Functional indicators of decomposition for monitoring ecosystem health in urban and agricultural wetlands

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Executive Summary

1. The assessment of the health of ecosystems has often previously neglected the overall functioning of a system by focusing on its structural components. Assessing ecological function, such as decomposition, provides a more accurate indication of the health of the entire water body than species composition alone. However, there are few rapid monitoring tools assessing ecosystem function, despite their utility for natural resource managers.

2. This research aimed to identify plausible rapid methods to quickly and efficiently monitor decomposition in urban and agricultural wetlands and to test their consistency in two regions. The research correlated water quality and sediment variables with three widely-established but resource-intensive measures of assessing decomposition over a 35-day period to identify potential rapid methods to monitor the ecological function of a wetland.

3. Across six wetlands, we found positive correlations between the various rates of decomposition and water pH, electrical conductivity, total nitrogen concentrations and the percentage of sediment that was less than 63 μm in size, but negative correlations with sediment pH. Microbial diversity was a general exception, tending to show opposite correlations to the wood, leaf litter and macroinvertebrate measures. No differences in decomposition were identified between urban and agricultural wetlands.

4. The indicators were broadly consistent with those identified in a confirmatory study in the Lower Lakes, South Australia. The consistency in identified indicators suggests that these are likely to be useful rapid indicators of decomposition in wetlands. Thus, these rapid indicators will allow managers to quickly assess ecological health of urban and agricultural wetlands and can be incorporated into a holistic functional assessment of wetland ecosystems.

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 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 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). It is becoming increasingly apparent, however, that structural indicators are not broad enough to reflect the complexity of an ecosystem (Xu, Jorgensen & Tao, 1999). For example, Mackie and Malmqvist (2009) state that

structural components such as the presence or absence of certain indicator species, may not affect ecosystem process rates, whilst disturbances may alter process rates but not organism assemblages. Likewise, Bunn and Davies (2000) found that primary production and community respiration were a better measure than macroinvertebrate assemblages for identifying increased turbidity and nitrogen enrichment. Thus it is becoming apparent that structural indicators may not be as reliable as once thought and a holistic approach is needed.

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 heterogeneous but unique ecosystems, whose biogeochemical and hydrological functions arise from a reliance on water (Maltby, 2009). 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 involves the complex break-down 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 break-down 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, common established measures to assess decomposition rates are costly and time-intensive, and often require specialised equipment and expertise. Such methods include a wood break-down assay (Arroita *et al.*, 2012), which uses mass loss as a surrogate measure of decomposition rate. Assays can assess break-down of entire logs (Ellis, Molles & Crawford, 1999), branches (Tank & Webster, 1998) or commercially-manufactured sticks such as tongue depressors (Aristi *et al.*, 2012). Break-down of leaf litter mass, measured using litterbags, and quantifying macroinvertebrate colonisation rates are another common method for assessing decomposition rates (Benfield, 2006). Another final 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 microbial utilization of a range of carbon substrates (McKenzie *et al.*, 2011).

Rapid indicators need to be variables that change reliably and predictably with the process of interest (Fairweather, 1999a). Previous studies have found that decomposition can be influenced by a variety of factors, including physico-chemical characteristics (e.g. Clapcott *et al.*, 2010) and nutrient levels (e.g. Tiegs *et al.*, 2013). For example, increased temperature and nutrient concentrations can accelerate decomposition, while lowered pH or increased salinity can inhibit decomposition rates (Lopes *et al.*, 2011; Young *et al.*, 2008).

Land-use type has also been shown to influence decomposition (Clapcott *et al.*, 2010; Imberger, Thompson & Grace, 2010). Agricultural land use often results in a decline in riparian vegetation, altering shading, insolation, water temperatures and dissolved oxygen concentrations (Hagen, Webster & Benfield, 2006). Nutrient levels generally increase due to fertiliser runoff and livestock in the surrounding catchment (Doledec *et al.*, 2006). Increased sedimentation, soil erosion and bank instability are also associated with surrounding agricultural land-use (Allan, 2004). Urbanisation also has 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). All of these factors may affect aquatic decomposition rates or change relationships with potential rapid indicators.

The objectives of this research were to identify indicators that could be used to rapidly assess the decomposition and thus the functioning of wetlands and to test the consistency of those indicators in two regions. To do this, we examined whether any of the possible rapid measures correlated with intensive measures of assessing decomposition rates, thus providing a reliable assessment of decomposition for southwestern Victoria, Australia. We also assessed whether land-use type influenced those correlations to determine whether different indicators would be needed in different

catchment types. Once basic indicators were identified, we repeated the experiment in the Lower Lakes, South Australia to assess the consistency of the indicators. We hypothesised that decomposition rates and the potential suitable rapid indicators would vary among land-use types and so that rapid indicators suitable for urban ecosystems may differ from those in agricultural ecosystems, but that indicators would be consistent across the regions sampled. Therefore, we aimed to provide natural resource managers and others with a rapid and reliable indicator of ecosystem functioning to assess decomposition in urban and agricultural wetlands.

2. Methods

2.1 Initial study area

This research was conducted over an austral summer, sampling from early January to late February 2014. Six perennial wetlands in the Glenelg-Hopkins catchment of southwestern Victoria (Fig. 1) were selected to have similar capacity, size, percentage of riparian vegetation, shading, macrophyte types, amount of exposed bare sediment and sediment grain size. Three wetlands, Mepunga, Glads Crossing and Cobrico Swamp (Table 1), had agricultural (generally cattle grazing) surrounding land use, with the remaining three, Lake Pertobe, Tea Tree Lake, and Lake Cobden, were in periurban landscapes (Table 1).

Aquatic and riparian vegetation was found at all wetlands. The tuberous root species *Triglochin procerum* was the most common vegetation type, except in Lake Pertobe. *Typha* spp., an erect 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*. Mepunga was the only site that had willows (*Salix* spp.) and the native floating fern *Azolla* spp. as part of the riparian and aquatic vegetation.

Two sites were selected within each wetland with a minimum of 30 m separating them, resulting in a total of twelve sites across the six wetlands. At each wetland, the sites were selected to quantify any small-scale differences within the wetland. 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. At each sampling event, two wetlands were sampled per day, over a three-day period in the order presented in Table 1.

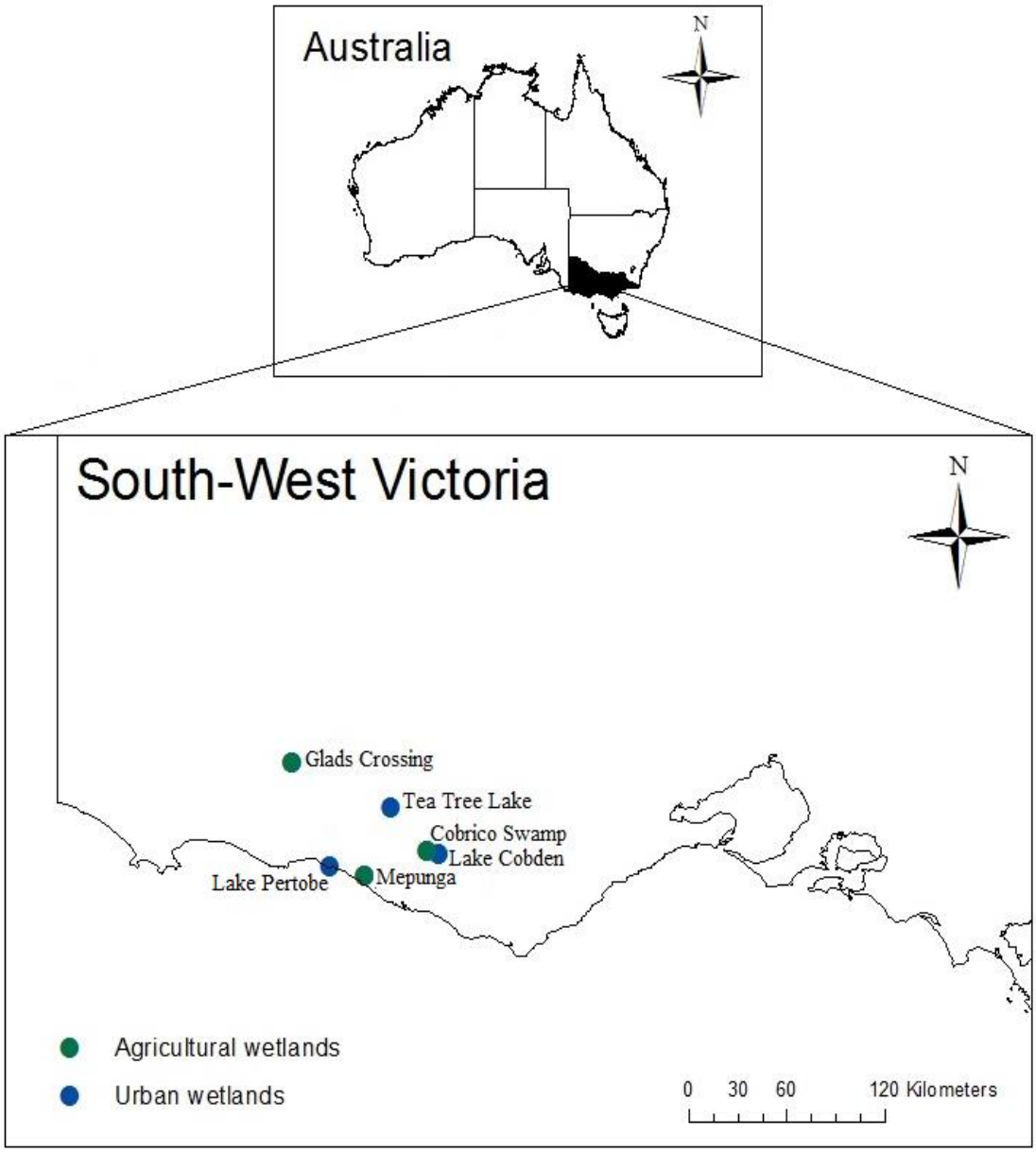


Fig. 1 Location and land-use of the six wetlands in southwestern Victoria. Mepunga, Glads Crossing and Cobrico Swamp have agricultural land-use as indicated by the green dots and Lake Pertobe, Tea Tree Lake and Lake Cobden have surrounding urban land-use as indicated by the blue dots. Litterbags were deployed at the three agricultural wetlands only.

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

Wetland	**Mepunga**	**Lake Pertobe**	**Glads Crossing**	**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
Litterbags sampled	Yes	No	Yes	No	Yes	No
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 vegetation	*Salix* spp.	*Phragmites australis*	*Triglochin procerum*	*Triglochin procerum*	*Typha* spp.	*Triglochin procerum*
Sediment exposed (%)	30	100	100	50	50	100

2.2 Established resource-intensive indicators

2.2.1 Wood break-down assay

Flat ashwood tongue depressors (150 x 18 x 1 mm in size, Beiersdorf, North Ryde, NSW, Australia, hereafter referred to as wood) were used in a standard wood break-down assay over 35 days, followed the methods of Aristi *et al.* (2012). Wood replicates were individually labelled, hole punched and dried at 70 °C for 72 hours, cooled in a desiccator and weighed (±0.0001 g; Aristi *et al.*, 2012). 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.

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

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. A second procedural control was exposed to the sediment for 20 minutes to control for abrasion during handling.

At Days 7 and 21, four wood experimental replicates were chosen at random to be gently removed from the sediment (Table 2), using the remaining replicates as spares to guard against potential future loss of samples. All remaining replicates were collected at Day 35. 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 per day.

Table 2 The timing of measurement (✓) of the potential rapid indicators (physico-chemical characteristics for the initial survey, nutrients and sediment characteristics), as well as when established resource-intensive indicators (wood break-down assays, microbial community function and data loggers) were deployed (×) and then collected (✓).

	Day				
Measure	**0**	**7**	**21**	**35**	**42**
Physico-chemical	✓	✓	✓	✓	✓
Nutrients		✓	✓	✓	
Sediment				✓	
Water level	✓	✓	✓	✓	✓
Wood	×	✓	✓	✓	
Microbial			✓		
Litterbags		×	✓	✓	
Data loggers	×				✓

2.2.2 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 (Biolog Inc., Hayward, California, USA). The plates had three replicates each, consisting of 31 carbon substrates and one control (i.e. a non-carbon substrate). The microbial sampling, extraction and plating followed the methods of McKenzie *et al.* (2011). At Day 21, 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® (Nasco, Fort Atkinson, Wisconsin, USA) in the dark on ice until extraction. The sub-sample corer was rinsed in water and washed in 100 % ethanol between replicates. After collecting the five experimental samples, a field procedural control

was conducted at each site by dipping the rinsed and ethanol washed sub-sample corer into the Whirlpak®, without taking a sediment core. This control was then treated 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 60 s, 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 square petri dish. Using an 8-channel micropipette, 100 µL was transferred into each of the 32 wells of the Biolog™ ECO plates for one replicate. Before plating, any electrical charge on the pipette tips was discharged by syringing and releasing the sample five times in the petri dish. All the microbial samples were plated in laboratory on the same day of collection.

After plating, the Biolog™ ECO plates were then incubated in the dark at 15 °C in a constant temperature cabinet for five days. Over the five days, microbes that can utilise a specific carbon source respire and precipitate a purple tetrazolium 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 hand scored by eye from 0 (no colour), 1 (lightest purple) to 4 (darkest purple) and was used a surrogate measure of microbial functional diversity (McKenzie et al., 2011).

2.2.3 Litterbag break-down assay

Macroinvertebrate and microbial decomposition rates within each wetland were also assessed using a litterbag method (Benfield, 2006). Green leaves of the emergent macrophyte *Phragmites australis* were collected during December 2013 from Lake Pertobe (Fig. 1). Leaves were transported to the laboratory, rinsed to remove any sediment or macroinvertebrates present and were then cut to a standard length of 10 cm to allow for easy handling and weighing. Leaves were placed into trays and oven dried at 60 ˚C for 72 h (Longhi, Bartoli & Viaroli, 2008).

Litterbags were deployed at each of the two study sites selected for the three agricultural wetlands. At each site, eight litterbags (35 x 50 mm, with a stretched mesh size of 4 mm) containing 10 ± 1 g of dried *P. australis* and eight control bags containing no leaf litter were concurrently deployed randomly along a 14-m transect, in two rows of eight and spaced a minimum of 50 cm apart. The 14-m transect encompassed the 7.5-m transect described above, extending evenly on either side. Each litterbag was folded in half, with the leaves in the half in contact with the sediment, and secured with two pegs on opposite corners of the bag. To enable re-location of bags, each bag was labelled and tied to a bamboo stake above the water with an additional label on the stake. At each site, an additional two litterbags containing 10 ± 1g of *P. australis* leaves were deployed for 20 minutes as procedural controls, to account for the possible handling losses of leaf mass during deployment.

Four litterbags and four controls at each site were collected after a 14-day deployment and the remaining bags were collected after 28 days (Table 2) using a dip net (250 μm mesh) to prevent the loss of macroinvertebrates and litter during the retrieval process. The contents of the dip net, as well as the bags themselves, were placed into zip lock bags and preserved in 70 % ethanol. Any natural accumulation of sediment, litter and invertebrates were thus also collected. The zip lock bags were then put on ice and transported back to the laboratory.

In the laboratory, *P. australis* leaves were removed and rinsed into a 250-μm sieve to remove any sediment or macroinvertebrates attached to the leaves. Litterbags were also rinsed into the sieve to remove fine organic material and macroinvertebrates. The washed *P. australis* leaves were placed into trays and dried at 60 ˚C for 72 h. Leaves were then placed into a desiccator to cool before obtaining dry weight (±0.0001 g). Remaining organic matter and sediment from each sample were oven-dried at 60 ˚C for 72 h. Samples were then placed in a desiccator to cool, before recording dry weight. Ash-free dry weight (AFDW) was obtained for each by heating each sample in a muffle furnace for 3 h at 550 ˚C, cooling the samples in a dessicator and then weighing. A 5-g sucrose control was also heated in the muffle furnace, to check the efficiency of the ashing process.

Three of the four replicate treatment and control litterbags were sorted to quantify macroinvertebrate assemblages. Where large numbers of macroinvertebrates were collected from litterbags, samples were split using a plankton splitter with a minimum of 12.5% of the original sample sorted, including at least 200 individuals per sample. Litterbags that were dry at the time of collection due to evaporation of the wetlands were not processed. All macroinvertebrates were then preserved in 70 % ethanol, identified to the lowest taxonomical level and assigned to an appropriate functional feeding group (Gooderam & Tyrslin, 2002).

2.3 Potential rapid indicators

2.3.1. Water physico-chemical variables

Electrical conductivity (EC, standardised to 25°C; μS cm^{-1}), turbidity (NTU), dissolved oxygen (DO; % saturation), pH and temperature (°C) were measured on each sampling event with a Yeokal 611 meter (Yeo-Kal Electronics, Brookvale, NSW, Australia) 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. The measurements were made at three evenly-spaced locations along each site at all sampling events (0, 7, 21, 35, and 42 days; Table 2) for all six wetlands. Issues were encountered with very low DO concentrations during early morning surveying, requiring 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 (YSI, Yellow Springs, Ohio, USA) 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. Water nutrient concentrations

Samples for 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 at the site. The 10-mL testing bottle was rinsed three times in wetland water before the sample was collected. These samples were then frozen until laboratory testing was possible. The Deakin University Water Quality Laboratory analysed the

collected water samples for total nitrogen (TN mg L^{-1}, detection limit 0.1 mg L^{-1} WQL-05), and total phosphorus (TP mg L^{-1}, detection limit 0.1 L^{-1} WQL-07) using NATA-accredited digestion, flow-injection analysis and spectrophotometric detection methods.

2.3.3 Water temperature

At each site, three HOBO® Pendant Data Loggers (Part # UA-002-64, Patent 6,826,664, Onset Computer Corporation, Bourne, Massachusetts USA) were also deployed in approximately 30 cm of water at Day 0. The loggers were evenly spaced within the site and pinned to the surface of the sediment with a metal tent peg. The data loggers recorded temperature (°C) every 30 minutes from the initial deployment period until collection at Day 42.

2.3.4 Sediment characteristics

Redox, temperature and pH readings were collected at each site. Redox and pH data were collected using a combined waterproof redox and temperature probe (HI98121, Hanna Instruments Inc) with the probe pushed into the sediment for one minute.

Sediment samples were collected from the remaining large cores after the microbial core samples had been extracted. Sediment samples were placed into a labelled, plastic zip lock bag, and were kept frozen until later analysis.

Wet weight (±0.0001 g) of sediments was recorded before each sample was dried at 105 °C for 36 hours. Dry weight was then recorded and percentage of moisture calculated. Samples were split in half to enable organic matter content and sediment size to be measured. For organic matter content, samples were treated as described above in Section 2.2.3.

In order to measure sediment size, samples were treated remove organic matter according to methods described by Bowman and Hutka (2002) prior to sieving. Approximately 200 mL of water was added and large pieces of organic matter were rinsed and removed. Hydrogen peroxide was then added in 10 mL increments and stirred with a glass rod periodically to encourage oxidation. Over a period of 24 to

86 hours, a volume of hydrogen peroxide was added (usually between 50 and 60 mL) to each sample. Samples that produced a substantial amount of froth and/or foam were also treated with a single drop of 2-octanol. When the hydrogen peroxide process was nearing completion, samples were heated to 45 °C to speed oxidation. Once oxidation was complete, samples were heated to 90 °C on a hotplate for 1 h to remove any remaining hydrogen peroxide from the sample. Once cooled, each sample was wet-sieved through a 63-µm sieve into a pre-weighed metal tray to separate the clay and silt fractions from the sand. Both were dried at 105 °C for 36 hours and weighed to obtain the relative fraction of clay and silt compared with larger fractions.

Once oxidation had concluded and the sample was deemed organic matter free, samples were heated to 90 °C on a hotplate for 1 hour to remove any remaining hydrogen peroxide from the sample. When cool, each sample was wet sieved through a 63-µm sieve into a pre-weighed metal tray to separate the silt and clay fractions from the sand. Both fractions were dried at 105 ˚C for 36 hours. However, many samples contained large amounts of organic matter which could not be broken down by the hydrogen peroxide. To rectify this, each sample was ashed in a muffle furnace for 3 hours at 550˚C to remove the remaining organic matter. Both fractions were then weighed using an electronic balance.

2.3.5 Water level

The change in the water level over the 42-day sampling period was also recorded. Bamboo stakes, which indicated the location of the wood samples and were the consistent point of monitoring for the water quality, were also used to measure the change in the water level over the sampling period. At each of the five stakes, evenly spaced along the site, the depth of water (mm) and the distance that the stake was from the water's edge (mm) were measured 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.

2.4 Statistical analyses

Multivariate statistical analyses were performed with PRIMER v. 6 (Clarke & Gorley, 2006) with the PERMANOVA+ add-on (Anderson *et al.,* 2008). PERmutational Multivariate ANalysis Of Variance (PERMANOVA) is a non-parametric, permutation-based method for assessing significance and, unlike traditional ANOVA, it makes few assumptions about the form of the data which makes it widely applicable in ecological studies, leading to greater confidence in interpretation of ecological datasets (Anderson *et al.,* 2008). Like ANOVA, continuous variables are able to be incorporated into PERMANOVA as a covariate in the analysis (abbreviated as PERMANCOVA).

2.4.1 Differences in land-use, time of decomposition and wetlands among intensive measures

The wood break-down assay was analysed using a univariate test of mass loss per day. No transformations or normalisation were required and a Euclidean distance similarity matrix was constructed. Procedural controls consistent had very low mass loss compared with treatment wood and were not considered consistent with handling error, although there was more variability in control mass loss on Day 35 retrievals. All remaining analyses were conducted on treatment wood only. A non-metric multidimensional scaling (MDS) plot was used to examine visual patterns in mass loss per day among the different wetlands, sites within wetlands, among differing sampling events and land-use types. Differences in mass loss were then tested using a four-factor PERMANCOVA (i.e. land-use [fixed factor, 2 levels], wetland [random, 6 levels] nested within land-use, site [random, 2 levels] nested within wetland and time of decomposition [covariate]).

Microbial functional diversity was also analysed using PERMANOVA in a multivariate analysis including each carbon source as a variable in the analysis. Here, a Bray-Curtis similarity measure was used, with a dummy variable (0.1) added to account for the zero-inflated structure of the data. Procedural and internal replicate controls were examined for any contamination prior to analysis of the replicates and any contaminated samples were excluded from analyses. Differences in the microbial functional diversity response were tested using a three factor PERMANOVA (i.e. land-use

[fixed factor, 2 levels], wetland [random, 6 levels] nested within land-use, site [random, 2 levels] nested within wetland.

Leaf litter mass loss was investigated for agricultural wetlands only. Differences in mass loss associated with procedural controls were tested using a three-factor PERMANOVA, including wetland (random factor, 3 levels), site nested within wetland (random, 2 levels) and treatment (fixed, 3 levels), on a Euclidean distance similarity matrix of untransformed data. Differences associated with time of decomposition were tested by excluding procedural controls and using mass loss as a rate per day as the unit of analysis. Here, a three-factor PERMANCOVA was used, including wetland (random, 3 levels), site nested within wetland (random, 2 levels) and time of decomposition as a covariate. For both, Monte Carlo simulations were used when the number of unique permutations for a given test was low (indicated by *P(MC)* in the results section).

Macroinvertebrates colonising the litterbags were analysed to identify differences among treatments (i.e. treatment *vs.* control, excluding procedural controls) using a five-factor PERMANCOVA (i.e. treatment [fixed factor, 2 levels], wetlands [random, 3 levels], sites nested within wetlands [random, 2 levels]), using both the time of decomposition and the amount of accumulated organic matter on the bag as covariates. Univariate analyses were conducted for each of total richness and total abundance (using a Euclidean distance similarity matrix on untransformed data). Multivariate analyses were conducted for assemblage composition using the analysis structure described above on a Bray-Curtis similarity matrix on log-transformed data with a dummy variable of 1 added.

2.4.2 Differences in land-use, time of decomposition and wetlands among rapid indicators

Water levels were not transformed. A Euclidean distance similarity matrix was constructed and the same four-factor PERMANCOVA that was used for wood mass loss was used to test for differences among land-use, wetlands, sites and time of decomposition.

Water quality variables (incorporating physico-chemical variables and nutrient concentrations) 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 PERMANCOVA (i.e. land-use [fixed factor, 2 levels], wetland [random, 6 levels] nested within land-use, site [random, 2 levels] nested within wetland, and sampling event [covariate]). For consistency with the other measures analysed, sampling event will be referred to as 'time of decomposition' throughout, and is equivalent to the time of decomposition of wood break-down. 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 Day 21 sampling event, so all analyses were also run excluding DO as a variable, to avoid any bias in the analyses. Results are only presented for those analyses including DO, unless results from analyses excluding DO varied substantially.

Differences in land-use type and wetlands in sediment size and organic matter content were tested using a two-factor PERMANOVA. This included land-use [fixed factor, 2 levels] and wetland [random, 6 levels] nested within land-use and was based on a Euclidean distance similarity matrix on untransformed data. Sediment temperature, pH and redox potential were measured on multiple sampling occasions, so were tested using a three-factor PERMANCOVA, including time of decomposition as a covariate to the analysis described for sediment size and organic matter content and using a normalised dataset.

2.4.2 Relationships between rapid indicators and intensive measures

A RELATE procedure was used to determine whether there was an overall correlation between the water quality, sediment characteristics and water levels with each of the resource-intensive measures individually. A second RELATE investigated correlations between resource-intensive measures and rapid water quality measures only (i.e. physico-chemical variables, water levels and nutrient concentrations) because of the higher level of replication among those variables. For microbial functional diversity only water quality measures from Day 21 (i.e. physico-chemical variables, water

levels and nutrient concentrations), were used as these coincided with the microbial sampling. A BEST procedure was then used to determine the strongest correlated water quality and sediment variables with the resource-intensive measures, and then the rapid water quality measures only. For the RELATE and BEST analyses for rapid water quality measures only, time of decomposition was included as a variable in the water quality dataset to account for differences in the time allowed for decomposition across different replicates. In all analyses, averages per site were used, given differences in the numbers of replicates of the various variables measured.

2.5 Testing of consistency of identified indicators

2.5.1 Confirmatory study area

We tested the consistency of the identified indicators of decomposition rates at six existing monitoring locations in the Lower Lakes, South Australia in September and October 2014 (Figure 2, Table 3). Contrasting land-uses were not part of the selection criteria given the lack of effect of land use on the initial study (see Section 3.1 below). All six locations are currently the focus of other monitoring programs: Boggy Creek, Hindmarsh Island; Warrengie; Nurra Nurra; Pt Sturt Lakeshore; Dog Lake, Tolderol Game Reserve; and Boggy Lake, Lake Reserve Road. Two sites were selected within each location using the same criteria as used for initial study in southwestern Victoria. Only two sampling events were undertaken (i.e. one for deployment and one for collection) and, at each sampling event, two locations were sampled per day, over a three-day period in the order presented in Table 3. Established resource-intensive indicators investigated tested excluded litterbags, given the lack of significant effect of treatment detected in the original study (see Section 3.1.3 below), but both wood break-down and microbial functional diversity were assessed for consistency of indicators in the confirmatory study area.

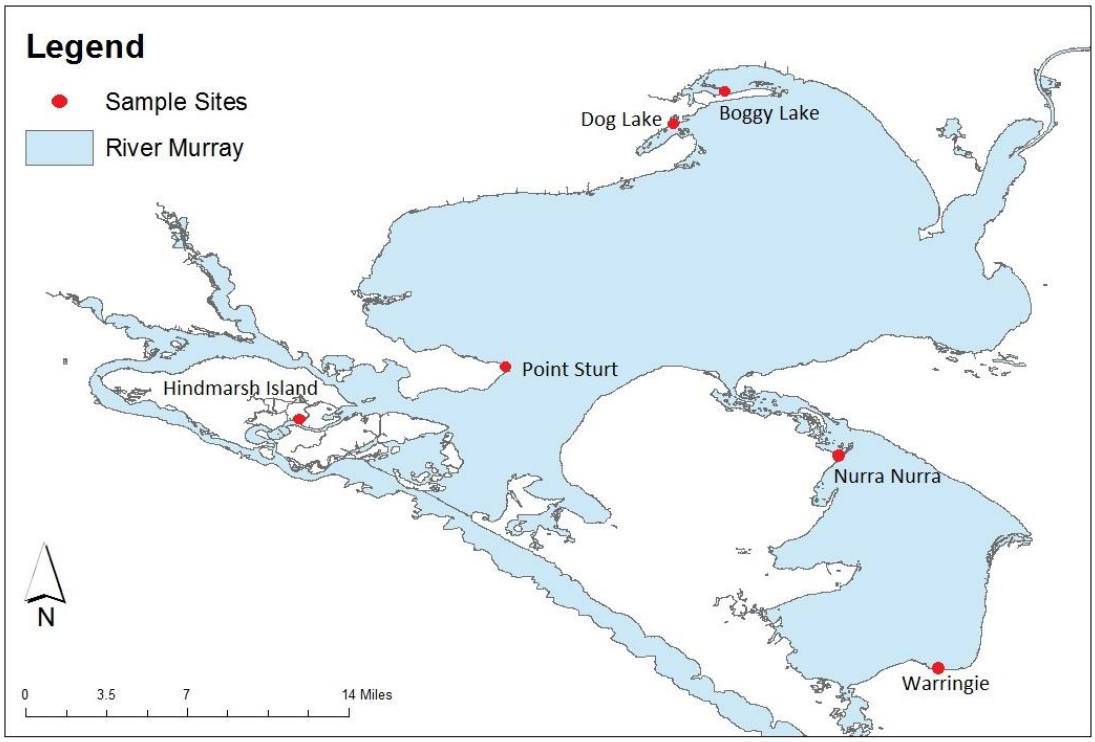


Figure 2. Six locations that were used to test the consistency of identified indicators in the Lower Lakes, South Australia

Table 2 Overview of the sampling dates, locations, and physical characteristics of the six locations used to test the consistency of the identified indicators

Location	Warrengie	Nurra Nurra	Point Sturt	Hindmarsh Island	Tolderol Game Reserve	Lake Reserve Road
Sampling event dates	24 September 29 October	24 September 29 October	25 September 30 October	25 September 30 October	26 September 31 October	26 September 31 October
General location	Warrengie	Nurra Nurra	Point Sturt	Boggy Creek	Dog Lake	Boggy Lake
Latitude	35° 41’ 37.7”S	35° 33’ 36.6”S	35° 30’ 3.73”S	35° 05’04.73”S	35° 21’ 45.97”S	35° 19’ 2.19”S
Longitude	139° 19’ 3.3”E	139° 158’ 37.9”E	139° 2’ 48.55”E	138° 55’ 12.97” E	139° 7’ 32.22”E	139° 14 0.4”E

2.5.2 Established resource-intensive indicators

2.5.2.1 Wood break-down assay

Five replicate pieces of wood were deployed in September using the same methods of preparation and placement as the initial southwestern Victorian study. The wood was retrieved 35 days later in October. At the start of deployment, at each site, procedural controls were undertaken as in the southwestern Victorian study. On the night of retrieval, the wood replicates and controls were initially oven-dried for 15 minutes in a household oven at 120 °C with the door to the oven left open to cool the temperature of the oven slightly. This was to dry the wood enough to prevent further decomposition until drying at 70 °C for 72 hours back in the laboratory at the completion of the field trip. Once oven dried in the laboratory, weight loss of the wood was determined as for the initial study.

2.5.2.2 Microbial functional diversity

The microbial functional diversity of the sediment at the different locations was measured using the same method as the initial southwestern Victorian study. Microbial sampling was conducted when the wood was retrieved (Day 35), with five replicate sediment cores and a field procedural control taken from each site. Samples were extracted on the same day as collection and incubated in the dark at 15°C in a portable constant temperature cabinet. Colour development was scored by eye after five days' incubation.

2.5.3 Potential rapid indicators

2.5.3.1 Water physico-chemical measures

Water physico-chemical measures were sampled in October when the wood was retrieved in the using the same procedure as the initial southwestern Victorian study. Unlike the previous study, a YSI-6050000 multi-meter (YSI Inc, Yellow Springs Ohio USA) was used to sample conductivity (μS cm^{-1} at 25°C), dissolved oxygen (% saturation), pH and temperature (°C), and turbidity was measured with

a Hach 2100Q Portab. Water depth and distance to edge was also measured using the same technique as the initial study.

2.5.3.2 Water nutrient concentrations

Samples for nutrient testing were collected on Day 35 using the same method as the initial southwestern Victorian study, with a single composite sample collected for each site.

2.5.3.3 Sediment characteristics

Sediment redox, temperature and pH were recorded at each site on Day 35. Redox and temperature were measured using the same methods as the initial southwestern Victorian study. Sediment pH was measured by collecting a small sediment sample and using paper pH indictor strips placed on the sediment and left for 5 minutes, because of issues with the reliability of the Hanna probes used in the initial southwestern Victorian study when used for sediment. The pH, as measured by the change in colour of the indicator paper, was scored using the provided colour chart. Sediment size and organic matter content was determined using the same sampling and analytical method as the initial study.

2.5.4 Statistical analyses

2.5.4.1 Differences in locations among intensive measures

The wood break-down assay was analysed as for the initial southwestern Victorian study, with the exception that land-use was not a factor and there was only one time, Day 35. Thus, differences in mass loss were tested using a two-factor PERMANOVA (i.e., location [random, 6 levels], site [random, 2 levels] nested within location).

Microbial functional diversity was also analysed using the same approach as the initial southwestern Victorian study. Differences in the microbial functional diversity were tested using a two factor PERMANOVA (i.e., location [random, 6 levels], site [random, 2 levels] nested within location).

2.5.4.2 Differences in locations among rapid indicators

The rapid indicator data were treated and analysed in the same way as for the initial southwestern Victorian study. After data were normalised, a Euclidean distance similarity matrix was constructed and the same two-factor PERMANOVA that was used for the intensive measures was used to test for differences among locations and sites.

2.5.4.3 Relationships between rapid indicators and intensive measures

RELATE procedures were used to determine whether there was an overall correlation between the dataset containing all of the rapid indicators from Day 35 with each of the two resource-intensive measures, individually. A BEST procedure was then used to identify which of the individual water quality and sediment indicators were best correlated each of the resource-intensive measures of decomposition.

3. Results

3.1 Established resource-intensive indicators

3.1.1 Comparison of wood break-down among land-use types, time of decomposition and 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 and were excluded from all analyses. There was also no contamination of the procedural controls, with very little mass loss recorded (0.01 ± 0.001 g), so they were excluded from further analysis.

Increasing mass loss occurred at all wetlands through time (Fig. 3a) but rates of decomposition were much higher during the first seven days of deployment than subsequently (Fig. 3b). Cobrico Swamp had the fastest rate of decomposition after 7 days (0.37 ± 0.04 % day^{-1}) while Lake Cobden had the fastest decomposition rate after 35 days (0.15 ± 0.01 % day^{-1}) and the fastest overall rate of decomposition (0.21 ± 0.02 % day^{-1}). The slowest overall decomposition rate was at Mepunga (0.15 ± 0.01 % day^{-1}) over the 35 days. Rates of decomposition were comparable in urban and agricultural wetlands, with both having an average rate of 0.18 ± 0.01 % day^{-1} (Fig. 3b).

There was a significant interaction in decomposition between wetlands and time of decomposition (pseudo-$F_{4, 132}$ = 5.03, P = 0.002). There were also significant differences among wetlands (pseudo-$F_{4, 6}$ = 31.36, P = 0.002) and with time of decomposition (pseudo-$F_{1, 133}$ = 150.75, P = 0.001; Fig. 4). In contrast, there was no statistically significant difference between the rates of decomposition at urban and agricultural wetlands (pseudo-$F_{1, 4}$ = 0.05, P = 0.841).

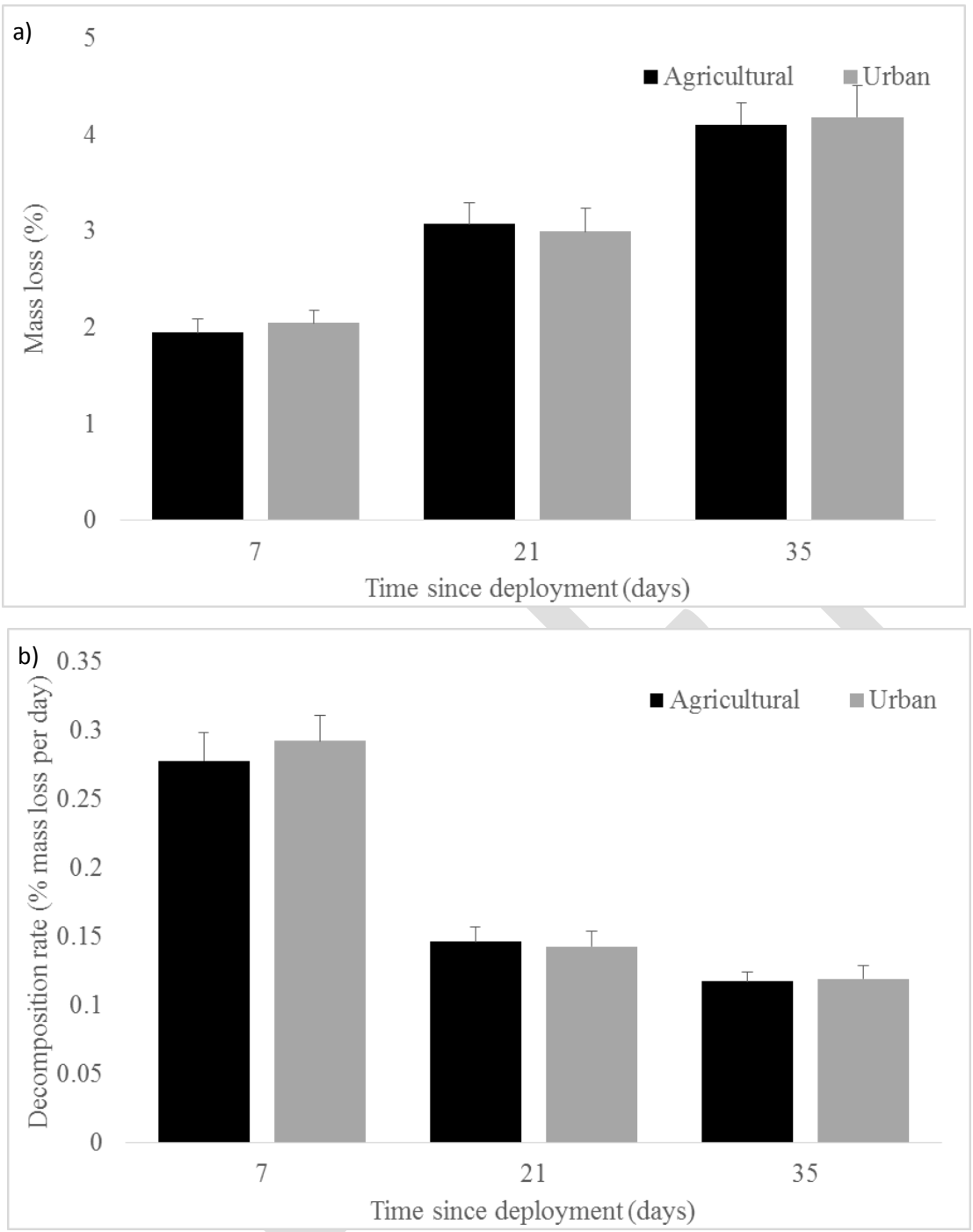


Fig. 3 a) Mass loss with length of deployment and b) decomposition rate per day for urban and agricultural wetlands. Figures shown are mean (+ SE) based on up to five replicate pieces of wood at each of two sites for three wetlands per land-use type (*n* = 156 in total). Urban wetlands were Lake Pertobe, Tea Tree Lake and Lake Cobden and agricultural wetlands were Mepunga, Glads Crossing and Cobrico Swamp.

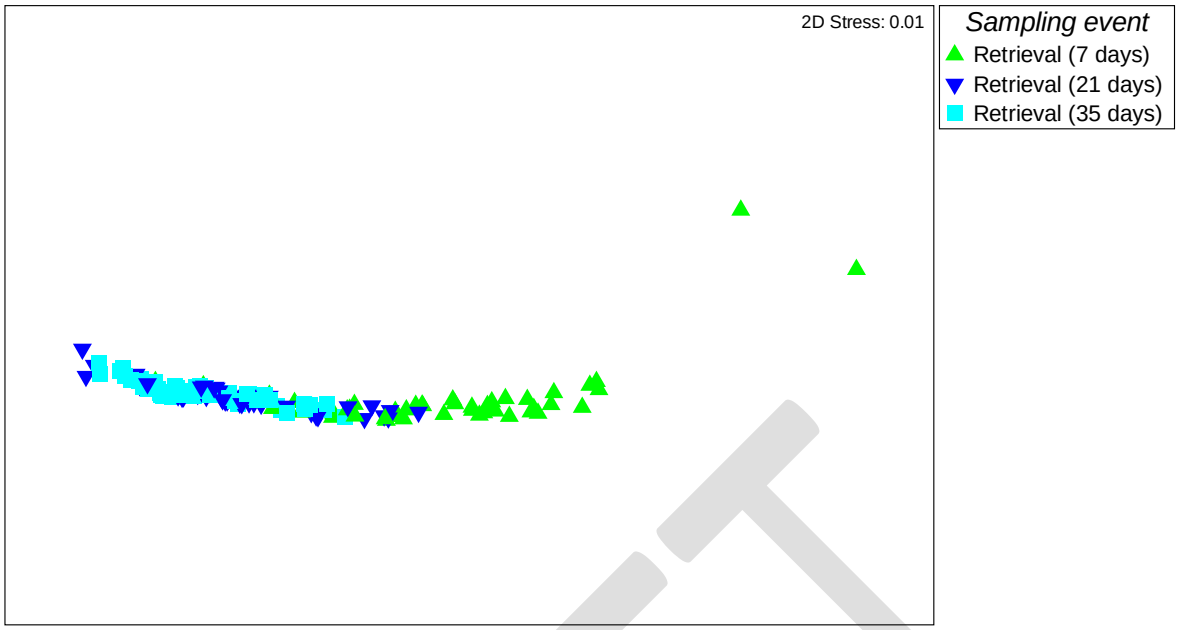


Fig. 4 MDS ordination plots illustrating the differences in wood mass loss as a rate per day (n = 229) among sampling periods (7, 21 and 35 days) at three agricultural and three urban wetlands. The plot is based on a Euclidean distance similarity matrix of untransformed data.

3.1.2 Comparison of microbial functional diversity among land-use types and wetlands

Procedural controls at both sites in Lake Pertobe and Site 2 in Cobrico Swamp were contaminated, with each site having a different single individual carbon source that was contaminated. All samples from these sites were excluded from further analysis. All procedural controls were then excluded from further analysis. The internal control (i.e. the well with no carbon source) for each replicate was contaminated for Site 1 at Cobrico Swamp, Site 2 at Glads Crossing and Site 1 at Lake Cobden and these replicates were also excluded from further analysis. This resulted 71 replicate readings of carbon source utilisation recorded for the 21-day sampling event.

There was little variation among number of wells indicating a consistent response from the microbes to the 31 carbon substrates available (i.e. indicating that the microbes were able to utilise that carbon source as a food, which is a surrogate for functional diversity of microbial community types) among sites or wetlands. The lowest number of carbons used was in Lake Cobden with 18 ± 2 and the highest

being Tea Tree Lake, with 25 ± 1 out of the 31 carbon sources available able to be utilised. This indicated that most of the carbon sources were able to be utilised by microbes, to some extent, in most wetlands. There was very little disparity found between the number of carbon sources used between urban and agricultural wetlands with values of 22 ± 1 for each land-use type.

There was limited variation in the intensity of carbon source utilisation between wetlands (a surrogate for the abundance of functional taxa)**.** The greatest average colour intensity was identified in Glads Crossing with a mean between two sites of 2.2 ± 0.1 and the lowest being in Lake Cobden (1.9 ± 0.1 across both sites). When considering the multivariate data showing the overall utilisation of each carbon source, there were significant differences among wetlands nested in land-use types for the PERMANOVA (pseudo-$F_{2, 4}$ = 4.36, *P(MC)* = 0.002; Fig. 5), but there were no significant differences among sites nested within wetlands (pseudo-$F_{4, 30}$ = 1.29, *P* = 0.116). Again, there was no significant difference identified between land-use types.

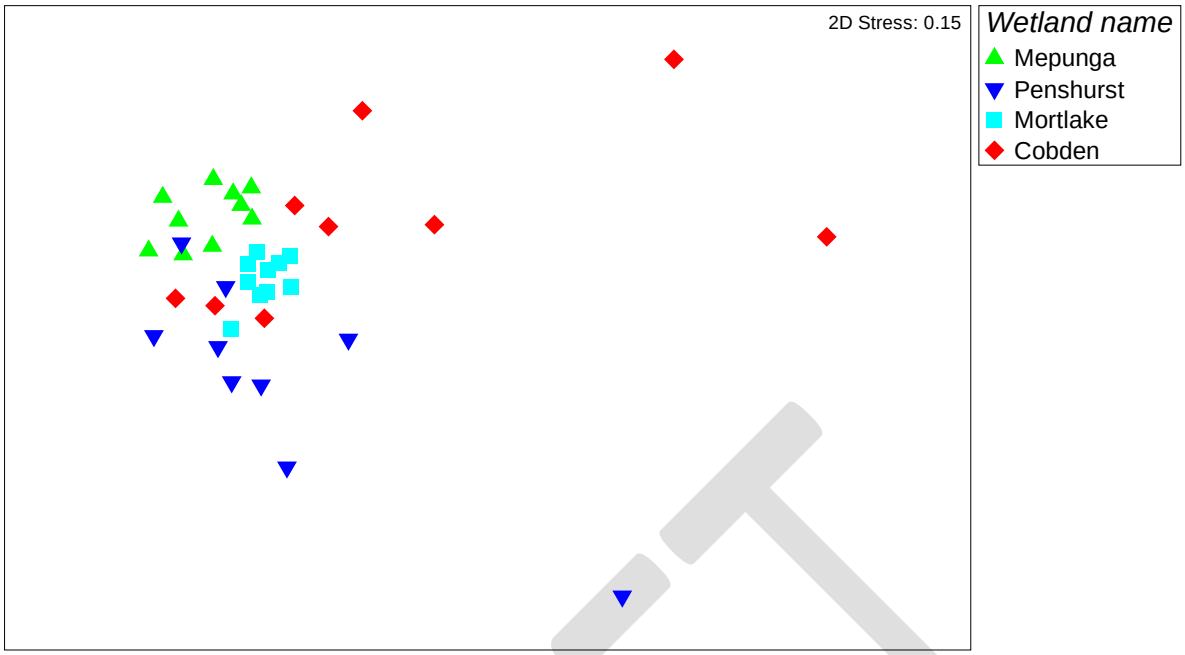


Fig. 5 MDS ordination plot illustrating the variability in microbial functional diversity in wetlands, among the four wetlands with viable microbial samples (*n* = 71). The plot is based on a Bray-Curtis similarity matrix with a dummy variable of 0.1 added.

3.1.3 Comparison of leaf break-down among wetlands and treatments

Procedural controls were deployed during the first retrieval period (i.e. Day 21) to measure the amount of leaf litter lost due to handling procedures. Losses were greatest at Cobrico Swamp, where there was a mean mass loss of 18.8 ± 2.3 %. Mepunga and Glads Crossing had similar losses of mass associated with the procedural controls, with mean values of 12.0 ± 1.7 % and 11.6 ± 2.0 %, respectively. Litter treatments from each wetland recorded more than twice the mass loss compared with that of the procedural controls. For the first retrieval event when the procedural controls were deployed, there were significant differences in mass loss among treatments (pseudo-$F_{1, 3}$ = 284.93, *P* = 0.019), as well as among wetlands (pseudo-$F_{2, 3}$ = 26.98, *P(MC)* = 0.014).

There were large decreases in the mass of *P. australis* leaves at all wetlands over the entire sampling period but decomposition rates declined through time (Fig. 6). Cobrico Swamp had the fastest decomposition rates at the first sampling event, with a mean rates of 3.4 ± 0.1 % day^{-1}. *P. australis*

leaves decayed slowest at Mepunga at 2.6 ± 0.03 % day^{-1}. Twenty-eight days after deployment, Cobrico Swamp and Glads Crossing had very similar decomposition rates at 2.1 ± 0.02 % day^{-1} and 2.1 ± 0.1 % day^{-1}, respectively, whilst leaves at Mepunga decayed at a rate of 1.8 ± 0.04 % day^{-1}. There was a significant interaction between the time since deployment and wetland (pseudo-$F_{2, 36}$ = 10.4, P = 0.001), indicating that there were inconsistencies in the rate of decay at the different wetlands tested.

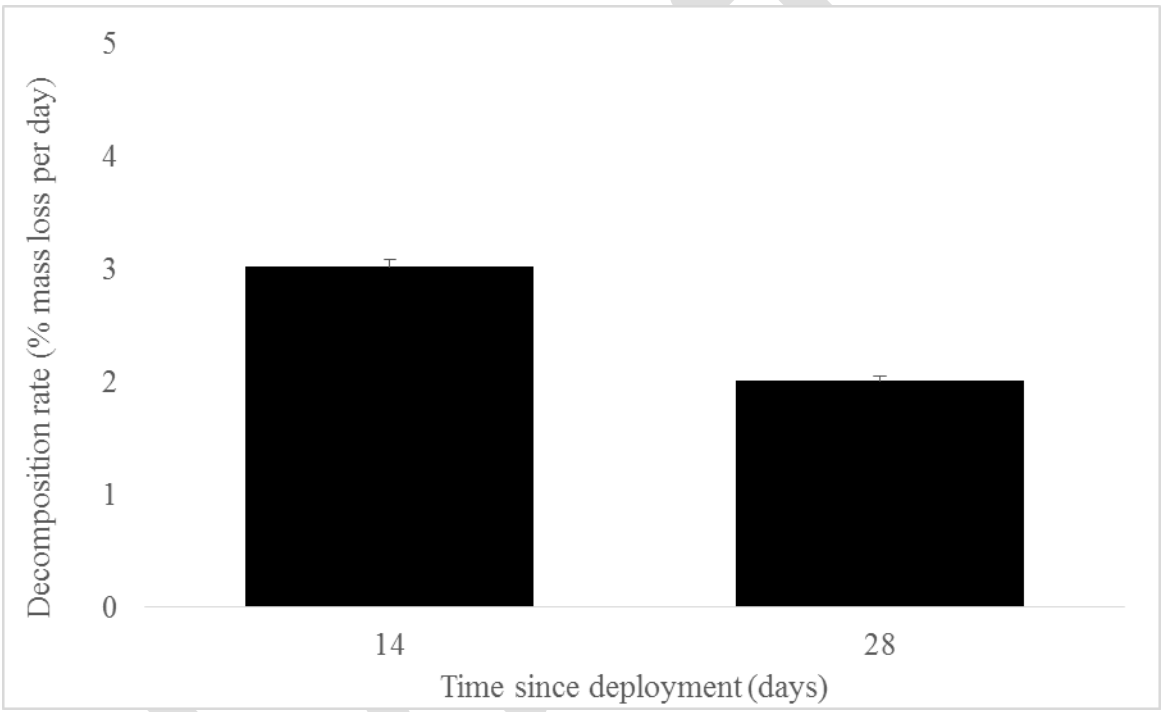


Fig. 6 Mean (+SE) decomposition rate (% mass loss per day) of *P. australis* leaves for each sampling event in three agricultural wetlands (n = 48). The agricultural wetlands were wetlands were Mepunga, Glads Crossing and Cobrico Swamp.

3.1.3 Comparison of macroinvertebrates colonising leaf litter among wetlands

Macroinvertebrate taxon richness was relatively similar among wetlands. At each of Mepunga and Cobrico Swamp, 31 different taxa were found over the entire sampling period. At Glads Crossing there were slightly fewer taxa, with only 20 taxa identified over the entire sampling period. A total of

14 taxa were collected from procedural controls. For taxon richness, there was a significant interaction between time of decomposition, sites within wetlands and treatments (pseudo-$F_{3, 20}$ = 3.69, P = 0.037) as well as a significant interaction between time of decomposition, wetland, treatment and the amount of accumulated organic matter (pseudo-$F_{2, 20}$ = 5.36, P = 0.022).

Over the study, a total of 110,914 macroinvertebrates were collected from litterbags, of which 258 individuals were collected from procedural controls. Microcrustaceans of the order Ostracoda and amphipods of the family Ceinidae were the most abundant macroinvertebrates with 40,134 and 37,500 individuals, respectively. Abundances of other taxa were much lower; the next most abundance taxon, chironomids of the family Chironominae (non-biting midges) had 15,188 individuals. Oligochaete worms and amphipods of the family Paramelitidae were also quite abundant with 7363 and 3115 individuals, respectively. All other taxa had abundances below 1000 individuals. All wetlands had similar dominant taxa, but Mepunga had the lowest macroinvertebrate abundances with only 8130 individuals collected, while Glads Crossing had the highest abundance with 76,084 individuals collected. Cobrico Swamp, unlike other wetlands, also had moderate abundances of the caddisfly Ecnomidae (802 individuals, absent elsewhere).

Total abundance, when analysed in a univariate analysis across the two retrieval events, showed significant interactions. These included interactions between the amount of accumulated organic matter, wetland and treatment (pseudo-$F_{2, 20}$ = 3.84, P = 0.042) and organic matter present and time of decomposition (pseudo-$F_{1, 20}$ = 9.39, P = 0.011), as well as significant differences among sites within wetlands (pseudo-$F_{3,20}$ = 5.7, P = 0.006). These results suggest high levels of small-scale temporal and spatial variability in macroinvertebrate abundances. Thus, there was no consistent pattern of more invertebrates (or more diverse invertebrates) utilising treatment litterbags (i.e. those with litter in them) than control bags.

The multivariate analysis, considering both the identity and the abundance of each family, differed from the univariate analysis in that, there was a significant interaction between time of decomposition and wetland (pseudo-$F_{2, 20}$ = 8.16, P = 0.001), and a significant main effect of site nested within

wetland (pseudo-$F_{3, 20}$ = 5.65, P = 0.001; Fig. 7). Litter and control treatment abundances were not significantly different (pseudo-$F_{1, 2}$ = 0.68, P = 0.559), nor was the accumulated organic matter a significant covariate (pseudo-$F_{1, 3}$ = 1.21, P = 0.326). Again, this suggests no pattern of invertebrates utilising treatment litterbags over control bags.

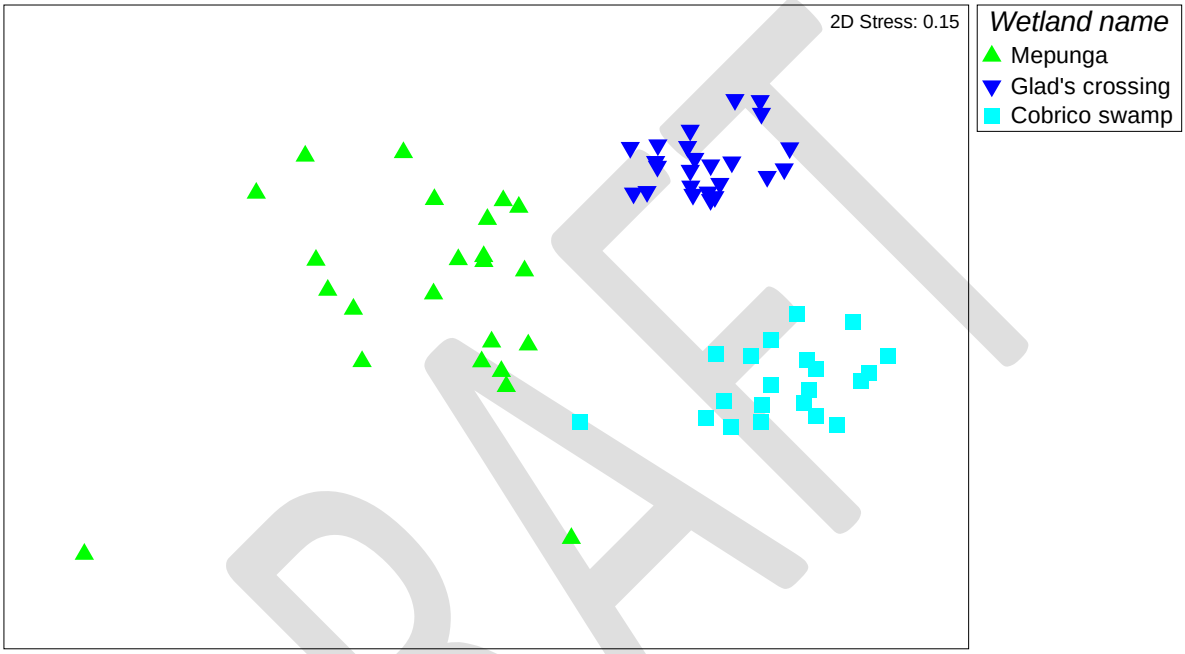


Fig. 7 MDS ordination plots illustrating the differences in macroinvertebrates colonising leaf litter among wetlands (n = 80). Three agricultural wetlands were included and the plot is based on a Bray-Curtis similarity matrix of log-transformed data with a dummy variable of 1 added.

3.2 Potential rapid indicators

3.2.1 Water quality

There were large variations in air temperature over the sampling period from 3.4 °C minimum to 44.0 °C maximum (www.bom.gov.au). High air temperatures caused an increase in water temperatures as the experiment progressed, and an associated reduction in the water levels at each wetland.

Most wetlands experienced severe declines in water level. Mepunga had the greatest decline, with depth falling by 28.0 ± 0.1 cm over the 35 days, but water levels dropped less at other wetlands, with Tea Tree Lake only decreasing in depth 0.8 ± 13.9 cm over the same timeframe. There were significant interactions in water level change between time of decomposition and wetlands nested in land-use types (pseudo-$F_{4, 276}$ = 145.04, P = 0.001) and time of decomposition and sites nested within wetlands (pseudo-$F_{6, 276}$ = 7.11, P = 0.001).

There was a large variation in the water temperatures recorded with HOBO® Data Loggers, over the 42-day sampling period at all wetlands. Lake Pertobe had the lowest temperature range, with water temperature varying between 7.1 and 50.0 °C, possibly due to canopy cover in the area sampled. Some loggers were exposed later in the study due to declining water levels, but the high temperatures reflect the conditions to which the various resource-intensive measures were exposed. The highest temperature range was found at Glads Crossing from 9.0 to 66.9 °C. Despite this, the variation in mean temperatures was quite low. All wetlands had similar mean temperatures, ranging from the lowest at Mepunga (20.73 ± 0.04 °C) to the highest at Tea Tree Lake (23.9 ± 0.04 °C).

From the water quality monitoring over the different sampling events, 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 (3341.3 ± 118.8 μs cm^{-1}). The highest average pH levels were found in Lake Cobden (8.86 ± 0.12), and the lowest at Mepunga (6.84 ± 0.09) over the entire sampling period. Turbidity also had quite a large range among wetlands. The lowest turbidity value over the entire

sampling period was recorded at Cobrico Swamp (18.3 ± 5.5 NTU) while the highest was recorded at Glads Crossing (332.9 ± 30.5 NTU). There was substantial 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 (17.6 ± 6.5 %) and the highest in Lake Cobden (142.9 ± 8.9 %) over the entire sampling period.

The nutrient concentrations (total nitrogen mg L^{-1} and total phosphorus mg L^{-1}), were generally above the nutrients 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 for each wetland across the three sampling events (Days 7, 21 and 35). Total phosphorus concentrations ranged from 0.09 ± 0.01 mg L^{-1} (Tea Tree Lake) to 0.80 ± 0.02 mg L^{-1} (Cobrico Swamp) across the time periods tested. Total nitrogen concentrations ranged from 1.23 ± 0.08 mg L^{-1} (Tea Tree Lake) to 3.38 ± 0.86 mg L^{-1} (Lake Pertobe).

There were statistically significant differences found among wetlands when examining physico-chemical characteristics, nutrient concentrations, and water level in a combined analysis including DO (pseudo-$F_{4, 6}$ = 11.79, P = 0.001; Fig. 8a). No significant differences were found between land use types (pseudo-$F_{1, 4}$ = 1.94, P = 0.18) or any other factor in the analysis. There were only slight differences in the results when DO was excluded (Fig. 8b), with a significant interaction between time of decomposition and wetland nested in land-use type becoming significant (pseudo-$F_{4, 11}$ = 2.54, P = 0.011).

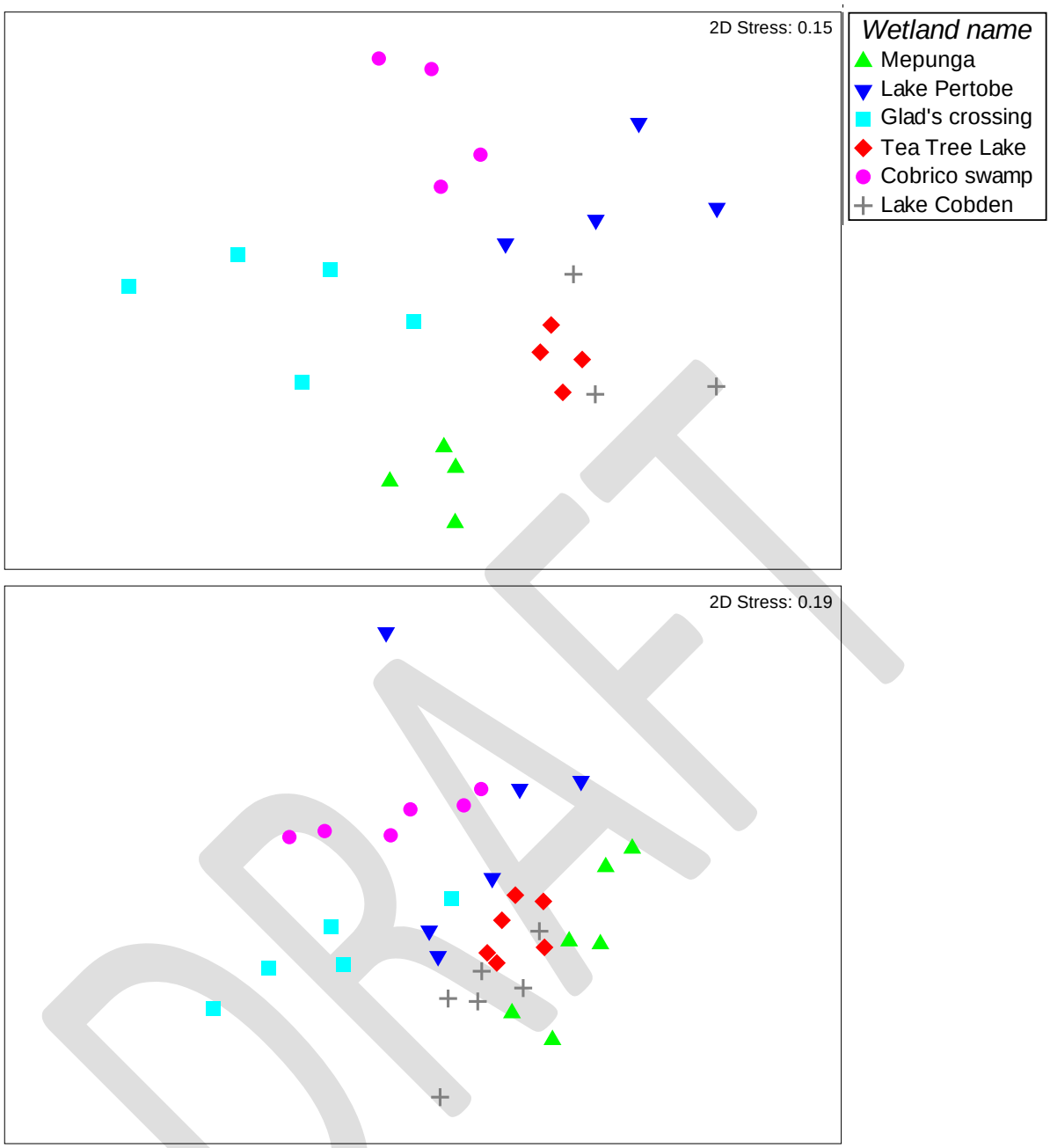


Fig. 8 MDS ordination plots illustrating the differences in the water quality parameters *(a)* with dissolved oxygen (%), (n = 24) and *(b)* without dissolved oxygen included (n = 35). The other water quality parameters, including electrical conductivity, turbidity, pH, temperature, total nitrogen, total phosphorus, and water level among wetlands over each sampling periods (7, 21 and 35 days). Both MDS plots are based on a Euclidean distance similarity matrix of normalised environmental data.

3.1.3 Comparison of sediment characteristics among wetlands

The sediment characteristics were recorded and samples collected at each site. Visually, there were notable differences between sediment size and colour characteristics. 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. There were significant differences in sediment size and organic matter content among wetlands (pseudo-$F_{4, 6}$ = 7.83, P = 0.002), but not among land-use types. There was also a significant interaction between time of decomposition and wetland nested in land-use type for sediment pH (pseudo-$F_{4, 12}$ = 3.11, P = 0.048), while sediment temperature and redox were significant different between land-use types (pseudo-$F_{1, 4}$ = 8.23, $P(MC)$ = 0.02).

3.3 Relationships between rapid indicators and intensive measures

All of the rapid indicators (sediment characteristics, physico-chemical parameters, nutrient concentrations, water levels) were compared with the resource-intensive measures of decomposition to identify the strongest correlations and thus possible rapid indicators of decomposition rates. The same analysis was performed with the rapid water quality dataset (excluding the sediment characteristics) and time of decomposition (number of days) (except for microbial functional diversity).

The rate of wood mass loss was significantly correlated overall with all rapid indicators as a whole dataset, including sediment characteristics (*Rho* = 0.289, P = 0.025). The best-correlated combination of variables was sediment size (as the percent fraction below 63 μm) and water pH (*Rho* = 0.468, P > 0.05; Table 4a), although this was not a statistically-significant relationship.

Table 4 Overall correlations between the potential indicators and the resource-intensive measures of decomposition in the BEST analyses for a) all rapid indicators, b) all water quality indicators including dissolved oxygen, and c) all water quality indicators excluding dissolved oxygen. The significant correlations are in bold font.

a)

		Combination of variables								
		Water variables				Sediment Variables				
		Total nitrogen	Temperature	Water pH	Electrical conductivity	Size	Redox	Organic matter content	pH	Temperature
Measure	*Rho*									
Wood	0.468			✓		✓				
Microbial	0.644	✓	✓		✓		✓			
Leaf litter	**0.986**		✓	✓				✓	✓	
Macroinvertebrate assemblages	**0.943**	✓				✓			✓	✓
Macroinvertebrate richness	0.825				✓			✓	✓	
Macroinvertebrate abundance	**0.968**	✓				✓				

b)

		Combination of variables							
		Total nitrogen	Total phosphorus	Temperature	Water pH	Electrical conductivity	Turbidity	Water levels	Time of decomposition
Measure	*Rho*								
Wood	**0.656**					✓			✓
Microbial	0.442	✓		✓	✓	✓			NA
Leaf litter	0.771		✓				✓		
Macroinvertebrate assemblages	**0.830**				✓	✓			
Macroinvertebrate richness	0.702					✓			
Macroinvertebrate abundance	0.543				✓			✓	

c)

		Combination of variables						
		Total nitrogen	Total phosphorus	Temperature	Water pH	Electrical conductivity	Water levels	Time of decomposition
Measure	*Rho*							
Wood	**0.537**				✓			✓
Microbial	0.590				✓			NA
Leaf litter	**0.764**					✓		✓
Macroinvertebrate assemblages	**0.701**		✓		✓			
Macroinvertebrate richness	0.305	✓	✓	✓				
Macroinvertebrate abundance	**0.602**				✓		✓	

The rapid water quality dataset, as a whole, showed no significant overall correlation with the rate of wood mass loss (*Rho* = 0.099, P > 0.05, including DO). However, the best-correlated combination of variables was time of decomposition and conductivity (*Rho* = 0.656, P < 0.001, Table 4b) when DO was included, or time of decomposition and pH (*Rho* = 0.537, P < 0.01, Table 4c) when DO was excluded from the analysis.

The microbial functional diversity was not significantly correlated with the dataset containing all rapid indicators (*Rho* = 0.055, P > 0.05). The best-correlated variables within that dataset were sediment redox, water pH, conductivity and total nitrogen (*Rho* = 0.644, P > 0.05, Table 4a). There was also no significant overall correlation between microbial functional diversity and the rapid water quality indicators when DO was included (*Rho* = -0.031, P > 0.05) or excluded (*Rho* = -0.005, P > 0.05). The best-correlated individual variables were water temperature, water pH, conductivity and total nitrogen (*Rho* = 0.442, P > 0.05, Table 4b) when DO was included and water pH (*Rho* = 0.590, P > 0.05, Table 4c) when DO was excluded.

For leaf litter decay rates, there was a statistically-significant relationship with the dataset containing all rapid indicators (*Rho* = 0.725, P = 0.013). There was an extremely strong correlation with the organic matter content of the sediment, sediment pH, water temperature and water pH, in particular (*Rho* = 0.986, P = 0.010, Table 4a).

There was also a strong overall relationship between decay rates and the rapid water quality dataset or excluding it (*Rho* = 0.34, P = 0.023), but not when DO was included (*Rho* = 0.331, P = 0.081). Litter decay rates were well correlated with individual variables, with the best-correlated variables being time of decomposition and conductivity (*Rho* = 0.764, P = 0.01, Table 4c) when DO was excluded, and total phosphorus and turbidity (*Rho* = 0.771, P = 0.090, Table 4b) with DO included.

All rapid indicators, as a whole dataset, was significantly correlated with the macroinvertebrate assemblages recorded (*Rho* = 0.857, P < 0.001). The best-correlated variables within that dataset were sediment size, sediment temperature, sediment pH and total nitrogen (*Rho* = 0.943, P < 0.05, Table 4a). In contrast, all rapid indicators were not significantly correlated with macroinvertebrate richness (*Rho* = 0.164, P > 0.05) nor abundance (*Rho* = 0.507, P = 0.06) although the latter was marginal. For richness, the best-correlated variables were sediment organic matter content, sediment pH and electrical conductivity (*Rho* = 0.825, P > 0.05, Table 4a), although this relationship was not statistically significant. For abundance, the best-correlated variables were sediment size and total nitrogen (*Rho* = 0.968, P = 0.01, Table 4a).

Similarly, the rapid water quality dataset was also significantly correlated with macroinvertebrate assemblages both when DO was excluded (*Rho* = 0.510, P = 0.01) and also when DO was included (*Rho* = 0.378, P = 0.043). The best-correlated variables (excluding DO) were pH and total phosphorus (*Rho* = 0.701, P < 0.05, Table 4c). With DO included, the best-correlated variables were pH and conductivity (*Rho* = 0.830, P < 0.05, Table 4b). The rapid water quality dataset was also significantly correlated overall with total abundance when DO was excluded (*Rho* = 0.304, P = 0.026), but not when DO was included (*Rho* = -0.009, P > 0.05). The variables best correlated with abundance were pH and the location of the water's edge both when DO was excluded (*Rho* = 0.602, P = 0.02, Table

4c) and included (*Rho* = 0.543, P = 0.310, Table 4b). In contrast, there was no overall relationships between the rapid water quality dataset and total richness excluding DO (*Rho* = -0.010, P > 0.05) nor when DO was included (*Rho* = 0.289, P > 0.05). The best-correlated variables (excluding DO) were water temperature, total nitrogen and total phosphorus (*Rho* = 0.305, P > 0.05, Table 4c), but this relationship was not statistically significant. When DO was included, the variable best correlated with richness was conductivity (*Rho* = 0.702, P > 0.05, Table 4b) but, again, this relationship was not significant.

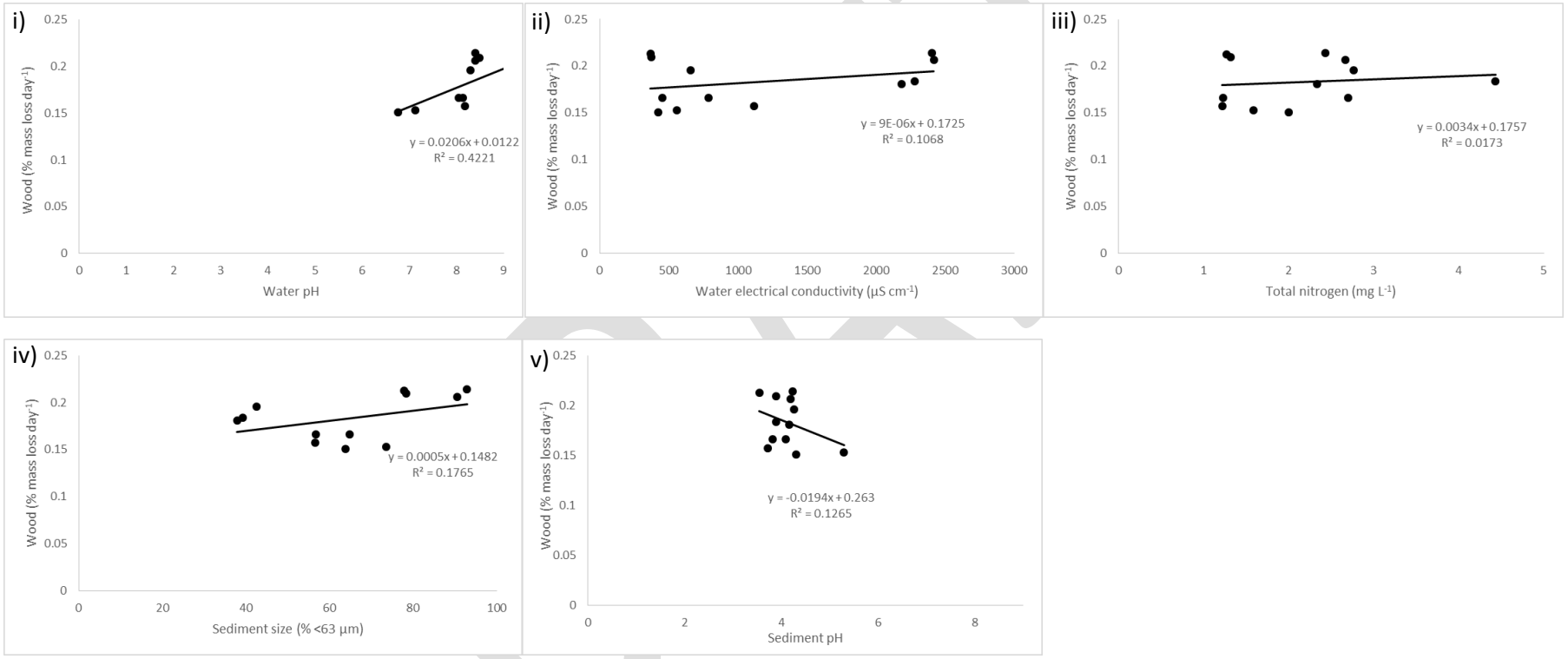
Overall, water pH, sediment pH, sediment size, total nitrogen and electrical conductivity were the most common indicators across the various resource-intensive measures of decomposition and analyses. In order to assess the general trends for each variable, we looked at the correlation of each with the various resource-intensive measures, in a series of univariate analyses (Fig. 9). In general, the correlations were relatively weak, reflecting the fact that it was the combination of these correlations that was identified as the strongest relationship for each of the resource-intensive measures described above. Nonetheless, the general trends here are of interest.

Relationships between the most common indicators and the various resource-intensive measures were largely consistent across those measures. Microbial diversity (i.e. the number of carbons able to be utilised, which was used as a univariate surrogate for the microbial functional diversity analysed above) was the exception, showing opposite patterns for all of the common indicators except for electrical conductivity. In general, there were positive correlations between the rate of decomposition and water pH, electrical conductivity, total nitrogen concentrations and the percentage of sediment less than 63 μm in size (Fig. 9). There were also general negative correlations with sediment pH, again with the exception of microbial diversity. Thus, there was a pattern for faster rates of decomposition for wood and litter break-down, with more diverse and abundant macroinvertebrates in more alkaline, slightly saltier waters, where there were higher nitrogen concentrations and smaller, more acidic sediments. These same conditions tended to result in lower microbial diversity. The only

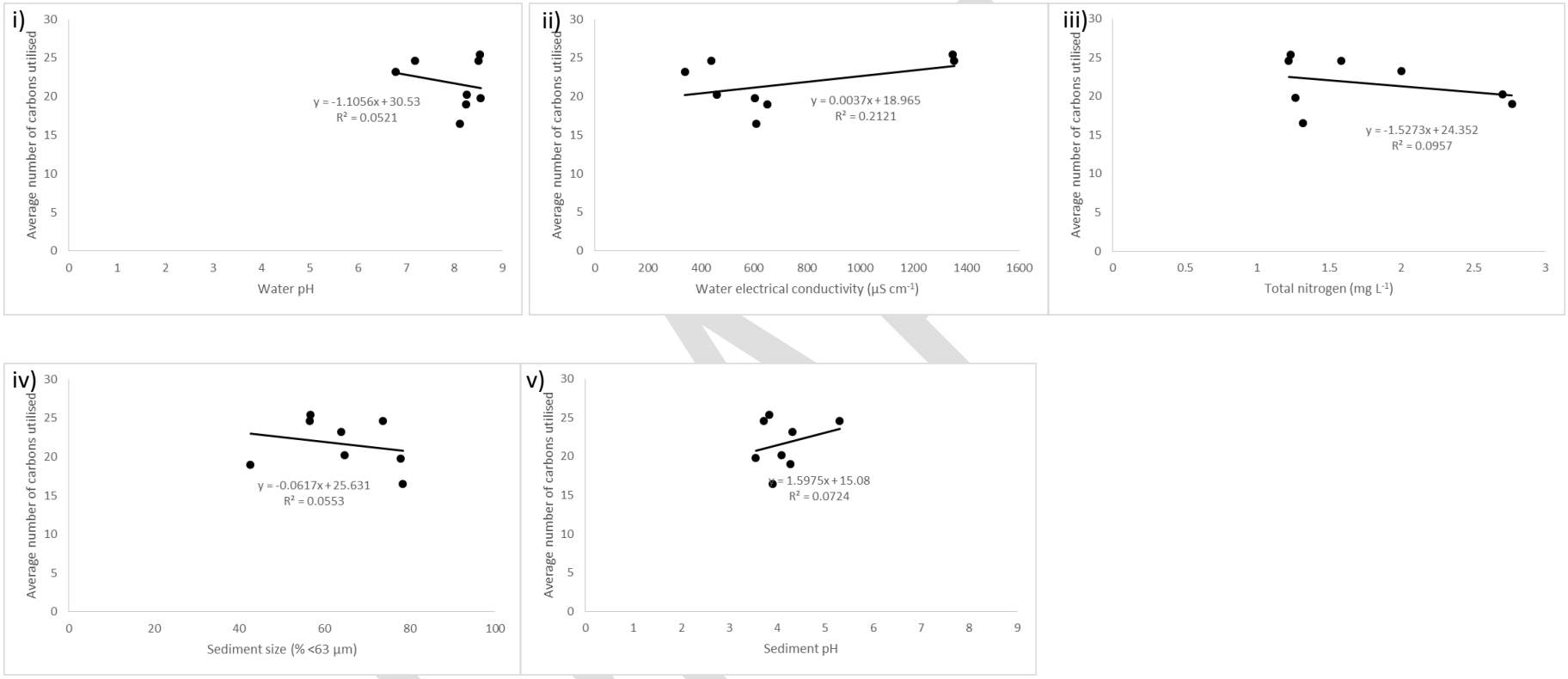
other exception was for macroinvertebrate abundances, which was negatively correlated with electrical conductivity.

Fig 9. Strength of the BEST correlations for *a)* rate of wood mass loss, *b)* microbial diversity, *c)* rate of leaf litter mass loss, *d)* macroinvertebrate diversity, and *e)* macroinvertebrate abundances, for each of *i)* water pH, *ii)* electrical conductivity, *iii)* total nitrogen, *iv)* sediment size, and *v)* sediment pH over the entire sampling event.

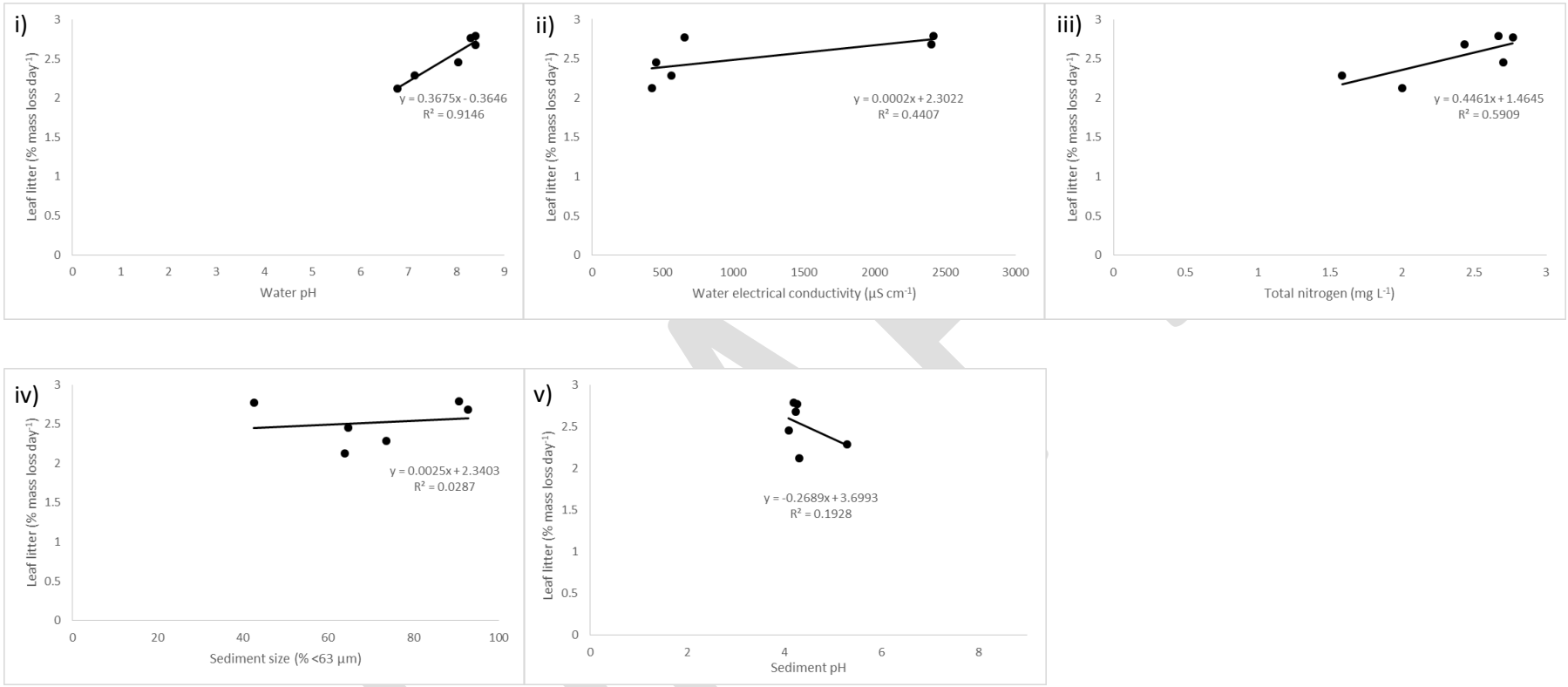
a)



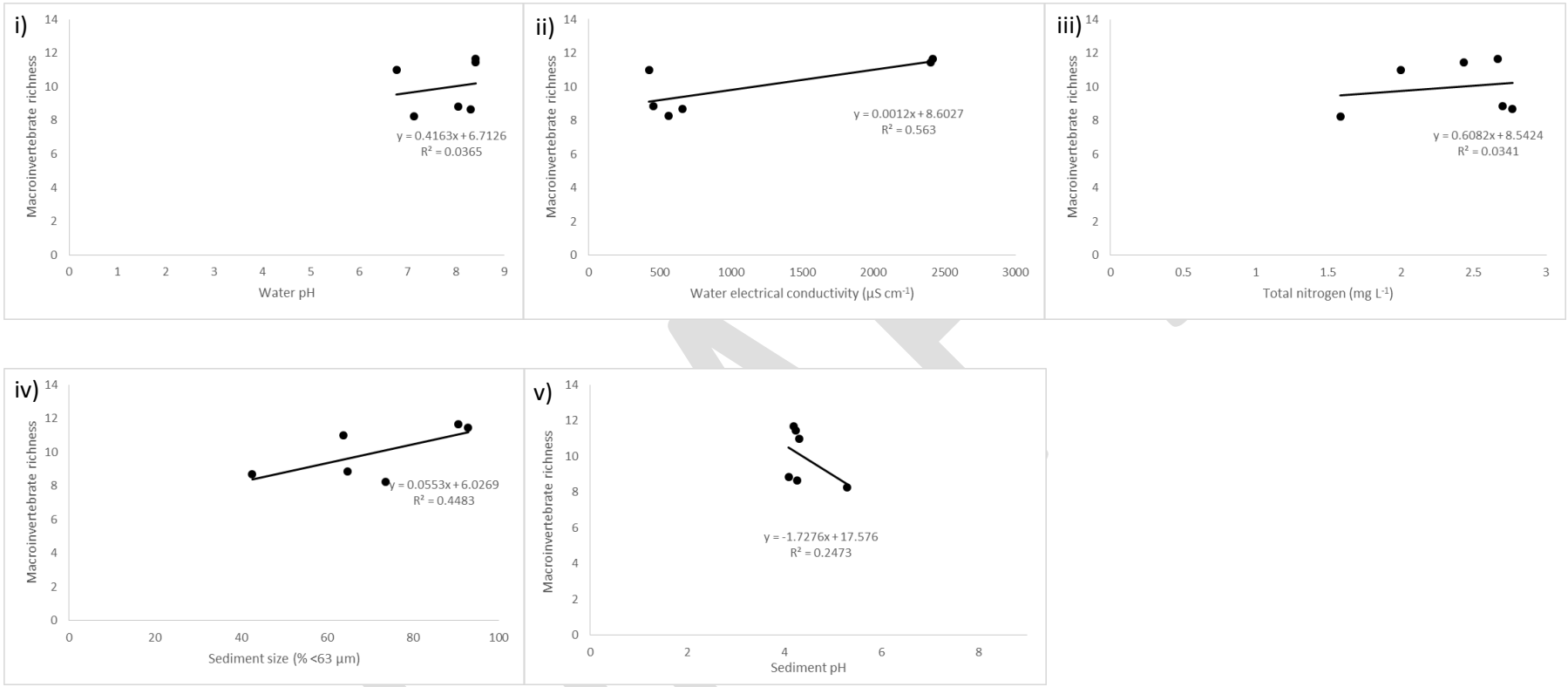
b)


i)
Average number of carbons utilised
y = -1.1056x + 30.53
R² = 0.0521
Water pH
ii)
Average number of carbons utilised
y = 0.0037x + 18.965
R² = 0.2121
Water electrical conductivity (µS cm-1)
iii)
Average number of carbons utilised
y = -1.5273x + 24.352
R² = 0.0957
Total nitrogen (mg L-1)
iv)
Average number of carbons utilised
y = -0.0617x + 25.631
R² = 0.0553
Sediment size (% <63 µm)
v)
Average number of carbons utilised
y = 1.5975x + 15.08
R² = 0.0724
Sediment pH

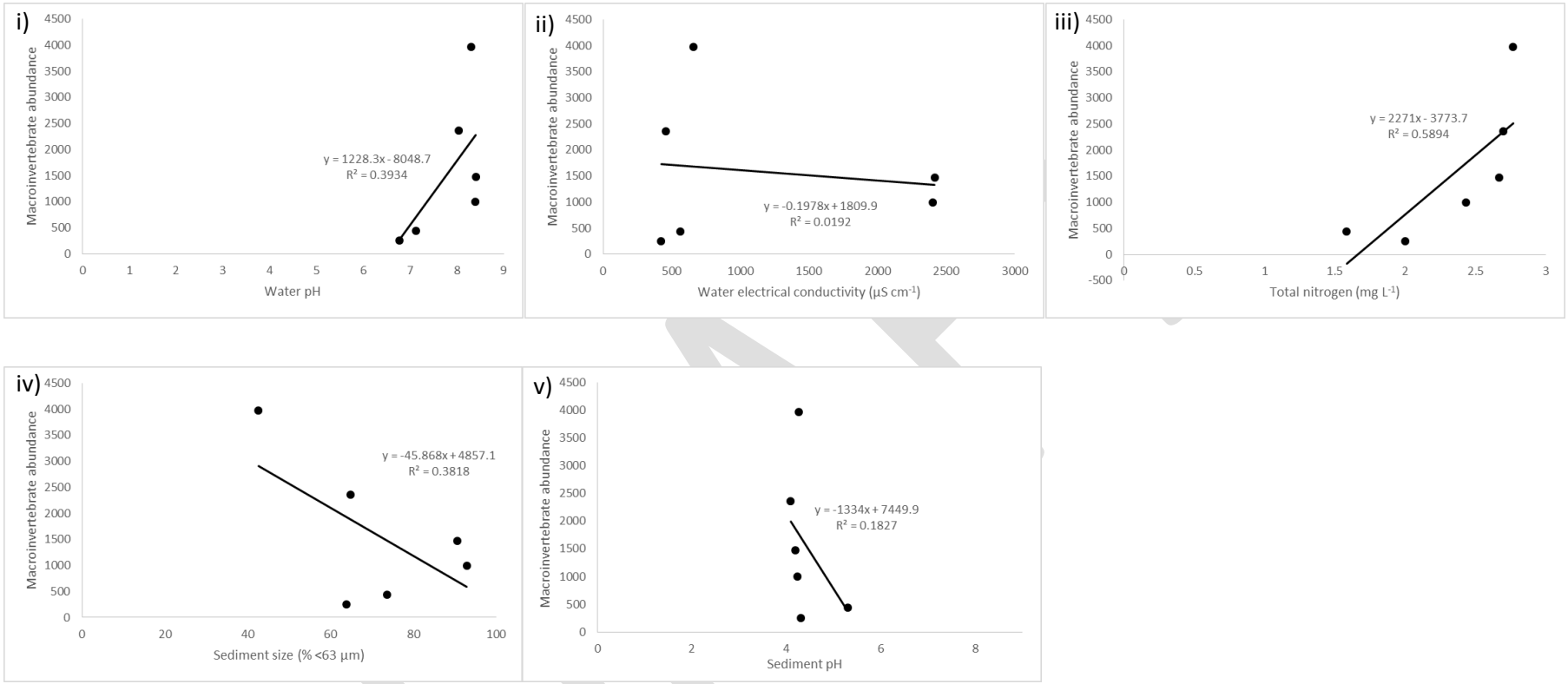
c)


i)
Leaf litter (% mass loss day-1)
y = 0.3675x - 0.3646
R² = 0.9146
Water pH
ii)
Leaf litter (% mass loss day-1)
y = 0.0002x + 2.3022
R² = 0.4407
Water electrical conductivity (µS cm-1)
iii)
Leaf litter (% mass loss day-1)
y = 0.4461x + 1.4645
R² = 0.5909
Total nitrogen (mg L-1)
iv)
Leaf litter (% mass loss day-1)
y = 0.0025x + 2.3403
R² = 0.0287
Sediment size (% <63 µm)
v)
Leaf litter (% mass loss day-1)
y = -0.2689x + 3.6993
R² = 0.1928
Sediment pH

d)


i)
Macroinvertebrate richness
Water pH
y = 0.4163x + 6.7126
R² = 0.0365
ii)
Macroinvertebrate richness
Water electrical conductivity (µS cm-1)
y = 0.0012x + 8.6027
R² = 0.563
iii)
Macroinvertebrate richness
Total nitrogen (mg L-1)
y = 0.6082x + 8.5424
R² = 0.0341
iv)
Macroinvertebrate richness
Sediment size (% <63 µm)
y = 0.0553x + 6.0269
R² = 0.4483
v)
Macroinvertebrate richness
Sediment pH
y = -1.7276x + 17.576
R² = 0.2473

e)


i)
Macroinvertebrate abundance
y = 1228.3x - 8048.7
R² = 0.3934
Water pH
ii)
Macroinvertebrate abundance
y = -0.1978x + 1809.9
R² = 0.0192
Water electrical conductivity (µS cm-1)
iii)
Macroinvertebrate abundance
y = 2271x - 3773.7
R² = 0.5894
Total nitrogen (mg L-1)
iv)
Macroinvertebrate abundance
y = -45.868x + 4857.1
R² = 0.3818
Sediment size (% <63 µm)
v)
Macroinvertebrate abundance
y = -1334x + 7449.9
R² = 0.1827
Sediment pH

3.4 Testing of consistency of identified indicators

3.4.1 Established resource-intensive indicators

3.4.1.1 Comparison of wood break-down among locations

Fifty-four pieces of wood were deployed across the six locations and twelve sites. Unfortunately some sites were interfered with during the 35-day deployment and only thirty-two pieces of wood were retrieved in total. All replicates were lost from Warringie Site 1 and one from Site 2, two were lost from Site 2 at Nurra Nurra, two were lost from Site 1 and four from Site 2 at Point Sturt, one was lost from Site 2 at Hindmarsh Island, four were lost from Site 2 at Tolderol Reserve, two from Site 1 and all from Site 2 at Lake Reserve Road. There was also no contamination of the procedural controls, with little mass loss recorded (0.08 ± 0.01 g), so they were excluded from further analysis.

The treatment wood loss across all six locations in the Lower Lakes ranged from 0.10 to 0.13 % day^{-1} (Fig. 10). One sample at Hindmarsh Island Site 1 had much higher rates of decomposition at 0.32 % day^{-1}, which had extremely high leverage on the overall results. As a result, this replicate was excluded to enable us to explore the remaining patterns in the results. With that replicate excluded, Hindmarsh Island Site 1 still had the highest rate of mass loss (0.13 ± 0.01 % day^{-1}). The lowest rate of wood loss was Tolderol Reserve (0.10 ± 0.01 % day^{-1}). There were no significant differences in the rate of mass loss over 35 days among the locations (pseudo-$F_{5,5}$ = 1.55, P = 0.351) or at sites within locations (pseudo-$F_{5,20}$ = 0.96, P = 0.47; Fig. 11).

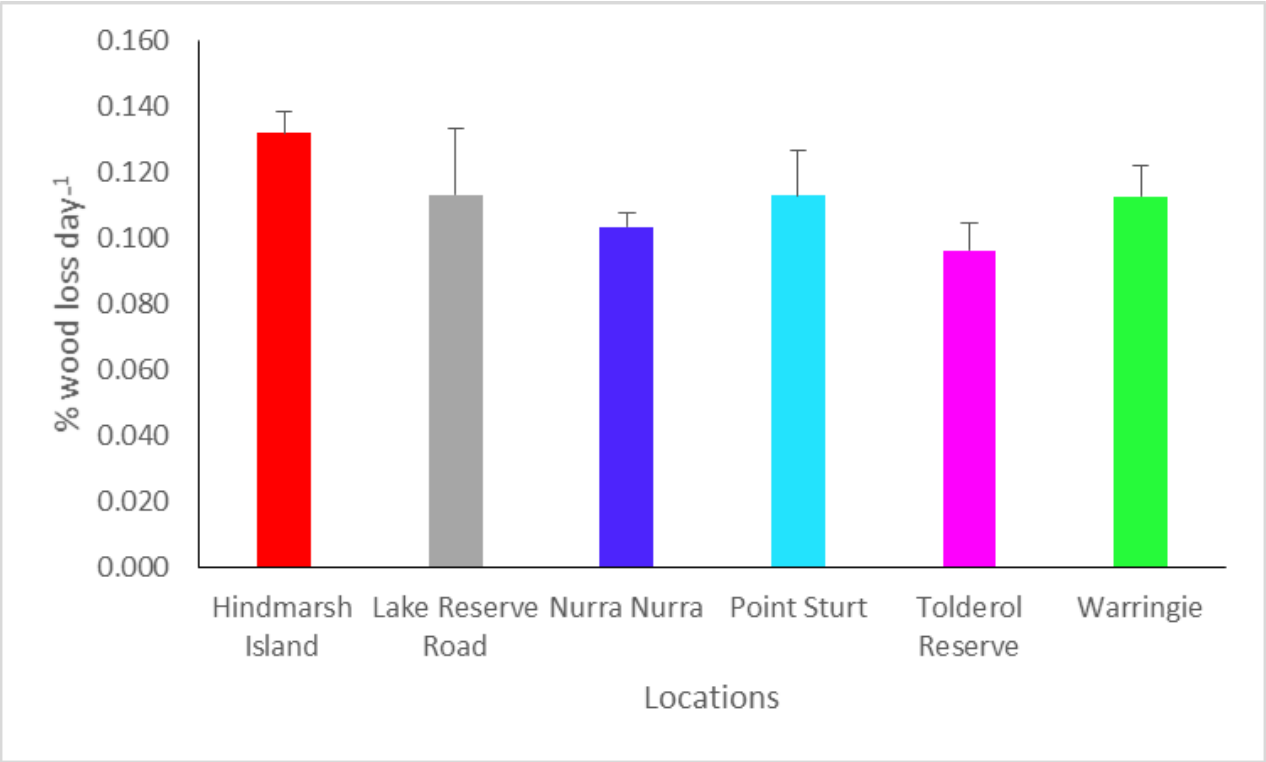


Fig. 10 Mean (+SE) decomposition rate (% mass loss per day) of wood for each location in the Lower Lakes, South Australia (*n* = 48).

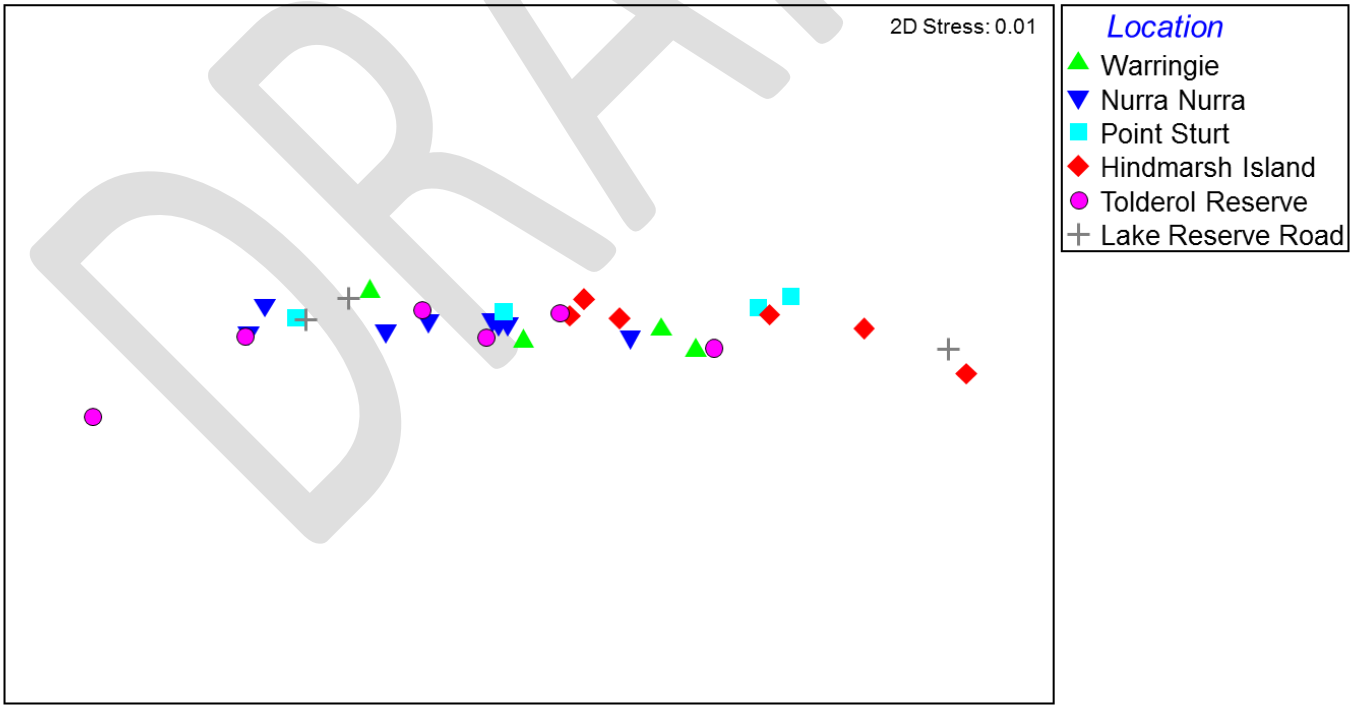


Fig. 11 MDS ordination plot illustrating the differences in wood mass loss as a rate per day (*n* = 32) among locations in the Lower Lakes. The plot is based on a Euclidean distance similarity matrix of untransformed data.

3.4.1.2 Comparison of microbial functional diversity among different locations

For microbial functional diversity, no procedural controls were contaminated and all sites could be used from the Lower Lakes. Only one sample (Hindmarsh Island Site 2, replicate 3) had contamination of the internal control and was, with the procedural controls, excluded from further analysis. This resulted 51 replicate readings of carbon source utilisation recorded for the 35-day sampling event across the six locations in the Lower Lakes.

The lowest number of carbons used was in Tolderol Reserve with 11 ± 1 and the highest was Warrengie, with 18 ± 1 out of the 31 carbon sources available able to be utilised. This indicated that there was variation among the locations and, at best, only a third of the carbon sources are able to be use by the microbial communities present. The lowest intensity across the six locations was Tolderol Reserve (0.54 ± 0.06) and the average intensity of carbon substrate use was typified by Warrengie (1.11 ± 0.10). This suggested that the microbial communities present were not fully able to utilize the individual carbons resulting in low colour intensity.

When considering the multivariate data including the overall utilisation of each carbon source, there were no significant differences among locations (pseudo-$F_{5, 6}$ = 1.97, P = 0.104; Fig. 12), or for sites nested within locations (pseudo-$F_{6, 47}$ = 1.30, P = 0.147).

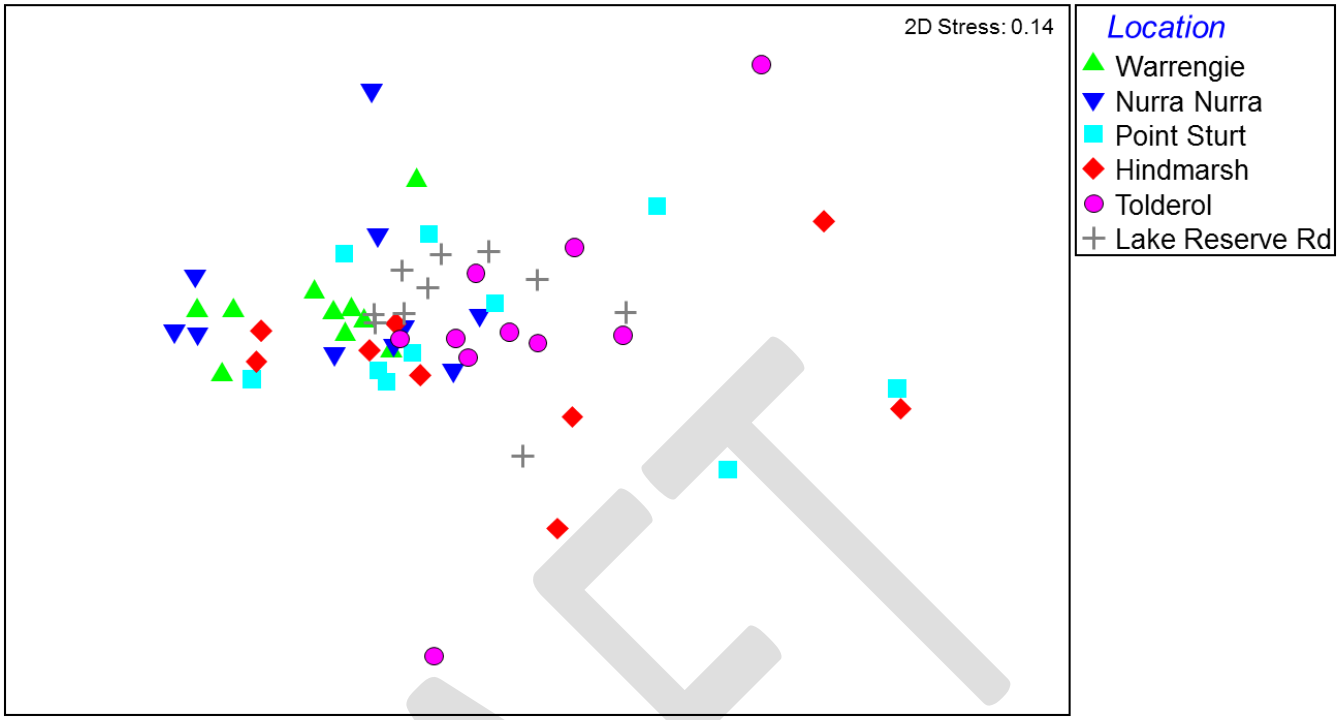


Fig. 12 MDS ordination plot illustrating the differences in carbon source utilisation (n = 59) among locations in the Lower Lakes. The plot is based on a Euclidean distance similarity matrix of normalised data.

3.4.2 Potential rapid indicators

3.4.2.1 Comparison of water physico-chemical and sediment characteristics among locations

For the Lower Lakes, because there were no differences in the number of sampling events among the potential rapid indicators, all sediment and water quality indicators were analysed simultaneously. These analyses indicated there were significant differences in the potential rapid indicators among locations (pseudo-$F_{5, 6}$ = 2.66, P = 0.001).

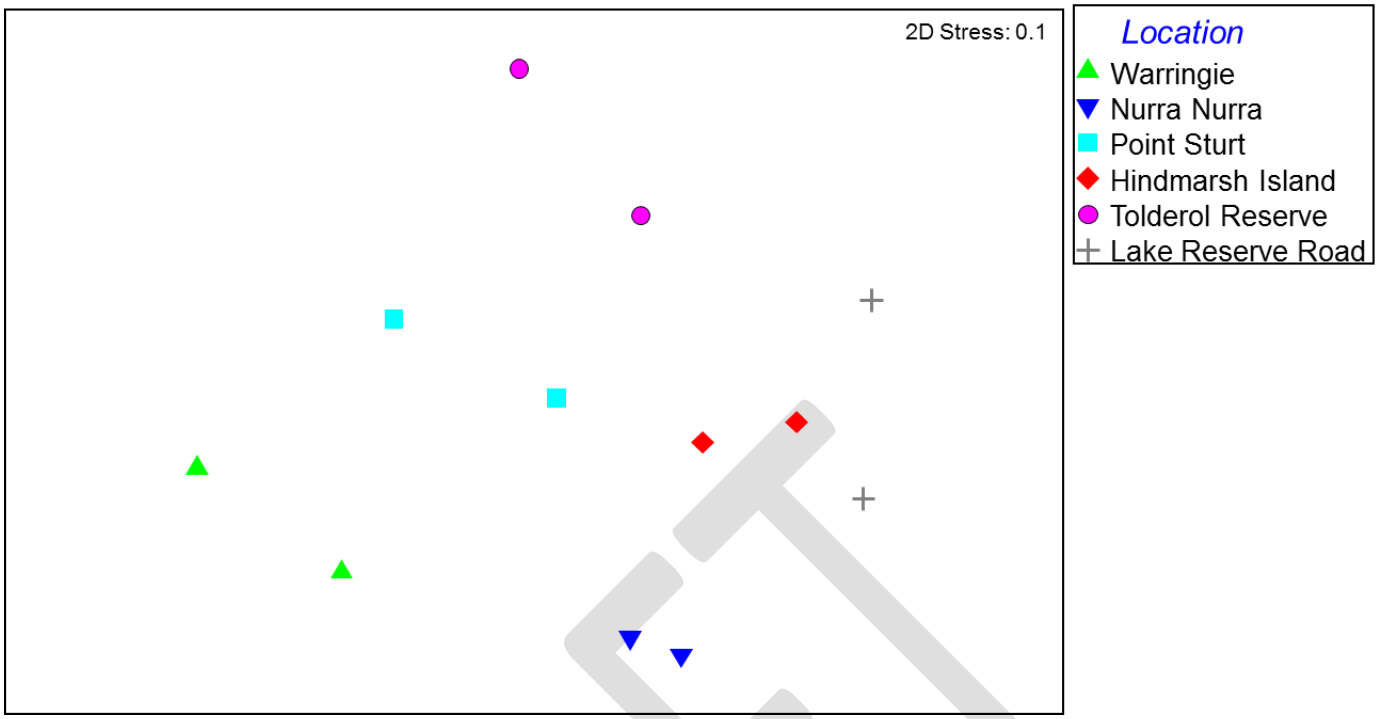


Fig. 13 MDS ordination plot illustrating the differences in potential rapid indicators in (*n* = 12) among locations averaged across sites in the Lower Lakes. The plot is based on a Bray-Curtis similarity matrix of untransformed data with a dummy variable of 0.1.

3.4.2.2 Relationships between rapid indicators and intensive measures

All rapid indicators as a whole dataset, including sediment characteristics, were not significantly corrected overall with the rate of wood mass loss (*Rho* = -0.102, P > 0.05). The best-correlated combination of variables within that dataset was water turbidity and depth, as well as sediment pH (*Rho* = 0.729, P > 0.05, Table 5).

All rapid indicators, as a whole dataset, were not significantly correlated with the microbial functional diversity (*Rho* = 0.211, P > 0.05). The best-correlated variables within that dataset were water pH, conductivity and depth, as well as sediment pH (*Rho* = 0.676, P = 0.02, Table 5).

Table 5 Overall correlations between the potential indicators and the resource-intensive measures of decomposition in the BEST analyses for all rapid indicators. The significant correlations is in bold.

		Combination of variables				
		Water pH	Electrical conductivity	Turbidity	Water depth	Sediment pH
Measure	*Rho*					
Wood	0.729			✓	✓	✓
Microbial	**0.676**	✓	✓			✓

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.

We hypothesised that one or more rapid measures would significantly correlate with decomposition rates and, therefore, act as a reliable indicator. This hypothesis was broadly supported by our findings, with a number of variables that were strongly correlated with decomposition rates, and broad consistency among the indicators that were identified in a second confirmatory study area. We also hypothesised that the best-correlated indicators would vary over land-use types, but our 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 Effectiveness of resource-intensive measures as functional indicators

Resource-intensive measures to assess decomposition rates have existed for many years (Latter & Howson, 1977). The wood break-down assay is a relatively 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). The significant differences found among wetlands for the wood break-down assay in this study reinforced its sensitivity to environmental conditions, and

identified relationships with water and sediment characteristics, allowed potential indicators to be identified.

Microbial community function was also found to be an effective way to assess the aerobic microbial assemblage within wetland soil and water, using commercially-available plates. 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 were differences in the microbial functional diversity among wetlands, with most carbon substrates able to be utilised in wetlands in southwestern Victoria. There was a smaller diversity of carbons able to be utilised and at a lower intensity at which they were used in South Australia, illustrating variation in microbial assemblage activity. While this measure was primarily conducted as part of the resource-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.

The final resource-intensive measure, litterbags have been used extensively in the past to determine decomposition rates within numerous lotic systems (Petersen & Cummins, 1974; Alvarez *et al.*, 2001; Moretti, Goncalves & Callisto, 2007), although few studies have used them for determination of wetland decomposition. In this study, we identified relatively high rates of handling loss of leaf litter via the procedural controls, although rates of mass loss through time were higher still. This high rate of handling loss may have been a result of drying the leaves before deployment which can increase fragmentation (Boulton & Boon, 1991). We originally hypothesized that that macroinvertebrate assemblages would be more diverse and more abundant in litterbags containing litter than in empty control litterbags, which would suggest that macroinvertebrates play a large role in the decomposition process in these wetlands. Our findings did not support this hypothesis. We found that there were no significant differences in abundances between the two treatments. This may suggest that macroinvertebrates play a reduced role in the decomposition process within these wetlands and that leaf mass loss is predominantly a result of microbial activity, although the large amounts of organic

material occurring naturally within wetlands may also have influenced the outcome (e.g. may have been more palatable). Thus, this technique was less useful as a method for measuring decomposition rates than the other methods used here.

The time and resources required to conduct these assays may also have implications for managers, as the manual labour costs involved were extensive, and thus constant monitoring of decomposition is not possible with these resource-intensive methods. So, while these techniques 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), hence confirming the need for rapid indicators of decomposition.

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.

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. For example, 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. Thus, robust benchmarking is required for similar systems in an Australian context.

4.2 Role of macroinvertebrates in decomposition

Macroinvertebrates have been studied extensively as structural indicators of ecosystem health (Feio *et al.*, 2010), but their functional role in processes such as decomposition has been less well-studied. Research has focused mainly on the extent of their role in decomposition within riverine systems (Bunn, 1988). Numerous studies have tried to correlate riverine macroinvertebrate abundances with decomposition rates but the results have been highly variable. Some studies found that macroinvertebrate abundances did indeed correlate with decomposition rates (Benstead, 1996; Iversen, 1975) whilst others found they did not correlate (Stockley, Oxford & Ormound, 1998; Dangles and Guerold, 1998). This study included the use of litterbags to investigate the role of macroinvertebrates in decomposition within wetlands. At all wetlands, the macroinvertebrate assemblages colonising litterbags were made up of taxa which are tolerant of poor water quality (Gooderham & Tsyrlin, 2002). These included chironomids (non-biting midges), amphipods, oligochaetes (worms) and ostracods (microcrustaceans). A study by Crown *et al*. (1992) in Western Australia, found similar macroinvertebrate taxon to this current study inhabiting nutrient-enriched wetlands. These taxa are known to feed on dead and decaying organic matter and contribute to the decomposition process within streams.

In this study, there were no significant differences in the abundance of macroinvertebrates between treatments, which could suggest that macroinvertebrates did not significantly contribute to decomposition within the wetlands. It could also suggest that the litterbag method may need to be

modified for use in lentic systems. The results identified an interaction between wetland and treatment. Glad's Crossing followed the expected pattern of higher abundances in bags containing leaf litter when compared to control bags. Mepunga and Cobrico Swamp exhibited the opposite pattern; abundances were greater in the control bags than in the bags containing litter. Studies which have found no correlation between macroinvertebrate abundances and decomposition suggested that litterbags could attract macroinvertebrates, as they provide shelter and/or a substratum to colonise (Webster & Simmons, 1978; Dangles, Guerold & Usseglio-Polatera, 2001). Dangles *et al.* (2001) conducted a study comparing macroinvertebrate abundances in litterbags and bags containing plastic strips. Dangles *et al*. (2001) found that the more particulate organic matter (POM) that accumulated on the bags, the higher the similarity between the litter and plastic strip treatments in terms of macroinvertebrate abundances. However, here, there was no consistent effect of the amount of organic matter accumulated on bags when this was included in the analyses as a covariate. Thus, results from this study cannot confirm that macroinvertebrates play a significant role in the decomposition of litter within wetlands.

4.3 Potential rapid indicators of decomposition

In exploring the main objective of this research, to identify a rapid indicator of decomposition, it was found that five indicators were most commonly correlated with decomposition rates: water pH; electrical conductivity; total nitrogen concentrations; the percentage of sediment less than 63 μm in size; and sediment pH. These potential indicators were broadly consistent between the initial and confirmatory study regions, although a loss of replicates in the latter (particularly for the wood break-down assay) is likely to have affected the power of the analyses, and may have masked some patterns.

Those indicators that were identified here are also broadly consistent with factors identified in previous studies as influencing decomposition rates. For example, higher nutrient concentrations have been shown to accelerate decomposition, while low pH and high salinity have been shown to inhibit

decomposition rates (Lopes *et al.*, 2011; Young *et al.*, 2008). 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) and sediment pH has been found to fluctuate with the decomposition of plant materials (Yan & Schubert, 2000; Tang & Yu, 1999). The only inconsistencies from these findings and ours was for microbial diversity, which tended to show opposite correlations with these patterns, and for electrical conductivity, where we found a positive correlation. We also found a weak negative correlation between increasing amounts of fine sediments and microbial diversity, in contrast to expectations.

Due to increasing concentrations of nitrogen, particularly in agricultural and urban wetlands (Paul, Meyer & Couch, 2006), we hypothesised that this variable might be an effective and important indicator. Total nitrogen was found to be strongly correlated with microbial community function and macroinvertebrate assemblages. Generally speaking, it was found that wetlands with high intensity utilisation of the carbon sources and more diverse and abundance macroinvertebrate assemblages had higher nutrient concentrations. This could mean that the organisms present had ideal levels of TN required to function, and/or that these microbes were capable of utilising the most complex carbons. Microbes and invertebrates 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).

As a result, some caution is needed in interpreting the relationships identified here. In another example, water pH was one of the variables identified as a potential rapid indicator. The change in pH over the sampling events was minimal and only small differences were found among most wetlands. Despite this, there was a general trend for decomposition rates to be highest for some measures in wetlands with the most neutral pH levels, such as Mepunga and Cobrico Swamp, which had the highest intensity of carbon source utilization. This suggests that aerobic microbes may have been

functioning most efficiently in wetlands at moderate pH levels. This is consistent with other research, which found the highest decomposition rates occurring in circumneutral systems (Dangles *et al.*, 2004). The wetlands assessed in this study, including those in South Australia, had relatively neutral to basic water pH levels. Further investigation into wetlands with acidic water 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 and macroinvertebrate functioning (Dangles & Chauvet, 2003, Dangles *et al.*, 2004, Niyogi, Lewis & McKnight, 2001). Such investigations may also confirm the observed pattern of higher decomposition at lower sediment pH levels, the opposite of what occurred for water pH. 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. Similar caveats exist for the relationships identified with other potential indicators (e.g. here the unexpected increase in decomposition rates with increased electrical conductivity) and thus broad-scale benchmarking would be desirable, as suggested above.

4.4 Testing consistency of identified indicators

There were no significant differences in decomposition among the six sites in the Lower Lakes as measured by either wood mass loss or microbial functional diversity. There were significant differences among the locations in their water and sediment characteristics. However, there were no significant relationships between the full suite of potential rapid indicators and wood mass loss or microbial functional diversity. The two different resource-intensive indicators of decomposition had different relationships with individual potential rapid indicators. Wood was most closely related to water turbidity and depth, and microbial to water pH and conductivity. Sediment pH was important for both intensive measures of decomposition. Thus, there were some differences in the best-correlated rapid indicators, but those that appeared were broadly consistent with the indicators identified for southwestern Victoria. This is encouraging for the use of the identified indicators more

broadly within semi-arid Australia, although additional benchmarking would again be of significant value.

4.5 Developing indicators across a range of land-use types

Agricultural and periurban 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 fertilizers (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 among 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.

4.6 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 we 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.

4.7 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 total nitrogen and pH. To ensure that pH is a suitable indicator year-round, repeat studies should be undertaken to account for seasonal variations. Testing pH and electrical conductivity across more extreme ranges of values would help to determine their versatility as rapid indicators. The initial study 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 consistency trial in the Lower Lakes was undertaken in spring and did not have such extreme temperatures but did show similar potential indicators, which is broadly encouraging.

There are also few studies which have looked at determining decomposition rates in pristine wetlands. This work should be of high priority to provide a benchmark of appropriate wetland function. Using rapid indicators of decomposition gives managers the ability to determine decomposition rates quickly and at low cost, providing information that can contribute to an evaluation of functional health. The results obtained are of little use if there is no baseline decomposition rates against which to compare.

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 pH, electrical conductivity, total nitrogen concentrations, the percentage of sediment less than 63 μm in size, and sediment pH being the most promising across the two study areas investigated. 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. However, further research is needed to determine the benchmark the ideal range of values for these indicators and to confirm their utility at broader spatial and temporal scales.

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