Litter%20Users%20Guide %20V4.pdf (verified April 6 2017)

NSW Agriculture (2002) Interpreting soil tests for calcium, magnesium and Ca:Mg ratios. , Acid Soil Action Leaf let 7, Published by NSW Agriculture,

North Central Victoria CMA (2016) Evidence of soil biological activity, In' Soil Health Guide, North Central Victoria'.

Reid G (2005) Soil biology basics Questions to ask about soil biology products, NSW Department of Primary Industries. Available online at

http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0004/41647/Questions.pdf (verified April 6 2017)

Reid G and Cox J (2005) Soil biology testing, NSW Department of Primary Industries. Available online at http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0018/41643/Soil_biology_testing.pdf (verified April 6, 2017)

Sanderman, J, Baldon J, Hwake B, Macdonald L, Massis- Puccini A and Szarvas S (2011) National Soil Carbon Research Programme: Field and Laboratory Methodologies, CSIRO, SA.

Stirling GR, Hayden H, Stirling M, Pattison T (2016) Soil Health, Soil Biology, Soilborne Diseases and Sustainable Agriculture: A Guide. CSIRO Publishing.

Soil quality website fact sheets Available online at www.http://soilquality.org.au/au/vic

- Making sense of biological indicators
- Microbial biomass
- Microbial biomass carbon
- Microbial activity
- Soil biological fertility
- Labile carbon
- Arbuscular mycorrhizas
- A practical test to assess the biological status of Australian grain-growing soils

Warn L (2014) Chicken litter: alternative fertiliser for pastures and ways to increase soil organic carbon. Publication No 14/067, Rural Industries Research and Development Corporation.

Appendix 1

Trial design and analysis using precision agriculture and farmer's equipment

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Background

Farmers are often interested in trialling a new technology before adopting it across the farm. If farmers have precision agriculture technology including yield monitors and variable rate controllers they can use it to conduct an on farm trial. They can use one new technology to test another.

To date, quite complex designs have been developed to provide farmers with the information necessary to run on farm trials (Bramley et al. 1999). Some of these approaches evolved from techniques developed to analyse large agronomic or variety trials and unfortunately they can be complex to implement, and analyse and interpret. In 2008 CSIRO and the Liebe Group attempted to run some trials using these complex techniques with farmer's machinery. Every trial failed; they are simply not farmer friendly. Replicated and randomised block designs were not designed to be planted with a 15 m seeder bar, managed with a 30 m spray boom and harvested with a 10 m front.

Paddock level experimentation differs from plot level experimentation in so far as the experiment is conducted in a commercial field that must return a profit with commercial equipment. The farmer is unlikely to allocate large areas to a treatment 'control' as this will generate sub optimal and uneconomic yields. The entire cropping operations must take place in a continuous manner (i.e. split plots are not allowed!) and be conducted in a manner that does not hinder conventional paddock operations, such as seeding, spraying and post emergent applications of fertiliser. Trial management should be integrated into normal paddock operations as farmers are too busy to deal with a complex trial that requires their attention at critical periods during the crops life.

To overcome some of these practical problems faced by farmers we outline a methodology that was successfully implemented by 4 farmers in the 2009 growing season using commercial seeding and harvesting equipment.

Principles of on farm trials

1. Few rather than more treatments

The most important process in experimentation is to ask an appropriate question and test it. Conventional experiments can be complex, where scientists may evaluate multiple rates of fertiliser on multiple crop species. In on farm experiments, where treatments must fit in with a commercial operation we recommend restricting the number of treatments to just one or two at most. The remainder of the paddock should be thought of as a control. A simple trial that generates a definitive outcome is better than a very complex and time consuming trial that confounds the issue.

When deciding on a treatment it is important to decide on a question that you want to ask and these are usually prefaced with words such as 'what, how, when or where'. For example:

- What effect does increased nitrogen have on grain yield?
- How will the crop respond to increased nitrogen?
- When will the crop yield more if nitrogen is increased (season)?

• Where will the crop yield more if nitrogen is increased (region)?

2. Go for large treatment differences

Once the question has been asked, it is important to make sure the treatment counts and is likely to change the yield of the crop. It is important to remember that the objective of a trial is to learn something about how the crop responds to inputs. To ensure this happens, the treatments must be large enough to bring about a change in crop yield. Even though the treatment might be uneconomic, it will provide insights into how the crop grows or how management should be changed in different regions in the paddock. Examples of treatments that will have an impact on crop yield if there is a deficiency or constraint might include:

- Increasing N by at least 20 kg/N/ha (ie ~ 50kg/ha of urea)
- Increasing P by at least 4 kg/P/ha
- Applying Gypsum at a rate of at least 2 t/ha
- Applying Lime at a rate of at least 2 t/ha.

3. Orientate the trial and treatments 'up and back'

If farmers have a yield monitor they will be able to identify zones in the paddock that are high yielding and zones that are low yielding. These areas may require different management strategies and may respond differently to the same treatment.

To explore this, the trial should be orientated to traverse the high zone and the lower or average yielding zone in the paddock, as indicated in Figure 1. It is also essential the sowing harvesting and treatments are all orientated in the same up and back manner. This is vital, as it facilitates an analysis known as a 'pair wise comparison or t-test' on the different zones within the paddock. This challenges the conventional wisdom of trial design, where researchers' would normally set the trial up in blocks on the good zone and the poor zone. However this approach keeps the trial design simple and ensures famers will be able to implement it with ordinary farm machinery.

The treatment should occupy at least two seeder bar widths (Figure 1). The location of the treatment must be recorded using a GPS so it can be overlayed on a yield map. The treatment should be located next to the control, which would often be the standard paddock management. This minimises the amount of the paddock that is 'experimental' and ensures the costs associated with running a trial are kept to a minimum. When the trial is harvested, it is important to keep the comb within the confines of the treatment and keep the comb full through the centre of the treatment, otherwise the yield information generated will be incorrect.



Figure 1. Orientation of strip trials across a paddock with high yielding and averaging yielding zones.

4. Analyse the trial data with a paired t-test or by eye.

It is important that data from each strip (control and treatment) are not averaged and simply compared. From the farmers perspective trial data can be analysed in formally or by eye. A paired t-test should be employed to formally analyse the trial. There is a lot of spatial information collected by the harvester and by pairing pixels adjacent to each other, it is possible to conduct a paired t-test across the whole strip, and separately on the low performing zone and the high performing zone. This is a powerful form of an analysis and is as statistically as robust as an analysis of variance.

The approach is demonstrated in figure 2 where each pair of pixels from the yield maps is treated as an experimental unit. One of the pixels is a control, the other is the treatment. Each pair of pixel provides a form of replication. Assuming a paddock is 500 m wide, and a yield is recorded every 10 m, there will be 50 pixels with control and treatment information. If this is split across high and low performing zones there should still be approximately 20 pixels for each zone with trial data.

From figure 2, the average difference between the treatment and the control is just 0.18 t/ha and using a paired t-test comparison, the difference is not significant (p = 0.09). When the low and zone is analysed separately, the difference between the treatment and the control was just 0.06 t/ha and not significant (p = 0.06). In contrast in the high zone the difference between the treatment and the control was 0.375 t/ha and highly significant (p = 0.004). In this instance, it is worth applying the treatment on the high zone.



Figure 2. An example of a paired t-test comparison of treatment means with the yields derived from multiple pixels in each zone.

Providing the trial has been conducted in an up and back manner and the treatments were chosen to generate a yield response, it may be sufficient to analyse the trial by eye and avoid formal statistical testing. To do this, simply zoom into the trial and carefully examine the yield in the treatment and the control. If the treatment is significant, a yield difference should be observed. It will be easier to identify treatment differences if the treatment is two seeder bar widths wide.

Summary

The approach presented here has been trialled with data farmers in the Eastern Wheatbelt, in the Northern Agricultural Region and in the South Coast. In one instance the trial was frosted and the trial was not harvested, but in other cases the trial was successfully completed. In 2009, we successfully implemented and obtained results from 4 farm trials. This compares favourable with the 4 trial failures in 2008.

In conclusion, when running a trial it is important to

- 1) Ask a question
- 2) Apply treatments that are likely to make a difference
- 3) Make sure the trial covers the range of soil types or zones of interest.
- 4) Sow and harvest the trial in the same direction
- 5) Ensure the trial is harvested where the comb does not enter the neighbouring treatment. This prevents the data becoming confounded by the experimental approach.
- 6) Analyse the data as individual pixels using a paired t-test or by eye. Strong treatment effects may even stand out by eye on some parts of the paddock.
- 7) Avoid combining data and simply comparing the means or averages of the two strips.

References

Bramley R, Cook S, Adams M and Corner R (1998) Designing your own on-farm experiments.

How precision agriculture can help. GRDC, Kingston.

Appendix 2

Websites which have a range of devices and sensors that are used to monitor soils and crops:

ICT <u>http://au.ictinternational.com/</u>

MEA http://mea.com.au/

Sentek http://www.sentek.com.au/

EnviroPro http://www.enviroprosoilprobes.com/