

Addition of Bulk Organic Matter to Acidic Pit Lakes May Facilitate Closure

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ABSTRACT

Macro nutrients (C, N, P) are essential for sustaining freshwater ecosystems. However, acidic pit lakes are often nutrient-poor as low pH levels and metal complexation (by Al, Fe and Mn) reduces dissolved P and C concentrations. In contrast, N is often not limiting as it is readily available from groundwater and blasting residues.

The Collie basin in Western Australia has a lake district of eleven acidic coal mine pit lakes (pH 3-7, low metals/metalloids and sulfate concentrations). Our previous researches at – microcosm, mesocosm and field scale demonstrated low C and P concentrations limit algal productivity. Addition of organic matter (in the form of terrestrial leaf litter) offered potential bioremediation benefits including habitat creation, sediment formation, the potential for sulfate reduction, and slow release of macronutrients to promote algal growth.

We tested the effect of organic matter additions of straw, lawn clippings and eucalypt leaves on acidic pit lake water and sediment in 1,200 L replicated mesocosms. We regularly measured water quality in each mesocosm over a year and concluded by measuring the development of macroinvertebrate communities. The highly labile C found in lawn clippings stimulated algal biomass, macroinvertebrate diversity and abundance, and improved water quality. Straw and eucalypt leaves did not alter water quality substantially but did increase abundance of macroinvertebrates.

Additions of nutrients, particularly as bulk organic matter increased macroinvertebrate abundance but also altered species composition from carnivorous highly mobile opportunists found in the controls to a range of herbivores/omnivores, while not necessarily altering water quality substantially. The presence of labile C (particularly in the grass) was able to increase algal biomass and improve pH. If conservation values are important for closure of pit lakes and eutrophication is not a risk, then addition of bulk organic matter (particularly labile forms) should be considered in closure plans. Organic matter could be actively added or passively through the catchment. As environmental improvements were seen in all treatments, adding many readily available organic matter types to pit lakes, may be helpful to achieving closure objectives associated with biodiversity.

Keywords: Macroinvertebrates, algae, ecosystem development, succession

INTRODUCTION

Natural lakes are increasingly degraded and lost through human activities. However, pit lakes resulting from the flooding of open-cut mine pits post-mining are creating new lakes (McCullough & Etten, 2011). Pit lakes can pose an environmental hazard to regional groundwater and surface waters, and nearby aquatic and terrestrial ecosystems (McCullough & Lund, 2006). Initially, pit lakes are geochemically controlled usually through acid and metalliferous drainage (AMD) from oxidation of sulfidic minerals in and around the lakes degrading water quality by increasing acidity, sulfate and metal/metalloid concentrations (Koschorreck & Tittel, 2007). Compared to natural aquatic systems, acidic pit lakes typically have greater depth, poor bankside stability, limited organic sediments, limited riparian vegetation cover and diversity, and low rate of allochthonous inputs (Van Etten, 2011; Lund *et al.*, 2013). The ecological development of pit lakes is poorly understood, but primary succession in terrestrial systems is largely driven by accumulations of organic matter and this is likely to be case in pit lakes. Poor water quality and low ecological values of acidic pit lakes may significantly reduce beneficial end use options for the lakes which might be otherwise possible.

Acidic pit lakes typically have low nutrient concentrations; especially of phosphorus and dissolved inorganic carbon, which may limit algal biomass (Nixdorf *et al.*, 2001). Although phosphorus is highly soluble under low pH conditions, it tends to co-precipitate with iron and aluminum in the water column or bind to the sediment (Kapfer, 1998). Dissolved organic carbon concentrations in the water are also often low due to limited allochthonous materials entering the lake from the catchment (Lund & McCullough, 2011). Acidic pit lakes often have adequate concentrations of nitrogen (left from blasting residue), but this nutrient predominantly exists as ammonia due to limited nitrification at low pH (Nixdorf *et al.*, 2001). Algal primary production is fundamental for lake ecological development leading to the establishment of a functional mature aquatic ecosystem (Kalin *et al.*, 2001; Lund & McCullough, 2011; McCullough & Etten, 2011). Additionally, algal biomass provides a substrate for in-lake alkalinity generation by microbes, and a binding site for metals (Dessouki *et al.*, 2005).

Carbon additions to a pit lake can also provide conditions and substrates suitable for the growth of sulfate reducing bacteria (SRB) (Frömmichen *et al.*, 2003). As SRB effectively reverse the AMD processes, they can substantially improve water quality in AMD affect pit lakes (McCullough & Lund, 2011). Furthermore, elevated metal ions may also be removed by direct sorption and complexation to internally-generated or added organic matter (Fyson *et al.*, 1998). Bioremediation by SRB activity shows some potential in the newer pit lakes in Collie which have higher sulfate concentrations (Read *et al.*, 2009). Readily available bulk organic materials such as mulch, hay, manure (cow) and sawdust have been found to be potentially useful for bioremediation in Collie (Thompson, 2000; Lund *et al.*, 2006). The comparatively low sulfate concentrations in Collie pit lakes ultimately limit the ability of SRB to improve the water quality of an entire pit lake but may produce local improvements within specific habitats within the lake (Lund *et al.*, 2006).

Closure criteria for pit lakes waters tend to focus on meeting water quality objectives for slightly disturbed environments (McCullough & Pearce, 2014). It is typically difficult to achieve such water quality closure criteria and active treatment of poor water quality is expensive and ongoing. In Collie pit lakes, there is a suggestion that additions of organic matter encourages algal growth and macroinvertebrate diversity and abundance despite little change in water quality (Lund *et al.*, 2006). We believe that with organic matter additions, there are broader opportunities for pit lakes to achieve biodiversity outcomes and ecological development trajectories to still meet closure criteria

defined by environmental values. Therefore, the aim of this project was to determine the effect of bulk organic material addition (straw, eucalypt leaves, and lawn clippings) on mine pit-lake water quality and aquatic biodiversity.

METHODS

Study site

The Collie pit-lake district is located within the Collie coal basin within the Collie River catchment. Collie township lies nearly 160 km south-southeast of Perth, and is the center of coal mining in Western Australia (Zhao *et al.*, 2009). The Collie Basin covers an area of approximately 225 km². Collie coal is a sub-bituminous coal with a relatively low sulfur content (0.3-0.9%), and low caking and ash (4-9%) properties (Le Blanc Smith, 1993). Low amounts of acidity are generated through pyrite oxidation, ferrollysis and secondary mineralization. This acidity is sufficient to generate low pH in pit lakes due to the low buffering capacity of the surrounding rock. There are currently 11 pit lakes in Collie; an overview of the entire lake district is provided in Lund *et al.* (2012). WO5H is one of the larger and more acidic lakes and was chosen as the source of water and sediment for this study.

Experimental Methods

In September 2012, SCUBA divers collected sediment from WO5H (top 0.2 m) in 20 L plastic containers at 2-3 m depth. In addition, a water tanker was used to collect ~12,000 L of WO5H water from the lake. Both water and sediment were taken to Perth within 24 h of collection and immediately transferred to mesocosms. The mesocosms were designed to mimic the littoral (<1 m deep) zone of WO5H. Twelve fiberglass mesocosms (diameter – 0.76 m and height – 0.80 m) with an individual capacity of 1,200 L were used for the experiment. Sediment was first added to all the mesocosms to a depth of 100 mm, followed by the WO5H water. Utilizing a randomized block design, three replicates of four treatments were randomly assigned. The treatments consisted of no additions (control), and additions of straw, eucalypt leaves and grass clippings. Grass clippings were obtained locally from a lawn mowing contractor, eucalypt leaves (*Eucalyptus* sp.) were obtained at Edith Cowan University, Joondalup campus during tree pruning operations, and straw was obtained from a pet supplier. All materials were allowed to dry in the sun for 2–3 days, before 3 kg was weighed and added to the appropriate mesocosm. This resulted in a layer approximately 50 mm deep for grass, and 0.3–0.4 m for eucalypt and straw across the bottom of the mesocosms (Figure 1). The mesocosm water volume was maintained at approximately 950 L, by rainfall and additions of reverse osmosis water to control for evaporation.

The mesocosms were monitored for changes in physico-chemical parameters initially every week for 6 months, then monthly until day 210, then at 3–4 month intervals for a total experimental duration of 421 days (18/9/2012–13/11/2013). A Datasonde 4a multi-parameter probe (Hydrolab, USA) was used to measure water temperature, pH, oxidation reduction potential (ORP), electrical conductivity (EC), dissolved oxygen saturation (DO), chlorophyll *a* and turbidity. Water samples were collected on five occasions (days 1, 38, 71, 113 and 421) from the surface (0.1 m deep) of each mesocosm. An aliquot of the water sample was filtered through 0.5 µm filter paper (Metrigard, Pall, USA), part of the filtered samples were acidified using analytical grade nitric acid (to achieve a pH of <2, approximately 1% w/v) for metal analysis (Al, Ca, Co, Cu, Fe, K, Mg, Mn, Na, Ni, and Zn) on

ICP–AES (Varian Vista-Pro, USA). Anions (SO_4^{2-} and Cl^-) were analyzed on filtered samples using ion chromatography (Metrohm 761 Compact, Switzerland). Ammonia/ammonium ($\text{NH}_4\text{-N}$), $\text{NO}_x\text{-N}$ (nitrate and nitrite) and filterable reactive phosphorus (FRP-P) in filtered and total nitrogen (TN-N) and total phosphorus (TP-P) on unfiltered samples were analyzed on an auto-analyzer (Skalar, USA). Dissolved organic carbon (DOC) was measured as non purgeable organic carbon and total organic carbon (TOC) in filtered samples using a TOC analyzer (Schimadzu TOC–V CSH, Japan). All analysis followed methods in APHA (1999).

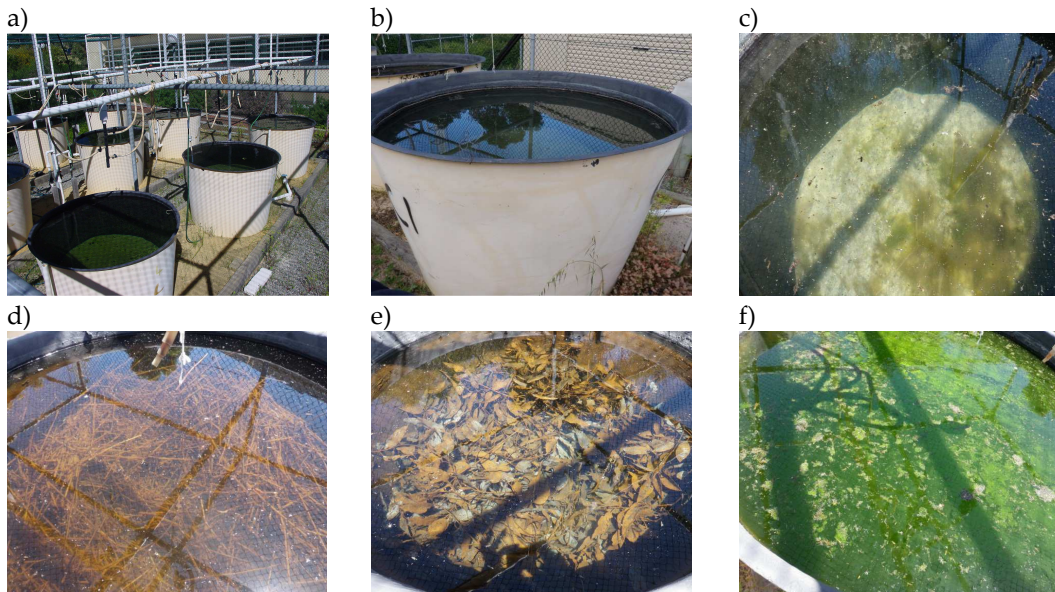


Figure 1. Photographs of the mesocosms in October 2012, showing a) experimental setup b) individual mesocosm, c) control, d) straw, e) eucalypt leaves and f) lawn clippings.

To avoid major disruption to the mesocosms, two surface small sweeps of the mesocosms for collection of macroinvertebrates were undertaken on days 38 and 86 using a small aquarium net (~500 μm mesh, 0.1 x 0.15 m). This was followed on day 421 by a sweep covering as much of each mesocosm as possible with a (0.22 x 0.22 m, 500 μm mesh) sweep net. Samples were preserved in 70% ethanol, sorted using a Bogarov trap and then counted and identified to broad taxonomic groups under a Olympus SZ-STU2 stereo microscope.

Water quality data from five water sampling occasions (days 1, 38, 71, 113 and 421), was ordinated using principal components analysis (PCA) in PRIMER (v6). We used a two-way ANOSIM on normalized data to determine if water quality was significantly different among treatments and times (excluding day 1, just after the treatments were added). We then analyzed treatments just for day 1 to establish that initial water quality parameters were similar across all mesocosms, regardless of treatment. Analyzed parameters included only those where more than half of the samples were above the detection limit – any values below detection were replaced with half the detection limit. Missing data were replaced by the average of any other data for that specific time and treatment, and auto-correlated parameters were reduced to a single parameter.

RESULTS AND DISCUSSION

Water Quality

At the beginning of the experiment (day 1), water quality among mesocosms was not significantly different (Global $R = 0.145$, $P > 0.05$), regardless of treatment (Figure 2). After day 1, treatments were significantly different overall (Global $R=0.714$, $P<0.05$) and between all treatment pairs (all $P < 0.05$, although straw x eucalypt $R = 0.296$, $P = 0.04$). Throughout the experiment, water quality among controls remained close to day 1 samples, with a slight divergence at the end of the experiment in day 71 (Figure 2). This shift is probably due to dilution by rainfall. The treatments separated from the controls on days 38 and 71, where grass generally was different to the other two treatments. On days 71 and 113, the treatments were similar to each other but distinct from the controls.

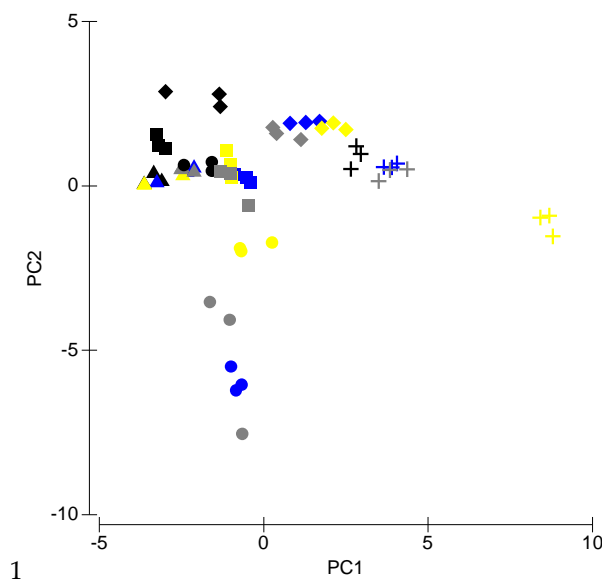


Figure 2. PCA of water quality data in mesocosms with acidic mine water over 14 months of incubation with bulk additions of carbon, 33.3% of the variability is associated with the first principal component axis (PC1) and 17% with PC2. *A priori* groups are time (by symbol) and treatment (by color). ▲ day 1, ● day 38, ■ day 71, ◆ day 113, + day 421, black = control, blue = straw, grey = eucalypt and yellow = grass

There were significant differences (Global $R=0.877$, $P<0.01$) between all sampling times (excluding day 1). Temperature of the mesocosms followed seasonal trends peaking at just over 30 °C (summer) and dropping to just less than 16 °C (winter). There were no important differences in temperature between any of the mesocosms. No evidence was seen of stratification in the mesocosms, presumably due to the shallow depth and wind driven mixing. Conductivity (mean±SE, 1.5 ± 0.01 mS cm⁻¹) follows a similar seasonal trend to temperature, reflecting largely evapoconcentration effects in summer (days 74-163) and dilution by rainfall in winter (days 256–347). Two notable trends were a slight increase in conductivity (of ~100 mS cm⁻¹) in the eucalypt treatment on days 60 and 64, and the lower conductivity from day 22 onwards in the grass treatment (of ~100 mS cm⁻¹) compared to the control and other treatments. No water samples were taken around days 60 and 64 so it is unclear what triggered the increase in conductivity for eucalypt leaves. The consistently lower conductivity seen for grass suggests binding of ions to leaves or ion precipitation may have been responsible.

The control remained aerobic (>60% saturation) throughout the experiment, but by day 20 dissolved oxygen in all treatments was less than 30% saturation (although grass by day 40 had started to return back to control levels). All treatments had returned to levels of dissolved oxygen similar to the controls after day 200. Biological oxygen demand caused by decomposition of the added organic matter was most likely responsible for reduced oxygen levels. Higher chlorophyll *a* concentrations in the grass-treated mesocosms are presumed to have increased oxygen concentrations as a result of photosynthesis. ORP follows the initial decline observed in dissolved oxygen for all the treatments, however by day 60 ORP in all treatments had returned to levels seen in the controls. On day 22, the mesocosms with straw had ORP of <-400 mV, the level at which methanogenesis (methane production) is possible, although by day 29 it had increased to >-103 mv.

pH was very stable at just over 3 for the entire experiment in eucalypt, straw and control treatments (Figure 3). Our previous mesocosm experiments using Collie pit-lake water have found pH has increased to circum-neutral in controls over the course of the experiment. At the time, we hypothesized that groundwater may be responsible for maintaining pH stability *in situ* (see Salmon *et al.*, 2008; Kumar *et al.*, 2011a). The current study used sediments collected from greater depths, 2-3 m compared to <1 m in the other studies, which may be more representative of lake sediment. It is possible that sediment processes in these sediments may be responsible for pH regulation (as per Peine *et al.*, 2000). Alternatively, the difference could be related to which lake the sediment was taken from i.e. WO5H compared to Lake Kerpwari.

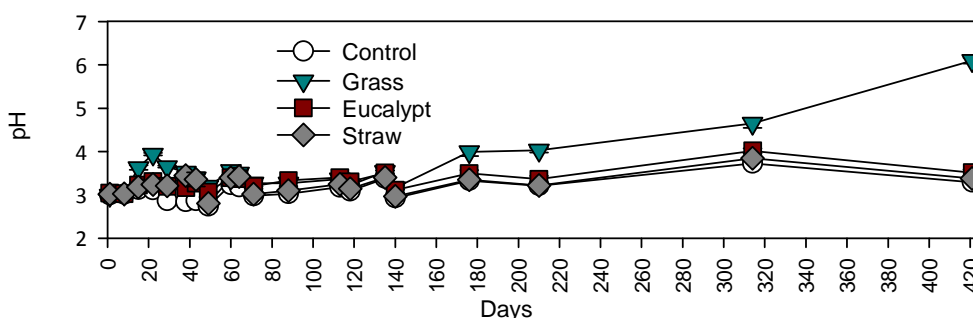


Figure 3. Mean (± SE) pH found in experimental mesocosms containing acidic mine-lake water collected from days 1 to 421.

Chlorophyll *a* concentrations were very low in the controls at <3 µg L⁻¹. Grass stimulated chlorophyll *a* production peaking at 332 µg L⁻¹ more than the other treatments; however eucalypt and straw also increased chlorophyll *a* slightly between days 60 and 140 compared to the control. It is likely that alkalinity generation by algae was partially responsible for the lawn clippings after day 140 increasing pH to >6 by the end of the experiment.

Variation in Mn, K, Na, Mg, and Ca concentrations were minimal among treatments but declined slightly over the course of the experiment likely due to dilution with rainwater (Figure 4). Concentrations of Al, Co, Ni and Zn dropped by an average of >40% over the experiment in all treatments and control, although declines were lowest in the control and highest for the grass treatment (>95%). Copper was low in all treatments (mean = 0.755 mg L⁻¹) but appeared to increase slightly over the experiment in the control (peak = 5.5 mg L⁻¹), the most likely source being release from the sediments. On day 38, Fe concentrations decreased for both controls and grass (mean = 0.9 mg L⁻¹) from an initial mean of 3.4 mg L⁻¹, and increased for eucalypt and straw (mean = 18.7 mg L⁻¹). By day 71, Fe concentrations were <1 mg L⁻¹ in all treatments and control. The day 38 peaks are

not easily explained by ORP/anoxia induced release from the sediment and this suggests that the Fe might have been released directly from the eucalypt leaves and straw in these treatments.

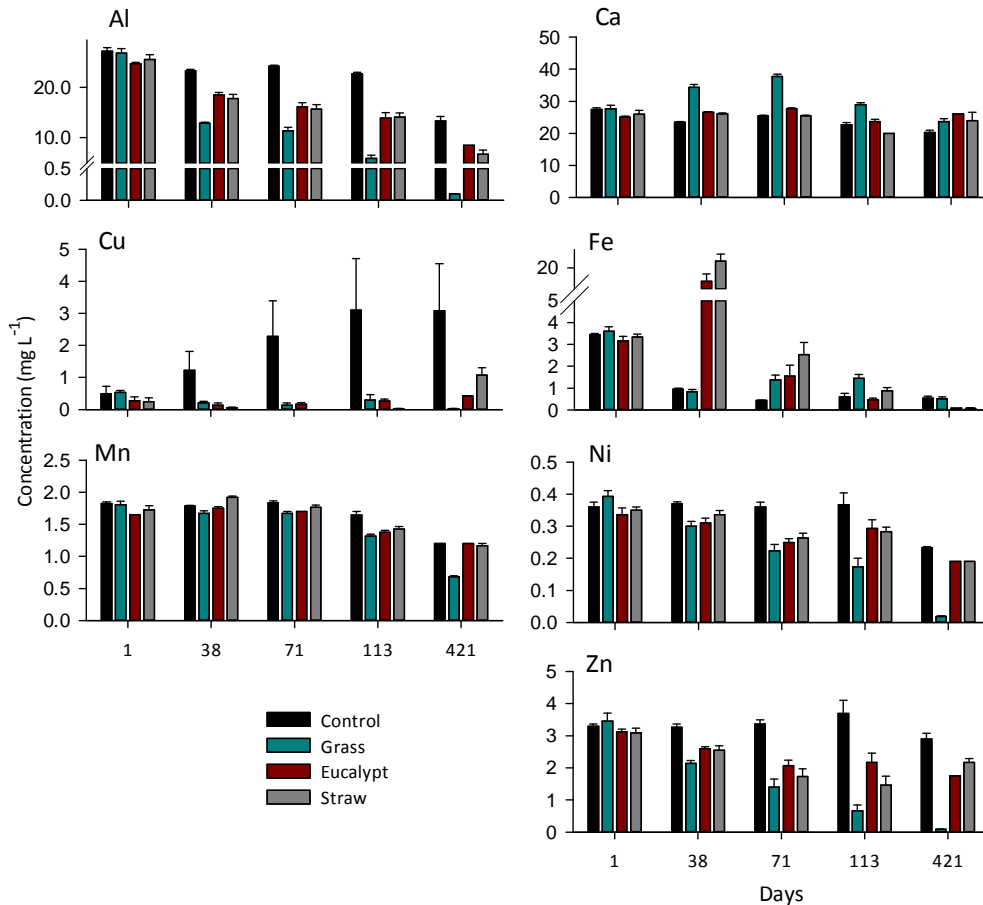


Figure 4. Mean (\pm SE) concentrations (mg L^{-1}) of select metals found in experimental mesocosms containing acidic mine-lake water collected from days 1 to 421.

Nitrification of ammonia to nitrate does not occur below pH 3 (Jeschke *et al.*, 2013) and initially the mesocosms were at pH 3. On day 1, $\text{NH}_4\text{-N}$ was present at a mean concentration of $517 \mu\text{g L}^{-1}$, with $\text{NO}_x\text{-N}$ concentrations at $68 \mu\text{g L}^{-1}$. Ammonification from the likely rapid breakdown of the grass produced exceptional high $\text{NH}_4\text{-N}$ concentrations in the grass treatment in days 38 and 71 exceeding $6000 \mu\text{g L}^{-1}$ (Figure 5). Over the course of the experiment, slight increases in pH appear to have allowed for nitrification of ammonia to NO_x , evidenced by the overall decline in mean $\text{NH}_4\text{-N}$ concentration to $69 \mu\text{g L}^{-1}$ and slight increase in NO_x to $88 \mu\text{g L}^{-1}$ with differences between treatments probably dependent on algal and possible sediment uptake. Kumar *et al.* (2011b) has shown that algal growth in Collie pit lakes is limited primarily by P availability. Although bulk organic matter is not typically rich in P, it does provide a potential source. Initially in the mesocosms FRP concentrations were $<14 \mu\text{g L}^{-1}$ and total P was below the detection limit of $20 \mu\text{g L}^{-1}$. FRP remained very low throughout the experiment ($<20 \mu\text{g L}^{-1}$) with the exception of a mean of $63 \mu\text{g L}^{-1}$ recorded for eucalypt on day 38. As the experiment progressed, total P increased above detection limits for all treatments, however on days 71 and 113, reached over $3500 \mu\text{g L}^{-1}$ in the

grass treatments. As FRP concentrations in the grass treatments remained low, it is likely that algal biomass was responsible for the high total P values. The apparent ability of grass to release P is an important additional benefit of using this material within a pit lake. However, the decline in total P on day 421 to near start conditions, suggests that P release from the organic material was exhausted. The highest peak concentrations of DOC and TOC were from eucalypt and straw (mean of 30 and 40 mg L⁻¹ for TOC respectively) on day 38, however these quickly dropped to <10 mg L⁻¹, with grass having the highest overall concentrations of DOC and TOC (mean 3.4 and 8.0 mg L⁻¹ respectively) after day 38.

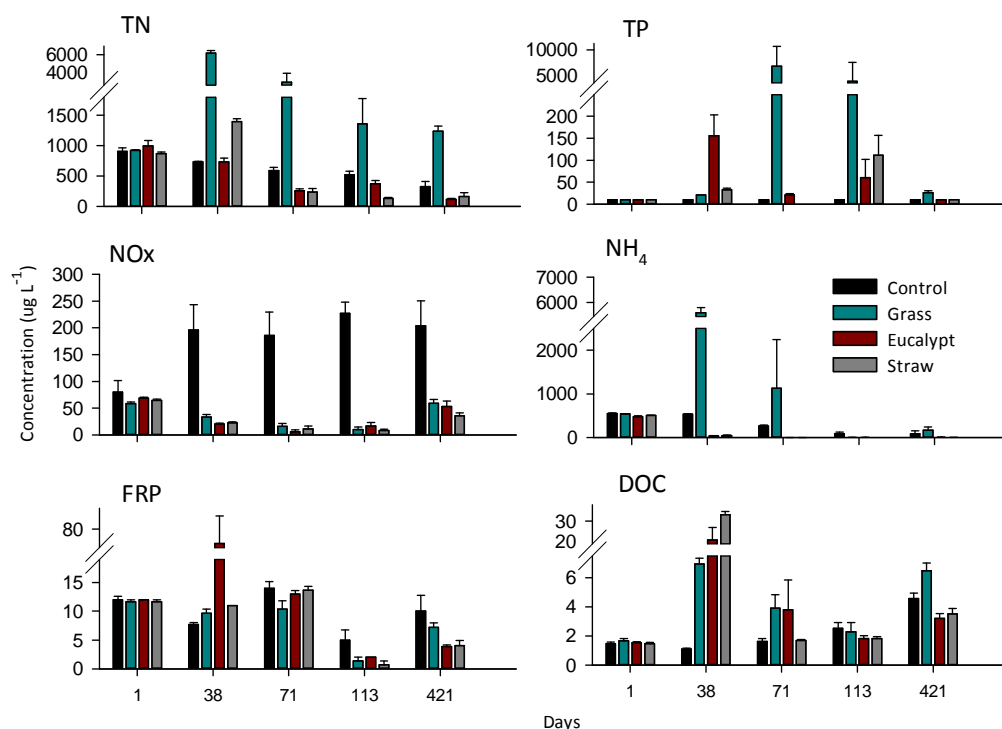


Figure 5. Mean (± SE) concentrations of nutrients in experimental mesocosms for days 1 to 421.

Chloride concentrations were consistent between treatments and as a conservative ion appeared to only change in relation to evapoconcentration and dilution by rainfall (Figure 6). Sulfate concentrations declined in all treatments and the control from mean of 830 μg L⁻¹ on day 1, with the control only showing a slight decline (mean 422 mg L⁻¹) and the highest declines seen for grass (mean 112 mg L⁻¹) on day 421 (Figure 6). This suggests that sulfate reduction/secondary mineralization was occurring in all the mesocosms (Triantafyllidis & Skarpelis, 2005), but adding C enhanced SRB activity and that the more labile the C the stronger the effect. Algae were observed growing in all of the mesocosms, which would have contributed labile carbon to the sediment. The grass treatment produced a phytoplankton bloom, measured as chlorophyll *a*, but the controls only produced benthic algae.

Biota

On day 38, all the mesocosms were dominated at the surface by mosquito larvae (Culicidae), with similar abundances and species. By day 86, saw the mosquito larvae replaced by Chironomidae larvae and pupae and Ceratopogonidae larvae and pupae with the occasional Coleoptera adult. All

mesocosms were very similar in species and abundance, although this may be an artifact of the limited sampling that was possible at this time. At the end of the control contained a large proportion of Coleoptera and mites (~40%) compared to the treatments (<2%). The presence of these hardy predatory species is common in pit lakes (Proctor & Grigg, 2006). Eucalypt and straw in contrast were dominated by Chironomidae (>95%), which are also hardy and common to many pit lakes. Grass was different with approximately 15% of the taxa recorded being Culicidae. Total abundance of macroinvertebrates increased with the treatments, with increases over the control of four fold, eight fold and 48 fold for eucalypt, straw and grass respectively. All the taxa collected were most likely colonists from nearby water bodies rather than introduced with the organic material or present in the water or sediment.

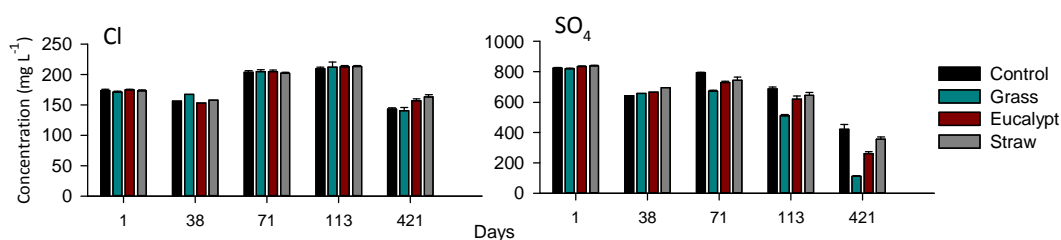


Figure 2. Mean (\pm SE) concentrations of Cl and SO₄ in experimental mesocosms from days 1 to 421.

CONCLUSIONS

Adding organic matter to highly acidic pit lake waters such as found at WO5H, resulted in no change in pH using more refractory C sources, but subtle improvements in other measured water quality parameters. Some of this improvement is likely due to secondary mineralisation and the sulfate reduction by SRBs. The more labile C produced by the grass treatment ultimately had the biggest impact on water quality and increased pH to 6. The grass also increased macroinvertebrate abundance. Although the taxa diversity (at this low level of taxonomic resolution) does not appear to have increased significantly in any of the treatments; there was a shift away from the predator dominated taxa seen in the control to more herbivorous/omnivorous taxa. Abundance of taxa also increased substantially in the treatments, particularly for the grass. These results suggest that when organic matter is added to pit lakes, more labile carbon sources may provide greater environmental value improvements. However, the results suggest that even refractory C sources such as straw have at least some environmental benefits. Although the quantities of organic material used in this study were very high (approximately 6 kg m⁻² dry material) compared to what might be achievable in the field, benefits are still likely at lower levels. As these organic matter additions are targeting biodiversity rather than treatment of water quality per se, restricting the material to the littoral zone would likely achieve positive benefits without requiring unrealistic quantities of material. Using pit lake catchments to help supply organic matter to the lakes (see Lund *et al.*, 2013) may be one way to achieve sufficient inputs of carbon over long term to compensate for biotic/abiotic losses. This increasing carbon enrichment may allow even an acidic pit lake to develop ecological values, as an alternative to water quality-driven closure objectives.

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