Examining potential functional indicators for monitoring ecosystem health in wetlands in south-eastern Australia

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Statement of Responsibility

This thesis is submitted in accordance with the regulations of Deakin University in partial fulfillment of the requirements of the degree of Bachelor of Environmental Science Honours. I, Hannah Harbourd, herby certify that the information presented in this thesis is the result of my own research, except where otherwise acknowledged or referenced, and that none of the material that has been presented for any degree at another university or institution.

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Date: 14th May, 2014

Abstract

1. Rapid monitoring tools to gauge the human impact on ecosystem health have been widely sought after and highly prioritized by environmental managers. The assessment of the health of an ecosystem has previously neglected the overall functioning of a system by focusing on its structural components. Functions occurring within a system such as decomposition provide a more accurate indication of the health of the entire water body.
2. The aim of this research was to determine possible rapid methods to quickly and efficiently monitor the function and health of wetlands with surrounding agricultural and urban land-use. This research involved comparing water quality variables with widely-established but time-intensive measures of assessing decomposition over a 35-day period to determine any correlation between the two and, thus, a potential rapid method to monitor the ecological function of a wetland.
3. Overall, across six wetlands, I found that water level had a negative relationship with decomposition; that is, there was an increase in decomposition with a decrease in water depth. Dissolved oxygen had a slight positive relationship, with the highest dissolved oxygen level coinciding with the fastest decomposition. pH influenced microbial community function, with the highest intensity of microbes being found at the most neutral pH levels.
4. These rapid indicators of decomposition will allow managers to quickly assess ecological health of urban and agricultural wetlands and contribute to the development of a holistic functional assessment of wetland ecosystems.

Key words: Functional assessment protocol, Shirley fabric, tongue depressors, Biolog^{TM} ECO plate, sediment, freshwater wetlands

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1. Introduction

In recent years there has been increasing deliberation over ecosystem health, as human dependence on the functioning of aquatic systems has become more widely understood (Xu *et al.*, 2005; Maltby, 2009; Su, Fath & Yang, 2010). Economic development is rapidly increasing on a global scale and so constant manipulation of every ecosystem on earth is occurring (Paul, Meyer & Couch, 2006). To maintain the health of ecosystems during this development, a method for the quick and efficient monitoring of some of our most important ecosystems is urgently required (Fairweather, 1999a). The overall concept of ecosystem health has been described as the state, condition or performance of an ecosystem with some desired endpoint (Rapport, Costanza & McMichael, 1998). It generally refers to the entirety of an ecosystem including both abiotic and biotic components of a landscape (Fairweather, 1999). It characterises the components of the ecosystem itself, however also highlights the services gained for human benefit (Maltby, 2009). Ecosystem health describes how the functioning of the ecosystem can deliver services beneficial to the human population, while still maintaining its health and the ability to renew and self-generate environmental outputs (Perrings, 2010).

The concept of ecosystem health has been widely understood and prioritised by environmental managers, leading to the need for a quick and efficient method for monitoring the condition of an aquatic system (Imberger, Thompson & Grace, 2010). Most current indicators of wetland health do not quantify the functioning of a given water body, but instead, use structural components (e.g. identity and abundance of different taxa) to determine the health of the system (Young, Matthaei & Townsend,

2008; Fuell *et al.*, 2013). Examples of structural indicators include the composition of macro-invertebrate communities (Young, Matthaei & Townsend, 2008; Clapcott *et al.*, 2012) and riparian vegetation cover (Burrell *et al.*, 2014). Such measures can be difficult to interpret because they only provide a one-off estimation of patterns and fail to provide a spatial and temporal scale of the processes under investigation (Imberger, Thompson & Grace, 2010).

As an alternative, measuring processes such as nutrient retention (Weisner & Thiere, 2010), ecosystem metabolism (Young & Collier, 2009) or decomposition (Tiegs *et al.*, 2013) provides a more accurate assessment of the functioning of a particular water body. This assessment can then be used by policy makers to highlight the extent to which a system has been altered from a comparable reference condition (Gessner & Chauvet, 2002; Fuell *et al.*, 2013). To be of value to managers, any indicator designed to quantify the functioning of a system should be quick, efficient, adaptable and robust (Imberger, Thompson & Grace, 2010). Such indicators need to be developed to allow rapid measurements, with deployment and collection being able to be conducted in quick succession and in a relatively uncostly manner. Most importantly, these indicators must also be clearly interpretable, and their validation is critical (Fairweather, 1999b).

This project focuses on the development of rapid indicators in wetland ecosystems. Wetlands are one of our most critical habitats and are vitally-important natural resources (Lifang *et al.*, 2009). They are heterogeneous but unique ecosystems, whose biogeochemical and hydrological functions arise from a reliance on water (Maltby, 2009). Wetlands occur in an extensive range of landscapes and may support shallow (generally < 2 m) standing water. They have substrates and biota adapted to flooding

and/or waterlogging and associated conditions of limited aeration (Maltby, 2009). These systems compose a large number of our freshwater storages, which are vital for the survival of life on earth (Islam, 2010). They have the ability to act as carbon sinks (Bernal & Mitsch, 2012), supplement groundwater (Lifang *et al.*, 2009), transform toxic substances (Zhou & Liu, 2005) and catchment nutrients (Shilla *et al.*, 2006), and provide habitat for endangered and native wildlife (Islam, 2010). There are many natural and human impacts on wetlands including hydrologic alterations, pollution inputs and vegetation damage (Li *et al.*, 2011), so a relevant indicator to determine the functioning, and therefore health, of these systems is critical.

A key function occurring within all water bodies including wetlands is decomposition. Decomposition, also known as mineralisation of organic material, is a function that supports many important values provided by wetlands, such as nutrient cycling, which supports higher primary and secondary production (Atkinson & Cairns, 2001). Rates of decomposition have been shown to influence nutrient availability (Neher *et al.*, 2003), primary production (Brinson, Lugo & Brown, 1981) and organic matter accumulation (Tanner, Sukias & Upsdell, 1998) in wetlands. Decomposition is a fundamental wetland process however it is largely understudied, and little information is available to predict the development of this process over time (Atkinson & Cairns, 2001). However, it is an important aspect of wetland ecosystems as it is the initial pathway for detritus to enter the ecosystem and thus a relevant way to assess the functioning of the system (Gessner & Chauvet, 2002).

The process of decomposition is the complex breakdown of organic matter. Organic matter is material made up of organic compounds that have come from the remains of once-living organisms in the environment and decomposition enables the transfer of

nutrients through an ecosystem (Knacker *et al.*, 2003). It is a fundamental aspect of an ecosystem; if it did not occur, all of the nutrients from the environment would be held within deceased organisms and no new life could be created (Odum, 1971). Decomposition of any resource is the outcome of three processes: leaching (transport through the soil profile and removal of unstable components) comminution (reduction in the particle size); and catabolism (the breakdown of complex molecules in the tissue, into smaller fragments via chemical processes) (Knacker *et al.*, 2003). This process occurs in a variety of sequences and can be immensely complex (Arroita *et al.*, 2012). The time period over which the different stages occur depends largely on multiple factors in the surrounding environment. This includes the physiochemical surroundings, nutrient quality and availability, and the microorganisms present in the surrounding landscape (Knacker *et al.*, 2003). However, the final rate of decomposition also depends on what is being decomposed, for example, leaf litter, wood, or decaying flora (Lecerf *et al.*, 2007).

Currently, the only established measures to assess decomposition rates are costly and time-intensive, and often require specialised equipment and expertise. One of these methods is a wood break-down assay (Arroita *et al.*, 2012), which uses mass loss as a surrogate measure of decomposition rates. A number of studies have looked at ways to assess wood break-down, including using entire logs (Ellis, Molles & Crawford, 1999), branches (Tank & Webster, 1998) or commercially-manufactured sticks such as tongue depressors (Aristi *et al.*, 2012). Another intensive method to measure decomposition rates uses standardised pieces of cotton (Boulton & Quinn, 2000). Cotton is a reliable standardised technique as it is composed of 95% cellulose, which constitutes the bulk make-up of plant litter (Latter & Howson, 1977). Therefore, a cotton strip assay assesses the cellulolytic activity by measuring the change in tensile

strength of the cotton as it decomposes in varying aquatic bodies (Tiegs *et al.*, 2013). Another intensive method, used more widely in soil science, involves assessing functional diversity of the microbial community that contributes to decomposition. This can be done by examining some of the carbon substrates utilised by those microbes (McKenzie *et al.*, 2011).

Potential rapid indicators need to be variables that reliably change with decomposition rates (Fairweather, 1999a). Previous studies have found that decomposition can be influenced by a variety of factors, including physico-chemical characteristics (Clapcott *et al.*, 2010; Dangles *et al.*, 2004; Serna, Richards & Scinto, 2013), nutrient levels (Tiegs *et al.*, 2013; Tate & Gurtz, 1986; Aristi *et al.*, 2012; Shilla *et al.*, 2006) and agricultural (Clapcott *et al.*, 2010) and urban (Imberger, Thompson & Grace, 2010) land-use types. These studies primarily found, for example, that increased temperature and nutrient concentrations accelerated the rate at which decomposition occurred, and factors such as lowered pH inhibited decomposition rates in aquatic systems.

Agricultural land use often results in a decline in riparian vegetation. Changes in riparian vegetation can alter the amount of shading, elevate insolation, increase water temperatures and reduce dissolved oxygen concentrations of the water body (Hagen, Webster & Benfield, 2006). Nutrient levels generally increase with agricultural land use due to fertiliser runoff as well as excretion by grazing livestock in the surrounding catchment (Doledec *et al.*, 2006). Increased sedimentation, soil erosion and bank instability are often negative outcomes of surrounding agricultural land-use (Allan, 2004). Urbanisation has also had many negative implications for nearby aquatic habitats (Imberger, Walsh & Grace, 2008). It has been found to alter nutrient

concentrations, water quality, and change biotic communities of urban ecosystems, predominantly due to storm water runoff through drainage systems (Imberger, Thompson & Grace, 2010; Walsh, Fletcher & Ladson, 2005).

Therefore, the objectives of this research are to identify indicators that could be used to rapidly assess the decomposition and thus the functioning of wetlands. In order to do this, I examined whether any of the possible rapid measures (i.e. water quality variables) correlated with intensive measures of assessing decomposition rates, thus providing a reliable assessment of decomposition. I also assessed whether land-use type influenced those correlations to determine whether different indicators would be needed in different catchment types.

I hypothesised that one or more rapid indicators measures would significantly correlate with decomposition rates, as measured by the intensive measures, thus making a suitable rapid indicator of wetland decomposition functioning. In addition, I hypothesised that decomposition rates and the potential suitable rapid indicators would vary among land-use types. Therefore, the rapid indicators for urban ecosystems may differ from those in agricultural ecosystems, but new methods would be available to provide managers and others with a rapid and reliable indicator of ecosystem functioning for decomposition.

2. Methods

2.1 Study area

This research was conducted over an austral summer, with the sampling undertaken from early January to late February 2014. Six perennial wetlands were selected in the Glenelg-Hopkins catchment of southwest Victoria (Fig. 1), all chosen due to similar features, including the capacity and size of the wetlands, percentage of riparian vegetation and wetland shaded area, macrophyte types, amount of exposed bare sediment, and sediment grain size. Three wetlands, Mepunga, Glads Crossing and Cobrico Swamp (Table 1), were chosen with a surrounding agricultural land use (generally cattle grazing). The other three, Lake Pertobe, Tea Tree Lake, and Lake Cobden (Table 1), were chosen to have surrounding semi-urban land use.

Aquatic and riparian vegetation was found at all wetlands. The tuberous root species *Triglochin procerum* (Sainty & Jacobs, 2003) was the most common vegetation type, with the exception of Lake Pertobe. In addition, *Typha* spp., a rigid native perennial (Sainty & Jacobs, 2003), covered the edges of Cobrico Swamp and parts of Tea Tree Lake. Lake Pertobe differed slightly with *Phragmites australis*, a native robust perennial (Sainty & Jacobs, 2003), instead of *Typha* spp. Mepunga was the only site with willows (*Salix* spp.) as part of the riparian vegetation. This wetland also had a large amount of the native floating fern *Azolla* spp., which was not so common in such quantities at other wetlands.

Table 1 Overview of the wetland sampling dates, location, land-use, and physical characteristics.

Wetland	**Mepunga**	**Lake Pertobe**	**Glads Grossing**	**Tea Tree Lake**	**Cobrico Swamp**	**Lake Cobden**
Sampling event dates	7, 14, 28 Jan, 11 & 18 Feb	7, 14, 28 Jan, 11 & 18 Feb	8, 15, 29 Jan, 12 & 19 Feb	8, 15, 29 Jan, 12 & 19 Feb	9, 16, 30 Jan, 13 & 20 Feb	9, 16, 30 Jan, 13 & 20 Feb
Location	Mepunga	Warrnambool	Penshurst	Mortlake	Cobrico	Cobden
Latitude	38° 26’ 10.54”S	38° 23’22.43”S	37° 51’ 12.73”S	38° 05’04.73”S	38° 18’27.70”S	38° 19’ 31.96”S
Longitude	142° 39’ 57.42”E	142° 28’26.97”E	142° 16’ 04.44”E	142° 48’ 40.53” E	143° 00” 43.80”E	143° 04” 29.65”E
Land-use	Agricultural	Urban	Agricultural	Urban	Agricultural	Urban
Elevation (m)	33	0	207	132	120	134
Size (Ha)	<1	19	1	2	3	1
Shading (%)	100	0	0	30	30	80
Dominant species	*Salix spp*.	*Phragmites australis*	*Triglochin procerum*	*Triglochin procerum*	*Typha spp*.	*Triglochin procerum*
Sediment exposed (%)	30	100	100	50	50	100

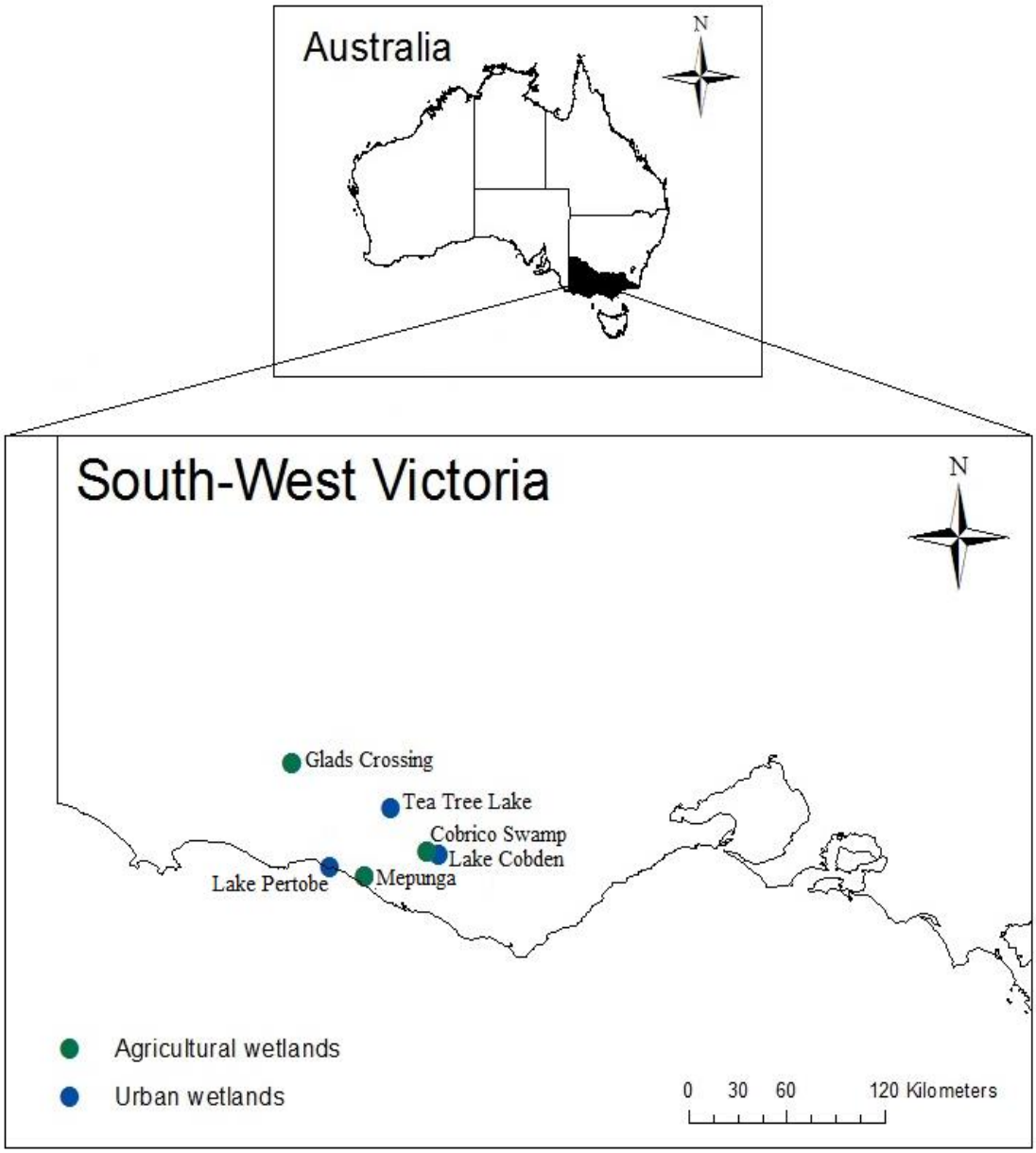


Fig. 1 Location and land-use of the six wetlands in southwest Victoria. Mepunga, Glads Crossing and Cobrico Swamp have agricultural land-use (green dots) and Lake Pertobe, Tea Tree Lake and Lake Cobden have surrounding urban land-use (blue dots).

2.2 Study sites

Two sites were selected within each wetland, resulting in twelve sites across the six wetlands. At each wetland, the two sites were selected to quantify any small-scale differences within the wetland. The sites were marked, with a minimum distance of 30 m separating the two sites. Each site was 7.5 m long, ran parallel to the bank of the wetland and, at the start of the study, had 25-30 cm of standing water (Appendix A). At each sampling event two wetlands were sampled per day, over a three-day period in the order presented in Table 1.

2.3 Potential rapid indicators

2.3.1. Physico-chemical characteristics

Electrical conductivity (EC, standardised to 25°C; μS cm^{-1}), turbidity (NTU), dissolved oxygen (DO% and mg L^{-1}), pH and temperature (°C) were measured on each sampling event with a Yeokal 611 meter in the middle of the water column. This varied from 10 to 30 cm of water depending on water level at the time of sampling as the wetland dried out. The measurements were made at three evenly-spaced locations along each site at all sampling event (0, 7, 21, 35, and 42 days; Table 2) for all six wetlands.

Very low DO concentrations during early morning surveying required the Yeokal meter to be recalibrated after each site. To better deal with this, after the 35-day point, two handheld 605000 YSI Professional Plus multi-parameter water quality meters were used to measure DO, using one meter per wetland for each day of sampling. For consistency, the Yeokal was used to measure all other variables over the entire sampling period.

2.3.2 Laboratory nutrient testing

Samples for laboratory nutrient testing were collected at 7, 21 and 35 days (Table 2). All samples for nutrient analysis were collected in the middle of the water column (5-15 cm of water) at the site. The 10-mL testing bottle was rinsed three times in wetland water before the sample was collected. These samples were then immediately frozen in an ENGEL car freezer and transferred to the laboratory freezer until laboratory testing was possible. The Deakin University Water Quality Laboratory tested the collected water samples total nitrogen (mg L^{-1}) (TN, using method WQL-05) and total phosphorus (mg L^{-1}) (TP, using method WQL-07).

2.3.3 Rapid nutrient testing

Nutrients were measured *in situ* at 35, and 42 days using a rapid VISOCOLOUR® ECO nutrient test kit (Table 2). The concentrations of nutrients including ammonium (NH_4), nitrate (NO_3), nitrite (NO_2) and phosphate (PO_4), and the metal iron (Fe) were measured at all wetlands. All samples for this rapid test kit (1 replicate per site) were collected in the middle of the water column (5-15 cm of water) at the site. The collection bottle (500 mL) was rinsed in wetland water three times before collection in the same general vicinity as the laboratory nutrient sample. The test kit analysis was done in the field at the time of collection.

2.3.4 Sediment analysis

Sediment samples were collected from the remaining large (5 cm diameter x 15 cm deep) cores after the microbial community samples had been extracted. Sediment samples (5 cm diameter x 5 cm deep = 98 mL), were placed into a labelled, plastic zip lock bag, and immediately frozen. Once back in the laboratory, the sediment samples were placed into another freezer for future analysis.

2.3.5 Water level

The change in the water level over the 42-day sampling period was also recorded. A bamboo stake, which indicated the location of the cotton and wood samples and the point of water quality monitoring, also indicated the change in the water level through time. There were five of these stakes evenly-spaced along the 7.5-m study site. The depth of water at the stake, as well as the distance that the stake was from the wetland bank, were recorded at each sampling event. When the water level declined to the extent that it retreated behind the bamboo sticks, this distance was also recorded to indicate where the water quality samples were taken.

Table 2 The timing of measurement of the potential rapid indicators (physico-chemical characteristics, lab nutrients and VISOCOLOUR® ECO), as well as when the established time-intensive indicators (cotton and wood break-down assays, microbial community function and data loggers) were deployed and then collected.

Measure	**Days**				
	0	**7**	21	**35**	**42**
Physico-chemical characteristics	✓	✓	✓	✓	✓
Lab nutrients		✓	✓	✓	
VISOCOLOUR® ECO nutrients				✓	✓
Sediment samples			✓	✓	
Water level	✓	✓	✓	✓	✓
Wood	✓	✓	✓	✓	
Cotton	✓	✓	✓	✓	
Microbial			✓	✓	
Data loggers	✓	✓	✓	✓	✓

2.4 Established time-intensive indicators

2.4.1 Data loggers

At each site, three HOBO® Data Loggers (Part # UA-002-64, Patent 6,826,664) were also deployed in approximately 30 cm of water at 0 days. These data loggers were evenly spaced to the left, right and middle of the 7.5-m sites and pinned to the surface of the sediment. The data loggers recorded temperature (°C) every 30 minutes from the initial deployment period. These were then collected at 42 days, and all data over this time period was downloaded for future analysis.

2.4.2 Wood break-down assay

2.4.2.1 Preparing the wood

Standard determination of wood break-down followed the methods of Aristi *et al.* (2012). Flat tongue depressors (hereafter referred to as wood) that were 15 cm in length, 1.8 cm wide and 0.1 cm high, made of ashwood, were used (Beiersdorf, North Ryde, NSW). The wood replicates were individually labelled with pencil, hole punched and dried at 70°C for 72 hours, cooled in a desiccator and weighed (±0.0001) (Aristi *et al.*, 2012). The replicates were then grouped and wrapped in aluminium foil and dry autoclaved at 121°C for 30 minutes. They were stored in clean plastic containers until deployment.

2.4.2.2 Deploying the tongue depressors

At the site, the wood was removed from the plastic containers and a sterilised waterproof tag was attached. Fifteen pieces of wood were paired with each of the fifteen rulers that had been deployed along the 7.5-m site in the wetland. The wood was gently placed edgeways-down in the sediment, with the length running parallel to the sediment surface, just under the sediment surface. String was looped around a hole in the wood and attached above the water level to a bamboo stick for re-location.

2.4.2.3 Wood retrieval

At the start of each retrieval sampling event, at each site, procedural controls were undertaken. One wood replicate per site was exposed to the air for ~20 minutes (air control) to control for possible atmospheric variations, and terrestrial microbial communities that may have come in contact with the wood at site. A second procedural control was exposed to the sediment for ~20 minutes, to control for abrasion during handling.

At 7, 21 and 35 days, four wood experimental replicates were then gently removed from the sediment (Appendix B). One wood replicate was left as a spare to protect against potential future loss of samples. The retrieved wood was rinsed in wetland water, and then placed in a zip lock bag, in the dark, on ice. On the night of retrieval (2-8 hours later) in the laboratory, the wood replicates were gently and individually washed with tap water, and then oven-dried at 70°C for 72 hours. After 72 hours, the wood was removed from the oven, placed in a desiccator and cooled, then weighed. Wood decomposition rates were then expressed as a percentage loss of the initial weight of the wood divided by the mass loss of the same piece of wood, for all samples collected at 7, 21 and 35 days sampling events.

2.4.3 Cotton strip assay

2.4.3.1 Preparing the cotton strips

This established method followed that of Boulton and Quinn (2000). Cotton strips were prepared from sections of standard Shirley Soil Burial test fabric (Shirley Dyeing and Finishing Ltd, Hyde, Cheshire). The cotton (4 cm weft x 5 cm warp) was cut and pieces were selected diagonally across the sheet so that no strip of cotton from the same replicate was chosen from the same warp or weft. The cotton strips, in groups of five replicates, were wrapped in aluminium foil and dry autoclaved at 121°C for 30 minutes. After autoclaving, the outside of the aluminium foil was patted dry with paper towel to remove excess water and

then packed in sterile (washed with 100% ethanol) plastic containers for deployment in the field.

2.4.3.2 Deploying the cotton strips

Cotton strips were deployed at 0 days. They were removed from their plastic containers in the field, and attached to the flat side of plastic 30-cm rulers with rubber bands. Prior to attaching the cotton, the rulers were dipped in 100% ethanol and then rinsed in the wetland water. Five strips of cotton were attached to each ruler. A small (1 cm) gap was left between each cotton strip to prevent the spread of bacteria or fungal colonies among individual pieces. The rulers were then placed edgeways-down in the sediment, with the top of the ruler about 1 cm below the sediment surface so that the cotton strips were in contact with the sediment on one side. To minimise physical abrasion on placing the ruler in the sediment, a separate ruler was held against the cotton strips during the placement process and then removed, leaving only the ruler with the cotton attached in the sediment. To ensure the relocation of the rulers was possible, string was looped around each ruler, labelled and attached the nearest bamboo stake. Fifteen rulers were deployed at each site. This included five replicates for each of three collection times. Rules were evenly spaced along the 7.5-m site.

2.4.3.3 Cotton retrieval

At the start of each retrieval sampling event, procedural controls were undertaken using the same method as the experimental samples. Five replicate strips of cotton on a ruler (air controls) were exposed to the air at the wetland site for ~20 minutes to control for possible atmospheric variation and terrestrial microbial communities that may have come in contact with the cotton at each site. In addition, another five replicate strips of cotton were attached to a ruler and placed in the sediment for ~20 minutes to control for abrasion during handling.

Cotton was retrieved at 7, 21 and 35 days after deployment (Table 2). Four, randomly selected, rulers were retrieved on each sampling event (Appendix C).

One ruler, with its cloth strips, was left in the wetland site in case of the loss of replicates. Loss of tensile strength due to handling was minimised by placing a ruler against the cotton strip ruler to remove it from the sediment. Each of the five cotton strips was then individually removed from the ruler and gently rinsed in wetland water. The groups of five strips were then placed in a zip lock bag in the dark on ice and were transported back to the laboratory on the same day. Once back in the laboratory (2-8 hours later), the strips were gently washed with tap water and then dried at 40°C for 24 hours (Tiegs *et al.*, 2013). Strips were then removed from the oven, placed in labelled plastic bags, and stored in a desiccator.

2.4.3.4 Tensile strength determination

All individual cotton strips, once dry, had their edges frayed, so that all strips were 100 threads wide (3 cm), allowing an equal section of the cotton to be tested. Immediately before testing, the strips were conditioned for 24 hours at 22°C and 61% humidity. Four testing strips were placed in the jaws of the Instron tensile tester, using a 30-kN load cell (Institute for Frontier Materials, Deakin University). The Instron jaws were lined with emery tape to prevent slippage and gripped approximately 1 cm of the ends of the strips. The gauge length (distance) between the jaws was set at 3 cm. Strips were pulled at a fixed rate of 100 mm minute^{-1} and the maximum tensile strength was recorded for each strip. Tensile strength loss was expressed as a percentage of the initial tensile strength (represented by an average of the controls, for the specific experimental sample) divided by the tensile strength loss of the experimental samples (as an average of 3 of the 5 strips that were on the specific ruler) for the 7 and 21 day sampling events (Tiegs *et al.*, 2013). However, in contrast to Tiegs *et al.*, (2013), the mass loss was expressed as the percentage loss of the specific sampling event (7

or 21 days), rather than average loss per day, to keep the calculations consistent with the wood.

Due to a mechanical malfunction the strips of cotton slipped during the testing process. To account for this, if the extension at break exceeded 11 kN, the samples were excluded to ensure only the most reliable readings were analysed.

2.4.3 Microbial functional diversity

The microbial functional diversity of the sediment in the different wetlands was measured based on carbon source utilisation, using Biolog™ ECO plates. The plates had three replicates each, consisting of 31 carbon substrates and one control (non-carbon substrate). The microbial sampling, extraction and plating followed the methods of McKenzie *et al.* (2011). At 21 and 35 days, five replicate sediment cores (5 cm diameter x 15 cm deep) were collected from each site. A sub-sample (2.2 cm diameter x 3 cm deep = 11.4 mL volume) core was collected from the centre of the larger core and stored in a sterile Whirlpak® in the dark on ice until extraction. The sub-sampler corer was rinsed in water and washed in 100% ethanol between replicates. A field procedural control was also conducted at each site by dipping the rinsed and ethanol washed sub-sample corer into the Whirlpak®, without taking a sediment core. After this, the control was treated according to the same procedure as the experimental samples.

In the laboratory, microbial extraction followed the technique described by McKenzie *et al.* (2011). This involved the addition of 100 mL of autoclaved distilled water and glass beads (6 beads, 4 mm diameter) into each of the Whirlpaks®. Samples were shaken vigorously by hand for 1 minute, and then put in the dark on ice for 15 minutes, to allow sediment to settle. A 15-20 mL sample of the water above the sediment in the Whirlpak® was then syringe-filtered (5 μm pore size) into a sterile petri dish. Using an 8-channel micropipette, 100 μL was

transferred into each of the 32 wells of the Biolog^{TM} ECO plates for one replicate. Before plating, the sample was syringed and released five times from the petri dish to remove any electrical charge on the pipette tips. All the microbial samples were plated on the same day as collection.

After plating, the Biolog^{TM} ECO plates were incubated in the dark at 15°C in a constant-temperature cabinet for five days. Over the five days, microbes that can utilise a carbon source respire and precipitate a purple dye, producing differing intensities of purple colour according to their ability to utilise each carbon source. The colour development in the different wells was then scored by eye from 0 (no colour), 1 (lightest purple) to 4 (darkest purple) and was used a surrogate measure of carbon source utilisation, assessing the functional diversity of microbial bacteria (Appendix D).

2.5 Statistical analyses

Multivariate statistical analyses were performed with PRIMER v. 6 (Clarke & Gorley, 2006) with the PERmutational Multivariate ANalysis Of Variance (PERMANOVA+) add-on (Anderson *et al.,* 2008). PERMANOVA is a non-parametric, permutation-based method for assessing significance and, unlike traditional ANOVA, it makes few, if any, assumptions about the form of the data which makes it widely applicable in ecological studies, leading to greater confidence in interpretation of ecological data sets (Anderson *et al.,* 2008).

Multivariate data included the physico-chemical characteristics, nutrients (rapid and laboratory), water level, cotton strip and wood break-down assays as well as the microbial community function data.

Firstly, water quality variables were normalised to remove the effect of differing scales of measurement and then a Euclidean distance similarity matrix was constructed. A non-metric multidimensional scaling (MDS) plot was used to examine visual patterns in water quality variability among the different wetlands, sites within wetlands, among differing sampling events and land-use types. Differences in water quality were tested using a four-factor PERMANOVA (i.e. land-use [fixed factor], wetland [random] nested within land-use, site [random] nested within wetland, and sampling event [random]). This analysis was conducted with the water quality, laboratory nutrients and water levels combined, then again for the VISOCOLOUR® ECO nutrients for the appropriate sampling events. Due to problems with some DO readings, two approaches were used for all analyses including DO. Firstly, only sites and times with reliable DO measurements were included. However, faulty DO readings were predominantly from the 21-day sampling event, so all analyses were also run excluding DO as a variable, to avoid any bias in the analyses. Results are presented only for those analyses including DO, unless results varied substantially.

The cotton strip and wood break-down assays were analysed using the same PERMANOVA structure in a univariate test of loss of tensile strength, or mass, respectively. Here, no transformations or normalisation were required and a Euclidean distance similarity matrix was constructed. Microbial community function was also analysed using the same PERMANOVA structure in a multivariate analysis including each carbon source as a variable in the analysis. Here, however a Bray-Curtis similarity measure was used rather than Euclidean distance, with a dummy variable added to account for the zero-inflated structure of the data.

All intensive measures were then examined in a combined analysis, with variables normalised to remove the effect of differing scales of measurement, and analysed as

described above. Microbial community function was included in this combined analysis as a mean number of substrates used and a mean intensity for each site. This same analysis was also conducted with just wood and microbial decomposition alone, without cotton included, to assess the impact of the less-reliable data due to mechanical malfunction.

A RELATE procedure was used to determine whether there was an overall correlation between the water quality, nutrients and water levels with each of the time-intensive measures individually, as well as when they were combined, firstly with all three intensive measures, then again with just the wood and microbial decomposition compared with the rapid indicator data. A BEST procedure was then used to determine the strongest correlated physico-chemical and nutrient variables with the time-intensive measures. Significance was determined by comparing the BEST values with critical values of a Spearman's rank correlation coefficient for the appropriate number of degrees of freedom. For both RELATE and BEST, time of decomposition was included as a variable in the water quality data set to account for differences in the time allowed for decomposition across different replicates.

The same analyses were then carried out on only the 27-day intensive measures data against the full suite of rapid indicator data, and then again for just the 35-day data against the full suite of rapid data, to simulate the data set that might be collected by a manager attempting to rapidly assess decomposition.

3. Results

3.1 Potential rapid indicators

3.1.1 Comparison of water quality among wetlands

There was large variation in air temperature over the sampling period from 3.9°C minimum to 43.6°C maximum (BOM, 2014). High air temperatures caused an increase in water temperatures as the experiment progressed and an associated reduction in water level at each wetland. Most wetlands experienced severe declines in water level. Lake Pertobe had the greatest decline, with depth falling by 23.0 ± 1.1 cm, but water levels dropped less at other wetlands, with Glads Crossing only decreasing in depth 11.9 ± 2.0 cm. There were significant differences in water levels across sampling events (pseudo-$F_{4, 16} = 8.57$, $P = 0.001$).

There was also large variation in water temperatures recorded with HOBO® Data Loggers over the 42-day sampling period at all wetlands. Mepunga had the smallest temperature range, varying between 6.2 and 50°C, possibly due to relatively high canopy cover due to the surrounding *Salix* spp. The largest temperature range was at Tea Tree Lake, from 7.7 to 64.0°C. Despite this, the variation in mean temperatures was quite low. All wetlands had similar mean temperatures, ranging from the lowest at Mepunga (20.7 ± 0.04°C) to the highest at Lake Cobden (23.9 ± 0.03°C). From the water quality monitoring, Mepunga had the lowest electrical conductivity of all wetlands (428.6 ± 24.5 μs cm^{-1}). The highest electrical conductivity was found at Cobrico Swamp (3361.4 ± 94.9 μs cm^{-1}). The highest average pH levels were found in Lake Cobden (9.1 ± 0.1), and the lowest at Mepunga (6.8 ± 0.1) over the entire sampling period. Turbidity also varied among wetlands. The lowest turbidity value over the entire sampling period was recorded at Cobrico Swamp (17.6 ± 4.7 NTU) while the highest was recorded at Glads Crossing (366.2 ± 30.8 NTU). There was variation in the dissolved oxygen (%) levels over the period of the day, generally lower DO

levels were found in the morning, with higher DO in the wetlands, sampled in the afternoon. The lowest dissolved oxygen concentrations were found in Mepunga (25.1 ± 7.5%) and the highest in Lake Cobden (162.6 ± 13.3%) over the entire sampling period.

The nutrient concentrations (TN and TP, mg L^{-1}), were generally above the guidelines for shallow inland lakes (TP = 0.1 mg L^{-1}, TN = 1.5 mg L^{-1}; EPA Victoria, 2003). Nutrients concentrations varied among wetlands, but were largely consistent across the different sampling events for each wetland. TP concentrations ranged from 0.05 mg L^{-1} (Tea Tree Lake) to 0.84 mg L^{-1} (Cobrico Swamp) across the time periods tested. TN concentrations ranged from 0.74 mg L^{-1} (Glads Crossing) to 7.60 mg L^{-1} (Lake Pertobe) (Appendix E).

There were statistically-significant differences among wetlands when examining physico-chemical characteristics, laboratory nutrients and water level in a combined analysis (pseudo-$F_{4,\ 8}$ = 3.00, P = 0.002; Table 1) and between sites nested within wetlands (pseudo-$F_{6,\ 8}$ = 3.80, P = 0.001; Table 1). There was also a significant interaction between wetlands and sampling events (pseudo-$F_{5,\ 8}$ = 10.625, P = 0.001; Table 1). No significant differences were found between land-use types (pseudo-$F_{1,\ 5}$ = 1.81, P = 0.21; Table 1, Fig. 1). There were only slight differences in the rapid indicator data when dissolved oxygen was excluded (Fig. 1), with sampling events becoming significant (pseudo-$F_{2,\ 8}$ = 3.607, P = 0.007; Table 1).

Table 1 Multivariate PERMANOVA results for differences in the rapid (water quality) and intensive measures (wood, cotton and microbial community function, as well as all intensive measures combined including and excluding cotton) showing the response of the factors Land use, Wetland nested within Land use, Site nested within Wetland and the Sampling Event. Significant results ($P < 0.05$) are shown in bold and non-significant results close to significant are underlined. N/a indicates no test was possible.

	PERMANOVA				
Measure	**Transformation**	**Factor**	**df**	**Pseudo-*F***	***P* (perms)**
Rapid measures (with DO)	Normalised	Land use	1,5	1.8129	0.21
		Sampling Event	2,5	2.2768	<u>0.069</u>
		Wetland (Land use)	4,8	3.0039	**0.002**
		Land use x Sampling event**	1,5	1.0522	0.386
		Site (Wetland)	6,8	3.8049	**0.001**
		Wetland x Sampling Event**	5,8	10.625	**0.001**
Rapid measures (excluding DO)	Normalised	Land use	1,5	1.6961	0.175
		Sampling Event	2,8	3.607	**0.007**
		Wetland (Land use)	4,14	5.2681	**0.001**
		Land use x Sampling event	2,8	1.0025	0.453
		Site (Wetland)	6,12	2.6896	**0.003**
		Wetland x Sampling Event	8,12	4.532	**0.001**
Wood	Untransformed	Land use	10, 5	0.48811	0.796
		Sampling Event	2,10	17.385	**0.001**
		Wetland (Land use)	6,14	2.1837	<u>0.06</u>
		Land use x Sampling event	2, 10	4.2882E-2	0.962
		Site (Wetland)	7,14	0.84788	0.563
		Wetland x Sampling Event	10,16	4.0864	**0.008**
Cotton	Untransformed	Land use	3,3	0.79656	0.57
		Sampling Event	1,2	7.7255	0.1091
		Wetland (Land use)	4,2	1.6458	0.304
		Land use x Sampling event	8,2	9.1983E-2	0.907
		Site (Wetland)	4,2	1.2303	0.5749
		Wetland x Sampling Event**	2,4	1.6458	0.304
Microbial	Untransformed	Land use	1,5	1.6314	0.212
		Sampling Event	1,4	2.1619	0.139
		Wetland (Land use)	5,10	1.9461	**0.018**
		Land use x Sampling event	1,4	0.46696	0.796
		Site (Wetland)	6,6	1.7863	**0.023**
		Wetland x Sampling Event	4,6	1.7544	**0.053**
All intensive measures	Normalised	Land use	2,3	0.87076	0.508
		Sampling Event	1,2	8.3885	<u>0.069</u>
		Wetland (Land use)	4,4	1.4098	0.266
		Land use x Sampling event	1,2	1.3515	0.363
		Site (Wetland)	4,1	2.456	0.3192
		Wetland x Sampling Event**	2,1	3.6886	0.138
Microbial and wood	Normalised	Land use	1,9	0.29047	0.79
		Sampling Event	1,2	2.5021	0.1603
		Wetland (Land use)	9,3	2.484	**0.04**
		Land use x Sampling event	n/a	n/a	n/a
		Site (Wetland)	9,3	0.3481	0.946
		Wetland x Sampling Event**	2,3	1.9286	0.185

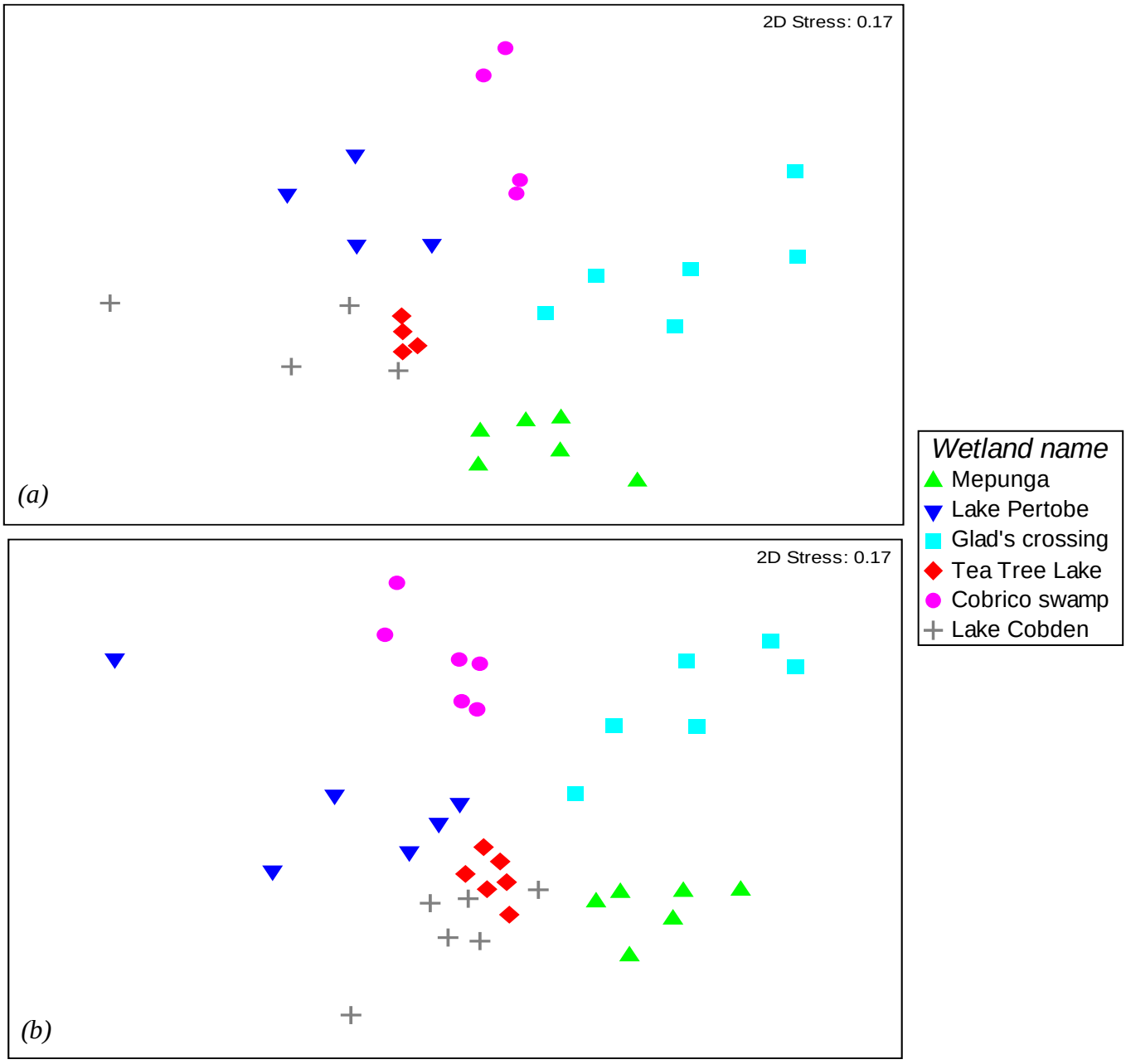


Fig. 1 MDS ordination plots illustrating the differences in the water quality variables *(a)* with dissolved oxygen (%), (*n* = 28) and *(b)* without dissolved oxygen included (*n* = 36). The other water quality variables included electrical conductivity, turbidity, pH, temperature, total nitrogen, total phosphorus and water level among wetlands over each sampling event (7, 21 and 35 days). Both MDS plots are based on a Euclidean distance similarity matrix of abundance data. All data were normalised.

3.1.2 Comparison of VISOCOLOUR® ECO nutrient testing among wetlands

The VISOCOLOUR® ECO nutrient test kit recorded a different pattern of nutrient concentrations compared with those measured in the laboratory. Phosphate was detected in the highest concentrations, with a maximum of 1.9 mg L^{-1} found in the agricultural wetland of Glads Crossing. Nitrite was detected at low levels relative to the guidelines (0.035 ± 0.008 mg L^{-1}) while ammonium had an overall mean concentration of 0.148 ± 0.020 mg L^{-1} across all wetlands and sampling events. There was no nitrate detected over the entire sampling period. When TN was calculated for the VISOCOLOUR® ECO, the mean value was 0.07 ± 0.01 mg L^{1} across all wetlands over 35 and 42 day sampling events, which was much lower than the laboratory nutrient findings. For TP there was 0.35 ± 0.12 mg L^{-1}, which was also lower than the laboratory nutrient concentrations. The metal Iron was found in all wetlands with an average concentration of 0.31 ± 0.06 mg L^{-1}. The only statistically-significant differences were among wetlands (pseudo-$F_{6, 8}$ = 2.46 P = 0.003).

3.1.3 Comparison of sediment characteristics among wetlands

The sediment characteristics were recorded and samples collected at each site, however, due to time constraints, there was no sediment analysis conducted and further analyses will occur for publication including grain size and organic content. Visually, there were notable differences between sediment size and colour characteristics (Appendix F). Lake Pertobe and Glads Crossing had extremely fine silty sediment, which made disturbance when wading in the wetland difficult to avoid. Mepunga had coarser sediment, which was stabilized by *Salix* spp. roots. The sediment was quite dark at Cobrico Swamp which had similar sediment to Mepunga, which was coarser and stabilised by the *Triglochin procerum* and *Typha* spp. roots. Tea Tree Lake had silt sediment which was a grey colour. Lake Cobden had a thick layer of leaf litter (~20 cm) on top of its very-fine sediment.

3.2 Time-intensive measures

3.2.1 Comparison of wood break-down across wetlands

A total of 227 tongue depressors were collected from 12 sites over 35 days. No replicates were lost during the course of the experiment but five pieces of wood had broken where they had been hole-punched and they were excluded from all analyses. There was also no contamination of the controls, with very little difference in the weight loss (0.46 ± 0.0098), so they were excluded from further analysis.

All wetlands showed increasing decomposition rates through time (Fig. 2). Glads Crossing had the fastest rate of decomposition after 7 days for the agricultural wetlands (2.63 ± 0.21%) increasing to 4.86 ± 1.05% after 35 days. The slowest decomposition rates for the agricultural wetlands were in Mepunga which had 3.99 ± 0.09% mass loss after 35 days.

Overall, the fastest decomposition rates for the urban wetlands were in Lake Cobden after 35 days (5.09 ± 0.03%). Tea Tree Lake had the slowest urban decomposition rate after 35 days with a mass loss of 2.63 ± 0.31%. Generally speaking, there were comparable decomposition rates found in urban and agricultural wetlands, with the highest average decomposition after 35 days being 4.06 ± 0.19% in urban wetlands and 4.31 ± 0.1% in agricultural wetlands (Appendix G). When the decomposition rates were analysed per day, after 7 days the measured rate was 0.31 ± 0.02% day^{-1}, 0.15 ± 0.03% day^{-1} after 21 days, and 0.12 ± 0.003 day^{-1} after 35 days, as an average across all wetlands.

There was a significant difference among sampling events (pseudo-$F_{2, 10}$ = 17.39, P = 0.001; Table 1). There were also significant differences among wetlands across the different sampling events (pseudo-$F_{10, 16}$ = 4.09, P = 0.008, Table 1). Again, comparable to water quality, there was no statistically-significant difference between the rates of decomposition at urban and agricultural wetlands (pseudo-$F_{10, 5}$= 0.49, P = 0.796; Table 1).

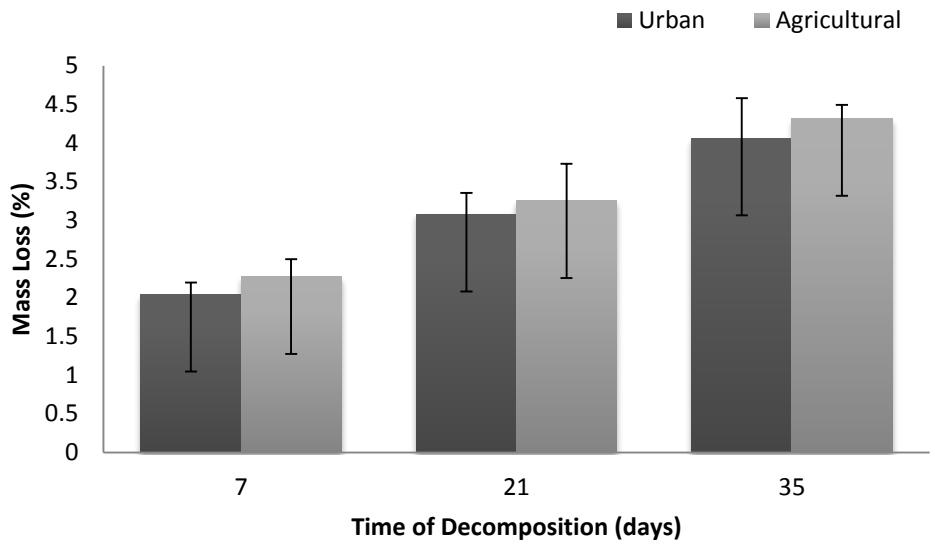


Fig. 2 Decomposition rates increasing monotonically through time for both urban and agricultural wetlands when measured using a wood break-down assay. These figures are based on mean and standard error values, from all urban (Lake Pertobe, Tea Tree Lake and Lake Cobden) and agricultural (Mepunga, Glads Crossing and Cobrico Swamp) wetlands over each sampling events (7, 21 and 35 days).

3.2.2 Comparison of cotton decomposition among wetlands

A total of 900 cotton strips were collected from six wetlands over the 35-day period. There were visual differences in decomposition among samples from each sampling event (Appendix C). Samples for 7 and 21 days were tested, however, all experimental samples from 35 days were too decomposed to test for tensile strength. This was expected due to the findings in previous literature (Tiegs et al., 2013), and was part of the rationale for also conducting a wood break-down assay. However, due to mechanical malfunctions with the Instron 1kN tensile tester, only 37 replicates experimental strips were statistically analysed.

Tea Tree Lake had to be completely excluded due to unreliable data. All remaining wetlands showed increasing decomposition rates through time (Fig. 3). After 7 days, the remaining urban wetlands, Lake Pertobe and Lake Cobden, were found to have the highest decomposition, 37.52 ± 6.09% and 21.99 ± 5.75% respectively. The lowest decomposition rates were found in the agricultural wetlands at Mepunga (22.55 ± 4.55%) and Cobrico Swamp (4.15%). After 21 days, decomposition rates increased in all wetlands examined. Lake Cobden was excluded after 21 days due to unreliable data. Lake Pertobe saw decomposition rates increase to 82.94 ± 4.41%, Mepunga to 86.79 ± 4.21% and Cobrico Swamp to 14.5 ± 1.34%. Overall, there was a larger difference in the land-use type for cotton (based on the four wetlands with reliable data over the entire period), with urban wetlands (82.94 ± 9.86%) having a higher decomposition rate after 21 days than the agricultural wetlands (65.09 ± 2.85%) (Appendix H). The cotton when examined for loss per day, after 7 days there was an average of 3.14 ± 0.82% loss day^{-1}, and after 21 days there was an average of 3.33 ± 0.15% loss day^{-1}. Cobrico Swamp had the lowest tensile strength loss by a considerable amount, of 0.59 ± 0% loss day^{-1} after 7 days and 0.69 ± 0.06% loss day^{-1} after 21 days.

There were no significant differences among wetlands nested within land-use types (pseudo-$F_{4, 2}$ = 1.65, P = 0.304; Table 1) or sampling events (pseudo-$F_{1.,2}$ = 7.73, P = 0.109; Table 1). Consistent with the other measures, there was no statistically-significant difference between the rates of decomposition at urban and agricultural wetlands (pseudo-$F_{3, 3}$ = 0.80, P = 0.57; Table 1), despite the trends noted above.

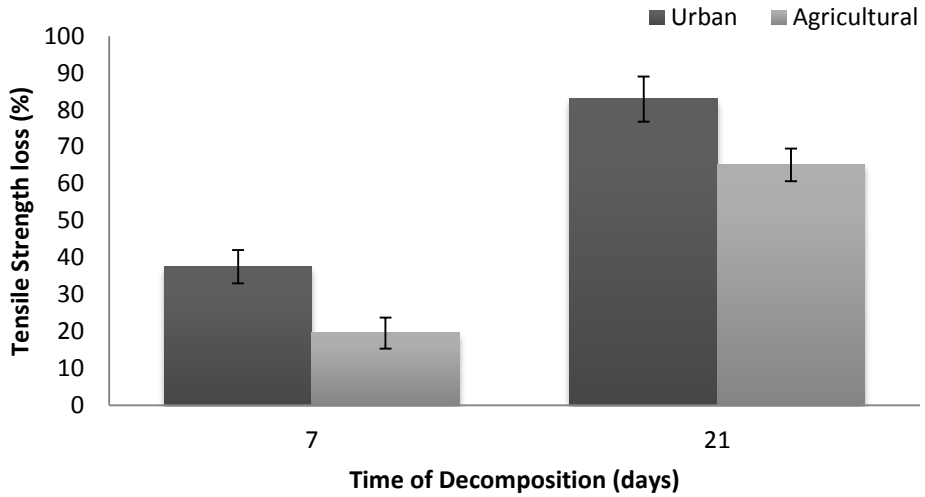


Fig. 3 Decomposition rates increased monotonically through time for both agricultural and urban wetlands when measured using a cotton strip assay. These figures are based on mean values, with standard error bars displayed from all agricultural wetlands (Mepunga, Glads Crossing and Cobrico Swamp) and all the urban wetlands examined (Lake Pertobe and Lake Cobden), over the sampling events (7 and 21 days).

3.2.3 Comparison of microbial community function among wetlands

There were 144 replicate readings of carbon source utilisation recorded for the 21- and 35-day sampling events (Fig. 4). When the procedural controls were analysed, no to very little contamination across all samples over both sampling events was found (0.06 ± 0.01).

There was very little variation among number of responses of the microbes (i.e. a surrogate for functional diversity of microbial community types) to the 31 carbon substrates available among sites or wetlands (Appendix J). However, there was more disparity at 21 days, with the lowest response occurring in Lake Pertobe with 24 ± 1 carbon sources used and the highest being Tea Tree Lake, where all 31 carbon sources available were utilised. At 35 days, the responses ranged from 28.5 ± 0.5 at Glads Crossing to 30.5 ± 0.5 at both Lake Pertobe and Cobrico Swamp. Tea Tree Lake still had a high number of carbon sources used (29.5 ± 0.5). Across both sampling events and all wetlands, the highest number of carbon sources utilized was at Tea Tree Lake (30.8 ± 0.8) and the lowest at Lake Pertobe (27.3 ± 1.9). This indicated that most of the carbon sources were able to be utilized by microbes, to some extent, in most wetlands. There was very little disparity found between the number of carbon sources used, between sampling events, or between urban and agricultural with values of 29.67 ± 0.44 and 29.5 ± 0.58, respectively, for land-use types at 35 days.

There was more variation in the intensity of carbon source utilisation between wetlands (a surrogate for the abundance or activity of functional taxa; Appendix I)**.** The greatest average colour intensity was identified in Cobrico Swamp (1.8 ± 0.1) and the lowest at Lake Cobden (1.2 ± 0.7), averaged over 21 and 35 days (Appendix I). Generally, Mepunga (1.7 ± 0.9) had the highest intensity carbon source utilisation and Lake Cobden (1.2 ± 0.7) had the lowest over the both sampling events. When considering the multivariate data showing the utilisation of each carbon source, there were significant differences among wetlands nested in

land-use types for the PERMANOVA (pseudo-$F_{5, 10}$ = 1.95, P = 0.018; Table 1), and sites nested within wetlands (pseudo-$F_{6, 6}$ = 1.79, P = 0.023; Table 1), as well as an interaction between wetlands and sampling events (pseudo-$F_{4, 6}$ = 1.75, P = 0.053; Table 1). Again, there was no significant difference identified between land-use types (Table 1).

3.2.4 Comparison of all intensive measures of decomposition across wetlands

In order to draw conclusions about the consistency across the different time-intensive measures, I conducted a combined analysis. As described in the Methods above, I firstly ran all the time-intensive methods including the cotton and then again excluding the cotton, to assess the impact of the missing data (Fig. 5). There were non-significant differences identified across wetlands when all intensive measures were combined (pseudo-$F_{4, 4}$ 1.41, P = 0.266; Table 1). However, when the cotton was excluded, there were significant differences found among wetlands nested in land-use types (pseudo-$F_{9, 3}$ = 2.48 P = 0.04; Table 1).

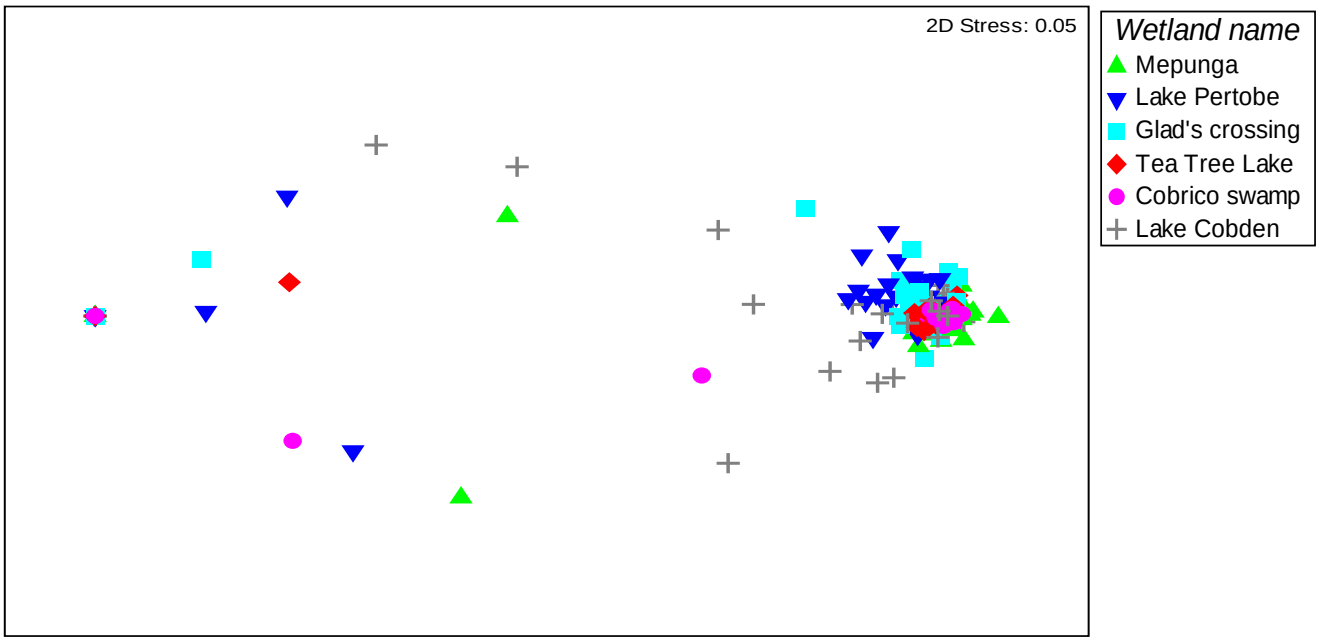


Fig. 4 MDS ordination plot illustrating the differences in the response of the microbial communities to the 32 available carbon substrates of the 21- and 35-day sampling events, among the six wetlands. The MDS plot is based on a Bray Curtis similarity matrix with an added dummy variable.

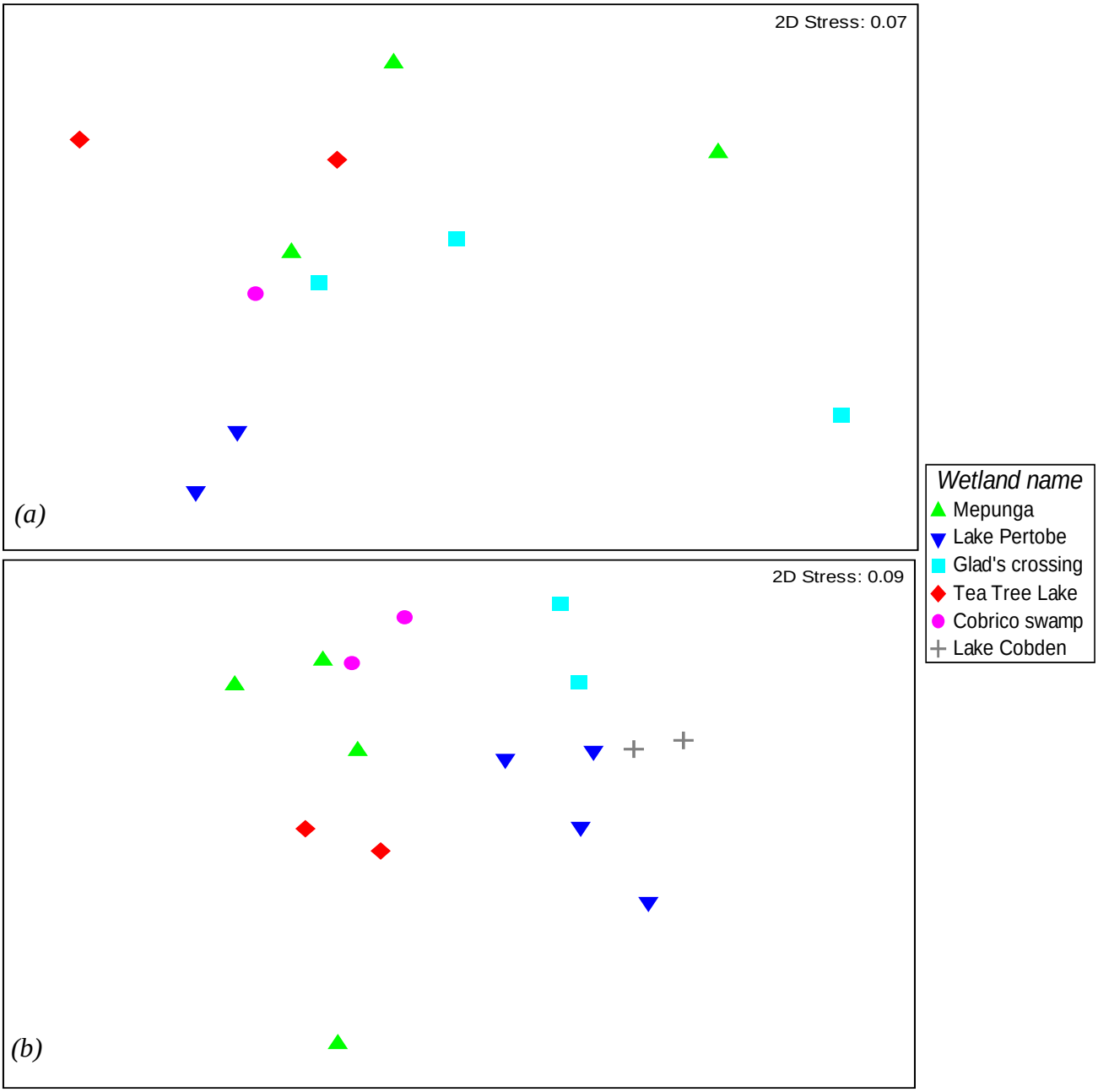


Fig. 5 MDS ordination plot illustrating the differences in the time-intensive measures in a combined analysis *(a)* with cotton included (7 and 21 days), (*n* = 11) and *(b)* excluding cotton (7, 21 and 35 days) (*n* = 16), across all six wetlands. Both MDS plots are based on a Euclidean distance similarity matrix. All data were normalised.

3.3 Relationships between rapid indicators and intensive measures

All of the rapid water quality measures (physico-chemical variables, nutrient concentrations, water levels) and time of decomposition (number of days) were then compared with the time-intensive measures of decomposition to identify the strongest correlations and thus possible rapid indicators of decomposition rates.

The rapid indicator dataset, as a whole, showed no significant overall correlation with the rate of wood decomposition (RELATE, *Rho* = 0.139, P > 0.05). However, the best-correlated combination of variables were dissolved oxygen, conductivity, turbidity, temperature and water level (BEST, *Rho* = 0.899, P < 0.05, Table 2). Similarly, for the cotton decomposition there was, overall, no significant relationship (RELATE, *Rho* = 0.466, P < 0.05) with the overall best-correlated variables including dissolved oxygen and water level (BEST, *Rho* = 0.754, P < 0.05; Table 2).

Overall, dissolved oxygen and water level were the rapid indicators that were most commonly correlated with both decomposition measures. In general, an increase in dissolved oxygen concentration tended to be associated with an increase in decomposition for wood, but no real pattern was observed with cotton decomposition rates (Fig. 6). For water level, a decrease in water depth tended to be associated with an increase in both cotton and wood decomposition rates (Fig. 6).

The microbial communities also showed no significant overall correlation with water quality variables (RELATE, *Rh o*= 0.324, P > 0.05, Table 2) with the best-correlated combination of variables including pH and total nitrogen (BEST, *Rho* = 0.393, P > 0.05, Table 2). The abundance of microbial communities tended to increase with an increase in pH, with no distinct pattern in microbial abundance with total nitrogen (Fig. 6). The intensity at which

microbes were found to be using the carbon sources tended to see a decrease in intensity as the pH became more basic, with the highest intensity of carbon sources being used found at a neutral pH. There was a slight positive relationship with total nitrogen and increase of the intensity at which the microbes were using the carbon sources (Fig. 6).

When all the intensive measures were combined and compared against all potential rapid indicator variables, there was again no overall statistically-significant correlation found (RELATE, *Rho* = 0.357, P > 0.05, Table 2). The strongest correlations were between temperature and time of decomposition and decomposition (BEST, *Rho* = 0.782, P < 0.05, Table 2). When the cotton was excluded from the combined analysis, there was also no significant relationship found with the rapid indicators overall (RELATE, *Rho* = 0.098, P > 0.05, Table 2) with total phosphorus, temperature, pH, turbidity, and water level being the best-correlated variables (BEST, *Rho* = 0.914, P < 0.05, Table 2).

3.4 Relationships between rapid indicators and intensive measures at 21 and 35 days

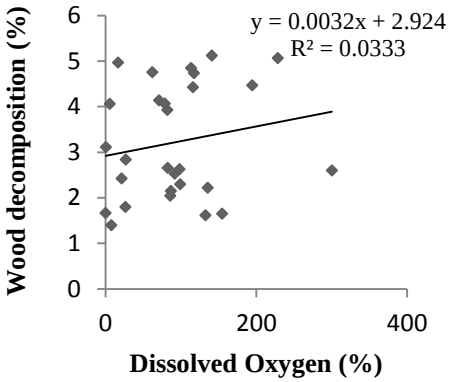
When assessing correlations at only 21 days, to simulate the circumstances of a rapid assessment, there were again no overall significant correlations found (RELATE, *Rho* = 0.661, P > 0.05, Table 2), potentially because of the very small samples size associated with the problems experienced with DO at the sampling event of 21 days. The best-correlated variables were found to be total nitrogen, turbidity and conductivity (BEST, *Rho* = 0.661 P > 0.05; Table 2). At 35 days only, there was also no overall significant relationship (RELATE, *Rho* = -0.114, P > 0.05, Table 2) between all rapid indicators and intensive measures, with the strongest-correlated variables being pH and conductivity (BEST, Rho = 0.224 P > 0.05; Table 2).

Table 2 Overall correlations between the rapid indicator data and the time-intensive measures of decomposition as identified using BEST analyses. The significant correlations ($P < 0.05$) are in bold font.

		Rapid indicators									
		Total Nitrogen	Total Phosphorus	Dissolved oxygen	Temperature	pH	Electrical Conductivity	Turbidity	Water level	Time of decomposition	
Measure	***Rho***										
Wood	**0.899**			✓	✓		✓		✓	✓	
Cotton	**0.754**			✓					✓		
Microbial	0.393	✓				✓					
Wood, cotton & microbial	**0.782**				✓					✓	
Wood & microbial	**0.914**	✓			✓	✓		✓	✓		
21 Days only	0.818	✓					✓	✓			
35 Days only	0.224				✓				✓		

Fig. 6. Scatterplots illustrating correlations for the most commonly-correlated variables identified, inlcuding *(a)* dissolved oxygen and *(b)* water level for wood, *(c)* dissolved oxygen and *(d)* water level for cotton, and *(e)* pH and *(f)* total nitrogen for microbial abundance, and *(g)* pH and *(h)* total nitrogen for microbial intensity over all wetlands and sampling events. Note differences in the scales of the x and y axes across the various panels.

(*a*)



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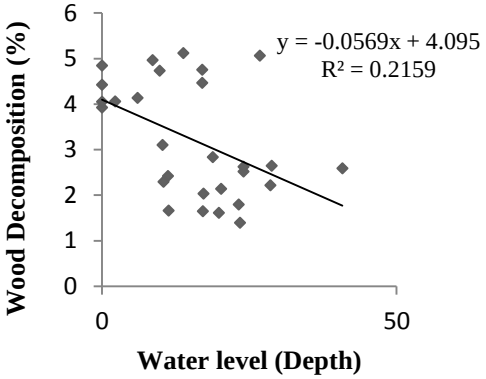

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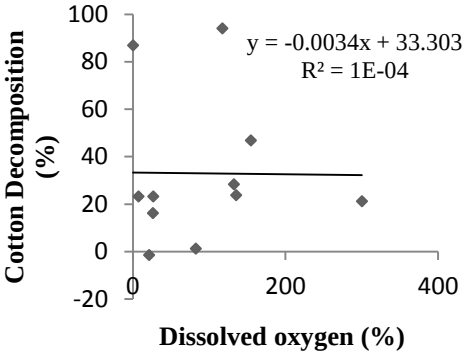

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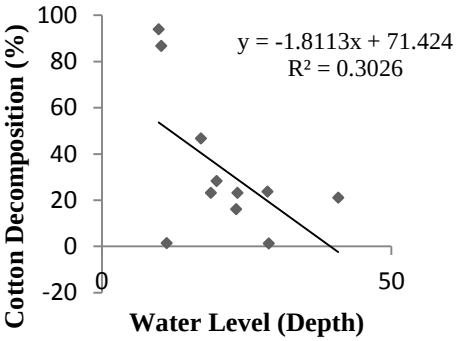
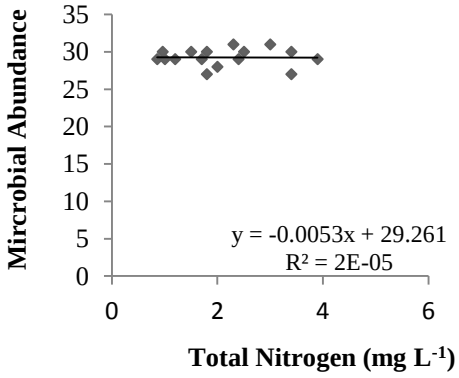
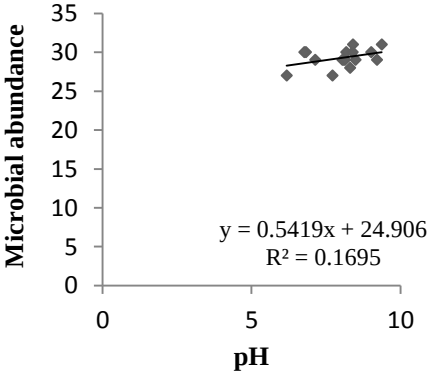


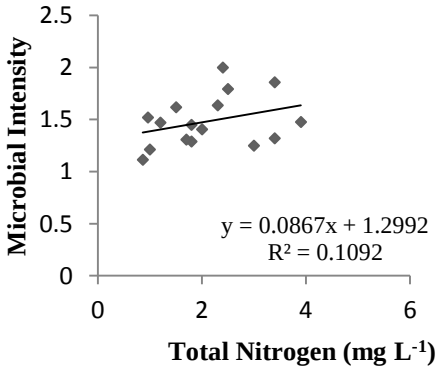
(*e*)



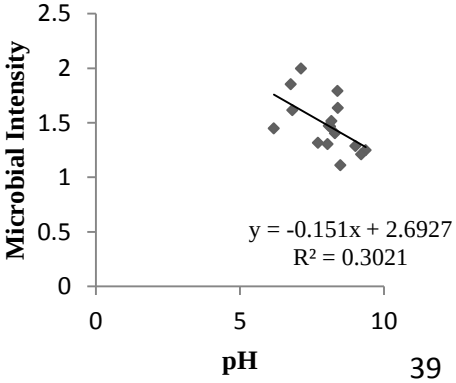
(*f*)



(*g*)



(*h*)



4. Discussion

Rapid monitoring tools to gauge the entirety of our effect on the environment are urgently needed (Rapport, Costanza & McMichael, 1998; Fairweather, 1999a). In particular, there are few tools that investigate the response of ecological functions for natural resource management (Landres, Morgan & Swanson, 1999). Therefore, the fundamental aim of this research was to identify possible rapid measures such as physico-chemical variables and nutrient concentrations that could have a strong relationship with decomposition; thus providing a reliable, rapid assessment of the likely decomposition occurring within a system.

I predicted that one or more rapid measures would significantly correlate with decomposition rates and, therefore, act as a reliable indicator. This was partially supported by my findings, with a number of variables that were strongly correlated with decomposition rates, but also requiring other highly-variable environmental factors (e.g. dissolved oxygen concentrations which vary on an hourly scale) to be considered before interpretation across a range of systems is possible. It was also hypothesised that the best-correlated indicators would vary over land-use types, but my findings did not align with this prediction. This was a beneficial outcome in terms of identifying reliable indicators, as it would make any identified indicator more versatile because it would be likely to apply over both land-use types.

4.1 Potential rapid indicators of decomposition

In exploring the main objective of this research, to identify a rapid indicator of decomposition, it was found that water level and dissolved oxygen were the two most strongly correlated variables. In this research, some of the wetlands most affected by changes

in water level over the course of the experiment had some of the highest decomposition rates. This pattern is in contrast to the findings of previous studies. Serna, Richards and Scinto (2013), for example, investigated the effect of water level on decomposition and found that a reduction in water level lowered decomposition rates. Van Der Valk, Rhymer and Murkin (1991) found that leaf litter species decomposed faster in a flooded water-level treatment when compared with litter that was not always submerged in water. This may be different to my study because, although some wetlands had dried to the extent that they no longer had water over the samples by day 35, the sediment remained waterlogged, allowing the sediment characteristics that enable aquatic microbial assemblages to be maintained (Duarte, Freitas & Cacador, 2012). For example, Van Der Valk, Rhymer & Murkin (1991) found that leaf litter dries out quickly if it is not inundated it, which negatively affects microbial populations. However, due to inevitable variations in water levels over different seasonal cycles, leading to changes in water temperature (Chimney & Pietro, 2006), for example, water level is not suited for use as a rapid indicator alone. Water level measurements could indicate variations in local conditions, however, it could not indicate the functioning of a system across a large spatial scale, and the climate conditions in different regions would need to be considered.

Dissolved oxygen was also significantly correlated with all intensive decomposition measures. Variables such as DO, however, are difficult to interpret due to diel fluctuations in respiration and photosynthetic rates of surrounding vegetation (Correa-Gonzalez *et al.*, 2014). DO levels are also strongly related to shading, and riparian vegetation cover (de Souza *et al.*, 2013). My sampling took place across the entire day in order complete all sampling in the available time period, and some wetlands were systematically examined in the morning (and were shown to have low DO levels) while others were examined in the afternoon, with relatively higher DO levels, and this may have influenced the relationship identified between DO and decomposition. I did, however, include the time of day of sampling as a co-variate in

the correlation analyses undertaken (not presented), but this did not alter the relationships identified. Despite this, and the fact that a reduction in DO levels is a bi-product of the stimulation of aerobic microbial activity due to oxygen consumption (Carvalho, Thomaz & Bini, 2005), DO could be used as one of a suite of variables that together could be a reliable indicator suite for decomposition, if other environmental factors are also considered. This is consistent with the findings of Carvalho, Thomaz and Bini (2005), who found that dissolved oxygen concentration decreased due to an increase in microbial activity.

Although these variables were strongly related to decomposition rates, this research was primarily aimed at identifying indicators that could be adaptable across a range of systems, without reliance on consistent climate conditions. I initially hypothesised that variables such as pH and nutrient concentrations had the potential to be more reliable indicators for monitoring wetlands in one-off, rapid assessments that would not be influenced by local climate conditions. These variables did have some correlation with the intensive measures of decomposition. pH was the variable with the strongest correlation with microbial community function. The change in pH over the sampling events was minimal and only small differences were found among most wetlands, which may explain non-significant nature of that correlation. However, the correlation can explain some observed patterns. For example, the relationship was strongest in wetlands with the most neutral pH levels, such as Mepunga and Cobrico Swamp, which had the highest intensity of carbon source utilisation, and indicates that the aerobic microbes were functioning most efficiently in wetlands at moderate pH levels. This is consistent with other research, which found the highest decomposition rates occurring in circumneautral systems (Dangles *et al.*, 2004). The wetlands assessed in this study had relatively neutral to basic pH levels. Further investigation into wetlands with acidic pH would be valuable, and possibly confirm pH as a useful indicator, as acidification has been widely reported to decrease decomposition due to the inhibition of microbial

functioning (Dangles & Chauvet, 2003; Niyogi, Lewis & McKnight, 2001). As a result, measuring pH over a wider range of values may prove to be a useful indicator of decomposition, as well as identifying aquatic systems that may be deteriorating in other ways. As such, pH should be widely monitored (Driscoll *et al.*, 2004).

It has been highlighted that nutrient loading has increased across the globe in aquatic systems and the impact of increased nutrient concentrations has become a crucial theme in aquatic ecology (Vitousek *et al.*, 1997; Woodward *et al.*, 2012). Due to increasing concentrations of nitrogen, particularly in agricultural and urban wetlands (Paul, Meyer & Couch, 2006), I hypothesised that this variable might be an effective and important indicator. TN was found to be strongly correlated with microbial community function. Generally speaking, it was found that wetlands with high intensity utilisation of the carbon sources had higher nutrient concentrations. This could mean that the microbes present had ideal levels of TN required to function, and/or that these microbes were capable of utilising the most complex carbons. Microbes are reliant on nutrients for consumption and energy requirements (Gulis & Suberkropp, 2003), and increased TN levels have been found to accelerate metabolism (Paul, Meyer & Couch, 2006). However, excessive nutrient enrichment can also be detrimental to microbial functioning capabilities (Hagen, Webster & Benfield, 2006). Measures of decomposition other than directly assessing microbial functioning were not strongly related to TN in this study. In particular, wetlands like Mepunga and Cobrico Swamp had relatively low decomposition rates but high nutrient concentrations, which may indicate that the bioavailability of nitrogen was lower in those wetlands in comparison to Lake Pertobe, which had high TN concentrations and high decomposition. Microbial activity has been found to be affected by bioavailability (Nielsen & Winding, 2002). Looking further into the different forms of nitrogen present in these wetlands was investigated in the last part of this research (VISOCOLOR ® ECO nutrients). However, time constraints meant limited replication, and

the accuracy of the test should be investigated, given differences between the nutrient concentrations recorded compared with those measured in the laboratory. Thus, additional investigation into the chemical speciation would give further insight into what the bioavailable forms of nitrogen in the wetland, and potentially strengthen the use of nitrogen concentrations as an indicator.

4.2 Developing indicators across a range of land-use types

Agricultural and urban land-use types were investigated within this research to identify potential rapid indicators for each of the two land-use types. Each type of land use has altered the surrounding environment of our once-natural wetlands in characteristic but different ways (Clapcott *et al.*, 2012) and so it seemed reasonable to expect decomposition rates and mechanisms to vary between the two. Previous studies have found differences in decomposition rates across the two (Gulis & Suberkropp, 2003). If this were the case for these wetlands, there may have been different indicators that would be more appropriate in one land-use type; however this study revealed no significant differences between decomposition rates and water quality variables between land-use types. Urban aquatic systems are generally affected by storm water run-off which leads poor water quality, nutrient influxes and the absence of native riparian vegetation (Paul, Meyer & Couch, 2006). Agricultural land use has also been found to affect the ecological health of a water body due to high sedimentation, soil erosion, bank instability, and runoff from the waste products of surrounding cattle and fertilisers (Hagen, Webster & Benfield, 2006). These differing inputs generally result in a change in the nutrient structure of the wetlands (Paul, Meyer & Couch, 2006) and would have been expected to alter decomposition rates. In addition, these inputs have been found to have flow-on effects within aquatic ecosystems, altering algal community composition and the productivity of in-stream fauna (Chessman, Hutton & Burch, 1992). The

fact that the nutrient concentrations were well above the EPA guidelines in some wetlands is most likely associated with the input of storm water and agricultural runoff into those systems, leading to similar levels of alteration in both land-use types. However, while there were no significant differences found between land uses, there were significant differences found between wetlands, and sites within wetlands, so further investigation into a larger number of wetlands with differing land-use would be ideal for further conclusions to be drawn about appropriate indicators in both land-use types.

The aim of this research was to identify interpretable and reliable indicators to be used across a range of systems within similar land-use intensities. However, in more degraded systems such as metropolitan areas with wetlands in close proximity to major cities (Imberger, Thompson & Grace, 2010) or wetlands with nearby cropping land use and high pesticide use (Clapcott *et al.*, 2010) for example, these indicators would need to be re-evaluated before we could be confident that they could be applied within such systems as a tool to assess their ecological functioning.

4.3 Effectiveness of time-intensive measures as functional indicators

Time-intensive measures to assess decomposition rates have existed for many years (Latter & Howson, 1977). Cotton strips have proven to be an effective, standardised measure of assessing decomposition (Tiegs *et al.*, 2013; Clapcott *et al.*, 2010; Lategan, Korbel & Hose, 2010). They were also appropriate for this research as they have been found to be influenced by environmental factors that affect microbial activity (Boulton & Quinn, 2000; Tiegs *et al.*, 2013). The cotton showed visual differences in decomposition through time and between wetlands. However, the resources required and time taken to prepare and undertake the assay were extensive. Mechanical malfunctioning that was not able to be fixed within the time

constraints of this project also limited the amount of reliable data that I could include in the analyses that are presented here. However, further analyses will be conducted prior to publication of this research. This may have influenced the results of this study as the replication within data set was lower than expected and I found no significant difference among wetlands or sampling events.

The wood break-down assay is a more recent development (Diez *et al.*, 2002) but is also a standardised method using a substrate commonly found in aquatic systems. It was easy to handle and a useful measure of ecosystem functioning as has been found in previous literature (Arroita *et al.*, 2012; Aristi *et al.*, 2012). Significant differences found among wetlands for the wood break-down assay in this study reinforced its sensitivity to environmental conditions. This assay was not as time-intensive as the cotton; however, it still required a large amount of preparation. The main benefit of using wood over cotton was that it relied on the use of a standard oven to measure weight changes and thus decomposition rates, a resource that is widely available, rather than a specialised tensile tester. My analysis of the wood assay did identify significant differences among wetlands and sampling events, allowing decomposition rates to be compared against the water quality variables. This change through time illustrated that these measures were a good indication of decomposition rates over the 35 days that this study occurred, and provided a good comparison for the rapid variables.

The time and resources required to conduct these assays may also have implications for managers, as the manual labour costs involved are extensive, and thus constant monitoring of decomposition is not possible with these methods. So, while these indicators do enable us to identify and compare decomposition occurring through time and identify differences among

wetlands, they are too time intensive, and are not practical for consistent monitoring (Fairweather, 1999a).

One final impediment to assessing decomposition in wetlands is the lack of a baseline against which to compare. In order to try and establish a desired level of decomposition occurring within any aquatic system, it would be desirable to conduct this research using a series of benchmarking reference wetlands, with natural surrounding land-use, so that these rapid indicators could be compared against ideal conditions and desirable ranges of decomposition rates could be identified. The break-down rates for wood in this research were considerably higher compared to that of previous research, which found rates of 0.00034 to 0.01647% day^{-1}, and 0.0011 to 0.0120% day^{-1} for Aristi *et al.* (2012) and Arroita *et al.* (2012), respectively. However, it should be noted that these comparable studies were conducted for a much longer time period and in streams as opposed to wetlands, which could account for the differences. The cotton decomposition rates identified by Tiegs *et al.* (2013), of 0.07 to 3.2% day^{-1} were quite similar to those found in this research, which may be due to the surrounding agricultural land use in both studies.

4.4 Technological advances in the use of microbial community function

Microbial community function was found to be an effective way to assess the aerobic microbial assemblage within wetland soil and water. This is able to be measured using commercially-available Biolog plates (Garland & Mills, 1991). The main benefits of this technique are that it is widely available and relatively simple to undertake, which makes it moderately practical and extremely replicable (Buyer & Drinkwater, 1997). This research found that there was no difference in the number of substrates that could be utilised by microbes among the different wetlands, with most carbon substrates able to be utilised in all

wetlands. However, there was more variation between the intensity at which the utilisation occurred, illustrating variation in microbial assemblage activity.

In some wetlands, I measured high decomposition rates but only low intensity of carbon source utilisation. This suggests that there may be a substantial assemblage of active anaerobic microbes within these systems. Such an assemblage could cause rapid breakdown of organic matter, as measured by the wood and cotton assays but would not be detected by the Biolog^{TM} analysis as it is an entirely aerobic test (Roling *et al.*, 2000).

For microbial community function, there were no significant differences found between land-use types or sampling events as a stand-alone factor. This indicates that if such a measure of carbon source utilisation were to be used, it should only need to be conducted at one time period, rather than replicated through time, which would add to the ease of use of the method for determining ecosystem functioning. Although this measure was primarily conducted as part of the time-intensive measures, it was quite rapid in comparison to the other intensive measures and could be used as a rapid indicator, if it were possible to facilitate the 5-day waiting period for the incubation to occur.

In stating this, there have been other advancements in technology that enable the use of once-complex analyses to be undertaken in a more cost-effective manner (Teske & Biddle, 2008). These include microbial analyses, as well as other methods such as gene technology (Teske & Biddle, 2008) and stable isotope analysis (Clapcott *et al.*, 2010) which could be used to monitor ecosystem functioning. The advancement of procedures such as stable isotope analysis reflect both the source and transformation of nitrogen (Sebilo *et al.*, 2003) and have been suggested as a surrogate measure of nutrient processing in stream catchments. Recent advances in high-throughput genetic sequencing (Hudson, 2008) have been suggested to result in a rapid, reliable approach to assess the ecological health of an environment (Chariton

et al., 2010). Therefore this is a field that requires more research and a comparison of expense versus the value of the data collected for assessments of ecological function.

4.5 Ecosystem health and the use of these indicators in its preservation

Ecosystem health as a whole has been identified as including the whole of the environment, comprising both abiotic and biotic components of the landscape (Fairweather, 1999a). Consequently, impacts on ecosystem health come from a collection of sources and the problems associated with ecosystems are broad and can be influenced by biophysical sciences, environmental management, health sciences and our socioeconomic ambitions (Fairweather, 1999b). This multifaceted concept of ecosystem health means that there are many factors that can contribute to a systems health or deterioration. The complex nature of an ecosystem is why this project focused on the functionality of a wetlands, as processes such as decomposition provide information about the variation at different spatial and temporal scales and specifically how the ecosystem responds to environmental changes (Young, Matthaei & Townsend, 2008). Previous structural aspects of an ecosystem, including the configuration of biological assemblages, are not indicative of entire ecosystem health and do not provide information about the services occurring within a system that are likely to be beneficial to humans (Arroita *et al.*, 2012). The idea of assessing any impact on the functionality of a system stems back to the use of a method to assess wetlands developed in Europe by Maltby (2009), the Functional Assessment Protocol. This procedure relies heavily on the identification of hydrogeomorphic units, which are areas of homogeneous geomorphology, hydrology and/or hydreogeology (Maltby, 2009). This is one form of rapid assessment of a wetland, which incorporates a number of different components from within the environment to give an overall assessment of ecosystem health. The multiple rapid indicators that I have identified could be incorporated as part of the functional assessment

that is currently being developed for management authorities to implement in southern Australian wetlands.

4.6 Limitations and future directions

Along with the limitations outlined above, this research would be interesting to conduct over a variety of wetlands across different seasons and regions, to determine the consistency of the indicators such as TN and pH. This project was conducted in a summer period, where air temperatures where quite high, which may have influenced results and long-term studies may have differing results for the correlation of water quality and decomposition, give temporal variation (Yang, Chen & Yang, 2012). The next step in this research is to undertake analyses using the sediment samples that were collected, which may identify additional rapid indicators (e.g. sediment grain size). Higher proportions of fine depositional sediment have been found to increase the rates at which decomposition occurs due an expansion in the surface area available for microbial biofilms to inhabit (Clapcott, 2007; Claret *et al.* 2001; Boulton and Quinn 2000). *S*oil organic matter concentrations will also be investigated, as they play a critical role in carbon and nutrient cycling (Herrick & Wander, 1997). Further investigation into sediment pH may also help identify possible rapid indicators, as soil pH has been found to fluctuate with the input of mineralisation of plant materials (Yan & Schubert, 2000; Tang & Yu, 1999). Therefore, these sediment characteristics of a wetland could act as indicators of decomposition and wetland functioning in conjunction with, or instead of, the water quality characteristics identified here.

It has also been mentioned by Boulton (1999) that there is no one indicator alone that can explain the aquatic ecological integrity, and that a combined use of several structural and functional variables provide a greater understanding of ecological health (Imberger,

Thompson & Grace, 2010). It would be interesting to compare the indicators identified here with sampling of macro-invertebrates that has also been conducted at the agricultural wetlands as a part of a related project, to determine whether these structural assemblages align with the functional indicators.

5. Conclusion

This research explored the use of rapid indicators to allow the quick and efficient monitoring of ecosystem functioning in wetlands. This study has verified that there are a number of potential rapid indicators that correlate with decomposition, with water level and dissolved oxygen being the most promising in local conditions, whereas pH and nutrient concentrations are likely to be most beneficial across a wider spatial scale. These functional indicators could therefore be used by management authorities to reliably predict the decomposition rates occurring within a system and effectively enable the monitoring of ecosystem health. These indicators could, potentially, with further research, help protect and manage vital water resources.

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7. Appendices

Appendix A. Set-up of study sites at *(a)* Lake Pertobe (Site 2), *(b)* Glads Crossing (Site 2)

(a)

(b)

Appendix B. Wood (tongue depressors) *(a)* before being deployed in the wetland and *(b)* after being retrieved from the wetland

(a)

(b)

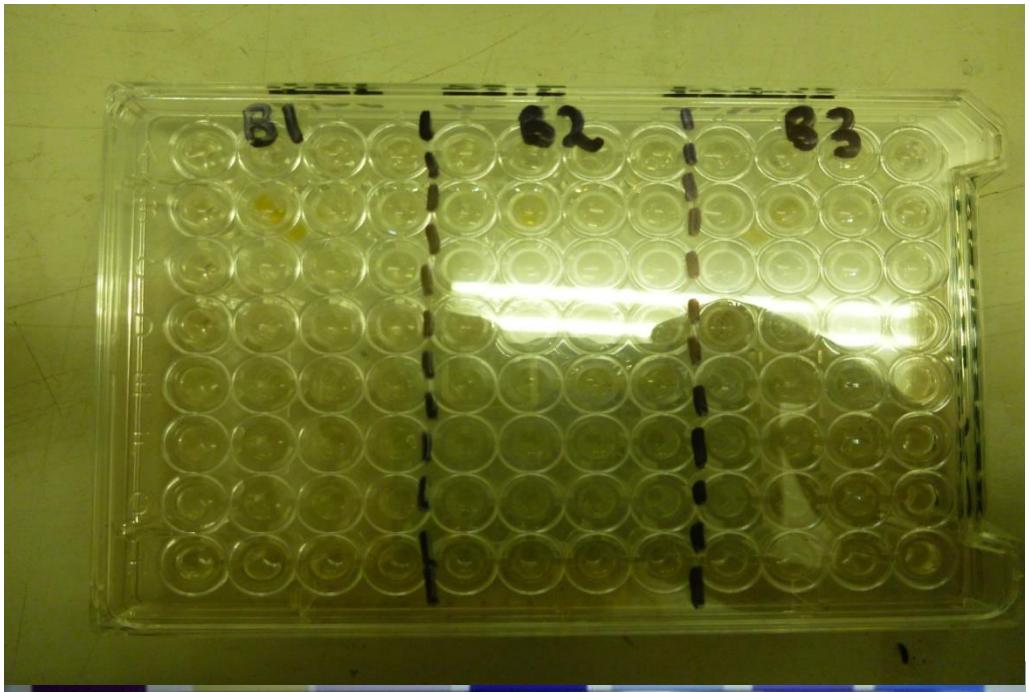
Appendix C. Cotton attached to the rulers *(a)* before being deployed in the wetland and *(b)* after being retrieved form the wetland (after 21 days).

(a)

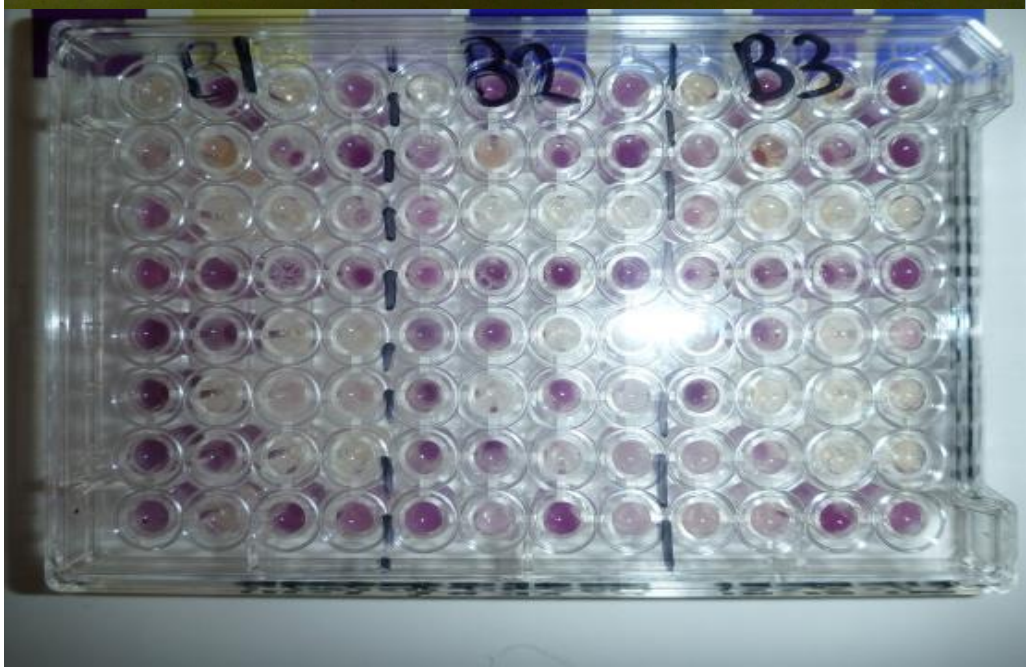
(b)

Appendix D. Biolog^{TM} ECO plate *(a)* before having sediment plated *(b)* after 5 days of incubation with sediment plated

(a)



(b)



Appendix E. Water quality variables measured at each site over the course of the sampling period as mean values with standard error displayed (physico-chemical, across 42 days, and TP and TN across 21 days).

Water Quality								
Wetland	**Site**	**Dissolved oxygen%**	**pH**	**Conductivity µS cm^{-1}**	**Temperature°C**	**Turbidity NTU**	**Total Nitrogen mg L^{-1}**	**Total Phosphorus mg L^{-1}**
Mepunga	1	20.77 ± 8.31	6.97 ± 0.07	460.53 ± 13.64	19.45 ± 0.51	3.27 ± 1.19	1.58 ± 0.45	0.22 ± 0.03
	2	11.49 ± 3.09	6.45 ± 0.08	323.07 ± 15.23	18.26 ± 0.48	27.32 ± 7.28	2.00 ± 0.76	0.20 ± 0.08
Lake Pertobe	1	119.10 ± 3.74	8.69 ± 0.11	1489.50 ± 185.83	28.16 ± 0.78	64.80 ± 7.48	2.20 ± 0.40	0.37 ± 0.15
	2	121.51 ± 5.97	8.71 ± 0.17	1508.08 ± 195.74	29.01 ± 1.00	87.38 ± 17.39	2.85 ± 0.15	0.28 ± 0.04
Glads Crossing	1	58.03 ± 10.68	8.31 ± 0.12	663.21 ± 64.77	17.51 ± 0.57	360.66 ± 41.56	1.95 ± 0.77	0.63 ± 0.09
	2	76.59 ± 14.17	8.56 ± 0.14	759.93 ± 27.47	21.08 ± 0.83	343.36 ± 48.04	2.21 ± 0.92	0.56 ± 0.09
Tea Tree Lake	1	90.53 ± 2.25	8.36 ±0.05	1290.55 ± 115.65	24.15 ± 0.76	33.98 ± 4.46	1.15 ± 0.05	0.07 ± 0.02
	2	86.23 ± 3.05	8.53 ± 0.07	1379.50 ± 50.44	24.51 ± 0.87	37.68 ± 4.43	1.08 ± 0.12	0.07 ± 0.01
Cobrico Swamp	1	74.50 ± 2.52	8.51 ± 0.17	3345.83 ± 174.87	20.78 ± 1.00	15.18 ± 6.38	2.35 ± 0.15	0.82 ± 0.00
	2	86.46 ± 8.07	8.36 ± 0.24	3336.75 ± 168.57	21.64 ± 0.92	21.49 ± 9.22	2.40 ± 0.10	0.84 ± 0.00
Lake Cobden	1	127.96 ± 13.30	8.69 ± 0.17	522.00 ± 26.61	23.89 ± 0.82	23.38 ± 7.74	1.33 ± 0.47	0.18 ± 0.09
	2	197.16 ± 6.30	9.46 ± 0.23	512.08 ± 26.98	24.60 ± 0.77	19.29 ± 6.04	1.25 ± 0.25	0.12 ± 0.06

Appendix F. Sediment cores taken from each wetland *(a)* Mepunga, *(b)* Lake Pertobe, *(c)* Glads Crossing, *(d)* Tea Tree Lake, *(e)* Cobrico Swamp, *(f)* Lake Cobden.

(a)

(b)

(c)

(d)

(e)

(f)

Appendix G. Wood decomposition over the 7, 21 and 35 day sampling events, for each site sampled. Mean and standard errors values are displayed.

		Wood decomposition		
Wetland	**Site Number**	**7 days**	**21 days**	**35 days**
Mepunga	1	1.39 ±0.21	3.11 ±0.36	3.93 ±0.66
	2	1.80 ±0.36	1.67 ±0.40	4.06 ±0.77
Lake Pertobe	1	1.62 ±0.19	3.61 ±0.60	4.85 ±0.58
	2	1.65 ±0.40	3.96 ±0.59	4.43 ±0.80
Glads Crossing	1	2.42 ±0.50	4.73 ±0.69	4.97 ±0.26
	2	2.84 ±0.66	4.47 ±0.44	4.76 ±2.13
Tea Tree Lake	1	2.15 ±0.21	2.47 ±0.84	2.63 ± 0.31
	2	2.04 ±0.17	2.41 ±0.06	2.30 ±0.08
Cobrico Swamp	1	2.65 ±0.52	3.04 ±0.41	4.14 ±0.41
	2	2.52 ±0.12	2.52 ±0.20	4.06 ±0.28
Lake Cobden	1	2.22 ±0.25	3.45 ±0.58	5.12 ±0.79
	2	2.60 ±0.43	2.58 ±0.34	5.07 ±0.74

Appendix H. Cotton decomposition over the 7 and 21 days sampling events, for each site sampled. Standard error is displayed only where there were multiple replicates available for analysis. n/a indicates that the cotton slipped at the jaws and there were no replicate samples for this site.

		Cotton decomposition	
Wetland	**Site Number**	**7 days**	**21 days**
Mepunga	1	23.15 ± 5.56	86.79 ± 4.21
	2	21.76 ± 11.23	n/a
Lake Pertobe	1	28.29 ± 7.73	78.69 ± 7.36
	2	46.76 ± 7.52	85.78 ± 6.06
Glads Crossing	1	24.33	94.02 ± 3.01
	2	34.54 ± 12.42	n/a
Tea Tree Lake	1	n/a	n/a
	2	n/a	n/a
Cobrico Swamp	1	4.15	14.46 ±1.34
	2	n/a	n/a
Lake Cobden	1	23.67	n/a
	2	21.15	n/a

Appendix I. Microbial intensity of carbon source use over 27 and 35 days for each site sampled. Mean values and standard errors are displayed.

		Intensity	
Wetland	**Site Number**	**21 days**	**35 days**
Mepunga	1	1.35 ± 0.14	1.81 ±0.15
	2	1.21 ± 0.15	1.65 ±0.16
Lake Pertobe	1	0.84 ± 0.14	1.29 ±0.17
	2	0.88 ± 0.15	1.25 ±0.19
Glads Crossing	1	1.10 ± 0.14	1.31 ±0.16
	2	1.23 ± 0.14	1.44 ±0.19
Tea Tree Lake	1	1.27 ± 0.17	1.47 ±0.17
	2	1.21 ± 0.14	1.52 ±0.17
Cobrico Swamp	1	1.39 ± 0.11	1.79 ±0.17
	2	1.58 ± 0.13	1.64 ±0.14
Lake Cobden	1	0.87 ± 0.10	1.11 ±0.11
	2	1.06 ± 0.15	1.21 ±0.14

Appendix J. Microbial abundance of carbon source use over 27 and 35 days for each site sampled.

		Abundance	
Wetland	**Site Number**	**21 days**	**35 days**
Mepunga	1	30	29
	2	27	30
Lake Pertobe	1	25	30
	2	23	31
Glads Crossing	1	27	29
	2	29	28
Tea Tree Lake	1	32	29
	2	32	30
Cobrico Swamp	1	28	30
	2	23	31
Lake Cobden	1	28	29
	2	26	29