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Managing the acidity of abandoned water filled coal mining voids in Collie (Western Australia) using organic matter

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USE OF THESIS

MANAGING THE ACIDITY OF ABANDONED WATER FILLED COAL MINING VOIDS IN COLLIE (WESTERN AUSTRALIA) USING ORGANIC MATTER

by

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Award of Master of Science (Environmental Management).

At the Faculty of Communication, Health and Science, Edith Cowan University, Joondalup.

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ABSTRACT

The effectiveness of using organic matter additions to increase pH in abandoned water filled coal mining voids in Collie was investigated. Previous studies have demonstrated that passing acidic drainage through organic matter increased the waters pH. Laboratory trials using intact sediment cores (collected from Ewington, Collie, Western Australia) were used to assess the effects of additions of hay, manure and mulched vegetation on pH, nutrients an sulphate levels. In a series of experiments the effect of different quantities of organic material, sulphate reducing bacterial inoculations and alkalinity generation were measured. A subsequent field experiment was conducted to examine the impact that manure and mulch had on mine void water in 18 *in situ* ponds constructed adjacent to Ewington.

It was concluded from the laboratory experiments that mulch and manure treatments were significantly better than hay as an organic matter addition for increasing the pH of acidified mine void water at Ewington. The manure produced the greatest increase in pH, although it contributed less to alkalinity than mulch; suggesting mulch has a greater ability to release carbonates than did manure. The manure and mulch additions increased the pH in the laboratory and field experiment by 0.5-1.5 pH units. This increase was sustained for most of the 21 week field experiment. An increase in pH in the control ponds was recorded over the last 8 weeks of the experiment which was probably due to the inflow of more alkaline groundwater through the substratum as a consequence of the winter rainfall. pH values measured in the experimental ponds 15 months after the commencement of the project indicated that the ponds treated with manure

maintained significantly higher pH levels than either the mulch or the control ponds. There was no difference between the pH values for the ponds treated with mulch and the controls suggesting that manure not only provided a greater increase in pH but also over a longer period.

The addition of organic matter also resulted in an increase in gilvin in the laboratory experiments. Low sulphate and sulphide levels were also recorded in all experiments before and after the introduction of organic materials into mine void water and ponds adjacent to Ewington indicating that sulphate reducing bacterial activity was not the cause for the increase in pH as occurred in other situations, but rather the addition of alkaline organic matter caused the increase in pH.

The addition of manure organic material was associated with an increase in the ortho-phosphate levels, resulting in an increase in chlorophyll *a* concentrations. This is believed to be the first stages of succession processes leading to the establishment of a biologically active wetland system. During this process the emerging ecosystem neutralises the acidic content of the water.

DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education; and that to the best of my knowledge and belief contain any material previously published or written by another person except where due reference is made in the text; or contain any defamatory material.

Signature [redacted]
Date 4-8-2000

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1.0 INTRODUCTION

Acid mine drainage or acid water pollution of mine sites is potentially the largest negative environmental impact resulting from coal mining (Harries, 1998a; Harries, 1998b; Lowson, Reedy and Beatie, 1993; Robertson, 1987; Ryan and Joyce, 1991). Well publicised examples of the problems caused by acid mine drainage in coal and other mineral mines within Australia include Rum Jungle in the Northern Territory, Captains Flat in New South Wales, Brukunga in South Australia, Mount Lyell in Tasmania and Mount Morgan in Queensland (Lawton, 1996; Zhou, 1994). The estimated operational cost for managing acid wastes in the Australian mining industry is $60 million per year (Harries, 1998a).

Rehabilitating a mine site is a major cost for mining companies. As an example of the scope of the rehabilitation costs, Western Main site near Lithgow in New South Wales spent 30% of the mine's profit on rehabilitation (Lawson, *et al.*, 1995). The initial poor condition of the mine was the result of over 50 years of mining that paid little regard for the environment. In late 1989, the pH of a drain at the pit top was 3.1, significantly below the required discharge level (Lawson *et al.,* 1995). Further investigations of the colliery revealed that the acid mine drainage was widespread with pH levels as low as 2.4 (Lawson *et al.,* 1995). The rehabilitation solution adopted was complex and involved a combination of different strategies, including constructing limestone weirs, underground pumping, decommissioning of surface tailings ponds, removal of surface reject coal stockpiles, isolation of known acid mine drainage 'hotspots' and community involvement with revegetation (Lawson *et al.,* 1995). Another example is the rehabilitating of the Rum Jungle Mine site in the Northern Territory. Between 1983 and 1986 the mine was rehabilitated by covering the waste rock dumps, relocating the tailing and heap leach material, treating water in the open-cuts, and redirecting surface flows at a total cost of $18.6 million (1986 dollars; Harries, 1996). At the Mount Lyell mine in Tasmania, the Office of the Supervising Scientist and the Tasmanian Department of Environment and Land Management jointly sponsored a $2 million remediation research and development program to identify cost effective remediation options to address the acid mine drainage problem (Needham and McBride, 1998; Waggitt and Jones, 1995).

In Western Australia, there are many procedures that need to be completed before any mining operation is allowed to commence. The major environmental component of this process is the drafting of a *Notice of Intent* that is submitted to the Department of Minerals and Energy for approval. This procedure includes obtaining approvals from the Department of Conservation and Land Management (flora and fauna requirements), the Water and Rivers Commission, the Department of Environmental Protection, the Aboriginal Affairs Department, and finally the Department of Minerals and Energy. The process of obtaining a mining lease in Western Australia is long and detailed, and explained in the "Guidelines to help you get Environmental Approval for Mining Projects in Western Australia" (*Dept. Minerals and Energy*, 1998).

Once a mining lease is approved the company must provide an unconditional performance bond. This "is a contract between the Minister for Mines and a third party of financial standing acceptable to the Minister, providing for the third party to unconditionally pay the agreed sum to the Minister on his request following the failure of the tenement holder to meet the previously agreed environmental commitments" (*Dept. Minerals and Energy*, 1998, p. 52). The bond is intended to give the State recourse to funds so that rehabilitation works can be undertaken on a mining tenement to meet the requirements of the environmental conditions placed on a tenement. This bond is normally between $5 000 and $15 000 per hectare depending on the level of impact. The bond covers all land required to be rehabilitated including waste dumps, tailings disposal facilities, stockpile areas, backfilled pits, hardstand areas, plant sites, haul roads, airstrips, accommodation areas and the safety zone around any abandoned open pit (*Dept. Minerals and Energy*, 1998). The bond is reassessed each year and adjusted on the basis of the potential rehabilitation that is reasonably expected. The magnitude of the rehabilitation costs together with the rehabilitation bond makes mining companies interested in long-term, cost effective rehabilitation solutions. Acid mine drainage is one such problem that requires a cost effective solution.

Acid mine drainage is defined as acidic drainage that occurs as a result of oxidation of in sulphidic rock exposed to air and water (Lowson *et al.*, 1993; Mills, 1997a). Acid mine drainage can emanate from surface and underground mine workings, waste and development rock, tailing piles and ponds (Comarmond, 1998; Mills, 1993). Acidic drainage occurs in many types of mining operations. The term acid mine drainage is often inappropriate since the problem is more frequently pollution of ground water coming from above ground deposits of mine wastes such as tailings and waste rock, rather than surface drainage (Ritchie, 1992). Acid mine drainage is typically the acidification of watercourses, but can also affect lakes and abandoned mine voids.

Acid mine drainage in Australia

Only limited research has been conducted in Australia on treating acid mine drainage. Most research has been conducted in the temperate zones of the northern United States (Tyrrell, 1996). Australia has diverse climatic conditions, (ranging from arid, to mediterranean and to monsoonal) not found in temperate northern United States. The cyclical wetting and drying pattern that is typical of northern Australia provides an environment suitable for cyclic oxidation and reduction. The acid drainage created during the drier months is more likely to be moved to adjacent areas during the high precipitation months. This weather pattern is seen at the Pine Creek goldfields site in the Northern Territory. Pine Creek has a dry season from April to October followed by a wet season from November to March. The wet season is typified by intense storms and monsoons; the average annual rainfall is 1147mm (Fawcett and Sinclair, 1996). In contrast Mount Lyell in Western Tasmania receives between 2500 and 3000 mm of precipitation annually (Robertson, *et al.*, 1997), with some precipitation on an annual average of 240 days (Johnston, *et al.*, 1997). At Mount Lyell the evaporation may exceed precipitation for only one or two months in each year, with excess water being lost to groundwater or surface water run off (Johnston, *et al.*, 1996). These high precipitation conditions create a high potential for sustained sulphidic run off. Northern United States remediation techniques like wastewater wetlands therefore perform differently in Australia, especially in low and sporadic rainfall conditions (Miedecke, 1989).

The arid to semi-arid climate and flat topography of much of Western Australia make it unlikely that the widespread acid drainage problems seen overseas or in the more temperate or tropical regions of Australia will develop here (Tyrell, 1996). The lack of water to transport contaminants means that impacts are likely to be localised, affecting only the site reclamation success, surrounding native vegetation and groundwater supplies (Tyrell, 1996). These impacts can, however, be severe and persistent. The limited annual rainfall and extensive periods of drought coupled with rare major rainfall events also mean that problems will take longer to develop, with the consequence that problem sites may not be recognised for decades (Biggs, 1998). Drying and wetting cycles, which are likely to occur in low rainfall areas, hamper acid and heavy metal removal processes. The potential for retained elements to be remobilised during a wet season after a long dry spell is also currently unknown (Tyrrell, 1996).

The variety of climatic conditions within Australia poses many potentially unique problems and little research has been done to develop ecologically sustainable solutions to acid wastewater resulting from mine site disturbances.

Coal mining

Coal is formed by the accumulation, alteration and compaction of plant remains in an anoxic reducing environment commonly found in swamps during the Carboniferous period (Plummer and McGeary, 1996; Skousen, 1995). Burial by sediment compresses the accumulated plant material, gradually driving out any water and volatile compounds. The coal changes from brown to the black form as the proportion of carbon in it increases (Plummer and McGeary, 1996).

Associated with coal formation is the mineralisation of pyrites and the accumulation of heavy metals. Pyrite or iron disulphide (FeS_2) is created when hydrogen sulphide (H_2S), formed by anaerobic bacterial reduction of sulphate anions in the water, combines with ferrous ions (Fe^{2+}). This process is commonly associated with coal deposits as the anoxic conditions favouring coal formation also favour pyrite mineralisation (Broughton and Robertson, 1992; Leeder, 1982).

Coal is mined by open-cut or underground shaft mining. In both types of mining large dumps of overburden (unwanted material), spoils and tailings, produced by mining processing, are removed leaving voids. Early open-cut and below ground coal mining operations in Australia (1850s - 60s) saw overburden dumped in areas surrounding the pit. This dumping tended to invert the soil profile causing the deeper soil strata to be placed on the surface. This deep soil that had previously been below the water table was then exposed to the air, exposing pyrite to oxygen, which, in the presence of water results in the release of sulphate ions and hydrogen ions (Chapman, 1994). It is still common practise to dump overburden near the mine pits. However, modern management techniques examine the acid generating properties of the soil and it is relocated accordingly to minimise acid drainage.

As mines increased in size, it became common practice not to backfill voids because of cost (Stedman, 1988). When the open-cut mining and dewatering operations ceased, the mining voids filled with water to the level of the water table, creating artificial wetlands (Stedman, 1988).

Brief history of Collie coal mining

Since the 1900s Collie coal has played a vital role in the economic development of Western Australia (Stedman, 1988). In the 75 years prior to 1965, gold and coal were the only significant minerals produced in Western Australia (Stedman, 1988). Coal was the major energy source used in the early development of Western Australia.

Early settlers realised the importance of coal and the Governor of the Swan River Settlement offered a reward in 1839 of 2560 acres of land for the location of "any considerable bed of coal" (Stedman, 1988, p. 2). Seven years later, the explorer A.C. Gregory claimed the reward after discovering the Irwin River coal seam 200 miles north of Perth. Other discoveries followed but there were few purchasers for the low grade coal and interest waned (Stedman, 1988). It was not until the 1880s and 1890s when there was a revival of interest in coal due to the increasing number of steamships arriving at Western Australian ports and the expansion of the Western Australian railroads. This led to the State Governor offering a £1000 reward for the discovery of a viable coal field within 50 miles of a declared port.

It is thought that the original discovery of the Collie river coal deposits in 1883 was by a stockman named George Marsh (Stedman, 1988). He picked up several dark coloured stones from the edge of a river pool and placed the stones on either side of his fire to support cooking utensils. Much to his surprise the 'stones' caught alight.

Trials using the coal were undertaken leading to the Engineer in Chief for the State C. Y. O'Connor, being enthusiastic regarding the potential of Collie coal. Over 1000 tonnes of Collie coal were mined by the end of 1894 (Stedman, 1988).

Initially all mining in the Collie basin was underground. The first open-cut coal mining operation was at Stockton, which was developed during World War II to supplement production from underground mines. There have since been 10 other open-cut coal mines opened in the Collie basin (Western Australian Forests Department, 1983; Stedman, 1988). In 1983, open-cut methods produced 80% of the coal in the Collie Coal Basin (Western Australian Forests Department, 1983).

During the 1940s and 1950s, when coal production rapidly increased at Collie, three major companies (Griffin Coal Mining Company, Western Collieries Ltd and Amalgamated Collieries Ltd.) operated open-cut and underground coal mines. At the end of 1960, Amalgamated Collieries ceased operation due to a dispute with the government (Stedman, 1988). At the time there was no requirement for them to undertake environmental rehabilitation of their mining areas.

There are five areas that Amalgamated Collieries mined using the open-cut method. They were Stockton (1943-1957), Wallsend (1946-1948), Black Diamond (1948-1953), Ewington No. 1 (1952-1959) and Ewington No. 2 (1960) (Stedman, 1988). The proximity of Wallsend to Collie meant the mining void could and was used as a rubbish dump after mining. It was later filled and landscaped to become a sports field (Stedman, 1988). The Black Diamond pit was divided into two lakes (Black Diamond A and Black Diamond B) with overburden from the western part of the pit (Stedman, 1988) resulting in one large and one small void. The smaller void is a shallow wetland while the larger void (Black Diamond) is a local recreation and swimming location. Stockton was allowed to fill and is used for water skiing. The two Ewington voids are located on private property. The larger of the two voids is commonly called Bluewaters (Ewington Open Cut No 1) and is a large lake up to 20 metres deep. The smaller void, simply is called Ewington (Ewington Open Cut No 2), is on average only 6 metres deep, with a maximum depth of approximately 11 m.

Chemistry of acid mine drainage

Acid mine drainage is the product of a chemical reaction between water (H_2O), oxygen (O_2), carbon dioxide (CO_2), and the relatively common iron-sulphur minerals, pyrite (FeS_2) and pyrrhotite (FeS), in the presence of bacteria (Mills, 1997a).

The process of acidification of water in mining voids has been described by numerous workers (Broughton and Robertson, 1992; Durkin and Herrman, 1994; Mills, 1997a, b) and it is a time dependant process controlled primarily by the:

- presence and nature of active sulphides,
- availability of water,
- availability of oxygen (convection and diffusion),
- iron oxidising bacterial action,
- surrounding temperature and pressure,
- pH or presence of base alkaline reactants (bacteria),
- physical characteristics of the substrate such as particle size, waste permeability, etc; and,
- surrounding biological factors.

(Comarmond, 1998; Fague and Mostyn, 1997; Mills, 1993, 1997a; Zhou, 1994)

The following four chemical equations demonstrate the processes:

In Equation 1.1, hydrogen ions (H^+), ferrous ions (Fe^{2+}) and sulphate ions ($SO_4{}^{2-}$) are produced by oxidation of pyrite.

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4{}^{2-} + 4H^+ \quad \textbf{(Equation 1.1)}$$

Ferrous ions (Equation 1.1) are then oxidised to form ferric ions (Fe^{3+}).

$$4Fe^{2+} + O_2 + 4H^+ \text{ (bacteria)} \rightarrow 4Fe^{3+} + 2H_2O \qquad \textbf{(Equation 1.2)}$$

Ferric ions (Equation 1.2) can then either be hydrolysed and form ferric hydroxide ($Fe(OH)_3$) and acidity (H^+; as shown in Equation 1.3), or the ferric ions can react with pyrite and act as an oxidant in generating greater amounts of ferrous ions, sulphate and acidity (as shown in Equation 1.4; Skousen, 1995). The rate limiting step in the overall process is the oxidation in solution of ferrous ion to ferric ion (Equation 1.2), whereas, the sulphide oxidation is relatively rapid (Skousen, 1995). However, iron oxidising bacteria greatly enhance and accelerate iron oxidisation which, in-turn, greatly speeds acid generation (Skousen, 1995). When the pH of water falls below about 4.5, sulphide dissolution becomes markedly faster; typically by five to one million times faster (Gray, *et al.*, 1990). This is due to the action of naturally occurring, acidophilic chemolithotrophic bacteria, which accelerate the oxidation of sulphides, sulphur or ferrous ion (Shuttleworth and Unz, 1988). They provide biochemically mediated reaction paths for the oxidation processes, with lower activation energies than the abiotic paths. This enables the bacteria to utilise the energy released by the oxidation process (Gray *et al.*, 1990). The most common of these bacteria are *Thiobacillus ferrooxidans* and *T. thiooxidans*, although numerous other bacteria have been isolated from mine waters (Gray *et al.*, 1990). While these bacteria are not catalysts by definition, they do act as accelerating agents if their habitat conditions are at, or close to, optimal and they are an important factor in the generation of acid mine drainage (Mills, 1997b). While bacteria can accelerate acid formation, the net effect of this oxidation of pyrites is the formation of two hydrogen ions for every mole of sulphate formed (King, *et al.*, 1974).

Once weathering has produced ferric ions, the ions can rapidly oxidise pyrite (Equation 1.4). Therefore, ferric ions cannot persist in the presence of pyritic minerals (Wilderman and Laudon, 1989).

$$Fe^{3+} + 3H_20 \rightarrow Fe(OH)_{3\,(s)} + 3H^+ \qquad \textbf{(Equation 1.3)}$$

$$14Fe^{3+} + FeS_2 + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \ \textbf{(Equation 1.4)}$$

(Gray, *et al.*, 1990; Skousen, 1995)

The natural pyrite ore has three well-defined morphologies; cubic pyrite, orthorhombic marcasite and framboidal amorphous material. These and other parameters such as the surface of the pyrite crystal having a passivating surface film and the pyrite having a variable elemental composition from $FeS_{2.00}$ to $FeS_{1.94}$ increase the complexity of the oxidation process (Lowson *et al.*, 1993).

If any of the processes represented by the equations above (1.1 – 1.4) were slowed or stopped, the generation of acid mine drainage would also slow or cease. Removal of air and/or water from the process, two of the three principal reactants, would stop pyrite from oxidising as occurs in nature. When pyrite is found in rock materials beneath the water table where oxidising conditions are limited, the pyrite remains unreacted. Only small amounts of pyrite are oxidised through natural weathering, thereby generating only small amounts of acidity. These small amounts of acid are naturally diluted or neutralised by surrounding rocks so that they are not seen or noticed. Mining often accelerates this natural process by exposing pyrite to oxygen and water.

Most commonly, it is sulphuric acid that increases the hydrogen ion content in mining voids (Equations 1.1 and 1.4). At low pH, the bacteria also contribute to the release of hydrogen ions by indirect oxidation of pyritic material via ferric ion, as well as by direct oxidation (Gray *et al.,* 1990). Pyritic waste generated during the mining of sulphide ore bodies contains other sulphide minerals, such as chalcopyrite (FeS.CuS) and pyrrhotite and/or sulphates [*e.g.* gypsum, ($CaSO_4.2H_2O$) or barite ($BaSO_4$)]. The ferric ion released by pyrite also accelerates the indirect bacterial oxidation of these minerals (Mills, 1997b). Although these minerals do not produce acid, they release heavy metals, exacerbating the adverse environmental impact of the acid mine drainage (Gray *et al.,* 1990). The kinetics of pyrite oxidation have been found to vary considerably, depending on the exact conditions and the source of the pyrite. This variability is a fundamental obstacle to developing methods or technology for preventing and mitigating acid mine drainage (Gray *et al.,* 1990).

Environmental impacts of acid mine drainage

The impacts of acid drainage on aquatic environments are manifested in changes in water quality and ecological functioning and integrity (Phillips, 1998). The environmental impact of acid mine drainage is dependant on the quantity of acid, produced and the amounts of base or alkaline material available. The consequences of acidic drainage depend on the pH, chemical composition and volume of the contaminated drainage, and the assimilative capacity of the receiving environment (Harries, 1996). The off-site impacts of sulphide oxidation in mine wastes at sites in arid regions can be different from those in high rainfall zones (Harries, 1996). In high rainfall zones there are often large amounts of acid mine drainage in stream run off and ground water. Whereas, in drier environments there is less flowing water, so the acid mine drainage is not transported or produced. This can cause intense acid problems when it does rain and large volumes of acidic water are created and transported in a short period of time.

Acid mine drainage contaminated groundwater with a low pH (1.9-2.3), high dissolved iron salts (concentration; TDS 15000 mg L^{-1}), high redox potential, high concentrations of metals (such as zinc, aluminium and cadmium) and high chemical oxygen demand is a hazard to human health, agriculture and the environment (Hancock, 1997; Whitehead, *et al.*, 1995; Woodin and Skiba, 1990). This particular 'toxic cocktail' of acidic water effects aquatic flora and fauna, and coats the riverbed with orange ferric hydroxides and a bacterial slime (Whitehead *et al.*, 1995).

Effects of heavy metals

Acid mine drainage can carry high concentrations of heavy metals leached from surrounding soil and rocks as the low pH increases metal solubility (Gyure *et al.* 1987; 1990; Kelly, 1988; Mills, 1997a). The types of metals leached depend on the soil and rock types that the acid mine drainage runs through or over. Metal cations commonly found in high concentrations in coal-formed acid mine drainage include nickel, copper, zinc, manganese, cadmium, aluminium, iron, vanadium, chromium and lead (Borg, 1987; Kelly, 1988).

Among the important stream biota that can be adversely effected by low pH and high metal concentrations are algae, the major primary producers in many streams. Bioaccumulation of metals in algae and bryophytes can expose fish and other consumers to concentrations of metals that are several orders of magnitude greater than the water in which they would normally live (Bailey and Stokes, 1985 cited in Engleman and McDiffett, 1996, p 67).

Effects of low pH

High acidity (pH < 3.0) kills plant life (Cline and Balla, 1976; Henriksen *et al.*, 1988), while moderately acid conditions (pH 3.0-4.5) will kill aquatic insects (Andersson and Danell, 1982). When acid mine drainage is severe, contact with the water can cause skin irritation to people (Howard *et al.*, 1989b). The acidic water can also attack cement culverts and bridge abutments resulting in a reduced life span for these structures (Fague and Mostyn, 1997).

Chemical conditions associated with acid mine drainage, such as high heavy metal levels and low pH are toxic to fish (Fague and Mostyn, 1997; Leivestad and Muniz, 1976; Woodin and Skiba, 1990). However, only a few cases of massive fish kills due to low pH have been documented (Leivestad and Muniz, 1976). Drainage across oxidised sulphidic sediments is believed to be the cause of stream acidification (Easton, 1989; Sammut, *et al.*, 1995; 1996) which has resulted in mass mortality of fish and other gilled organisms in some of Australia's coastal tributaries and estuaries when prolonged dry periods have been broken by heavy rainfall (Brown, 1983; Easton; 1989). Acidity disrupts the mechanisms by which fish maintain their ionic balance of internal fluids causing a loss of body salts, especially sodium. Newly hatched and young fish are extremely sensitive to pH changes in water (Woodin and Skiba, 1990). Fish lay fewer eggs in acidic water and many of these eggs die (Woodin and Skiba, 1990). Aluminium in acidic water damages the gills of fish and they become covered in mucus and the fish suffocate (Woodin and Skiba, 1990). There is also a reduction in productivity associated with acid mine drainage due to a loss in nitrogen, phosphorous and carbon sources in the water.

Control and prevention of acid mine drainage

One feature of acid mine drainage is that the chemical and bacterial mechanisms responsible for its formation are auto catalytic; once mine waste becomes acidic the problem tends to worsen and is difficult to stop (Environmental Mining Council of British Columbia, 1998; Gray *et al.*, 1990). Often all that can be done is to treat the drainage to remove acid and dissolved metals, or to isolate the wastewater from the environment in a zero-discharge or controlled discharging facility (Gray *et al.*, 1990). The large volumes of wastewater involved, often being released over many centuries, can make any mitigative action expensive (Gray *et al.*, 1990). Preventative action is preferable, especially when planning new facilities and management processes for pyritic wastes.

Much of the effort to control acid mine drainage in the past has been directed at treating the symptoms rather than controlling the problem at the source (Axler *et al.*, 1998; Perry and Kleinmann, 1991). Prevention of acid generation is the most desirable approach with the aim to eliminate any one of the three components of acid generation (Durkin and Herrmann, 1994; Silver, 1989). These three components are the mineral substrate, the oxidant and the biological catalyst. Elimination of these components may be achieved by:

- removal of the pyrite source (Hester and Associates, 1984),
- rendering acid generation minerals inactive by the development of surface coatings (Brooks, 1998; Currey, 1998; Flyn, 1969; Stiller, 1982),
- exclusion of water (Orr and Veivers, 1997; Robertson, 1987),
- control of biological oxidation,
- controlling the temperature (Robertson, 1987, 1988; Robertson and Barton-Bridges, 1988),
- exclusion of oxygen (Barton-Bridges and Robertson, 1989a, b; Fawcett and Sinclair, 1997); or
- addition of a base substance (Robertson, 1987, 1988).

Where it is not possible to prevent the generation of acid mine drainage, the next most desirable approach is to prevent acid and acid product migration into the environment. Since water is the mode of transport, the approach typically relies on the prevention of water entry to the waste pile. Control of water exiting from the pile is of little value, since in the long-term all water entering the pile must exit, unless a strategy is put in place for its long-term storage.

The control requirements are as follows:

- diversion of all surface water flowing towards the pile,
- interception or isolation of groundwater flow towards the pile, or
- prevention of infiltration of precipitation into the pile (Robertson, 1988).

If acid generation and migration from the source has taken place, the remaining option is to collect and treat the contaminated effluent. This control measure is the least attractive because of the obvious disadvantages in maintaining a treatment process in the long-term. The high risk of failure of any long-term treatment system in the long term is an additional disadvantage. This control technique is suitable for the short-term, particularly when used in conjunction with one or both of the preceding methods of control (Robertson, 1988).

Remediation and amelioration strategies for treating acid mine drainage

Many techniques have been devised for the amelioration of acid mine drainage problems; these include liming, filtration through biological materials or wetlands (Brodie *et al.*, 1989; Henrot *et al*, 1989; Microbial Technologies, 1997; Reed *et al.*, 1988; Robertson, 1987; Robertson and Barton-Bridges, 1988, 1990; Street and Titmus, 1982; Wilderman and Laudon, 1989), use of bactericides (Sobek, 1987), surface coatings on the sulphides (Dugan, 1987; Roberston, 1988; Silver, 1989), engineered covers (Dugan, 1987; Roberston, 1988; Silver, 1989), flooding (Robertson, 1988), development of raised bogs (Brown, 1991, cited in Shelp, Chesworth and Spiers, 1996, p. 425) and an *in situ* electrochemical method using aluminium and zinc sacrificial anodes (Shelp, *et al*., 1996). Most of these pose problems with cost effectiveness and longer-term maintenance issues.

One of the most promising developments in the treatment of acid mine drainage is the recognition of the role of natural processes in the removal of acidity, sulphate and metals from drainage waters. Biological sulphate reduction has been identified as one of the major contributors to the amelioration process in wetlands (Broughton and Robertson, 1992). Over time, the low pH of waters created by acid mine drainage may be neutralised by anaerobic microbial activities (Gyure *et al.,* 1990; Kelly *et al.* 1982).

Sulphate reducing bacteria

Sulphate reducing bacteria remove contaminants by oxidising matter and using sulphate as an electron acceptor (Hedin *et al.,* 1988). Sulphate reducing bacteria are strictly anaerobic and are severely inhibited by even small amounts of oxygen. They require an organic carbon source (Hammack and Edenborn, 1991) and the redox potential to be less than –100 *m*V (Lyew *et al.* 1994; Lyew and Sheppard, 1997).

The sulphate reduction reactions are as follows;

$$SO_4^{2-} + 2CH_2O \text{ (i.e. organic matter)} + 2H^+ {}_{\text{(bacteria)}} \rightarrow H_2S + 2H_2O + 2CO_2 \text{ (pH} < 7.0) \quad \textbf{(Equation 1.5)}$$

$$SO_4^{2-} + 2CH_2O \text{ (i.e. organic matter)} + OH^- {}_{\text{(bacteria)}} \rightarrow HS^- + H_20 + HCO_3^- \text{ (pH} > 7.0) \quad \textbf{(Equation 1.6)}$$

(Jones and Chapman, 1995)

As indicated in Equations 1.5 and 1.6, the removal of sulphate results in a decrease in acidity (Wendt-Potthoff and Neu, 1998). Sulphur is released as hydrogen sulphide gas or it remains in the wetland as metal sulphides, polysulphides, elemental sulphur, iron monosulphides and pyrite (Hedin *et al.* Hammack, 1988). The released hydrogen sulphide gas reacts with heavy metal ions to form insoluble metal sulphides that can easily be separated from a solution (Kim *et al.,* 1999), however, the metal sulphide precipitation depends on the inflow concentrations and pH (Hedin *et al.,* 1988; 1994). This reduction process also produces alkalinity (HCO_3^-) that decreases acidity and raises pH (Hedin *et al.,* 1988). Bicarbonate ions produced during the sulphate reducing bacteria reaction buffer the solution pH to between 6 and 7 (Kim *et al.,* 1999). This buffering will also cause some metal ions to precipitate as insoluble hydroxides.

Dvorak, *et al.* (1992) reported that pilot-scale reactors filled with spent mushroom compost and sulphate reducing bacteria lowered concentrations of the metals aluminium, cadmium, iron, manganese, nickel and zinc by over 95% in the waste water, while completely neutralising the acidity of contaminated water. Hammack and Edenborn (1992) found that the rate of sulphate reduction and metal removal was increased by a factor of 10 following the addition of lactate, which is a preferred carbon source for many sulphate reducing bacteria. During sulphate reduction in acidified bacterial cultures, the pH of the medium increases by between one and two pH units and black iron sulphate deposits are formed (Gyure *et al.,* 1987; Johnson *et al.,* 1997).

The reduction of sulphate to hydrogen sulphide (H_2S) is brought about by specialised anaerobic bacteria from the genera; *Desulfovibrio* (five species) and *Desulfotomaculum* (three species; Broughton and Robertson, 1992). These bacteria are all heterotrophic organisms and have a respiratory metabolism in which sulphates, sulphides and/or other reducible sulphur compounds serve as the final electron acceptors, with the resulting production of H_2S (Broughton and Robertson, 1992; Johnson *et al.,* 1997). The organic substrates for these bacteria are generally short chain acids such as lactic and pyruvic acid (Broughton and Robertson, 1992). In nature, these substrates are provided through fermentation activities of anaerobic bacteria on more complex organic substrates, such as rotting vegetation (Broughton and Robertson, 1992). Sulphate reduction by bacteria occurs in the epilimnetic and hypolimnetic sediments of lakes (Kelly *et al.,* 1982; Rudd, *et al.*, 1986a).

Recently, sulphate reduction has been recognised as a potential acid mine drainage treatment process in its own right (Christensen, *et al.,* 1996; Lyew and Sheppard, 1999). Natural *in situ* sulphate reduction has been identified in an underground mine in Norway (Broughton and Robertson, 1992; Herlihy and Mills, 1985). This presents the potential for instituting a relatively low maintenance biological treatment system in abandoned underground and open-cut voids that not only modifies the effluent pH, but also extracts the soluble metal content as stable sulphide compounds (Broughton and Robertson, 1992). The reducing conditions under which these sulphide metal compounds are deposited, also provides an ideal long-term storage facility for the preservation of these compounds (Broughton and Robertson, 1992). Rudd *et al.*, (1986a) found that organic sulphur formation was probably the most important long-term source of sulphur to the sediments.

Bacterial sulphate reductions require hypoxic conditions and an oxidisable organic substrate. There are other anoxic bacterial reactions that require an organic substrate. These are iron and manganese reduction and methanogenesis (Kelly and Chynoweth, 1981). All of these anoxic reactions compete for the available organic substrate, however, as sulphate concentrations increase, sulphate reduction increases to the point where bacterial sulphate reducers tend to dominate (Kelly *et al.*, 1982; Lyew and Sheppard, 1999). The sulphate reduction out competes the methanogenesis due to a higher redox potential.

Rudd, *et al.* (1986b) provided a conceptual model of the fate of the end products of sulphate reduction after observing the natural sulphur accumulation in four lakes in southern Norway (Figure 1.1).

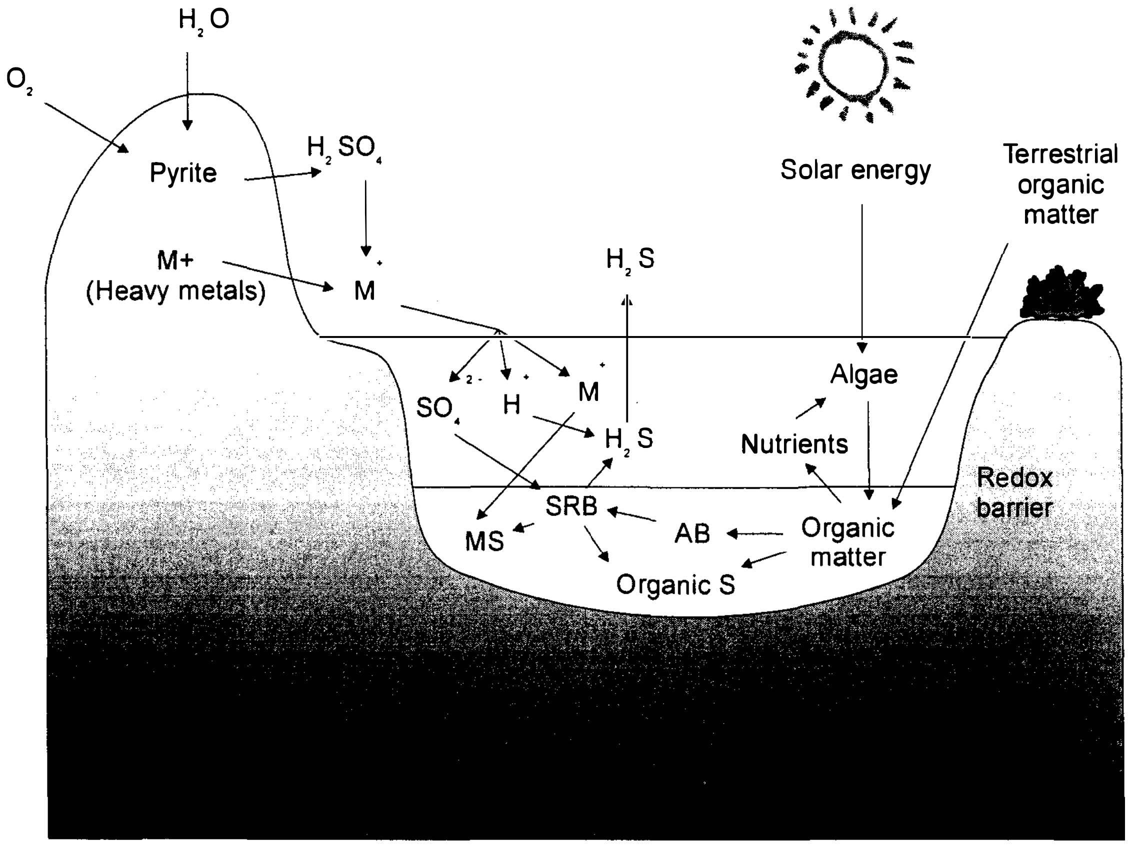


Figure 1.1. The formation of acid mine drainage and its natural amelioration in mining pit lakes.
(Adapted from Rudd *et al.*, 1986b; Kelly, 1988; and King *et al.*, 1974)
SRB = Sulphate reducing bacteria, AB = Anaerobic bacteria, MS = Metal sulphides.

A key feature of this model (Figure 1.1) is the input of organic matter into the water creating a source of nutrients for algae. Algal formation then continues the production of organic matter potentially creating a self-sustaining system.

While recognition is growing that a "walk-away" situation cannot be achieved for every site, the technical and financial liabilities of maintaining a chemical treatment plant (*i.e.*, liming) in perpetuity have increased the interest in developing effective long-term passive care options. The use of sulphate reducing bacteria to increase the pH represents an inexpensive biological alternative to chemical neutralisation of acidic lakes (Gyure *et al.,* 1987; Tsukamoto and Miller, 1999).

Wetlands

Sulphate reducing bacteria solutions are generally applied in two ways, as treatment for drainage waters or as an in-void treatment. Drainage treatment typically involves passing acid mine drainage water through a wetland system while an in-void treatment seeks to recreate natural wetland conditions within the void.

Acid mine drainage remediation by passive systems was first documented by Huntsman, *et al.*, (1978), and Wieder and Lang (1982) in Ohio and West Virginia respectively. Since the 1970s a variety of passive treatment systems have been developed that do not require continuous chemical inputs and that take advantage of naturally occurring geochemical and biological processes to cleanse contaminated mine waters. Observations of natural *Sphagnum* moss wetlands ameliorating water quality stimulated the idea that engineered wetland systems might be used for the treatment of acid mine waters (Gazea, *et al.*, 1996). Similar studies by Brooks, *et al.,* (1985) demonstrated acid mine drainage amelioration in *Typha* wetlands. Some wetland plants show long-term adaptation to low pH and high metal concentrations, whereas, in other cases acid mine drainage eventually degrades the quality of natural wetlands (Skousen, 1997). Artificially constructed wetlands for water treatment might provide a low cost and low maintenance treatment of acid mine drainage (Kleinmann, 1991; Skousen, 1997). Hedin *et al.* (1994) described the goal of wetland treatment systems "to enhance the natural amelioration processes so that they occur within the treatment system, not in the receiving water body". This area of wetland remediation has been the focus of most passive remediation research over the past 10 years.

Passive treatment of acid mine drainage represents an alternative to conventional neutralisation techniques with alkaline reagents such as lime, limestone, magnesia and others. Passive systems typically require less input of energy, reagents and manpower, and present lower operational and maintenance costs than conventional treatment schemes. Thus, they may be employed during the mine operation as well as in the post-closure period.

The cost of anoxic lime drain treatment, field observations of the improvement of acid mine drainage upon passage through naturally occurring *Sphagnum*-dominated wetlands, and the suggestion that constructed wetlands might provide a 'low cost, low maintenance' alternative to conventional chemical treatment, sparked considerable enthusiasm in the coal and consulting industries (Klienmann, *et al.*, 1983; Wieder and Lang, 1982). Although hundreds of wetlands were constructed in the United States between 1984 and 1988 to treat acid mine drainage, the treatment effectiveness continues to be variable and generally unpredictable (Gazea *et al.,* 1996; Wieder, *et al.*, 1990; Wieder, 1994). *Sphagnum* moss proved highly sensitive to transplanting, abrupt water chemistry changes and increased accumulation of iron. At most sites the moss died within one growing season (Gazea *et al.,* 1996). Despite these negative results, research on alternative types of constructed wetlands continues (Mitsch and Wise, 1998; Skousen, 1997). Recent research indicates anaerobic processes are more important than the type of organic matter used in applied passive treatment systems for coal acid mine drainage (Gazea *et al.,* 1996).

Most of the constructed wetlands were not installed for research purposes and because hydrologic conditions were generally unknown in some instances, apparent improvements in the water quality of mine drainage may have resulted from the dilution with rainwater or the influx of good quality groundwater (Wieder *et al.,* 1990). Most data collected for this type of treatment were due to regulatory requirements and only water chemistry data from periodic sampling of the discharge exists. These data typically do not contain information about the metal accumulation in the wetland (Wieder *et al.,* 1990).

Even after the initial dilution is accounted for, constructed wetlands can have a considerable effect on the acidity of acid mine drainage and the concentrations of dissolved metals (Tyrrell, 1996). Wetlands for wastewater treatment use biological, chemical and physical features of natural wetlands to improve water quality (Tyrrell, 1996). Wetlands serve as sinks, sources and transformers of chemicals. Changes depend on the wetland type, hydrology and annual climatic variation (Mitsch, 1992). Wetlands can alter and trap pollutants and contaminants (Hammer, 1990). Some of these features include filtering of suspended material, metal uptake into live roots and leaves, adsorption and exchange by plants, soil and other biological materials; abiotic or microbially-catalysed metal oxidation and hydrolysis reactions in aerobic zones, and microbially-mediated reduction processes in anaerobic zones (Gazea *et al.,* 1996). Mechanisms of metal retention within wetlands include; formation and precipitation of metal oxides and hydroxides, formation of metal sulphides, organic complexation reactions, exchange with other cations on negatively-charged sites, and direct uptake by living plants (Skousen, 1997). Consequently wetlands, natural and constructed, are treating various wastewater types throughout the world.

Research has been conducted on the design parameters necessary for effective wetland construction for acid mine drainage treatment or wastewater (Hedin and Nairn, 1990; Kepler, 1990). However, research into the usefulness and benefit of wetland plants is limited. While wetlands that do not contain vegetation and use a variety of substrates have been proposed or shown to be successful (Stark, *et al.*, 1996; Wieder, 1989), vegetation can be important in the development of constructed wetlands. The presence of plants can reduce channelisation and increase the residence time of the acid mine drainage in the wetland (Demchik and Garbutt, 1999). Although most acid mine drainage treatment in wetlands is by microbial processes, plants can take up minor amounts of heavy metals (Demchik and Garbutt, 1999) and stimulate microbial communities by producing a carbon source, adding oxygen and providing a physical site for microbial attachment to the roots (Skousen, *et al.*, 1992). Plants also provide habitat for wildlife and aesthetic value (Skousen *et al.*, 1992).

Passive treatment alternatives

Three types of passive technologies have been developed for the treatment of acid mine drainage; aerobic wetlands, compost or anaerobic wetlands, and anoxic lime drains. In aerobic wetlands, oxidation reactions occur, and metals precipitate as hydroxides and oxyhydroxides that are suited to waters that have net alkalinity. Compost wetlands promote anaerobic bacterial activity resulting in sulphate reduction, the subsequent precipitation of metal sulphides and the generation of alkalinity. Anoxic limestone drains generate alkalinity and can be used for the pre-treatment of the acidic mine waters (Gazea *et al*., 1996). Acid water is passed through a limestone bed under anoxic conditions to prevent armouring of the limestone.

The applicability of each of the three passive technologies mainly depends on the composition of the mine waters under treatment and they are most often effective when used in combination (Gazea *et al.,* 1996; Skousen, 1997). Based on field observations, it is recommended that anoxic lime drains should be a pre-treatment stage aiming to add alkalinity to the water, followed by an aerobic wetland to remove as much iron as possible through oxidation (Gazea *et al.,* 1996). Directing the partially treated water flow through a series of anaerobic cells with composted organic substrate will result in increasing the pH to near neutral and in reducing the other metal levels to values close to environmentally permissible limits (Gazea *et al.,* 1996).

An effective treatment system must in most cases do two things; firstly, remove metals from the water by precipitation, oxidation or reduction. Secondly, raise the pH to a level in which fish, insects and a new functional ecosystem can be established.

Anaerobic organic substrate systems (or compost wetlands)

Anaerobic systems require the mine water to flow through a body of organic material (normally about 1 m thick) under anaerobic conditions (Robinson and Robb, 1995). Anaerobic wetlands generally contain a layer of limestone in the bottom of the constructed wetland. The limestone is overlain by organic material and wetland plant species planted into the organic substrate (Figure 1.2; Robb and Robinson, 1995; Skousen, 1997). These systems are used when the water has a net acidity. Alkalinity must be introduced into the system before dissolved metals will precipitate (Skousen, 1997). Compost wetlands generate alkalinity through a combination of bacterial activity and limestone dissolution that in turn neutralise the acidity contained in the influent waters. This is important for the long-term viability of the proposed treatment. Anaerobic wetlands are applicable to the treatment of mine waters containing ferric iron or aluminium, and acidity higher than 300 mg L^{-1} $CaCO_3$. The sulphate reducing bacteria require a rich organic substrate in which anoxic conditions will develop (Skousen, 1997). Typical substrates used in these wetlands include low cost natural products and wastes such as spent mushroom compost, horse and cow manure, hay, peat, wood chips and sawdust (Skousen, 1997). Spent mushroom compost containing 10% dry weight limestone is also a commonly used substrate (Skousen, 1997). Compost substrates with low calcium carbonate content can be supplemented with limestone.

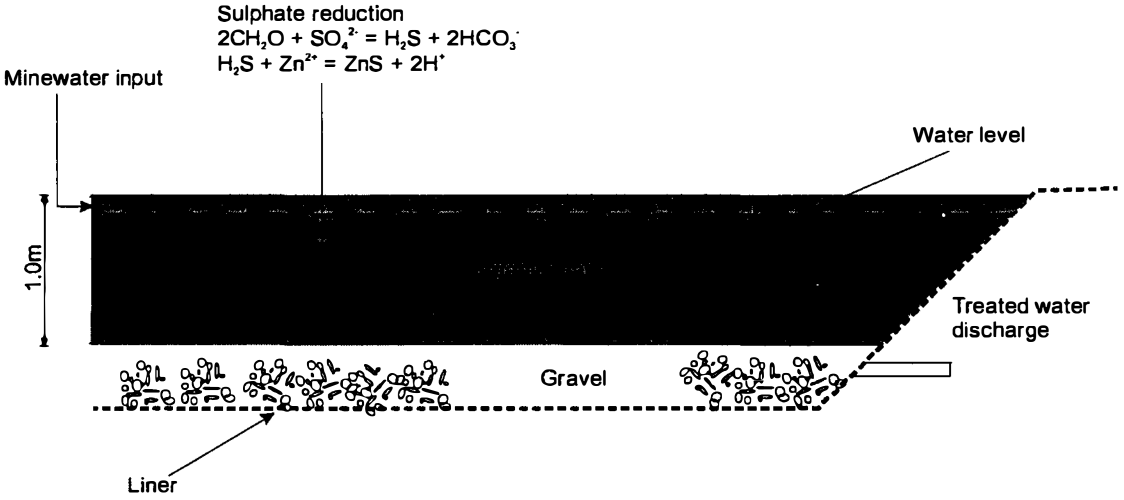


Figure 1.2 Design of an anaerobic wetland.
(from Robb and Robinson, 1995)

The propagation of plants on the surface is discouraged so as to avoid the penetration of the cell surface by the plant roots and the subsequent injection of excess oxygen into the substrate, destroying the anaerobic conditions. This would cause a problem with competing aerobic and anaerobic microenvironments that will ultimately reduce the performance of the sulphate reducing bacteria and therefore the treatment system (Robinson and Robb, 1995). Mine water with a high sulphate content infiltrates through the thick, permeable organic matter sub-surface sediment and becomes anaerobic due to high biological oxygen demand.

Several additional treatment mechanisms function in anaerobic wetlands compared to aerobic wetlands. These include metal exchange, formation and precipitation of metal sulphides, microbially generated alkalinity due to reduction reactions and continuous formation of carbonate alkalinity due to limestone dissolution under anoxic conditions (Figure 1.2; Robb and Robinson, 1995; Skousen, 1997).

These design parameters represent current methodologies that will be potentially replaced with more efficient techniques currently under development (Gazea *et al.,* 1996). Research is underway testing successive alkalinity producing systems (Gazea *et al.,* 1996; Skousen, 1997). These experimental systems combine anoxic lime drain technologies with sulphate reduction mechanisms; they promote vertical water flow through rich organic substrates into limestone beds beneath the organic compost. The problems associated with dissolved oxygen and ferric iron contents on anoxic lime drains are eliminated in successive alkalinity producing systems; ferric iron is reduced to a ferrous form and dissolved oxygen is removed from the water within the organic substrate prior to its treatment through the limestone bed (Gazea *et al.,* 1996).

Successive alkalinity producing systems

Successive alkalinity producing systems have been identified as the most applicable technology for the treatment of low acid mine drainage load/low flow point sources (Johnston, *et al.*, 1996). Successive alkalinity producing systems combine the use of anoxic lime drain technology and anaerobic compost wetland technology (Johnston *et al.,* 1996; Skousen, 1997). The treatment effectiveness within a passive treatment system is based on the residence time of acid mine drainage within the system (Johnston *et al.,* 1996). Successive alkalinity producing systems consist of a series of alkaline producing and settlement components to progressively raise the pH, remove metals from solution and precipitate as sediments (Johnston *et al.,* 1996). In a successive alkalinity producing system, acidic water is piped into a holding tank and then pumped into anaerobic organic matter underlain by limestone. Below the limestone is a drainage pipe where the water is directed into an aerobic wetland. The compost layer ensures that the discharge will be anoxic upon entering the limestone treatment component, altering the redox state of the iron load, and acting as a potential metal sink for sulphide precipitates. The limestone layer provides alkalinity and an additional pH adjustment (Johnston *et al.,* 1996; Skousen, 1997). Hamilton, *et al.* (1999) system comprised of five aerobic reed beds, an anaerobic cell and an aerobic rock-filter (Hamilton *et al.,* 1999). It was found that this system significantly reduced heavy metal levels (iron, arsenic, zinc, cadmium, copper and manganese) and levels of sulphate (Hamilton *et al.,* 1999).

***In situ* void treatment**

Acidity of mining void lakes will typically decrease as a consequence of a slow increase in biological activity (Gyure, *et al.*, 1987). Based on 25 years of data, Cambell and Lind (1969) found that acidic mine lakes with a greater input of organic matter, such as leaves and grass, exhibited a higher pH than lakes with little organic matter input. Kalin and Geller (1998) report that limnologists often presume that an autochthonous succession from young (i.e., mining lakes) lakes to mature lakes exists, resulting in lakes with accumulated sediments and a well-developed ecosystem. The inputting of organic matter just increases the rate of this autochthonous accumulation.

Organic matter might therefore be used to increase the presence of sulphate reducing bacteria and to treat water effected by acid mine drainage (Tuttle, *et al.*, 1969a). Subsequent studies by Tuttle *et al.,* (1969b) found the placement of sawdust in acidic lakes can increase the population of sulphate reducing bacteria by producing a low redox potential and by providing a source of organic nutrients to supply energy and carbon (Tuttle, *et al.*, 1969b). The improved conditions for sulphate reducing bacteria resulted in the removal of sulphate from the water and producing an accompanying increase in pH. The productivity of acidic mine lakes with low supplies of allochthonous nutrients becomes dependent on carbon formed by autochthonous means (Gyure *et al.,* 1987).

Bechard, *et al.* (1994) developed a low maintenance microbial treatment system that used alfalfa as an organic substrate. Whereas, the use of hay, timothy hay and straw failed as treatment systems after about three weeks.

Organic matter additions will supply nutrients for algal growth increasing in primary productivity and therefore increasing the supply of organic matter to the sediments, which will contribute further to the process of sulphate reduction. Nutrients are often limited in waters affected by acid mine drainage due to the high levels of cations that quickly precipitate out any available nutrients, therefore there is typically little available carbon, nitrogen and phosphorus in the lake waters (Gyure *et al.*, 1987). Nutrient availability is more important than pH in limiting photosynthetic activity in lakes (Gyure *et al.*, 1987).

Organic matter additions can contribute in other ways to increasing the pH in the lakes. When organic matter is spread evenly over the sediment a capping effect can limit oxygen reaching pyrite in the sediments; creating the anaerobic environment necessary for the functioning of sulphate reducing bacteria. If acidic groundwater is entering the lakes it will have to pass through the organic matter layer prior to entering the water column. Any sulphate reducing bacteria in the sediments will probably increase the pH of the groundwater. Bacterial sulphate reduction can also cause the amelioration of the polluting effects of acid mine drainage by precipitating metals such as sulphides (*e.g.*, zinc sulphides and copper sulphides; Johnson *et al.*, 1997). If the pH of the acid lakes is increased by the addition of organic matter, any metals within the water column are likely to precipitate out and become bound to the sediments as their solubility is reduced at a higher pH (Johnson *et al.*, 1997).

Establishing sulphate reducing bacteria in the sediments of acid lakes by the introduction of nutrients to convert sulphuric acid to hydrogen sulphide gas and metal sulphides has four potential benefits;

- reducing the acidity of the water,
- actively binding soluble heavy metals into insoluble precipitates,
- offering a low cost, self-sustaining alternative; and
- being effective regardless of groundwater or sediment contributions to acidity.

Sulphate reducing bacteria thrive only in anoxic conditions and the addition of a layer of organic matter across the sediment can create these conditions (Schindler, 1996).

Australian Coal Association Research Program

A research group funded by the Australian Coal Association (Australian Coal Association Research Program; ACARP) is attempting to develop appropriate low cost, low maintenance solutions for rehabilitating acidic flooded coal mine voids of the Collie area (Phillips *et al.,* 1999). The aims of this study are to contribute to the development of technology and guidelines for the treatment of the lakes that form in mine voids as a result of seepage, run-off and residual effluent drainage. It is proposed to stock these voids for aquaculture after rehabilitation is complete (Phillips *et al.,* 1999). The ACARP project is being undertaken by a team comprising of staff from three educational institutions (Curtin University of Technology, Edith Cowan University and the South-West College of TAFE), two coal mining companies (The Griffin Coal Mining Company Ltd and Wesfarmers Coal Pty. Ltd.) and two government departments (Conservation and Land Management and Western Australian Department of Minerals and Energy; Biggs, 1998; Phillips *et al.,* 1999). A staged approach has been adopted by ACARP and currently the research program is its 3rd stage of development. A series of simultaneous and sequential investigations have been incorporated into the three stages of the program. These include investigation of:

- the geology, geochemistry and hydrogeology of the area,
- low cost water pumping techniques,

- amelioration of acid run off from coal mine dumps,
- passive mine drainage treatments and aquatic vegetation strategies,
- use of bacterial strategies for increasing pH in acidic voids; and,
- determining the tolerance and potential for restocking of the lakes with fish and crustaceans.

(Biggs, 1998; Phillips *et al.,* 1999)

This thesis addresses a sub-component of the bacterial strategies sub-program investigating the use of bacteria to increase water pH.

Study aims

Worldwide considerable research has been undertaken into the use of organic matter for treating point source acid mine drainage. Aside from a few small scale experiments by Tuttle *et al.*, (1969a, b) and Gyure *et al.,* (1987), very little research has been undertaken to rehabilitate acidic voids using organic matter. In WA, the climate tends to reduce the influence of acid mine drainage point sources and increase the significance of diffuse sources. This limits the usefulness of techniques such as successive alkalinity producing systems. In Collie the mine voids are primarily isolated and there is no obvious flow between voids or across the landscape.

This study aims to investigate the potential for *in situ* additions of organic matter to increase pH in flooded coal mine voids in Collie (WA).

Specifically:

- to test a range of locally available organic matter types for their effectiveness in increasing pH within Collie void;
- to determine the mechanisms responsible for any changes seen in pH when organic matter was added;
- to examine the longer term effects on pH, nutrients and sulphate levels in a field situation similar to a Collie mining void when an economically reasonable quantity of organic matter is added.

2.0 STUDY SITE

Collie

Collie is situated about 160km S.S.E. of Perth and 55 km east of Bunbury (33° 22' 116° 09' Figure 2.1). The town of Collie has a resident population of about 9500 people and is located on the north western rim of the Collie Basin. The major land uses in the area are coal mining, power generation and agriculture which are the cornerstones of the local economy (Collie Water Advisory Group, 1996). The recreation and nature conservation values of the surrounding State forest areas are highly regarded along with the recreational opportunities provided in the area. Collie has a mediterranean climate with 75% of its rainfall occurring in the five months from May to September (Collie Water Advisory Group, 1996).

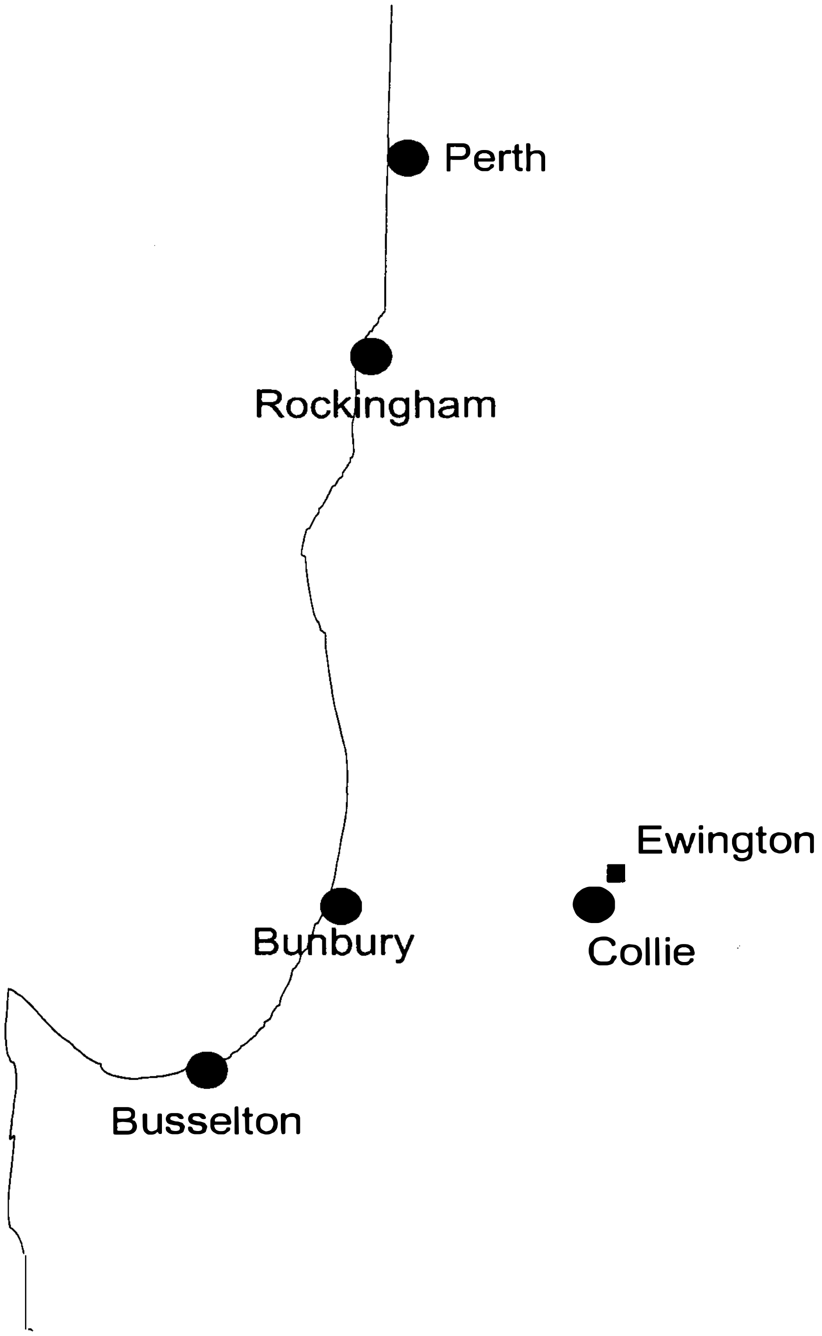


Figure 2.1 Location of Collie.
(Readers Digest Australia, 1994)

Geology of the area

The Collie Basin is a small sedimentary basin occurring in the Collie River catchment (Collie Water Advisory Group, 1996). It occupies about 10% of the river catchment and is approximately 27 km long and up to 13 km wide and has an area of about 225 km^2. The basin contains large coal and groundwater resources which are being mined (Collie Water Advisory Group, 1996). The Collie basin is divided into two sub basins – Cardiff and Premier (Collie Water Advisory Group, 1996). The coal seams do not outcrop and all knowledge of the coal sequence is derived from borehole data and mining exposures with open-cut and underground mines (Stedman, 1988).

The Premier sub basin contains seams in three separate horizons. The uppermost coal unit, Muja, includes nine named seams (Ate, Bellona, Ceres, Diana, Eos, Flora, Galatea, Hebe, Iona; Stedman, 1988). All seams are mined in the Muja Open Cut to a depth of over 200 metres, which necessitates dewatering operations. The underlying Premier coal member also contains nine named seams, three of which are mined at the Chicken Creek Open Cut to the east of Muja. The same coal bearing unit was mined at the Ewington mine site.

Ewington: the study site

Ewington is on private property and there is no general public access to the site. As Amalgamated Collieries Ltd., the miners of Ewington, no longer exist, any rehabilitation of Ewington and the surrounding area is now the responsibility of the private landowner who is leasing the land from the State government (Stedman, 1988).

Ewington is the focus void of the ACARP research program due to its small size and lack of public access. Therefore it was chosen as the focus for this study. Ewington has a relatively low pH (3.3-4.6) due to acid drainage from the surrounding overburden dumps and from groundwater inflow (Phillips, *et al.*, 1999). Eroded steep cliffs on the southern and south-eastern bank surround part of the perimeter of the lake. This erosion would suggest that the lake receives run off after heavy rains across overburden dumps, potentially increasing the acidity of the lake. Overburden dumps mostly surround Ewington. There is also an acidic seep on the south-eastern bank throughout the winter-spring period (Phillips *et al.*, 1999).

Ewington was mined to a depth of 10 - 15 m (Figure 2.2; Western Australian Forests Department, 1983). Erosion and sedimentation processes are likely to have reduced the angle of the sides, nevertheless, their gradient is still steep leaving only a narrow littoral zone around most of its edges. Overburden dumps surrounding Ewington are generally poorly vegetated and consequently the supply of allochthonous nutrients into the lake is probably small.

There are 25 species of plants within 10m of the water in Ewington (Phillips *et al.*, 1999); most of which are endemic to the area. The rushes and sedges are sparsely distributed with a concentration on the eastern shallow wing (Phillips *et* *al.,* 1999). The groundcovers are sparse, especially on the southern bank (Figure 2.3).

The pH of Ewington varied little throughout 1998 except for a couple of occasions when minor changes were noted. Conductivity appeared to vary on a seasonal basis due to evapo-concentration in summer and dilution in winter. The changes in conductivity are small in magnitude (870-1618 $\mu s\ cm^{-1}$) and are unlikely to have any effects on the voids biota (Phillips *et al.,* 1999). Ewington water becomes stratified based on temperature in summer although only the deepest section is affected. Slight declines in dissolved oxygen were noted in summer in the bottom waters, probably associated with the hypoliminion in the deepest section (Phillips *et al.,* 1999).

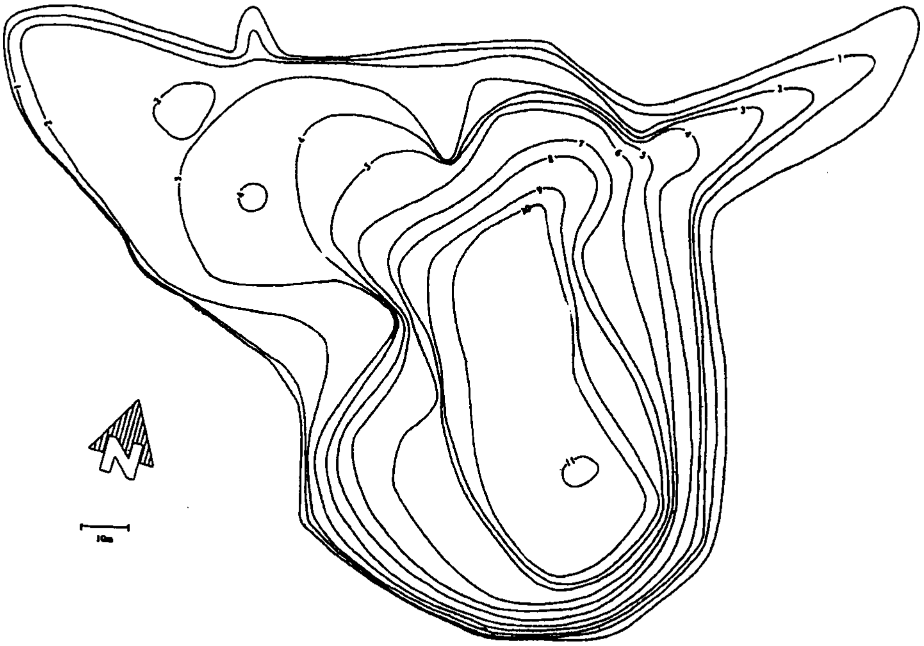


Figure 2.2 Bathymetry of Ewington.

(from Phillips *et al.*, 1999)

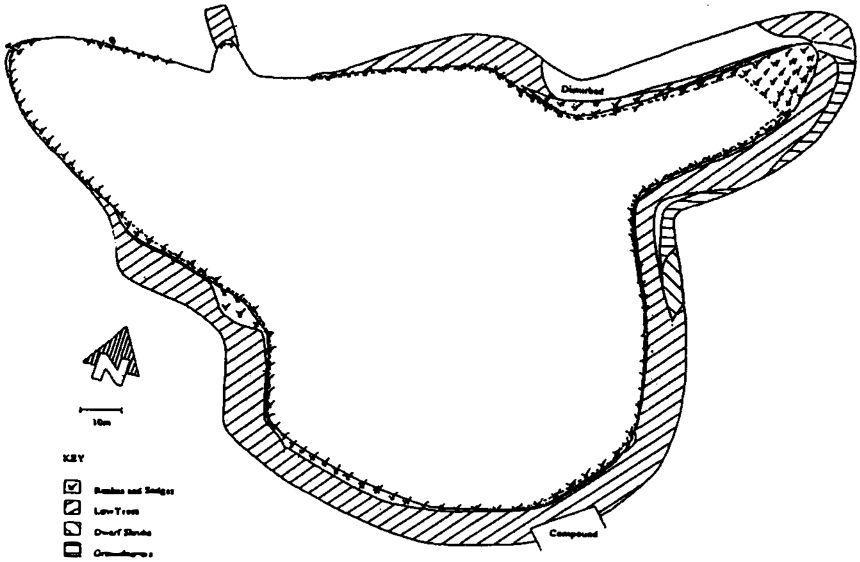


Figure 2.3 Peripheral vegetation map showing distribution of rushes, sedges and ground cover.

(from Phillips *et al.*, 1999)

3.0 METHODS

A pilot experiment (in appendix), three laboratory experiments followed by a field experiment were used in this study. The laboratory experiments were designed to test, which organic matter types are the most effective, if the quantity of organic matter added made any difference to the pH change, and the mechanisms responsible for the changes in pH. The field experiment tested the organic matter types effectiveness *in situ* at Ewington over a five month period. The approach taken by the study in relation the laboratory experiments is shown in Figure 3.1.

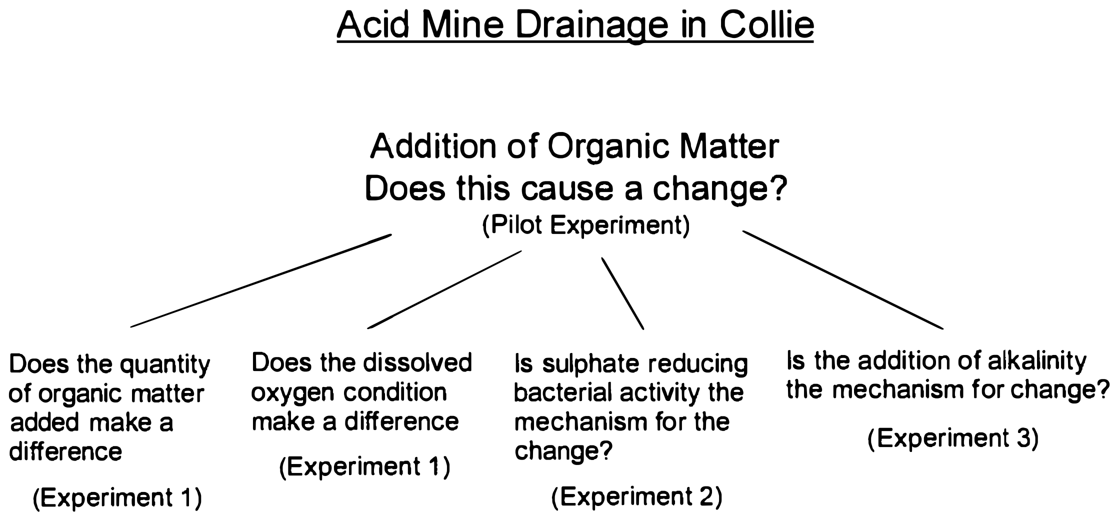


Figure 3.1 The approach taken by the study.

This chapter describes the methods that are common to both the laboratory and field experiments described in the subsequent chapters.

Selection of organic matter types

The three types of organic matter (manure, hay, and mulch) were chosen for this research project based on their availability and cost. If organic matter additions are a solution to the acidic voids problem in Collie, then there will need to be very large quantities available at an economically affordable price. If this is not possible it may become uneconomic and unfeasible for mining companies to utilise the remediation method. Hay was obtained from bales bought at the Ewington property, and the mulch was shredded vegetation that is commonly used in home gardening and was supplied by Greenwaste Services, Unit 11/16 Dellamarta Road, Wangara, WA. The cow manure was made into a slurry by adding water to raw cattle faeces obtained from a Collie dairy farmer. The cattle faeces were about 6 months old when collected.

Measurement of physio-chemical properties

In this study a variety of parameters were measured in both the laboratory and field experiments.

Conductivity

Conductivity was measured in the laboratory and field experiment using a Yeo Kal Model 611 Intelligent Water Quality Analyser or Wissenschaftlich-Technische-Werkstätten (WTW) 330 conductivity field meter. Measurements were automatically standardised at 25 °C. Meters were calibrated before use.

Alkalinity

Alkalinity is a measure of acid-neutralising/buffering capacity. It is the sum of all the titratable bases. The measured value may vary significantly with the end-point pH used (APHA, 1989). Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known (APHA, 1989).

Alkalinity was determined by titration (APHA, 1989) of a 25 mL sample with a standard solution (approx 0.003N) of sulphuric acid to a pH of 4.5, detected using a WTW 330 pH meter. The alkalinity is reported in terms of $CaCO_3$.

pH

pH was measured using a Yeo Kal Model 611 Intelligent Water Quality Analyser or WTW 330 field pH meter. Measurements were automatically corrected for temperature and meters were calibrated before use.

Dissolved oxygen

Dissolved oxygen levels in natural and waste waters are influenced by the physical, chemical and biochemical activity in the water body (APHA, 1989). Measurements of dissolved oxygen were taken using a Yeo Kal Model 611 Intelligent Water Quality Analyser or WTW OXI 320/set. Meters were calibrated before sampling, and measurements were recorded in mg O_2 L^{-1} and as a percentage of total saturation (% saturation).

Sulphate

Water samples used to measure sulphate content were stored at 4 °C to avoid the conversion of SO_4^{2-} to S^{2-} (APHA, 1989). Sulphate was measured using the barium chloride turbidimetric method (APHA, 1989). All measurements were made at 20 ± 2 °C. A sample of 100 mL of water was placed in an Erlenmeyer flask and a 20 mL aliquot of buffer solution A (APHA, 1989) was added and mixed at a constant speed. While it was mixing "a spoonful of barium chloride crystals ($BaCl_2$)" (APHA, 1989, p. 4-208) was added and stirred for 60 seconds. After the stirring period had ended the turbidity was measured at 420 nm on a spectrophotometer (Shimadzu) after five minutes (APHA, 1989). The sample colour and turbidity were corrected against a blank sample from the same water by not adding any $BaCl_2$ crystals.

Sulphide

Water samples (250 mL) were collected and preserved by adding 10 drops of zinc acetate (2M; APHA, 1989). Sulphide measurements were made using a Sure-flow ™ combination silver/sulphide electrode and an Orion 290 field meter (Orion Research, 1998). A Sulphide Anti-Oxidant Buffer II (SAOB II) solution was prepared using 40 g L^{-1} of sodium hydroxide (NaOH), 160 g L^{-1} sodium salicylate ($C_6H_4(OH).COONa$) and 36 g L^{-1} ascorbic acid ($C_6H_6O_6$; Orion Research, 1998). A stock solution of sodium sulphide was made by adding 100 g of reagent grade

NaS.9H_2O in 100 mL of distilled, deaerated water. A sulphide standard was prepared weekly by pipetting 10 mL of the stock solution into a 1 L volumetric flask. A 500 mL aliquot of the SAOB II buffer solution was added and the solution made up to 1 L with distilled, deaerated water. The water was deaerated by bubbling the water with nitrogen gas through an air stone for 15 minutes. It was then checked using a WTW 330 OXI meter to make sure the dissolved oxygen level was < 0.01 mg L^{-1}. The exact concentration of sulphide was determined by titrating 10 mL of this sulphide solution with 0.1M lead perchlorate using a millivolt electrode as an end point indicator. The concentration was calculated using the following equation:

$$C = 3206\,(V_t/V_s) \qquad \textbf{(Equation 3.1)}$$

C = concentration as ppm sulphide,

V_t = volume of titrant at end point,

V_s = volume of standard (10 mL; Orion Research, 1998).

Other standards were prepared by serial dilution of the weekly standard. The meter was then calibrated using a three-point calibration method (Orion Research, 1998).

The measured concentration of sulphide in parts per million (ppm) was recorded from the Orion 290a meter. The recordings were taken from a 25 mL sample, which had been diluted 1:1 with the SAOB II buffer solution and mixed at a constant rate by a magnetic stirrer. Deaerated, distilled water was always used to prevent the oxidation of sulphide during the measurements.

Gilvin

Gilvin is a term used to describe the intense brown colour formed by humic/fulvic acids in aquatic systems at concentrations that can effect the attenuation of photosynthetically available radiation (Lund and Ryder, 1997). It was measured to provide an indication of the level of dissolved carbon present in the water. It can also provide an indication of the aesthetic appeal of the water. Gilvin was measured by determining the absorbency of filtered (Whatman ® GF/C) water at 440 nm and multiplying by a correction factor (2.303; Wrigley, *et al.*, 1988). A correction factor was then used to correct the absorbency for one metre (i.e., if 1cm cuvettes were used, the absorbency was multiplied by 2.303 * 100, to make up a one metre light path; Equation 3.2). Measurements were made using 4 cm cuvettes in a spectrophotometer (Shimadzu model). A blank of distilled water was used to zero the spectrophotometer prior to measurement.

$$\text{Gilvin } (_{g440}\ m^{-1}) = \text{Abs}_{440} * 2.303 \qquad \textbf{(Equation 3.2)}$$

Filterable reactive phosphorous, nitrite/nitrates, ammonia

Water samples (250mL) were filtered using Whatman ® GF/C papers. The water samples were then frozen and sent to the Marine and Freshwater Research Laboratory Environmental Science (MAFRL) at Murdoch University, Western Australia for analysis. This is an NATA accredited research laboratory. The ammonia was measured on a Lachat Automated Flow Injection Analyser (Lachat Instruments QuickChem Method 31-107-06-1-A (29th Jul 1994). The filterable reactive phosphate was measured on a Lachat Automated Flow Injection Analyser (Lachat Instruments QuickChem Method 31-115-01-3-A (17th Aug 1994) and the nitrate plus nitrite was measured using a Lachat Automated Flow Injection

Analyser (Lachat Instruments QuickChem Method 31-107-04-1-A (18th Jul 1996).

4.0 LABORATORY EXPERIMENTS

Introduction

Studies designed to evaluate the effectiveness of wetland systems constructed for mine drainage treatment should desirably monitor hydraulic fluxes, water chemistry and wetland substrate chemistry (Henrot, *et al.* 1989). However, cost and personnel commitment required for this type of research are often in excess of available resources. In this situation, small scale laboratory studies can be useful for evaluating the processes involved in chemical modification of mine drainage in wetland systems instead of more expensive field monitoring programs. Laboratory studies provide the ability to control hydraulic flux and water chemistry, minimising sources of variability, which often make interpretation of field research difficult (Henrot, *et al.,* 1989). Lyew and Sheppard, (1997), Chermack and Runnells, (1997) and Wieder, Linton and Heston (1990) all used laboratory mesocosms or cores for research to examine the effects of sulphate reducing bacterial action or chemical cap development on acid drainage environments. Cores represent an artificial microcosm that attempt to replicate conditions in a waterway in a laboratory situation. As cores are taken from the lake and transported to the laboratory, there is always the potential for the disturbance of the water column and associated substrate to alter the active biological processes in the core, such that they no longer replicate the lake situation. It is difficult to replicate light conditions, water inflow, and biological activity that occur in a lake in a core transported to the laboratory. Cores therefore can only ever approximate conditions of the lake. Cores enable the researcher to set up manageable experiments that might provide an indication of what could occur in an *in situ* experimental situation.

A pilot study and three sequential experiments were conducted to determine the effectiveness of different organic matter additions (i.e., manure, hay and mulch). The pilot experiment was designed to quantify the changes in pH with three organic treatments of acidic void water. The research protocol and results from the pilot study are contained in appendix 1 and are not described in this section because the outcome from this experiment was very similar to the results for experiment 1.

To address the first aim of the project, a series of three sequential experiments were undertaken. Experiment 1 tested the effectiveness of two quantities of selected organic matter on pH and sulphate reducing bacterial activity. As sulphate reducing bacterial activity appeared low experiment 2 tested the effects of sulphate reducing bacteria and inoculations when organic matter was added to cores. As pH improvements were recorded with the addition of organic matter experiment 3 attempted to determine the importance of alkalinity generated by the organic matter on changes in pH.

Methods for Laboratory experiments

The three experiments all used intact cores and the oxygenated conditions described below. A pilot study (see Appendix 1) demonstrated the practicality of using this experimental setup.

Intact cores

Cores containing water and substrate from Ewington were collected using perspex tubes (600 mm high and 100 mm in diameter). These perspex tubes were pushed into the sediment by hand to a depth of 200 mm and a stopper placed on top of the tube. The vacuum created in the tube when it was lifted enabled an intact sample of sediment and the water column to be removed. A stopper was inserted in the bottom of the tube before it was transported to the laboratory. A stopper in the top of the core minimised gas exchange with the ambient atmosphere. When in the laboratory, the sediment section of each core was wrapped in black plastic to exclude light and hence photosynthetic activity in the sediment (Figure 4.1). Cores were then left in a controlled temperature laboratory (20 ± 2 °C) for two days to settle prior to experimentation.

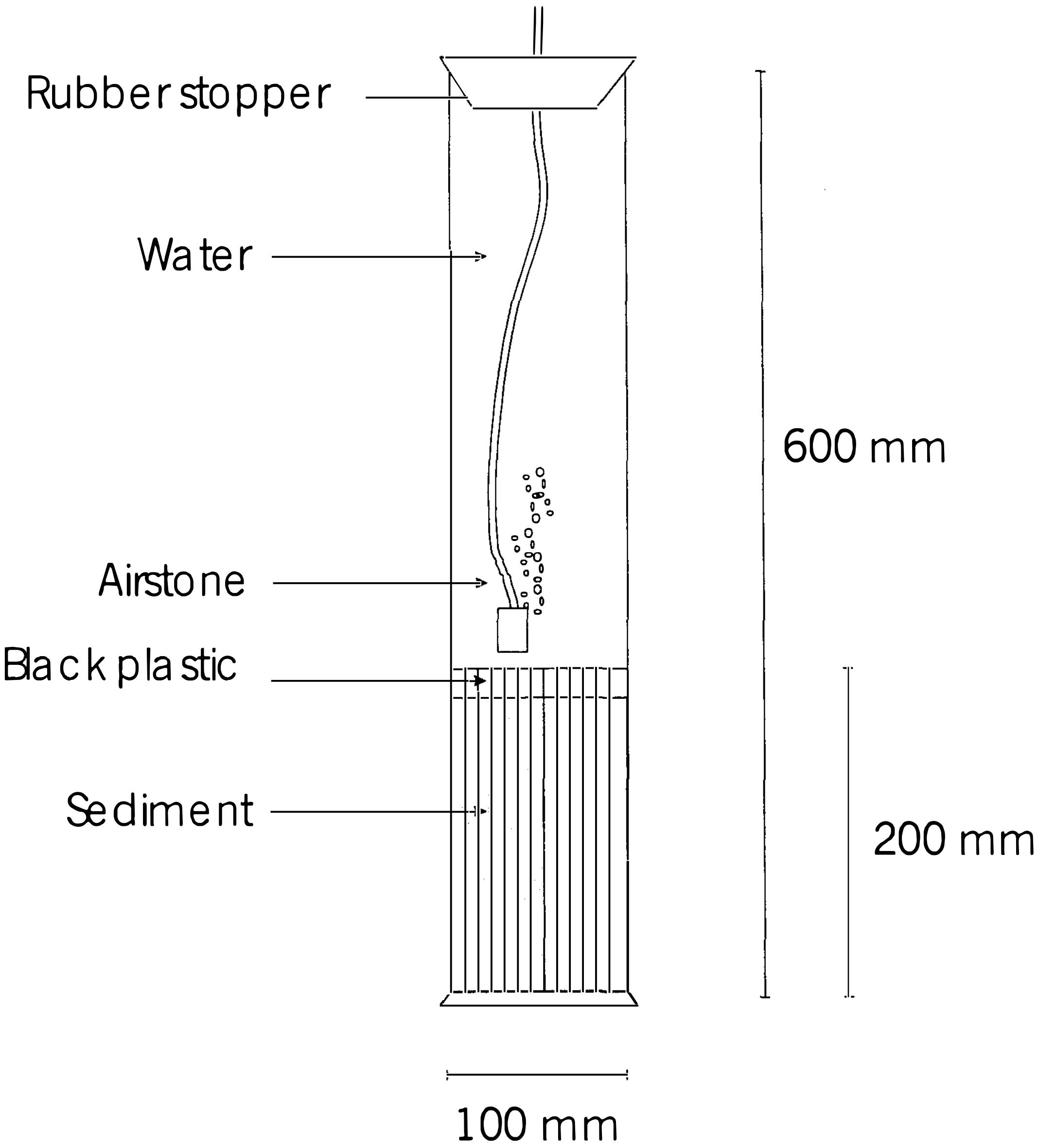


Figure 4.1 Schematic design of a core used in the laboratory experiments.

Dissolved oxygen conditions

Sulphate reducing bacteria are strictly anaerobic and are severely inhibited by even small amounts of oxygen. Water chemistry and biota changes in the intact cores were therefore examined under two different aeration conditions, hypoxic and oxic, to see if sulphate reducing bacteria are responsible for any pH changes.

To create the hypoxic conditions (< 2 mg O_2 L^{-1}) in the cores, dry nitrogen gas was bubbled through the water column for 15 minutes for the first two days after any treatments were added, and then each sampling day (different for each experiment) to evacuate dissolved oxygen and to maintain core water oxygen levels below 2 mg O_2 L^{-1}. Water in the oxic cores was bubbled with atmospheric air, in the same pattern as the hypoxic cores, to maintain the dissolved oxygen content above 5 mg O_2 L^{-1}. An airstone was used to increase bubble surface area and improve sequestration of air into the water column. Hypoxic conditions were used instead of complete anoxia due to the difficulties experienced in the pilot experiment in maintaining the dissolved oxygen levels at zero.

Experiment 1 – Quantity and type of organic matter needed to produce an affect

Ninety cores collected on the 4/8/98 were divided into two groups; 45 cores with oxic conditions and 45 to hypoxic conditions. Within each group, six replicates of the three treatments (hay, mulch and manure) by two quantities (50 cm^3 and 100 cm^3) and six controls were randomly assigned on day 0 (Table 4.1). These two organic matter quantities were chosen because the equivalent amount to 50 cm^3 in a perspex tube when spread evenly over the lake bottom was considered an economically feasible quantity of organic matter, if it was to be used in a rehabilitation situation. One hundred cm^3 was used to determine what advantage would be gained by a two fold increase in organic matter. Only five replicates of 100 cm^3 organic matter treated cores were used because I was limited to transporting ninety cores from the study site to the laboratory.

Table 4.1 Design of Experiment 1: Core allocations to the three organic matter treatments and control.

	$50cm^3$ of Organic Matter		$100cm^3$ of Organic Matter	
	Oxygen concentration			
Organic Matter	Oxic (>5mg O_2 L^{-1})	Hypoxic (<2mg O_2 L^{-1})	Oxic (>5mg O_2 L^{-1})	Hypoxic (<2mg O_2 L^{-1})
Control	6	6	6	6
Manure	6	6	5	5
Hay	6	6	5	5
Mulch	6	6	5	5

The intervals of measurements for temperature, dissolved oxygen and pH were days –1, 0, 1, 2, 4, 6, 9, 11, 13, 17, 23, 29, 37 and 43. Sulphate, sulphide and gilvin were measured at days –1 and 43.

Experiment 2 – Testing for sulphate reducing bacteria

Thirty-six cores were collected from Ewington (24/11/98) and transported to the laboratory on the same day. All cores were kept under hypoxic conditions. Twelve cores were randomly assigned as controls, 12 treated with 50 cm^3 of manure and 12 treated with 50 cm^3 of mulch. In each block of 12 cores, six were inoculated with sulphate reducing bacteria (Table 4.2).

Table 4.2 Design of Experiment 2: Core allocation to the two organic matter treatments and control.

	Oxygen concentration	
	Hypoxic (<2mg O_2 L^{-1})	
Organic Matter	Inoculum added	No Inoculum
Control	6	6
Manure	6	6
Mulch	6	6

The sulphate reducing bacteria were obtained by centrifuging (3000 rpm, 2 minutes) a sample of the top 5 cm of sediment taken from Lake Joondalup, until 50 mL of supernatant was obtained. Lake Joondalup produces hydrogen sulphide gas at times of the year indicating that it is a good source of sulphate reducing bacteria (M. Lund, pers. comm. November, 1998). The supernatant was then injected into the sediment of the cores. At all stages of collection, preparation and injection of the inoculum, exposure to oxygen was minimised by use of a nitrogen atmosphere gas chamber. Dissolved oxygen levels and pH of the water column were monitored for days –1, 1, 2, 8, 14, 20, 23, 28, 34, 41 and 48. At the end of the six-week period a sodium sulphate (Na_2SO_4) aliquot was added to each sample to bring sulphate levels to approximately 800 mg L^{-1}. This was used to test whether sulphate reducing bacteria were present in the sediment. If they were present, a black sulphide precipitate would be evident (Lyew and Sheppard, 1999). Sulphate, sulphide and gilvin were measured six weeks after the sodium sulphate was added.

Experiment 3 – Measuring alkalinity production

Thirty six cores of doubly, deionised water were prepared with no sediment on 7/6/99. Eighteen of the cores were kept under hypoxic conditions and 18 cores under oxic conditions. Within each block of 18 cores, six were left as controls, six were treated with manure and six were treated with mulch (Table 4.3). Fifty cubic centimetres of manure or mulch was added to each treatment tube.

Table 4.3 Design of Experiment 3: Core allocation to two organic matter treatments and control.

	Oxygen concentration	
Organic Matter	**Oxic** (>5mg O_2 L^{-1})	**Hypoxic** (<2mg O_2 L^{-1})
Control	6	6
Manure	6	6
Mulch	6	6

Dissolved oxygen levels and pH of the water columns were monitored on days –1, 1, 3, 7, 10, 15, 22, 28, 35 and 42. Water in the cores was sampled to measure sulphate, sulphide, gilvin and alkalinity for days –1, 22 and 42.

Data analyses for laboratory experiments

Data reporting

Core experiments

Means and standard errors (se) are reported for all data from each experiment. These data are generally reported in Appendix 2. Summary tables of these more detailed tables are incorporated into the text. The results also present a *grand* *mean* for each treatment using the data points after the effect of the treatment had stabilised. A visual observation of the plotted data was used to determine when the effect of the treatment had stabilised in each experiment. The mean for the pH and dissolved oxygen are reported for days 9 to 43 for experiment 1; and days 14 to 48 for experiment 2; and days 7 to 42 for experiment 3.

Repeated measures MANOVA

A repeated measures MANOVA (Hair, *et al.* 1995) was used to determine differences among treatments in all experiments. The repeated measures MANOVA was calculated using the data points after the cores or ponds had stabilised. The same set of data points were used to calculate the *grand means* for stabilised cores and ponds over a period of time (see earlier). Interaction effects were routinely monitored in the statistical analysis. They are only referred in the subsequent text if they interaction was significant at the $\propto = 0.05$ level.

A Tukey test was used to determine post hoc differences between organic matter treatments, and organic matter treatments and controls that are observed in the MANOVA. The statistical analysis was generally performed after the data had stabilised into a regular pattern, however, this was not always the case as total stabilisation for some experiments were not evident for the duration of the experiment. For example, pH might slowly increase over the duration of the experiment. This would generally be detected in the Wilks' Lambda test of significance for repeated measures. Where a significant difference was found between organic matter treatments, and organic matter treatments and control, the post hoc Tukey test enabled a week-by-week examination of where the differences occurred. If there was a trend, for example, a slow increase in pH for the treatments and not the control, then this would be detected from the Tukey test and is reported.

Power analysis

If a particular statistical test indicated no significant difference it might be because there is no real difference or because the study design makes it unlikely that a real effect would be detected (Inouye, 1997). Small samples sizes and wide variability within treatments make it statistically more difficult to detect difference between treatments when they actually exist. Statistical power indicates the probability of obtaining a statistically significant result, when there is a real difference in the treatments (type II errors). Acid mine drainage remediation is expensive, and researchers and mine management would not like to discard a potentially cheaper solution to the problem simply because it was unable to be statistically demonstrated using stringent criteria when further exploratory investigation may provide a more conclusive positive result. A power analysis was calculated for the experimental results using SPSS. Power analysis results were routinely considered in the data analyse and are presented in most summary tables to assist the reader to interpret the results.

Results

Experiment 1

pH

A significant increase in pH of between 1-3 units was recorded for mulch, manure and hay over the 57 day experiment (Figure 4.2). The largest increase was seen for manure {4.40 (± 0.032) to 7.09 (± 0.033)}, followed by mulch {4.39 (± 0.03) to 6.85 (± 0.03)}. In both cases pH had increased and stabilised after 10 days. Hay produced a more variable response with a final value over 0.5 of a pH unit below the other organic matter types {4.51 (± 0.065) to 6.39 (± 0.035)}.

The 100cm^3 quantities of manure produced significantly different final pH values to that for 50cm^3, however, the differences were relatively small {50cm^3; 7.10 (± 0.02); 100 cm^3; 7.31 (± 0.03); Figure 4.2; Table 4.4}. No significant difference was found between pH values for different quantities of hay {50cm^3; 5.94 (± 0.05); 100 cm^3; 5.80 (± 0.06)} or mulch {50cm^3; 6.54 (± 0.03); 100 cm^3; 6.61 (± 0.03)} (Figure 4.2; Table 4.4).

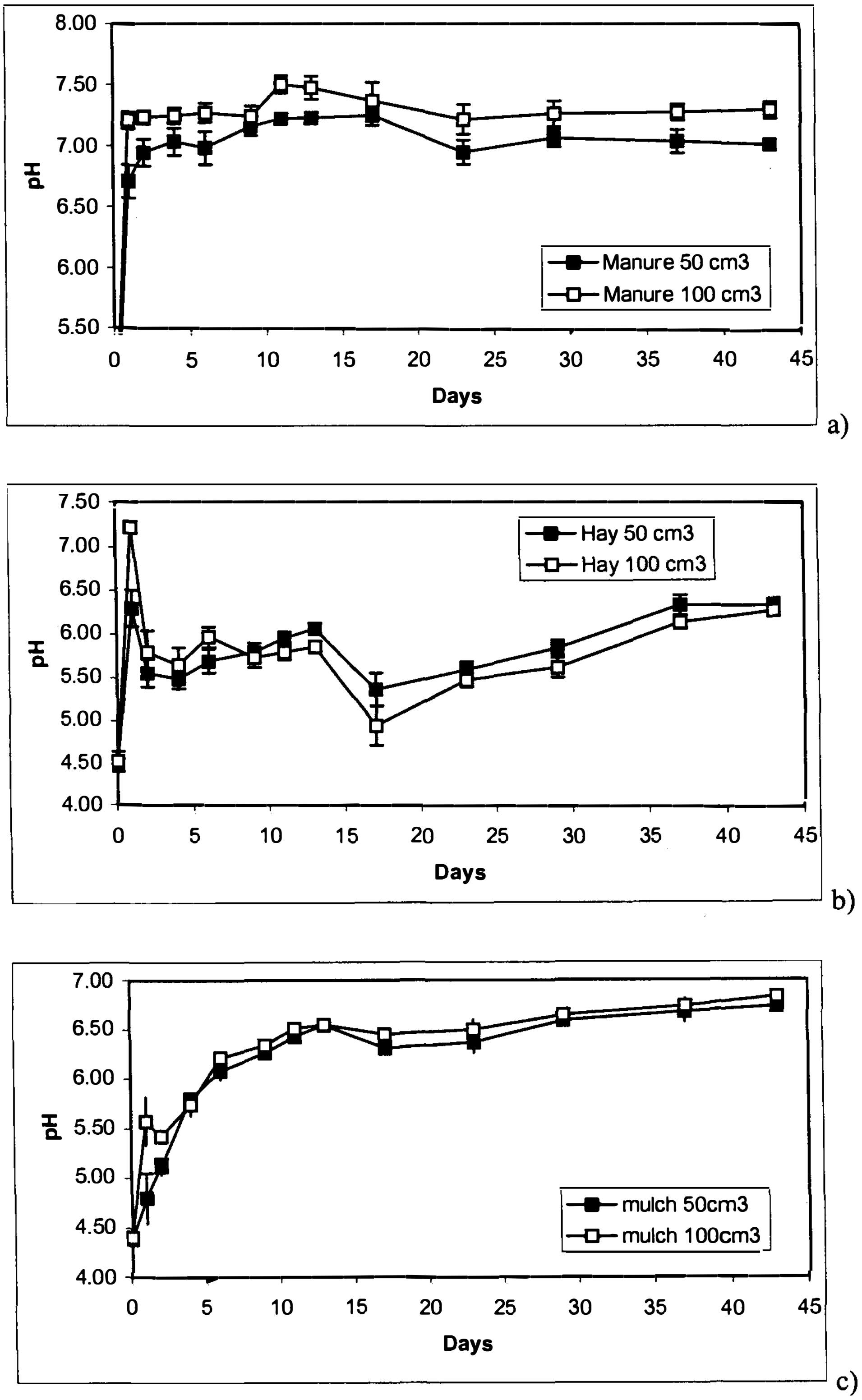


Figure 4.2 Experiment 1: Comparison of changes in mean pH values for the three treatments over the duration of the experiment showing the effects of quantity for manure (a); hay (b); and mulch (c).
Standard error bars are shown for each sampling day, n = 6 for each treatment.

There was no significant difference in pH values between aeration conditions for the control or mulch treatment but there was a significant difference between aeration conditions for the manure {hypoxic, 7.10 (± 0.06); oxic, 6.85 (± 0.06)} and hay treatments {hypoxic, 5.62 (± 0.06); oxic, 5.98 (± 0.05); Tables 4.6}.

Dissolved oxygen

The control cores were hard to keep below the desired 2 mg O_2 L^{-1} for the hypoxic conditions. Mean dissolved oxygen concentrations in the hypoxic control cores was 2.43 (± 0.07) mg O_2 L^{-1} and in the oxic control cores 4.08 (± 0.09) mg O_2 L^{-1}. Minor oxygen leakage around the rubber stoppers is the suspected reason for the increased oxygen concentrations in the hypoxic cores.

The dissolved oxygen levels for the two aeration conditions remained significantly different for the control cores, however, the lower level was not anoxic (Figure 4.3). The three treatment conditions all showed hypoxic conditions generally < 0.2 mg L^{-1} with the addition of organic matter (Figure 4.3).

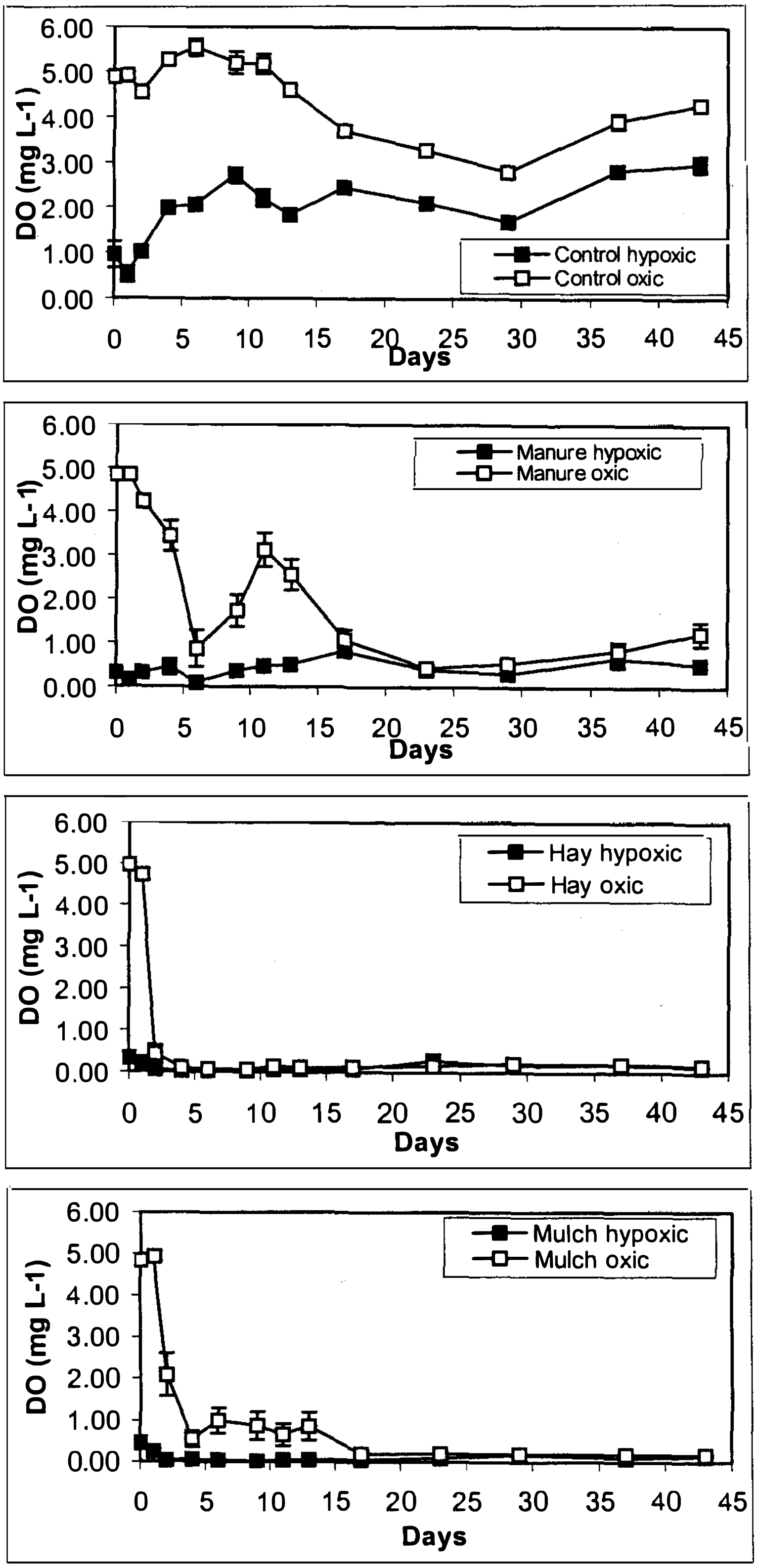


Figure 4.3 Experiment 1: Comparison of changes in mean dissolved oxygen levels (mg L^{-1}) over the duration of the experiment showing the interaction between aeration condition for each of the treatments and the control. Standard error bars for each sampling day, n = 6 for each treatment.

There was no significant change in dissolved oxygen levels for the manure or hay over the period of the experiment (Table 4.4) but there was a significant decrease in the dissolved oxygen levels for the mulch {from 2.64 (± 0.05) mg O_2 L^{-1} at day 1 to 0.40 (± 0.18) mg O_2 L^{-1} at day 43}. Higher quantities of organic matter reduced dissolved oxygen levels for manure and mulch treatments {manure - 50 cm^3, 1.53 (± 0.12) mg O_2 L^{-1}; 100 cm^3, 1.03 (± 0.13) mg O_2 L^{-1}; mulch - 50 cm^3, 0.87 (± 0.11) mg O_2 L^{-1}; 100 cm^3, 0.45 (± 0.11) mg O_2 L^{-1}}.

Sulphate and sulphide

Water for all experiments came from the same source {sulphate; 0.81 (± 0.14) mg SO_4 L^{-1}; sulphide; 67.60 (± 16.17) ppb}. There was no significant difference in the sulphate or sulphide levels between the organic matter treatments when comparing aeration or quantity (Table 4.4). Although sulphate levels in all treatments were generally low, a post hoc Tukey test on day 43 results showed that sulphate levels for manure {3.25 (± 0.141) mg SO_4 L^{-1}} and hay {3.41 (± 0.505) mg SO_4 L^{-1}} treatments were approximately twice as high as those for the mulch treatment {1.32 (± 0.662) mg SO_4 L^{-1}} and the controls {0.81 (± 0.141) mg SO_4 L^{-1}; Table 4.4}.

For sulphide, there was a significant difference among treatments, and a significant difference between quantities (Table 4.4; Figure 4.8). On day 43 the manure {496.0 (± 71.4) ppb} and hay {412.9 (± 118.1) ppb} treatments had significantly higher sulphide levels than the mulch {216.6 (± 51.1) ppb} treatment and the control {132.8 (± 26.4) ppb}. The post hoc Tukey test showed that when more organic matter was added there was a significant increase in the sulphide levels for manure and hay (Table 4.4; Figure 4.4); the corresponding sulphate levels were low (Table 4.4). This suggests sulphate is being converted to sulphide by sulphate reducing bacterial activity, at least in the early phase of the experiment. Neither the aeration conditions nor the quantity of organic matter added made any difference to the level of sulphate reduction.

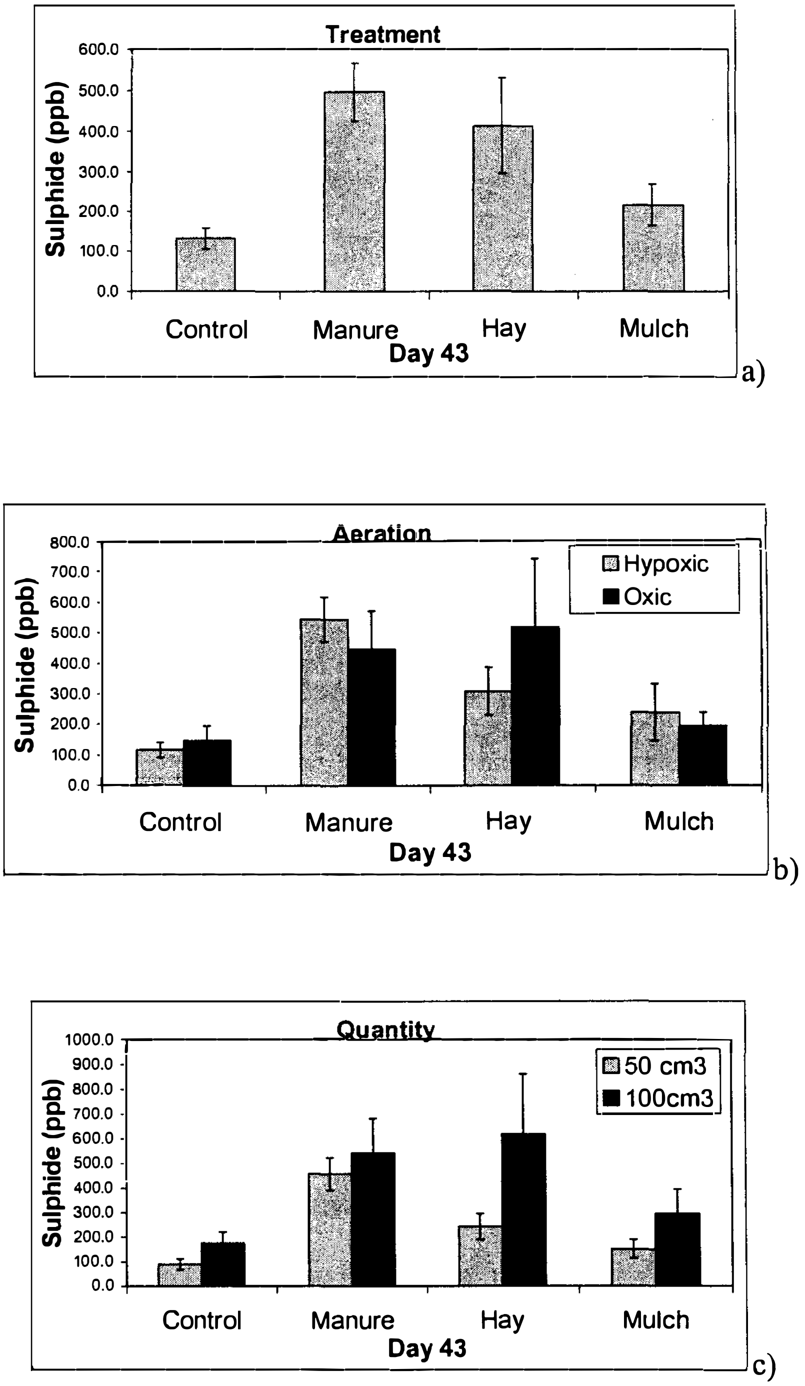


Figure 4.4 Experiment 1: Comparison of sulphide levels (ppb) among treatments (a); between aeration conditions (b); and between quantities (c). Mean sulphide level on day 0 (before treatment) was 2.4 (± 1.33) ppb. Standard error bars are shown for each sampling day, n = 6 for each treatment.

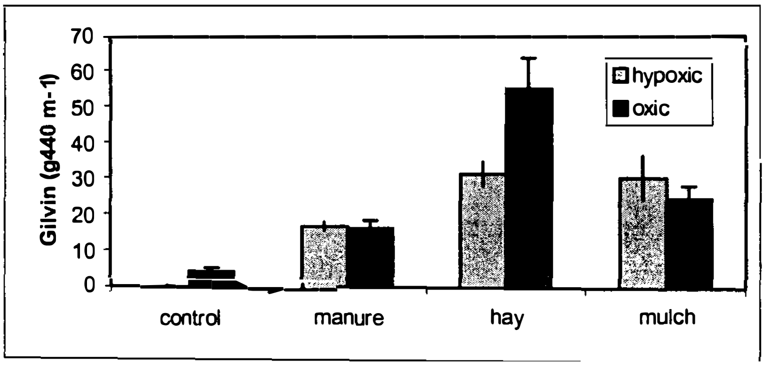
Gilvin

A significant increase was recorded in gilvin values for the three treatments over the duration of the experiment. Mean gilvin values (g440) for day 43 for the three treatments and control were: control 3.11 (± 0.10) $_{g440}$ m^{-1}; manure 50.68 (± 0.57) $_{g440}$ m^{-1}; hay 38.23 (± 1.06) $_{g440}$ m^{-1}; and mulch 53.10 (± 1.26) $_{g440}$ m^{-1} compared with 2.82 (± 0.610) $_{g440}$ m^{-1} at the beginning of the experiment.

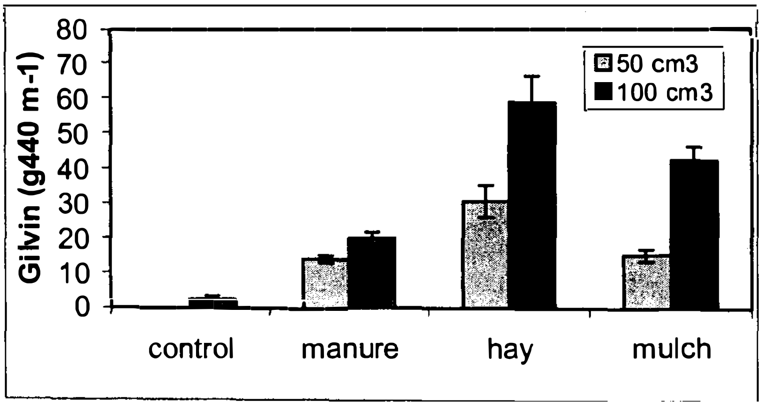
There was a significant interaction between treatments and quantities, and treatment and aeration (Table 8.7; Figure 4.5) for gilvin values which means that further statistical analysis is not possible for the time series data. The hay {55.76 (± 8.574) $_{g440}$ m^{-1}} treatment had higher gilvin levels for the oxic conditions than for hypoxic conditions {31.74 (± 3.380) $_{g440}$ m^{-1}}, whereas, for the other organic matter treatments and control, the hypoxic condition had higher gilvin levels {manure hypoxic - 16.89 (± 1.201) $_{g440}$ m^{-1} vs oxic – 16.46 (± 2.081) $_{g440}$ m^{-1}; mulch hypoxic – 30.87 (± 6.183) $_{g440}$ m^{-1} vs oxic 24.90 (± 3.651) $_{g440}$ m^{-1}; control hypoxic – 0.17 (± 0.302) $_{g440}$ m^{-1} vs oxic 4.19 (± 0.958) $_{g440}$ m^{-1}; Figure 4.9}. Gilvin levels were higher when increased amounts of organic matter were added {manure 55.34 (± 4.21) $_{g440}$ m^{-1} to 80.32 (± 7.02) $_{g440}$ m^{-1}; hay 123.27 (± 8.49) $_{g440}$ m^{-1} to 202.39 (± 34.47) $_{g440}$ m^{-1}; and mulch 61.85 (± 7.22) $_{g440}$ m^{-1} to 171.19 (± 15.56) $_{g440}$ m^{-1}}. An ANOVA on day 43 day data indicated a significant difference ($F_{3,86} = 31.6$, $P < 0.001$) among treatments and the controls for gilvin values. A subsequent post hoc Tukey test indicated a significant difference in gilvin levels between the control and treatments, and among some treatments {hay 43.75 (±

5.205) $_{g440}$ m^{-1} > mulch 27.89 (± 3.564) $_{g440}$ m^{-1} > manure 16.67 (± 1.173) $_{g440}$ m^{-1} > control 2.18 (± 0.646) $_{g440}$ m^{-1}}.

The hay treatment had a strong unpleasant odour and a slimy film formed on the surface of the cores after about three days. It took the mulch about three days to settle to the bottom and settlement rate increased when the water was slightly agitated as in pilot experiment.



a)



b)

Figure 4.5 Experiment 1: A comparison of gilvin values between aeration conditions (a) and between quantities of organic matter (50 vs 100 cm^3; b). Standard error bars are shown for each sampling day, n = 6 for each treatment.

Table 4.4 Experiment 1: Mean and standard errors for pH, dissolved oxygen, sulphate, sulphide and gilvin for days 9 to 43.

* indicates significant values at $p \le 0.05$; SO_4 - sulphate; S^{2-} - sulphide; sulphate, sulphide and gilvin for day 43 (see methods section for details on the calculation of means). D - days; T - treatments; A - aeration conditions; Q- quantity.

	Treatment	Aeration	Quantity	$\bar{x}$ ± se	D x T	D x T x A	D x T x Q	D x T x A x Q	Tukey test results at last sampling day				
					F value	*F* value	*F* value	*F* value		Control	Manure	Hay	Mulch
pH	Control	<2 mgL^{-1}	50 cm^3	4.62 ± 0.079	264.3*	6.942*	1.795	0.392	Control				
		<2 mgL^{-1}	100 cm^3	4.89 ± 0.108									
		>5 mgL^{-1}	50 cm^3	4.96 ± 0.106									
		>5 mgL^{-1}	100 cm^3	5.04 ± 0.073									
	Manure	<2 mgL^{-1}	50 cm^3	7.23 ± 0.032					Manure	*			
		<2 mgL^{-1}	100 cm^3	7.49 ± 0.042									
		>5 mgL^{-1}	50 cm^3	6.96 ± 0.028									
		>5 mgL^{-1}	100 cm^3	7.13 ± 0.038									
	Hay	<2 mgL^{-1}	50 cm^3	5.77 ± 0.076					Hay	*	*		
		<2 mgL^{-1}	100 cm^3	5.58 ± 0.092									
		>5 mgL^{-1}	50 cm^3	6.11 ± 0.040									
		>5 mgL^{-1}	100 cm^3	6.02 ± 0.049									
	Mulch	<2 mgL^{-1}	50 cm^3	6.57 ± 0.044					Mulch	*	*	*	
		<2 mgL^{-1}	100 cm^3	6.52 ± 0.032									
		>5 mgL^{-1}	50 cm^3	6.52 ± 0.037									
		>5 mgL^{-1}	100 cm^3	6.63 ± 0.041									

Table 4.4 cont.

	Treatment	Aeration	Quantity	$\bar{x}$ ± se	D x T	D x T x A	D x T x Q	D x T x A x Q	Tukey test results at last sampling day				
					F value	*F* value	*F* value	*F* value		Control	Manure	Hay	Mulch
DO (mg L^{-1})	Control	<2 mgL^{-1}	50 cm^3	2.26 ± 0.092	512.2*	36.781*	8.796*	0.836	Control				
		<2 mgL^{-1}	100 cm^3	2.60 ± 0.089									
		>5 mgL^{-1}	50 cm^3	4.15 ± 0.140									
		>5 mgL^{-1}	100 cm^3	4.00 ± 0.119									
	Manure	<2 mgL^{-1}	50 cm^3	0.66 ± 0.068					Manure	*			
		<2 mgL^{-1}	100 cm^3	0.32 ± 0.046									
		>5 mgL^{-1}	50 cm^3	1.73 ± 0.170									
		>5 mgL^{-1}	100 cm^3	0.99 ± 0.164									
	Hay	<2 mgL^{-1}	50 cm^3	0.11 ± 0.012					Hay	*	*		
		<2 mgL^{-1}	100 cm^3	0.14 ± 0.038									
		>5 mgL^{-1}	50 cm^3	0.14 ± 0.011									
		>5 mgL^{-1}	100 cm^3	0.16 ± 0.015									
	Mulch	<2 mgL^{-1}	50 cm^3	0.15 ± 0.067					Mulch	*	*	*	
		<2 mgL^{-1}	100 cm^3	0.27 ± 0.095									
		>5 mgL^{-1}	50 cm^3	0.09 ± 0.010									
		>5 mgL^{-1}	100 cm^3	0.11 ± 0.028									

Table 4.4 cont.

	Treatment	Aeration	Quantity	$\bar{x}$ ± se	D x T	D x T x A	D x T x Q	D x T x A x Q	Tukey test results at last sampling day				
					F value	*F* value	*F* value	*F* value		Control	Manure	Hay	Mulch
SO_4 (mg L^{-1})	Control	<2 mgL^{-1}	50 cm^3	0.88 ± 0.222	7.454*	1.720	1.043	0.485	Control				
		<2 mgL^{-1}	100 cm^3	0.65 ± 0.118									
		>5 mgL^{-1}	50 cm^3	1.14 ± 0.497									
		>5 mgL^{-1}	100 cm^3	0.57 ± 0.115									
	Manure	<2 mgL^{-1}	50 cm^3	2.40 ± 0.526					Manure	*			
		<2 mgL^{-1}	100 cm^3	3.02 ± 1.683									
		>5 mgL^{-1}	50 cm^3	3.54 ± 0.346									
		>5 mgL^{-1}	100 cm^3	4.25 ± 0.908									
	Hay	<2 mgL^{-1}	50 cm^3	2.51 ± 0.674					Hay	*			
		<2 mgL^{-1}	100 cm^3	4.17 ± 1.642									
		>5 mgL^{-1}	50 cm^3	3.06 ± 0.638									
		>5 mgL^{-1}	100 cm^3	3.91 ± 0.501									
	Mulch	<2 mgL^{-1}	50 cm^3	0.59 ± 0.107					Mulch		*	*	
		<2 mgL^{-1}	100 cm^3	3.14 ± 2.409									
		>5 mgL^{-1}	50 cm^3	0.64 ± 0.047									
		>5 mgL^{-1}	100 cm^3	0.70 ± 0.211									

Table 4.4 cont.

	Treatment	Aeration	Quantity	$\bar{x}$ ± se	D x T	D x T x A	D x T x Q	D x T x A x Q	Tukey test results at last sampling day				
					F value	*F* value	*F* value	*F* value		Control	Manure	Hay	Mulch
S^{2-} (ppb)	Control	<2 mgL^{-1}	50 cm^3	60.33 ± 18.724	273.87 ± 51.3	273.87 ± 51.3	273.87 ± 51.3	273.87 ± 51.3	Control				
		<2 mgL^{-1}	100 cm^3	175.50 ± 29.208									
		>5 mgL^{-1}	50 cm^3	119.25 ± 39.635									
		>5 mgL^{-1}	100 cm^3	175.95 ± 90.811									
	Manure	<2 mgL^{-1}	50 cm^3	444.23 ± 114.73					Manure	*			
		<2 mgL^{-1}	100 cm^3	664.80 ± 59.170									
		>5 mgL^{-1}	50 cm^3	471.67 ± 76.210									
		>5 mgL^{-1}	100 cm^3	418.70 ± 276.12									
	Hay	<2 mgL^{-1}	50 cm^3	114.02 ± 14.943					Hay	*			
		<2 mgL^{-1}	100 cm^3	540.60 ± 94.955									
		>5 mgL^{-1}	50 cm^3	371.33 ± 76.812									
		>5 mgL^{-1}	100 cm^3	693.70 ± 502.64									
	Mulch	<2 mgL^{-1}	50 cm^3	96.33 ± 27.532					Mulch		*		
		<2 mgL^{-1}	100 cm^3	410.40 ± 184.41									
		>5 mgL^{-1}	50 cm^3	204.90 ± 66.141									
		>5 mgL^{-1}	100 cm^3	180.98 ± 67.507									

Table 4.4 cont.

	Treatment	Aeration	Quantity	$\bar{x}$ ± se	D x T	D x T x A	D x T x Q	D x T x A x Q	Tukey test results at last sampling day				
					F value	*F* value	*F* value	*F* value		Control	Manure	Hay	Mulch
Gilvin (g_{440} m^{-1})	Control	<2 mgL^{-1}	50 cm^3	0.54 ± 0.571	102.660*	14.365*	16.178*	6.708*	Control				
		<2 mgL^{-1}	100 cm^3	-0.21 ± 0.140									
		>5 mgL^{-1}	50 cm^3	3.71 ± 0.392									
		>5 mgL^{-1}	100 cm^3	4.67 ± 1.948									
	Manure	<2 mgL^{-1}	50 cm^3	15.07 ± 1.772					Manure	*			
		<2 mgL^{-1}	100 cm^3	19.08 ± 1.012									
		>5 mgL^{-1}	50 cm^3	12.60 ± 1.057									
		>5 mgL^{-1}	100 cm^3	21.08 ± 3.512									
	Hay	<2 mgL^{-1}	50 cm^3	25.86 ± 3.947					Hay	*	*		
		<2 mgL^{-1}	100 cm^3	38.79 ± 4.078									
		>5 mgL^{-1}	50 cm^3	35.78 ± 8.280									
		>5 mgL^{-1}	100 cm^3	79.74 ± 6.165									
	Mulch	<2 mgL^{-1}	50 cm^3	15.07 ± 3.491					Mulch	*	*	*	
		<2 mgL^{-1}	100 cm^3	49.84 ± 5.203									
		>5 mgL^{-1}	50 cm^3	15.86 ± 1.441									
		>5 mgL^{-1}	100 cm^3	35.75 ± 4.028									

Table 4.5 Experiment 1: MANOVA, Wilks' Lambda and Tukey test results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen, D – Days, T – Treatments, A - Aeration Conditions, 1- Control; 2 – Manure; 3 – Hay; 4 – Mulch, Obs. Power is provided at both the 95 and 90% levels.

		MANOVA					Wilks' Lambda				
Experiment 1		*F* value	Hyp./error df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * T	39.17	3/16	0.000	1.000	1.000	0.254	15/33.5	0.186	0.640	0.769
	D * T * A	0.740	3/16	0.544	0.170	0.280	0.244	15/33.5	0.164	0.660	0.785
DO	D * T	46.10	3/16	0.000	1.000	1.000	0.170	15/33.5	0.050	0.810	0.890
	D * T * A	0.430	3/16	0.736	0.120	0.200	0.170	15/33.5	0.046	0.815	0.900
Sulphate (mg L^{-1})	D * T	6.403	3/16	0.005	0.916	0.963					
	D * T * A	0.962	3/16	0.435	0.215	0.333					
Sulphide (ppb)	D * T	1.586	3/16	0.232	0.338	0.478					
	D * T * A	0.099	3/16	0.959	0.064	0.123					
Gilvin ($_{g440}$ m^{-1})	D * T	90.316	3/16	0.000	1.000	1.000					
	D * T * A	3.979	3/16	0.011	0.814	0.890					

Table 4.5 continued, **Tukey test results represented by *P* values.**

		Day 8			Day 12			Day 16			Day 26			Day 34			Day 42		
		1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
pH	**2**	0.000		0.000	0.000		0.000	0.000		0.000	0.001		0.000	0.000		0.000	0.001		0.001
	3	0.993	0.000		0.997	0.000		0.975	0.000		0.057	0.000		0.999	0.000		0.992	0.001	
	4	0.000	0.000	0.001	0.006	0.001	0.010	0.007	0.003	0.015	0.558	0.016	0.004	0.191	0.031	0.149	0.095	0.105	0.154
DO	**2**	0.000		0.625	0.000		0.551	0.000		0.905	0.000		0.849	0.000		0.978	0.000		1.000
	3	0.000	0.625		0.000	0.551		0.000	0.905		0.000	0.849		0.000	0.978		0.000	1.000	
	4	0.000	0.641	1.000	0.000	0.453	0.998	0.000	0.912	1.000	0.000	0.934	0.934	0.000	1.000	0.985	0.000	1.000	1.000
Sulphate (mg L^{-1})	**2**																1.00		0.706
	3																0.686	0.706	
	4																0.035	0.033	0.004
Sulphide (ppb)	**2**																0.419		0.991
	3																0.279	0.991	
	4																0.966	0.686	0.512
Gilvin ($_{g440}$ m^{-1})	**2**																0.000		0.025
	3																0.000	0.025	
	4																0.000	0.858	0.004

Experiment 2

This experiment had two aims; 1, to determine if low levels of sulphate reducing bacterial activity as seen in experiment 1 was due to the lack of sulphate reducing bacteria, or 2, whether it was due to low sulphate levels, or a combination of both.

pH

There was a significant difference among organic matter treated cores and the control cores for pH, but no significant difference between inoculation conditions (Table 4.6, Figure 4.6). The post hoc Tukey test indicated that the two organic matter treatments {manure; 6.80 (± se0.03) and mulch; 6.76 (± 0.03)} had significantly higher pH values than the control {5.44 (± 0.08)}, but there was no significant difference between the two treatments (Figure 4.6). Thus the increase in pH in the treatments in experiments 1 and 2 were not due to the lack of sulphate reducing bacteria.

Dissolved oxygen

There was no significant difference in dissolved oxygen levels among treated cores, although the treated cores had significantly lower levels of dissolved oxygen that the controls. There was no significant difference between inoculated conditions for treated cores.

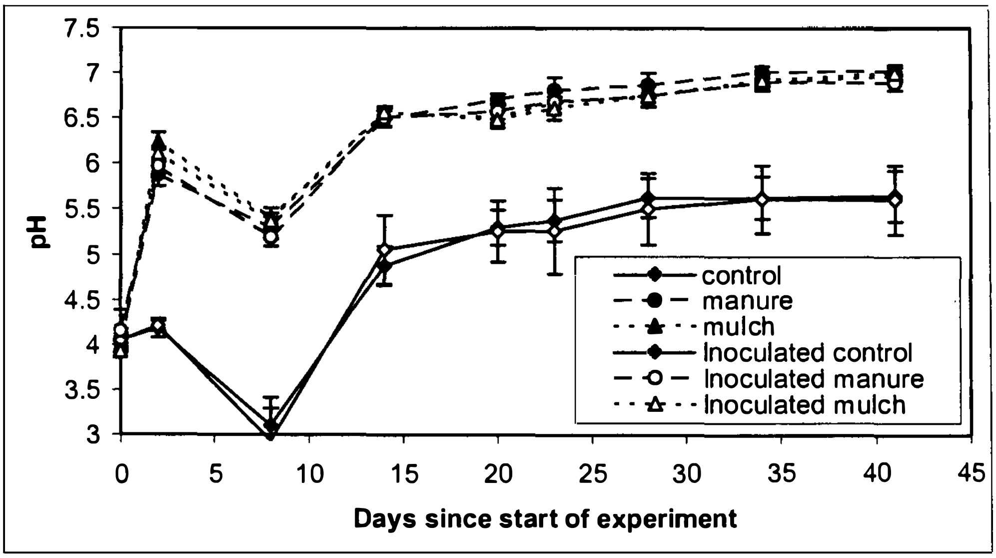


Figure 4.6 Experiment 2: A comparison of changes in mean pH values for organic matter treatments and controls for the duration of the experiment, showing differences between inoculated and un-inoculated conditions. Standard error bars are shown for each sampling day, n = 6 for each treatment.

Sulphate and sulphide

After the sulphate inoculation, there was a significant difference among the two organic matter treatments and the control for the sulphate, but there was no significant difference between the different inoculation conditions (Table 4.6). The post hoc Tukey test indicated that after the sulphate inoculation the control had a significantly higher sulphate level {2100.23 (± 161.96} mg SO_4 L^{-1}} than the manure and mulch treatment {19.02 (± 6.35) mg SO_4 L^{-1}; and 40.93 (± 12.57) mg SO_4 L^{-1} respectively; Table 4.6}. This indicates a reduction of sulphate in the treated cores.

For sulphide, there was no significant difference among the organic matter treatments and no significant difference among inoculation conditions after the sulphate inoculation {control no inoculation - 0.03 (± 0.033) ppb; control inoculation – 2.23 (± 2.333) ppb; manure no inoculation – 2.08 (± 1.110) ppb; manure inoculation – 0.90 (± 0.682) ppb; mulch no inoculation – 8.30 (± 4.567) ppb; mulch inoculation – 1.95 (± 1.254) ppb; Table 4.6}.

All of the cores containing organic matter produced a small amount of black precipitate, showing that there was a small amount of bacterial activity in all the cores (Gyure, et al., 1987; Johnson, et al., 1997). However, there was no observable difference in the amount of black sulphide precipitate between the inoculated and non-inoculated cores. pH for the treatment cores increased in both the inoculated and non-inoculated cores. Thus, the increase in pH values for the treatments in experiment 1 was not due to a lack of sulphate and the consequential lack of sulphate reducing bacterial activity.

Gilvin

The changes in gilvin values were similar to those observed in experiment 1 with the control being significantly less than the treated cores. There was no significant difference among inoculation treatments (Table 4.6).

Table 4.6 Experiment 2: Summary of mean and standard errors for pH, dissolved oxygen, sulphate, sulphide and gilvin.
* indicates significant values at $P \leq 0.05$; SO_4 - sulphate; S^{2-} - sulphide; sulphate, sulphide and gilvin are on last day only. D - days; T - treatments; I - inoculation conditions.

	Treatment	Inoculation	$\bar{x}$ ± se	D x T	D x I	D x T x I	Tukey test results at last sampling day			
				F value	*F* value	*F* value		Control	Manure	Mulch
pH	Control	No inoculum	5.45 ± 0.093	34.639*	0.092	0.029	Control			
		Inoculum	5.42 ± 0.139							
	Manure	No inoculum	6.84 ± 0.046				Manure	*		
		Inoculum	6.75 ± 0.042							
	Mulch	No inoculum	6.76 ± 0.040				Mulch	*		
		Inoculum	6.75 ± 0.044							
DO (mg L^{-1})	Control	No inoculum	1.79 ± 0.102	8.420*	0.035	1.454	Control			
		Inoculum	1.27 ± 0.112							
	Manure	No inoculum	0.77 ± 0.107				Manure	*		
		Inoculum	0.92 ± 0.129							
	Mulch	No inoculum	0.43 ± 0.111				Mulch	*		
		Inoculum	0.68 ± 0.146							

Table 4.6 cont.

	Treatment	Inoculation	$\bar{x}$ ± se	D x T *F* value	D x I *F* value	D x T x I *F* value	Tukey test results at last sampling day	Control	Manure	Mulch
SO_4 (mg L^{-1})	Control	No inoculum	2156.22 ± 287.851	149.046*	0.097	0.116	Control			
		Inoculum	2044.24 ± 176.945							
	Manure	No inoculum	14.79 ± 5.541				Manure	*		
		Inoculum	23.25 ± 11.825							
	Mulch	No inoculum	42.06 ± 16.068				Mulch	*		
		Inoculum	39.80 ± 20.895							
S^{2-} (ppb)	Control	No inoculum	0.03 ± 0.033	2.010	0.977	1.910	Control			
		Inoculum	2.23 ± 2.233							
	Manure	No inoculum	2.08 ± 1.110				Manure			
		Inoculum	0.90 ± 0.682							
	Mulch	No inoculum	8.30 ± 4.567				Mulch			
		Inoculum	1.95 ± 1.254							
Gilvin (g_{440} m^{-1})	Control	No inoculum	2.56 ± 0.761	34.50*	0.217	4.201*	Control			
		Inoculum	7.52 ± 3.999							
	Manure	No inoculum	18.95 ± 2.051				Manure	*		
		Inoculum	14.70 ± 3.364							
	Mulch	No inoculum	18.93 ± 1.699				Mulch	*		
		Inoculum	16.30 ± 1.723							

Table 4.7 Experiment 2: MANOVA, Wilks' Lambda and Tukey test results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen, D – Days, T – Treatments, A – Aeration conditions, 1- Control; 2 – Manure; 3 – Hay; 4 – Mulch, Obs – Observed.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error Df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * T	264.3	3/74	0.000	1.000	1.000	0.059	21/195.8	0.000	1.000	1.000
	D * T * A	6.942	3/74	0.000	0.973	0.988	0.192	21/195.8	0.000	1.000	1.000
	D * T * Q	1.795	3/74	0.156	0.449	0.581	0.659	21/195.8	0.096	0.905	0.950
	D * T * A * Q	0.392	3/74	0.795	0.124	0.208	0.848	21/195.8	0.946	0.401	0.537
DO	D * T	512.2	3/74	0.000	1.000	1.000	0.051	21/195.8	0.000	1.000	1.000
	D * T * A	36.781	3/74	0.000	1.000	1.000	0.363	21/195.8	0.000	1.000	1.000
	D * T * Q	8.796	3/74	0.001	0.941	0.971	0.571	21/195.8	0.007	0.981	0.992
	D * T * A * Q	0.836	3/74	0.478	0.223	0.335	0.690	21/195.8	0.188	0.853	0.917
Sulphate (mg L^{-1})	D * T	7.454	3/74	0.000	0.083	0.148					
	D * T * A	1.720	3/74	0.170	0.432	0.564					
	D * T * Q	1.043	3/74	0.378	0.272	0.393					
	D * T * A * Q	0.485	3/74	0.694	0.144	0.235					
Sulphide (ppb)	D * T	5.623	3/74	0.002	0.934	0.967					
	D * T * A	0.876	3/74	0.457	0.233	0.346					
	D * T * Q	0.878	3/74	0.457	0.233	0.347					
	D * T * A * Q	0.210	3/74	0.889	0.088	0.157					
Gilvin ($_{g440}$ m^{-1})	D * T	102.660	3/74	0.000	1.000	1.000					
	D * T * A	14.365	3/74	0.000	1.000	1.000					
	D * T * Q	16.178	3/74	0.000	1.000	1.000					
	D * T * A * Q	6.708	3/74	0.000	0.969	0.986					

Table 4.7 continued, **Tukey test results represented by *P* values.**

		Day 9			Day 11			Day 13			Day 17			Day 23			Day 29			Day 37			Day 43		
		1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
pH	**2**	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00
	3	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00	
	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.02	0.00
DO	**2**	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.19	0.00		0.01	0.00		0.00	0.00		0.00
	3	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.19		0.00	0.01		0.00	0.00		0.00	0.00	
	4	0.00	0.01	0.13	0.00	0.00	0.51	0.00	0.00	0.01	0.00	0.00	0.98	0.00	0.04	0.89	0.00	0.01	1.00	0.00	0.00	0.98	0.00	0.00	1.00
Sulphate (mg SO_4 L^{-1})	**2**																						0.003		1.00
	3																						0.002	1.00	
	4																						0.88	0.03	0.02
Sulphide (ppb)	**2**																						0.004		0.86
	3																						0.04	0.86	
	4																						0.84	0.04	0.24
Gilvin (g_{440} m^{-1})	**2**																						0.00		0.00
	3																						0.00	0.00	
	4																						0.00	0.00	0.00

Table 4.8 Experiment 2; Control data only. MANOVA and Wilks' Lambda test results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen (mg L^{-1}), D – Days, T – Treatments, A – Aeration Conditions.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * A	1.26200	1/22	0.290	0.179	0.280	0.091	7/16	0.000	1.000	1.000
DO	D * A	128.624	1/22	0.000	1.000	1.000	0.079	7/16	0.000	1.000	1.000

Table 4.9 Experiment 2; Manure data only. MANOVA and Wilks' Lambda test results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen, D – Days, Q - Quantity, A – Aeration Conditions.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * A	30.525	1/18	0.000	0.999	1.000	0.255	7/12	0.007	0.931	0.975
	D * Q	12.352	1/18	0.002	0.913	0.958	0.477	7/12	0.161	0.495	0.655
	D * A * Q	0.831	1/18	0.374	0.139	0.227	0.463	7/12	0.141	0.522	0.680
DO	D * A	26.394	1/18	0.000	0.998	0.999	0.126	7/12	0.000	1.000	1.000
	D * Q	10.954	1/18	0.004	0.879	0.938	0.432	7/12	0.103	0.583	0.736
	D * A * Q	1.993	1/18	0.175	0.267	0.389	0.507	7/12	0.208	0.442	0.602

Table 4.10 Experiment 2: Hay data only. MANOVA and Wilks' Lambda test Results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen (mg L^{-1}), D – Days, Quantity, A - Aeration Conditions.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * A	62.354	1/18	0.000	1.000	1.000	0.141	7/12	0.000	0.999	1.000
	D * Q	10.697	1/18	0.004	0.871	0.933	0.622	7/12	0.451	0.279	0.423
	D * A * Q	0.963	1/18	0.339	0.153	0.247	0.532	7/12	0.254	0.400	0.559
DO	D * A	0.470	1/18	0.502	0.100	0.173	0.618	7/12	0.442	0.284	0.420
	D * Q	0.610	1/18	0.445	0.115	0.194	0.401	7/12	0.073	0.647	0.789
	D * A * Q	0.720	1/18	0.407	0.127	0.211	0.615	7/12	0.436	0.287	0.431

Table 4.11 Experiment 2: Mulch data only. MANOVA Wilks' Lamda test results.
See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen (mg L^{-1}), D – Days, Q - Quantity, A – Aeration Conditions.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * A	0.284	1/18	0.601	0.080	0.144	0.103	7/12	0.000	1.000	1.000
	D * Q	1.834	1/18	0.192	0.250	0.368	0.331	7/12	0.029	0.797	0.899
	D * A * Q	0.698	1/18	0.414	0.124	0.207	0.430	7/12	0.101	0.587	0.739
DO	D * A	8.289	1/18	0.010	0.778	0.870	0.436	7/12	0.107	0.575	0.729
	D * Q	6.609	1/18	0.019	0.682	0.796	0.364	7/12	0.046	0.727	0.852
	D * A * Q	6.867	1/18	0.017	0.698	0.809	0.507	7/12	0.208	0.442	0.602

Experiment 3

Alkalinity

The alkalinity for day 0 was the same for each core because the water (DI) came from the same source. A significant difference developed among treatments by the end of the laboratory trial (day 42). The post hoc Tukey test results showed the mulch treatment {151.33 (± 6.181) mg $CaCO_3$ L^{-1}} had a significantly higher alkalinity than the control {20.22 (± 2.808) mg $CaCO_3$ L^{-1}} and manure {93.40 (± 3.242) mg $CaCO_3$ L^{-1}} treatment, and the manure treatment had a higher alkalinity than the control (Table 4.12). The addition of organic matter therefore probably contributed to the increase in pH in the treated cores, however, there must have been other contributors as the pH was higher in the manure than in the mulch, whereas the alkalinity was higher in the mulch than in the manure.

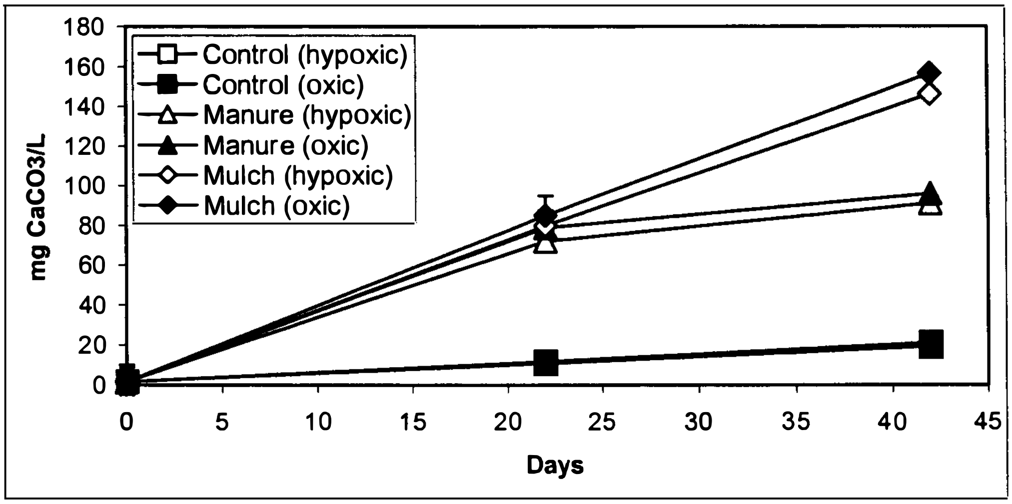


Figure 4.7 Experiment 3: A comparison of alkalinity values (mg $CaCO_3$ L^{-1}) between treatments under different aeration conditions.
Standard error bars are shown for each sampling day, n = 6 for each treatment.

pH

The pH for organic matter treated cores increased over the first couple of days and then decreased again to a slightly higher pH than the original levels (Table 4.8). There was a significant difference in pH values among treatments with a post hoc Tukey test indicating that the pH of the two organic matter treatments {mulch 6.68 (± 0.024) and manure 7.08 (± 0.024)} were significantly ($P < 0.05$) higher than the control between days 22 and 42 {6.55 (± 0.021)}, and for days 7 to 42 the manure treatment had a significantly higher pH than the mulch treatment (Table 4.12). The magnitude of change in pH for the treatments was similar to Experiment 2.

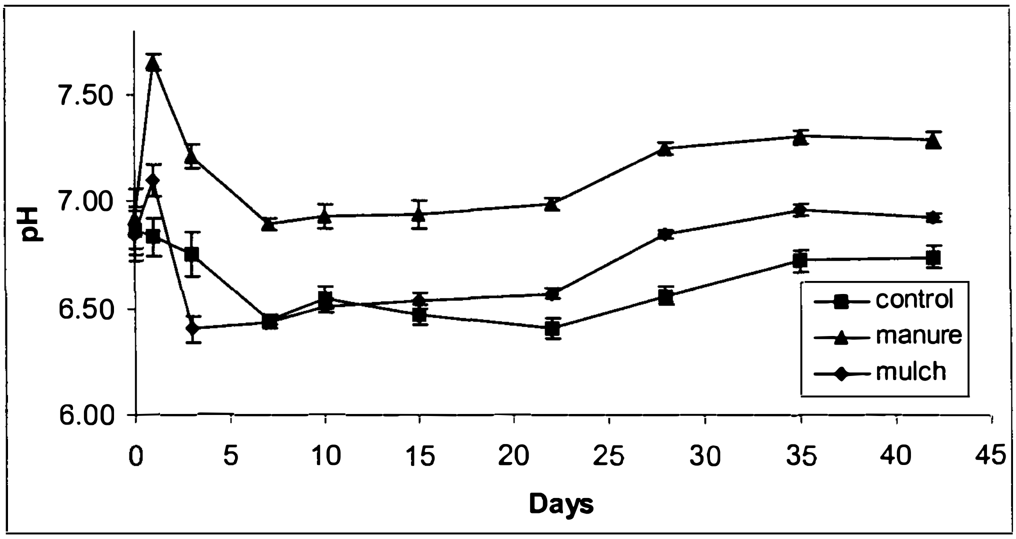


Figure 4.8 Experiment 3: A comparison of pH values between treatments. Standard error bars are shown for each sampling day, n = 6 for each treatment.

Sulphate and sulphide

There was no significant difference among treatments or aeration condition for sulphate (Table 4.12), and no sulphide was present on day 42 for any treatments or the control. Small amounts of sulphate were found after organic matter was added to the cores; the mean sulphate levels at day 42 were; control – 2.47 (± 1.099) mg SO_4 L^{-1}; manure – 4.20 (± 1.544) mg SO_4 L^{-1}; mulch – 5.33 (± 2.706) mg SO_4 L^{-1}. As no sulphide was evident it was concluded there was no sulphate reducing bacterial activity present.

Table 4.12 Experiment 3: Summary of mean and standard errors for pH, dissolved oxygen, sulphate, and alkalinity.

* indicates significant values at $P \leq 0.05$; SO_4 - sulphate; sulphate, sulphide and alkalinity are on last day only. D - days; T - treatments; A - aeration conditions.

	Treatment	Aeration	$\bar{x}$ ± se	D x T	D x T x A	Tukey test results at last sampling day			
				F value	*F* value		Control	Manure	Mulch
pH	Control	<2 mgL^{-1}	6.63 ± 0.029	153.102*	2.370	Control			
		>5 mgL^{-1}	6.48 ± 0.025						
	Manure	<2 mgL^{-1}	7.18 ± 0.026			Manure	*		
		>5 mgL^{-1}	6.99 ± 0.036						
	Mulch	<2 mgL^{-1}	6.71 ± 0.029			Mulch	*	*	
		>5 mgL^{-1}	6.65 ± 0.039						
DO (mg L^{-1})	Control	<2 mgL^{-1}	3.03 ± 0.149	277.243*	3.195	Control			
		>5 mgL^{-1}	4.69 ± 0.176						
	Manure	<2 mgL^{-1}	1.80 ± 0.146			Manure	*		
		>5 mgL^{-1}	3.30 ± 0.197						
	Mulch	<2 mgL^{-1}	0.25 ± 0.028			Mulch	*	*	
		>5 mgL^{-1}	1.27 ± 0.181						

Table 4.12 cont.

	Treatment	Aeration	$\bar{x}$ ± se	D x T *F* value	D x T x A *F* value	Tukey test results at last sampling day			
							Control	Manure	Mulch
SO_4 (mg L^{-1})	Control	<2 mgL^{-1}	3.88 ± 1.787	0.538	0.577	Control			
		>5 mgL^{-1}	1.06 ± 1.148						
	Manure	<2 mgL^{-1}	2.92 ± 1.638			Manure			
		>5 mgL^{-1}	5.48 ± 2.673						
	Mulch	<2 mgL^{-1}	6.35 ± 5.310			Mulch			
		>5 mgL^{-1}	4.31 ± 1.901						
Alkalinity (mg CaCO3 L^{-1})	Control	<2 mgL^{-1}	19.05 ± 5.942	187.192*	0.201	Control			
		>5 mgL^{-1}	21.00 ± 3.737						
	Manure	<2 mgL^{-1}	90.90 ± 5.512			Manure	*		
		>5 mgL^{-1}	95.90 ± 3.658						
	Mulch	<2 mgL^{-1}	146.16 ± 7.614			Mulch	*	*	
		>5 mgL^{-1}	156.50 ± 9.971						

Table 4.13 Experiment 3: MANOVA, Wilks' Lambda and Tukey test results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen, D – Days, T – Treatments, 1- Control; 2 – Manure, 4 – Mulch.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * T	34.639	2/30	0.000	1.000	1.000	0.502	12/50	0.092	0.787	0.877
	D * I	0.092	1/30	0.764	0.060	0.115	0.837	6/25	0.572	0.260	0.388
	D * T * I	0.029	2/30	0.971	0.054	0.106	0.864	12/50	0.984	0.158	0.259
DO mg L^{-1}	D * T	8.420	2/30	0.001	0.947	0.976	0.523	12/50	0.124	0.749	0.849
	D * I	0.035	1/30	0.835	0.054	0.106	0.903	6/25	0.840	0.154	0.253
	D * T * I	1.454	2/30	0.250	0.286	0.411	0.892	12/50	0.995	0.130	0.220
Sulphate (mg L^{-1})	D * T	149.046	2/30	0.000	1.000	1.000					
	D * I	0.097	1/30	0.757	0.061	0.116					
	D * T * I	0.116	2/30	0.891	0.066	0.125					
Sulphide (ppb)	D * T	2.010	2/30	0.152	0.382	0.516					
	D * I	0.977	1/30	0.331	0.160	0.253					
	D * T * I	1.910	2/30	0.166	0.365	0.498					
Gilvin ($_{g440}$ m^{-1})	D * T	34.50	2/30	0.000	1.000	1.000					
	D * I	0.217	1/30	0.643	0.074	0.136					
	D * T * I	4.201	2/30	0.019	0.719	0.819					

Table 4.13 continued, **Tukey test results represented as *P* values.**

		Day 14		Day 20		Day 23		Day 28		Day 34		Day 41		Day 48	
		1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
pH	**2**	0.000		0.000		0.000		0.000		0.000		0.000		0.000	
	4	0.000	0.933	0.000	0.678	0.000	0.901	0.000	0.955	0.000	0.968	0.000	0.991	0.000	1.000
DO (mg L^{-1})	**2**	0.546		0.071		0.042		0.017		0.275		0.082		0.018	
	4	0.002	0.033	0.000	0.016	0.000	0.124	0.004	0.826	0.163	0.949	0.289	0.767	0.033	0.965
Sulphate (mg L^{-1})	**2**													0.000	
	4													0.000	0.986
Sulphide (ppb)	**2**													0.986	
	4													0.183	0.241
Gilvin ($_{g440}$ m^{-1})	**2**													0.000	
	4													0.000	0.887

Table 4.14 Experiment 3: Control data only. MANOVA and Wilks' Lambda test results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen, D – Days, I – Inoculation.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * I	0.005	1/10	0.944	0.050	0.101	0.617	6/5	0.778	0.106	0.196
DO	D * I	1.973	1/10	0.190	0.246	0.370	0.465	6/5	0.529	0.162	0.282

Table 4.15 Experiment 3: Manure data only. MANOVA Wilks' Lambda test results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen, D – Days, I – Inoculation.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * I	1.117	1/10	0.316	0.160	0.259	0.754	6/5	0.928	0.078	0.149
DO	D * I	0.209	1/10	0.657	0.070	0.131	0.438	6/5	0.481	0.176	0.303

Table 4.16 Experiment 3: Mulch data only. MANOVA Wilks' Lambda test results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen, D – Days, I – Inoculation.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error Df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * I	0.012	1/10	0.914	0.051	0.102	0.681	6/5	0.859	0.091	0.172
DO	D * I	0.554	1/10	0.474	0.104	0.181	0.582	6/5	0.726	0.116	0.212

Table 4.17 Experiment 3. MANOVA, Wilks' Lambda and Tukey test results.

See methods section for description of repeated measures analysis calculations; DO – Dissolved oxygen, D – Days, T – Treatments, A – Aeration Conditions, 1- Control; 2 – Manure; 4 – Mulch.

		MANOVA					Wilks' Lambda				
		F value	Hyp./ error df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * T	153.102	2/30	0.000	1.000	1.000	0.231	12/50	0.000	0.999	1.000
	D * T * A	2.370	2/30	0.111	0.442	0.577	0.439	12/50	0.032	0.883	0.940
DO ($mg\ L^{-1}$)	D * T	277.243	2/30	0.000	1.000	1.000	0.023	12/50	0.000	1.000	1.000
	D * T * A	3.195	2/30	0.550	0.567	0.696	0.201	12/50	0.000	1.000	1.000
Sulphate ($mg\ L^{-1}$)	D * T	0.538	2/30	0.590	0.130	0.217					
	D * T * A	0.577	2/30	0.568	0.137	0.226					
Alkalinity ($mg\ CaCO_3\ L^{-1}$)	D * T	187.192	2/28	0.000	1.000	1.000					
	D * T * A	0.201	2/28	0.819	0.078	0.143					

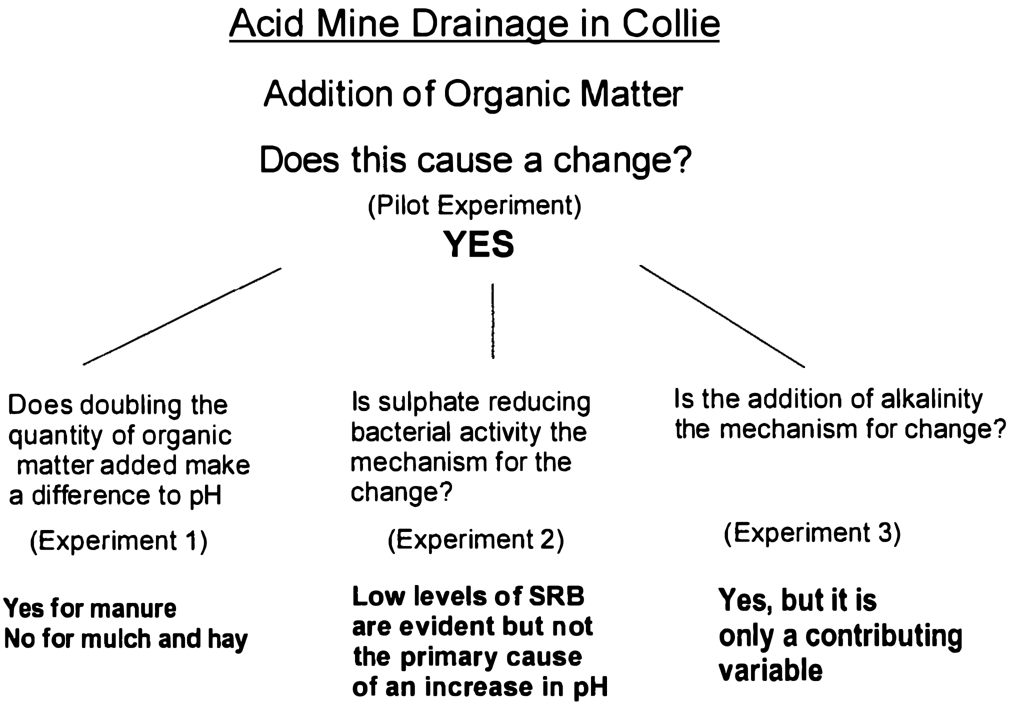
Table 4.17 continued, **Tukey test results represented by *P* values.**

		Day 7		Day 10		Day 15		Day 22		Day 28		Day 35		Day 42	
		1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
pH	**2**	0.000		0.000		0.000		0.000		0.000		0.000		0.002	
	4	1.000	0.000	0.404	0.000	0.242	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DO	**2**	0.000		0.000		0.000		0.000		0.000		0.000		0.000	
	4	0.000	0.006	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sulphate (mg L^{-1})	**2**													0.820	
	4													0.563	0.903
Alkalinity (mg $CaCO_3$ L^{-1})	**2**													0.000	
	4													0.000	0.000

Summary and discussion of laboratory experiments

The abandoned mine voids in Collie represent a unique situation. Throughout the world acid mine drainage sites typically have high concentrations of heavy metals, and high sulphate levels (Hancock, 1997; Whitehead, *et al.,* 1995; Woodin and Skiba, 1990) Voids near Collie do not show either of these characteristics; they are low in sulphate and have low concentrations of heavy metals (M. Lund, pers. comm., January, 1999). This series of laboratory experiments was designed to test whether the addition of organic matter increased void water pH as was indicated by (Tuttle *et al.*, 1969 a,b; Gyure *et al.,* 1987). If it did, was there a difference between the types of organic matter and was suphate reducing bacterial activity the primary cause. If sulphate reducing bacteria was not the cause was there another possible explanation for the increases in pH with the addition of organic matter.

The addition of manure and mulch increased the pH of the cores compared to the control, and the addition of the hay also increase pH but not to the same level as the other two organic materials under both aeration conditions. The addition of similar volumes of manure and mulch resulted in a greater increase in pH for the manure treatment compared to the mulch treatment under both aerated and hypoxic conditions. These results conform with those of Tuttle *et al.*, (1969 a, b) and Gyure, *et al.*, (1987) who found that the pH of acid water increased when it was passed through organic matter.



Acid Mine Drainage in Collie

Addition of Organic Matter

Does this cause a change?

(Pilot Experiment)

YES

Does doubling the quantity of organic matter added make a difference to pH

(Experiment 1)

Yes for manure
No for mulch and hay

Is sulphate reducing bacterial activity the mechanism for the change?

(Experiment 2)

Low levels of SRB are evident but not the primary cause of an increase in pH

Is the addition of alkalinity the mechanism for change?

(Experiment 3)

Yes, but it is only a contributing variable

Figure 4.9 The approach taken by the study.

Doubling the quantity of organic matter additions from 50 to 100 cm^3 per core produced significantly higher pH values for manure (50 cm^3 - 7.1 compared with 7.31 for 100 cm^3). There was no difference in pH values between the quantities for the mulch and hay treatments. This suggests that there is an asymtopic affect for the introduction of mulch and hay in increasing the pH. Increased quantities of manure and hay increased gilvin values. There are two likely mechanisms to explain these results; 1) increased amounts of organic matter provided more nutrients and carbon sources in the manure (but not the hay and mulch) for the sulphate reducing bacteria to consume, resulting in a greater level of sulphate reduction, hence an increased pH; or 2) the addition of more manure increased the quantity of introduced alkalising substance to the water column which increased the pH. Whereas increasing the quantity of mulch and hay did not increase the quantity of alkalising substance available to neutralise the acid. These alternative hypotheses were tested in experiments 2 and 3 respectively.

The volume of mulch and hay per unit volume of acidic water required before the increases in pH cease is unknown but is between the equivalent of adding 50 and 100 cm^3 or organic material to a 100 mm diamater core filled with 400 mm of void water. Additional experiments are required to determine the exact quantity of mulch or hay neccessary to obtain the maximum increase in pH as there is no literature that discusses the affect of quantity of organic matter additions on changes in pH.

For all organic matter treated cores the dissolved oxygen levels were low (mean; 0.45 mg O_2 L^{-1} with a range of 0.01 to 4.51 mg O_2 L^{-1}) indicating these cores were mostly hypoxic. Doubling the quantity of organic matter also significantly decreased the dissolved oxygen levels for all treatments. Kim, *et al.*, (1999) suggested that in mine waste water the biological oxygen demand of organic compounds added to a system would exceed the available oxygen and exhaust the oxygen supply, rendering the environment anaerobic. The low level of dissolved oxygen in the organic matter treated cores is therefore probably explained by this high biological oxygen demand. For experiments 1 and 2, the dissolved oxygen levels on the last day for the control cores for the two aeration conditions were about 4 mg O_2 L^{-1}. The interval between bubbling cores with nitrogen for the hypoxic and compressed air for the aerated cores increased so the dissolved oxygen level in the cores might represent a more natural level. This meant that the dissolved oxygen levels for the control aerated and hypoxic cores decreased and increased, respectively, because the gases bubbled in the cores had leaked past the rubber stopper seals.

Void water has very low gilvin levels. The addition of organic matter significantly increased gilvin levels and the colouration in the cores. As might have been expected, doubling the quantity of organic matter increased the levels of gilvin. Gilvin levels for the mulch and manure treatments were significantly higher than for hay and the control indicating that these two treatments will most probably give the void water more colour.

The major cause of acidic mine voids throughout the world is acid mine drainage (Gray, 1998). Often associated with acid mine drainage are high sulphate levels due to the oxidation of pyrite (FeS_X). If sulphate reducing bacteria were responsible for the pH increase, a high level of sulphate and sulphide would normally be present. However, the levels of sulphate present in the core samples used in the laboratory experiment were low ($\approx$ 4 mg SO_4 L^{-1}; Sass, *et al.,* 1997). Doubling (50 to 100 cm^3) the quantity of organic matter added to the cores made no significant difference to the level of sulphate indicating none of the organic matters contained dissolved sulphate. However, the higher quantity of organic matter increased sulphide levels in treated cores. The levels of sulphide for the manure and hay were higher than for the control, and the manure treatment was higher than for the mulch at the end of Experiment 1. This suggests that there might have been low levels of sulphate reducing bacterial activity and the bacteria had depleted the available sulphate. This would explain the low sulphate levels and higher levels of sulphide precipitate.

Six weeks after the sodium sulphate aliquot was added to the treatment and control cores in experiment 2, the sulphate levels were higher in the control cores than in both inoculated and non-inoculated treatments cores; there was no significant difference between the inoculation conditions in the sulphate levels. All of the cores containing organic matter also produced a small amount of black precipitate. This provides futher evidence of low levels of sulphate reducing bacterial activity in all of the cores (Gyure, *et al.* 1987; Johnson, *et al*., 1997). The lack of a significant difference in pH values between the inoculated and uninoculated cores after the sulphate aliquot was added was probably due to the already available sulphate reducing bacteria in the treated cores having converted all the available sulphate into sulphide and additional sulphate reducing bacteria could provide no increase in pH as there was no available sulphate for it to react with.

Manure released about the same quantity of alkalinity into the cores as the mulch treatment after 22 days. However, after 22 days the mulch treatment continued to release alkalinity at the same rate whereas the increase in alkalinity for the manure cores was comparatively less. Mulch may have more alkalising capacity per unit volume than manure. In the mulch the alkalinising material may not be as readily available for release under low pH compared with manure and may be more dependant on time, bacterial processes or a higher pH. This might be why mulch had a greater ability to release carbonates for the duration of the experiment. If the pH in cores containing organic materials primarily increased as a consequence of the introduction of alkalising substances, then it would be expected the pH value in the mulch would have been higher than the manure. This was not the case, as the mean pH in the manure cores was 7.08 compared with the mulch cores of 6.68. These results indicate that the alkalising affect contributes to the increase in pH, but other biological or chemical processes are also involved and make the difference between the mulch and manure cores.

In summary, the addition of organic materials into intact cores from Ewington increased the pH. Significant increases in pH experienced in the treated cores could not be explained adequately by the low level of sulphate reducing bacteria acting on the available carbon source (i.e., organic matter). This is different to the studies reported by Tuttle *et al.*, (1969 a, b) and Gyure, *et al.*, (1987) where sulphate reducing bacteria were the primary cause of the increase in pH. The organic material introduced into the cores contained alkalising substances that contributed to the increase in pH. Similar volumes of manure and mulch introduced into cores larger increases in pH for manure, and both pH values, these increases were greater than for hay. Doubling the volume of mulch added to cores did not increase the pH whereas it did for manure and hay. In the alkalising experiment, similar volumes of mulch and manure added to cores resulted in mulch releasing more alkalising material but manure providing the higher pH. It is therefore obvious that other unknown chemical or biological processes resulted in the manure treated cores to having a higher pH value than the mulch treated cores.

5.0 FIELD EXPERIMENT

Introduction

Mesocosms are often used for testing (verifying) laboratory results in field situations. Wieder, *et al*., (1990), and Street and Titmus (1982) both used mesocosms experiments to examine acid mine drainage issues. Street and Titmus (1982) used a partitioned lake and Wieder, *et al*., (1990) used six model wetlands to test dynamics in wetlands exposed to synthetic acid mine drainage. Wieder, *et al*., (1990) suggested that the use of mesocosms could be useful in generating site-specific data that can be applied to the formulation of cost-benefit analyses to compare a proposed wetland treatment system with alternative chemical methods for treating acid mine drainage.

Based on the first laboratory experiment it was concluded that mulch and manure treatments were better than hay for increasing the pH of acidified Collie mine void water. The hay treatment was therefore not included in the field experiment. It was also concluded that the dissolved oxygen levels in the ponds could not be controlled (based on lab experiment) because it was not possible to seal the ponds from atmospheric air. Results from the laboratory experiments indicate that hypoxic conditions created by biological oxygen demand would provide a suitable environment for sulphate reducing bacteria should this be a primary agent for increasing pH in *in situ* ponds. However, sulphate reducing bacteria was not expected to be a primary mechanism for pH change in the ponds based on laboratory experiments.

The aim of the field experiment was to determine a) the effect that the addition of vegetation mulch and cattle manure have on the pH of lake water in a situation similar to that in Ewington; b) the longevity of the treatment affects in a field situation; and c) whether experimental results using intact cores in a laboratory can be used to predict the outcomes of similar experiments in ponds in a field situation. If this were the case then researchers could be more confident about predicting likely outcomes of *in situ* rehabilitation programs based on the relatively inexpensive laboratory experiments.

Methods

Mesocosms

In the field experiment 18 ponds, approximately 2 x 3 m and with a water depth of 1 m, were excavated next to Ewington (Figure 5.1). Six ponds were treated with mulch and six with manure. The remaining six ponds were left as controls. Site constraints meant that a row of 5, a row of 9 and a third row of 4 ponds were excavated adjacent to the lake. Where possible the field experiment was designed so that a pair of control's or a pair of similar organic matter treatments were placed next to each other and spread systematically along the rows, to minimise the effect of changes in soil texture and contamination by groundwater inflow (Figure 5.1). The sides of the ponds were stabilised with polypropylene sandbags to prevent collapse of the sidewalls and the potential contamination of the next pond. Sandbags were soaked in Ewington for 24 hours prior to use and were filled with the sand extracted from each hole so as to minimise the potential contamination of each pond by soil from another source. A slurry of sediment from Ewington was pumped into each pond to a depth of approximately 100 mm to ensure that as far as possible each pond replicated the real void ecosystem. The pump moved water and sediment at a fixed rate and the continual movement of the suction pipe over a new piece of the bottom of Ewington meant that if all the ponds were filled to the same depth, a comparable amount of slurry was obtained for each pond. Ground water also infiltrated into the 18 ponds and combined with the water pumped to fill the ponds to a depth of approximately one metre a couple of days after they were constructed. Three days were allowed for the sediment to settle and for the ponds to become stable before the organic material was introduced.

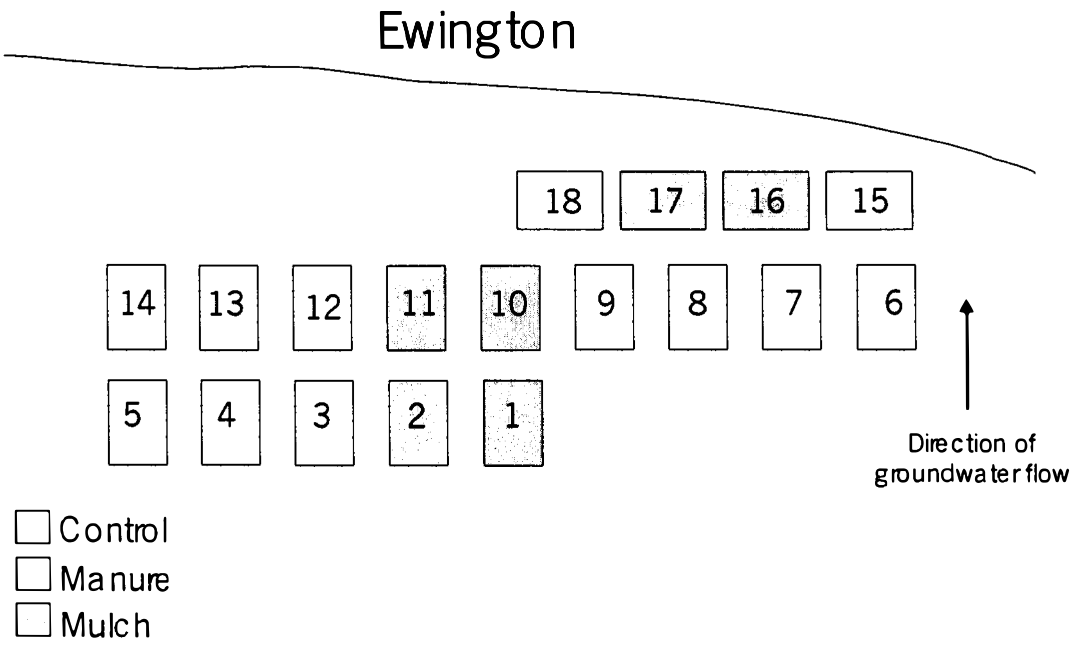


Figure 5.1 Layout of the ponds adjacent to Ewington showing the pairing of similar organic matter types for the field experiment.

The centre of each pond (top and bottom) and Ewington (two metres from bank, one metre deep adjacent to the ponds) were measured for pH, dissolved oxygen, conductivity, temperature, redox potential. In addition, pond turbidity, salinity, sulphide, sulphate, heavy metals, water nutrients (nitrate/nitrite, FRP and ammonia), chlorophyll *a*, and water depth were monitored. Samples were taken for each pond weekly for the first month and then fortnightly for the following four months. The field trial was conducted between January and July 1999, between 0930 and 1200 hours.

Measurement of physical, chemical and biotic properties of the ponds

Depth

The water depth of the ponds was measured using a fibreglass tape measure attached to a plumb bob. Measurements were taken at the centre of the pond, which was presumed to be the deepest part of the excavated voids.

Temperature

Temperature of the water column in the ponds was measured using a thermistor incorporated into the probe of either the Yeo Kal Model 611 Intelligent Water Quality Analyser, a WTW OXI 320/set, WTW 330 pH meter or a WTW 330 conductivity meter depending on the availability.

Turbidity

The turbidity was measured *in situ* for the ponds using a turbidity probe on a Yeo Kal Model 611 Intelligent Water Quality Analyser. The meter was calibrated before use.

Macro-invertebrates

The macro-invertebrate sampling was performed using a 25 x 25 cm, 500 µm Fresh Water Biological Association (FBA) sweep net. Two minutes of intense sweeping (top layers of sediment and in the water column) was used to estimate macro-invertebrate family richness and presence/absence. The invertebrates were put in plastic bags with water from the ponds. The samples were then live sorted in a white plastic tray, for 15 minutes by hand that evening and subsequently stored in 70% ethanol for later identification. Samples were identified to Family level using Davis *et al.* (1997) and counted using a dissecting microscope.

Chlorophyll *a*

Assessing concentration of chlorophyll *a* is the most common method for estimating phytoplankton biomass and is less time consuming than phytoplankton counts and simpler than other productivity methods (Axler and Owen, 1994). The concentration of chlorophyll *a* has also been shown to relate to primary productivity in water and can be used to assess the physiological health of algae by examining its degradation products (the phaeopigments, phaeophytin-*a* and phaeophorbide-*a*; Axler and Owen, 1994).

A 250 mL water sample was taken from each pond and filtered (Whatman ® GF/C). The filter paper was then frozen. Thawed filter papers were placed in centrifuge tubes to which 10 mL of N, N – Dimethylformamide (DMF) was added. The centrifuge tubes were stored in the dark at 4 °C for 24 hours. The absorbency of the DMF was then determined at 750 and 665 nm using a spectrophotometer (Shimadzu). The sample was then acidified using 200 μL of 0.2M hydrochloric acid (HCl). The acid was added directly into the cuvette. Another reading was then made at 750 and 665nm.

The chlorophyll level ($\mu g\ L^{-1}$) was then calculated by:

$$28.43 * \{(665B - 750B) - (665A - 750A)\} * 10 / (vol * cuv) = [chl\ a]$$

(Equation 5.0)

A = After acidification

B = Before acidification

vol = Volume of water sample in litres

cuv = light path of cuvette in centimetres (APHA, 1989).

Phytoplankton

The phytoplankton levels were measured by collecting six replicates of 1.77 litres of pond water from the vertical column (50 mm core of 900 mm in length) and pouring it through a phytoplankton net. The six water samples from each pond where each stored in a McCartney vial and preserved with 10 ml of 70% histoethanol. Vials were allowed to settle for 40 days. The surface one mL from each vial was removed every 4 days. The remaining one mL from each vial was placed on Sedwick Rafter slide and examined under a microscope. The number of phytoplankton were counted per unit area, averaged and multiplied to give an estimate of phytoplankton numbers per litre (APHA, 1989).

Data Analysis

For the field experiment, means and standard errors are presented for each sampling day and the *grand means* and the repeated measures MANOVA results are reported for days 83 to the end of sampling at day 153 for pH, dissolved oxygen (% saturation and mg O_2 L^{-1}), conductivity, temperature and depth. The mean values are reported for redox potential (ORP), turbidity and salinity for the combined data for days 111 to 153. The days are different here because the equipment was not available for some sampling days. Data are reported separately for top and bottom water samples. The mean for the top and bottom samples were averaged to give a mean (mid-level) value for each pond and used in the statistical analyses.

Data for days 83 to 153 for pH, dissolved oxygen, conductivity, temperature, chlorophyll *a* and depth, and days 111 to 153 for ORP, turbidity and salinity were used to calculate a *grand mean* for the period. Data for each pond for each of these sampling days were used in the repeated measures MANOVA and the post hoc Tukey test to determine statistical differences between controls and various treatments, and between treatments. Summary data for each treatment and each sampling day are presented in the appendix.

Long term monitoring data

Regular monthly monitoring of a selected deep-water site near the centre of Ewington has been in progress since April 1997. Before 1997, a few infrequent measurements were taken by Mills (1995) and Lisa Edwards, an Edith Cowan University honours student during 1995. Measurements included the recording of pH and conductivity at the top and bottom of the water, and dissolved oxygen and temperature at a mid-water point. These data are used to display temporal changes in the physical and chemical properties of the lake and to demonstrate that the chemistry of the constructed ponds acted in a similar manner to the void.

Results

Rainfall

Rainfall during the period of the field experiment increased after day 97 and continued for the rest of winter (28 April 1999; Figure 5.2).

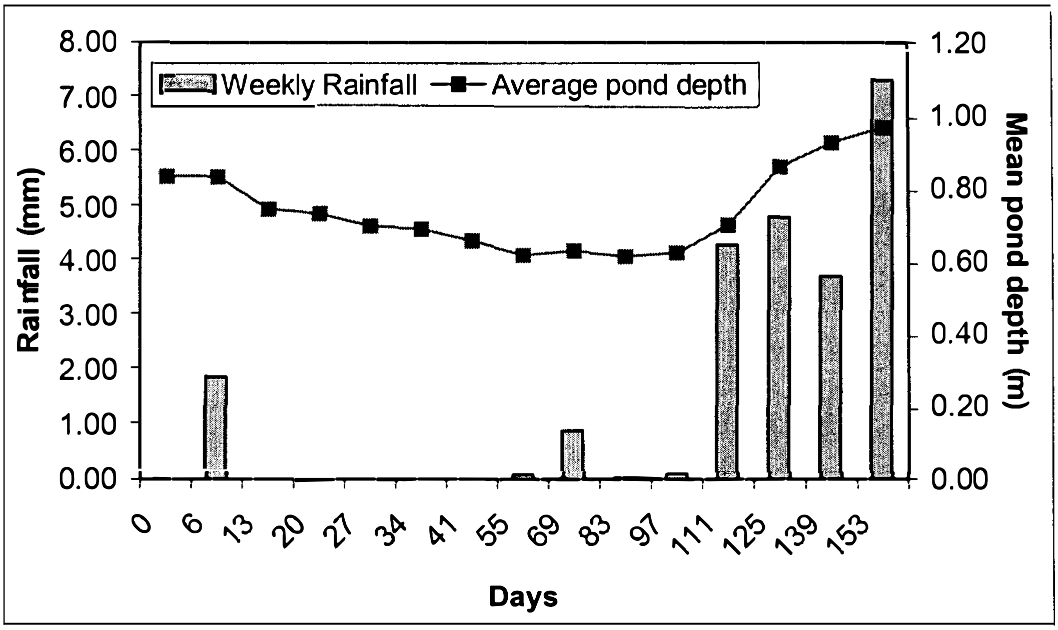


Figure 5.2 Weekly rainfall compared with changes in mean pond depth during the period of the field experiment.

Rainfall data collected at Griffin Coal, Collie (approx 15 km from study site).

Depth

The mean depth of the ponds for days 83 to 153 were; controls 0.77 (± 0.031) m; manure 0.72 (± 0.032) m; and mulch 0.86 (± 0.032) m. Depth changed as evaporation and precipitation altered the surface water run off and level of the watertable (Figure 5.2). The water levels in the ponds dropped gradually till about day 97, then increase till the end of sampling at day 153. The increase in depth after day 97 corresponds with the commencement of the winter rains (Figure 5.2).

Do the mesocosms closely parallel the lake?

A strong correlation exists between the data for the *in situ* control ponds and Ewington over the period of the field experiment for pH, dissolved oxygen levels, conductivities, sulphate, sulphide, nutrient and phytoplankton levels (see results below, Figures 5.3 and 5.7, Tables 5.1, 8.14 to 8.18) indicating that the ponds are a reasonable indicator of the Ewington environment. The ponds are, however, more responsive to changes in rainfall and temperature than Ewington, probably due to their smaller size and shallow depth.

Water temperature

There was a decrease in pond water temperature from day 83 to 153 (Table 5.3). At the bottom of the water column, the mean temperature was generally about 0.5 °C cooler than at the surface for all the ponds. There was no significant difference in water temperature among treatment ponds (Table 8.17; Figure 5.3) and the pattern of temperature change for the ponds correlated strongly with the water temperature pattern reported from Ewington since January 1997 (Figure 5.4). The mean temperature from April 1991 to August 1999 for Ewington was 17.9 (± 0.76) °C (Figure 5.6), with the warmest water in February and coolest in July. As the pattern of water temperature for the control and treatment ponds and the lake are similar. As a consequence temperature is not considered to be a variable likely to influence comparisons for physical or chemical processes.

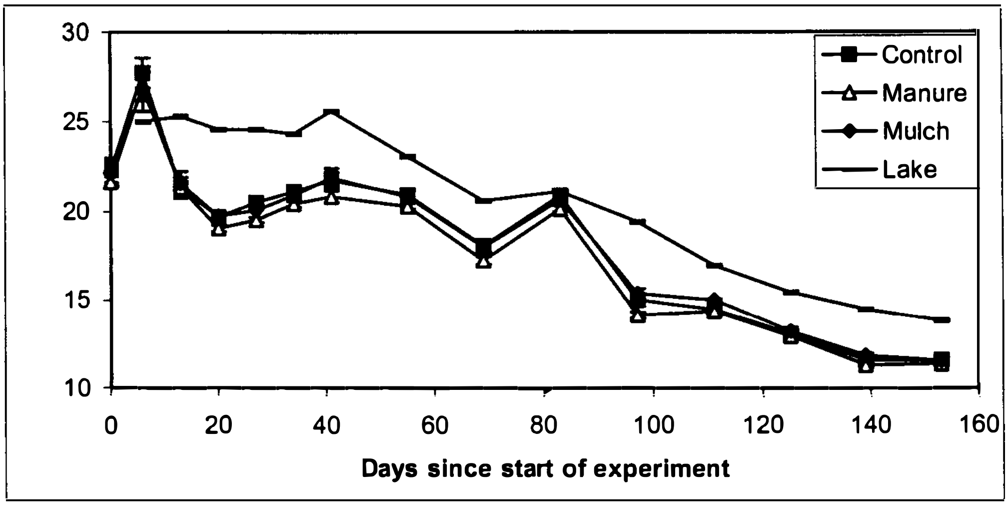


Figure 5.3 Changes in mean pond water temperature for the three treatment conditions and Ewington for the duration of the experiment.
Standard error bars are shown for each sampling day, n = 6 for each treatment.

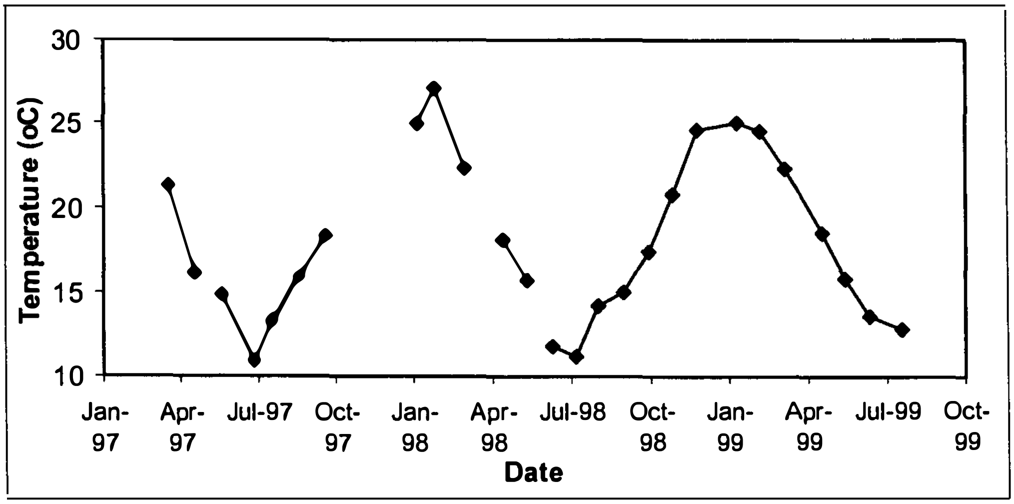


Figure 5.4. Changes in Ewington water temperature from January 1997 to August 1999.

pH

There was no significant ($P < 0.05$) difference between the top and bottom pH and there was no significant change in pond water pH values after day 83 (Table 5.1). The mean pH for the top and bottom of the water column in each pond for days 83 to 153 for the two organic matter treatments and control were; control 5.16 (± 0.199); manure 5.95 (± 0.127); and mulch 5.75 (± 0.107). There was a significant ($P < 0.05$) difference among pH values for treatments and controls.

Fluctuation in pH in Ewington showed the same pattern as was evident in the treatment ponds (Figure 5.5). The pH values for manure and mulch ponds were consistently higher (> 0.5 to 1.0) than for the control ponds for all days except day 153; these values were inturn was higher (< 0.25 to 0.8) than Ewington. The pH of pond water increased for the two organic matter treatments and the control from day 97 until day 139 (Figure 5.5). There appeared to be an inflow of ground water into the ponds as indicated by the changing pond depths associated with increased rainfall. It was concluded that the increase in pond water pH after day 97 was associated with the steady inflow of more pH neutral ground water after periods of rain. The height of the bund around each pond meant there was little chance of surface water running into any of the ponds and thus changing the pond water pH. The decrease in pH from day 139 to day 153 goes against this trend. The heavier rains in the last couple of weeks before the experiment concluded might have flushed considerably increased amounts of ground water into the lake returning it to its more characteristic low pH levels. Figure 5.6 shows substantial fluctuations in lake pH over a period of two years but there are no data on changes in pH for ground water in the vicinity of Ewington.

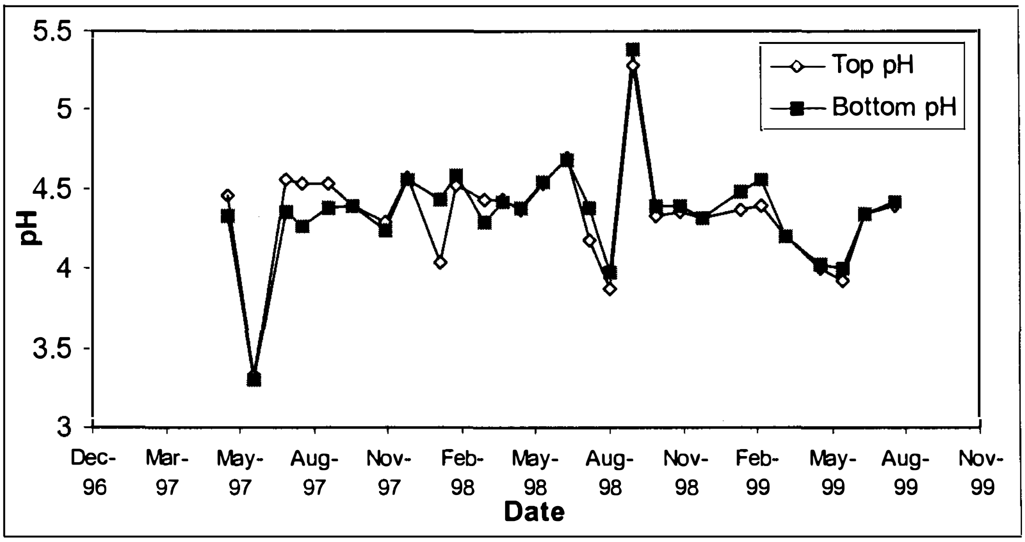


Figure 5.6 Temporal changes in the pH for Ewington from January 1997 till August 1999

The post hoc Tukey test indicated that the manure treatment had a significantly higher pH than the control for days 83 to 139, whereas the pH in the mulch treatment was only significantly higher than the control for days 83 and 97 (Table 5.1). The pH for the control ponds increased at a more rapid rate than in the treatment ponds toward the end of the field experiement (control ponds increased from 4.52 on day 83 to 5.33 on day 153; mulch ponds increased from 5.56 to 5.62; manure ponds increased from 5.66 to 5.89). At day 153 there was no significant difference between the pH for the control and treatment ponds. An unscheduled opportunity arose on 20 March 2000 to revisit Ewington and record pH values in the ponds. The mean pH values for the control ponds was 4.31 (± 0.056), the mulched ponds was 4.48 (± 0.125) and the manure ponds was 5.78 (± 0.29). An ANOVA on these data indicated a significant difference ($F_{2,15} = 18.83$, $P < 0.001$)

among treatments and the control ponds. A post hoc Tukey test indicated that there was no significant difference between the mulch and control ponds but the pH in the manure ponds was higher than in the mulch and the control ponds. These data indicate the increase in pH values for the manure treatment have been sustained over a period from January 1999 to March 2000, covering a complete seasonal cycle.

The mean surface pH value for Ewington from May 1993 to August 1999 was 4.31 (± 0.049); the mean bottom pH value from April 1995 to August 1999 was 4.35 (± 0.059) (Figure 5.6). pH occasionally showed a substantial shift from the norm. One possible explanation for these shifts in pH is the changing rate of ground water inflow after winter rainfall. Similar variations were also evident in the ponds constructed adjacent to Ewington and monitored over a period of six months.

Table 5.1. Comparison of means and standard errors for pH for days 83 to 153 for two treatments, the controls and Ewington.

	Treat't	Day 83	Day 97	Day 111	Day 125	Day 139	Day 153
		$\bar{x}$ ± se	$\bar{x}$ ± se	$\bar{x}$ ± se	$\bar{x}$ ± se	$\bar{x}$ ± se	$\bar{x}$ ± se
pH	Control	4.52 ± 0.110	4.68 ± 0.106	5.08 ± 0.139	5.45 ± 0.100	5.72 ± 0.118	5.53 ± 0.177
	Manure	5.66 ± 0.125	5.63 ± 0.121	5.91 ± 0.140	6.17 ± 0.107	6.45 ± 0.099	5.89 ± 0.124
	Mulch	5.56 ± 0.151	5.46 ± 0.159	5.75 ± 0.151	5.95 ± 0.132	6.16 ± 0.139	5.62 ± 0.149
	Lake	4.26	4.34	4.7	4.87	5.11	4.6

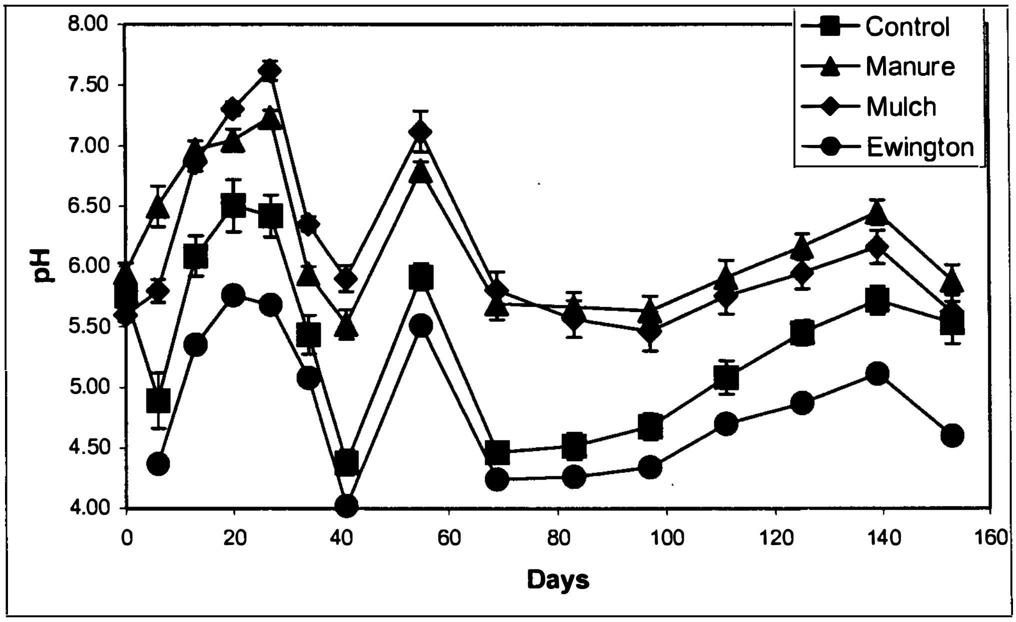


Figure 5.5 Changes in the mean pH for pond water for the two treatments, control, and Ewington for the duration of the experiment.
Standard error bars are shown for each sampling day, n = 6 for each treatment.

Dissolved oxygen

The pattern of results for dissolved oxygen measured in mg O_2 L^{-1} and as a percentage of saturation are the same (Tables 5.2, 8.15 and 8.16). The dissolved oxygen levels increased during the field experiment but only after the rainfall increased (about day 97). A post hoc Tukey test indicated that the dissolved oxygen levels in the mulch ponds were significantly lower than the control (except for day 153; mulch 0.35 (± 0.044) mg O_2 L^{-1} and control; 7.09 (± 0.879) mg O_2 L^{-1}) and lower than the manure (4.08 (± 0.915) mg O_2 L^{-1}) until day 111. After day 111 there was no significant difference between the control and manure treatment nor the manure and mulch treatments (Figure 5.7). The dissolved oxygen levels in Ewington for days 83 to 153 were 8.56 (± 0.875) mg O_2 L^{-1}. This is higher than the levels experienced in the ponds. The dissolved oxygen levels at the bottom of the water columns were always lower than the surface dissolved oxygen levels.

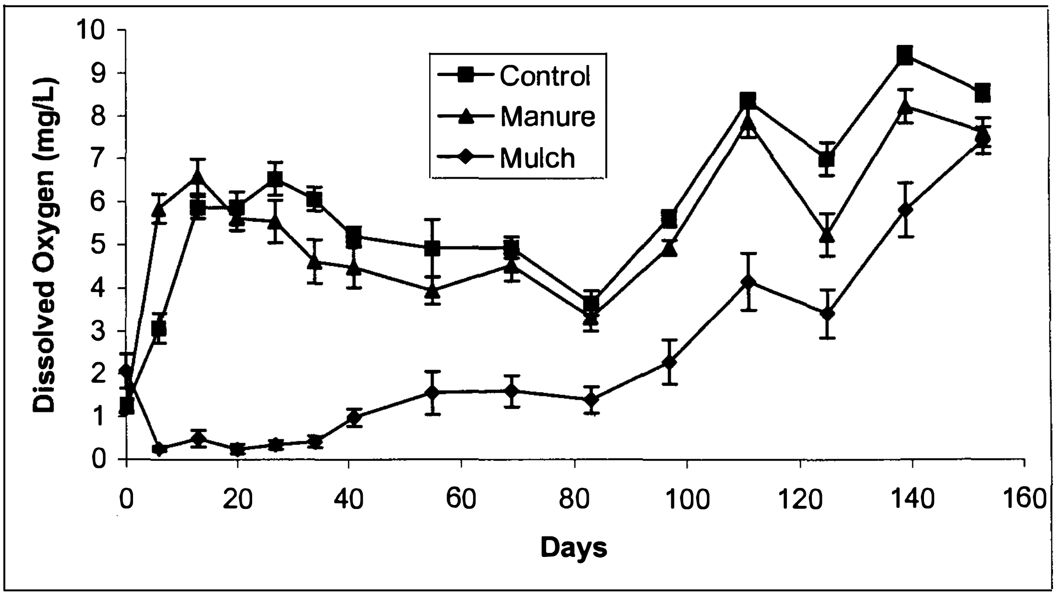


Figure 5.7 Changes in the mean dissolved oxygen (mg O_2 L^{-1}) content for the two treatments and the control ponds for the duration of the field experiment
Standard error bars are shown for each sampling day, n = 6 for each treatment.

Conductivity

Water at the bottom of the control and manure ponds had a mean conductivity 50 µs cm $^{-1}$ higher than the surface. This was not the case for the mulch treatment (see appendix Table 8.18). There was no significant ($P < 0.05$) difference in conductivity between the treatments and control (Table 5.2).

The conductivity in the control ponds was not significantly different to the conductivity in Ewington over the same 6 month sampling period till August 1999 {Ewington; 1099.14 (± 26.285) µs cm $^{-1}$}, however, wide fluctuation in the results might have obscured any differences. The conductivity for the ponds and Ewington were generally highest at the end of summer (February/March) and decreased as the rainfall increased in winter. The conductivity for the ponds becomes variable after day 97 (Figure 5.8) most probably because of the inflow ground water resulting from increased rainfall (Figure 5.2). The rainfall decreases the conductivity by introducing freshwater to the surface ground water, which flows into the ponds.

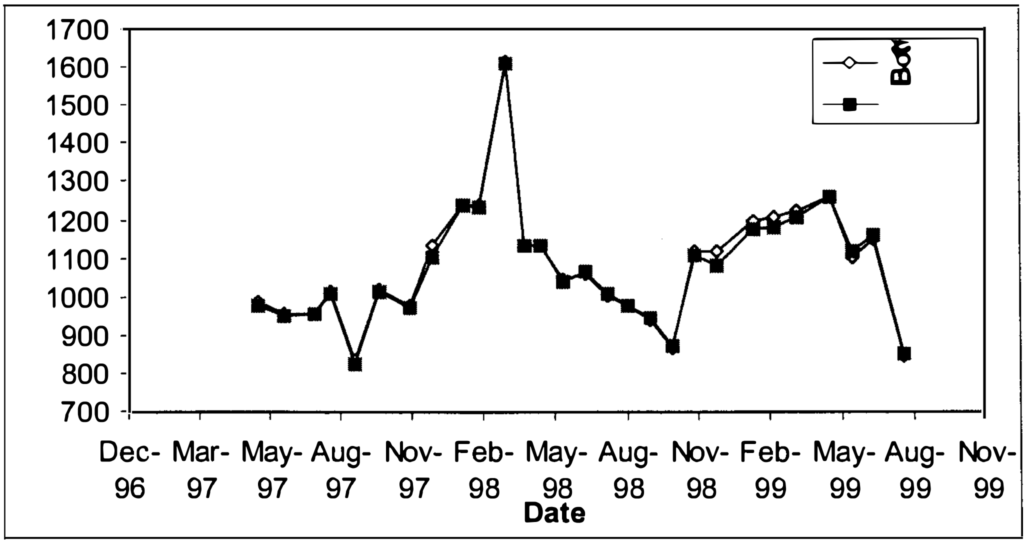


Figure 5.8 Conductivity changes for Ewington (µs cm^{-1}) from January 1997 to August 1999.

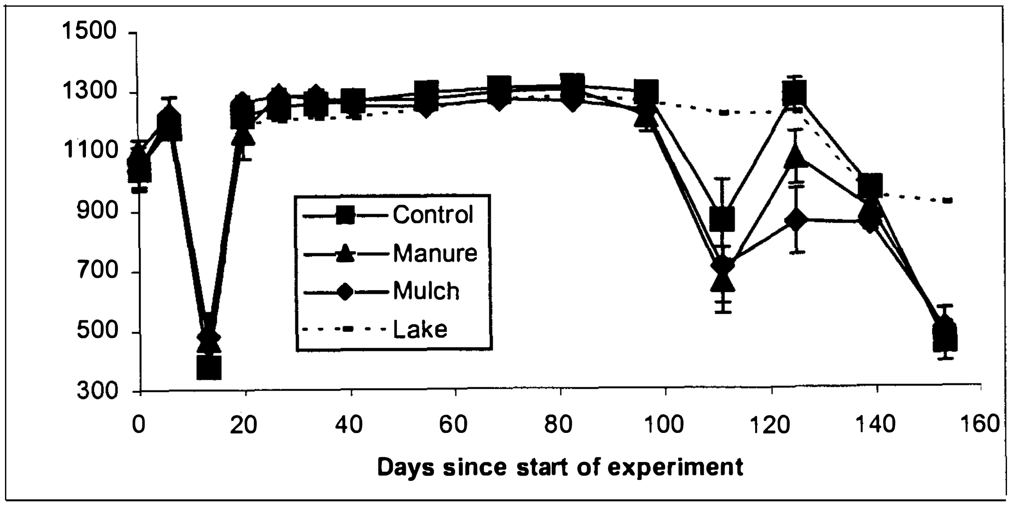


Figure 5.9 Changes in mean conductivity (µs cm^{-1}) for the treatment the control ponds, and Ewington for the duration of the experiment. Standard error bars are shown for each sampling day, n = 6 for each treatment.

Turbidity

As might be expected, the turbidity at the bottom of the water column was significantly higher than at the top for all treatments and the controls. There was no significant difference in turbidity between the treatments and control (control - 42.85 (± 18.9) ntu; manure - 25.75 (± 11.8) ntu; mulch - 45.08 (± 26.3) ntu; Table 5.3; Figure 5.10). Turbidity was not measured every sampling day as the equipment wasn't always available.

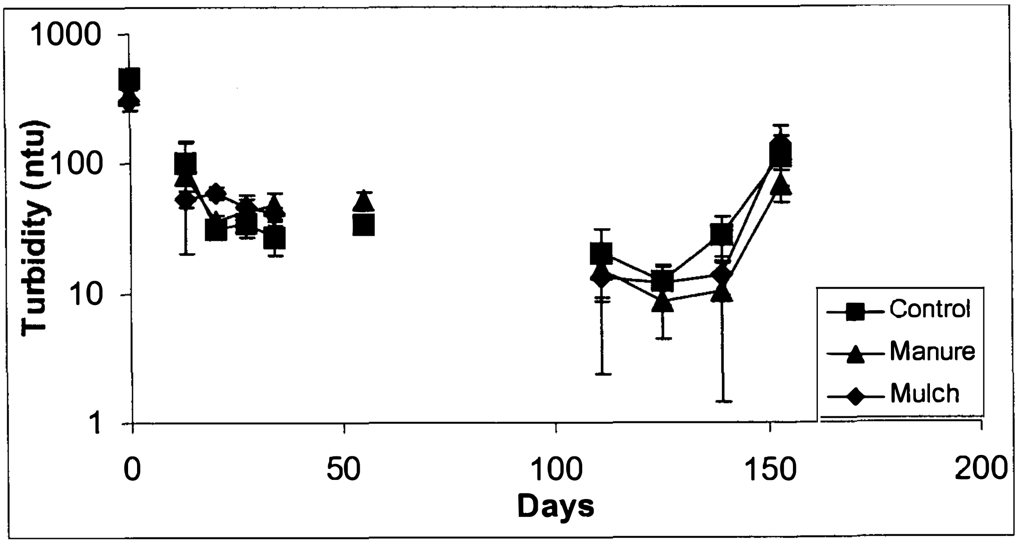


Figure 5.10 Changes in mean pond water turbidity (ntu) for the treatments and control for the duration of the experiment.

Standard error bars are shown for each sampling day, n = 6 for each treatment.

Oxidation reduction potential

At the bottom of the water column the control redox potential was generally twice as high as the surface, whereas for the organic matter treatments the redox potential was more uniform across the water column. There was no difference in the redox potential among the organic matter treatments and controls (manure – 160.81 (± 6.64) *m*V; mulch – 167.46 (± 13.18) *m*V; control – 197.16 (± 5.563) *m*V; Table 8.20) and no significant change in the potential over time (Table 8.20; Figure 5.10).

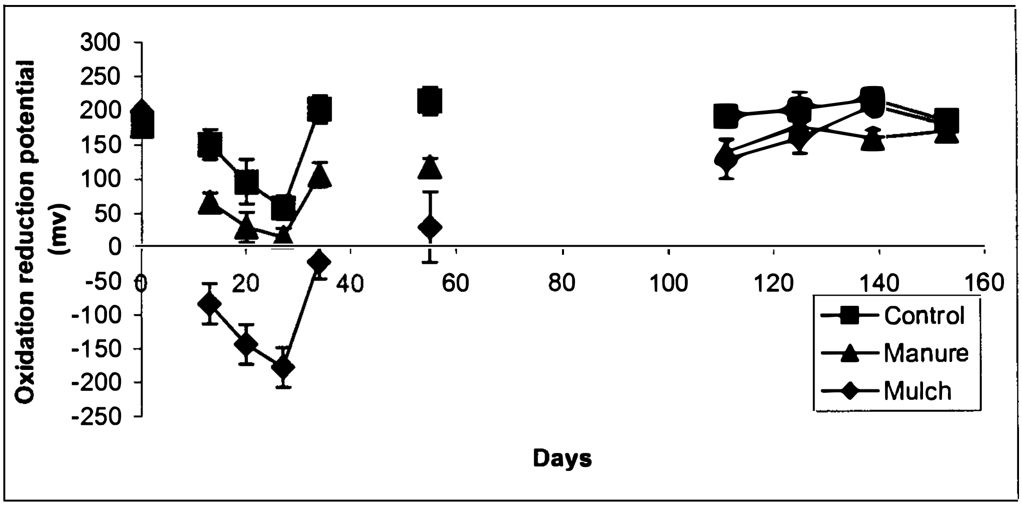


Figure 5.11 Changes in mean redox potential for the treatments and the control for the duration of the experiment.
Standard error bars are shown for each sampling day, n = 6 for each treatment.

Gilvin

There was no significant change in gilvin values between days 83 and 153 (Table 5.2). The mean gilvin values for days 83 to 153 for the two treatments and control were; control 1.07 (± 0.002) $_{g440}$ m^{-1}; manure 4.05 (± 0.002) $_{g440}$ m^{-1}; and mulch 3.79 (± 0.003) $_{g440}$ m^{-1}. There was no significant difference in gilvin values among the organic matter treatments or control (Figure 8.23; Table 5.2). Variation between ponds and consideration of the statistical power of the analysis would suggest a visual inspection of the data might be more informative. Toward the end of the trail there appear to be no differences between the two treatments which were in turn slightly higher than the control.

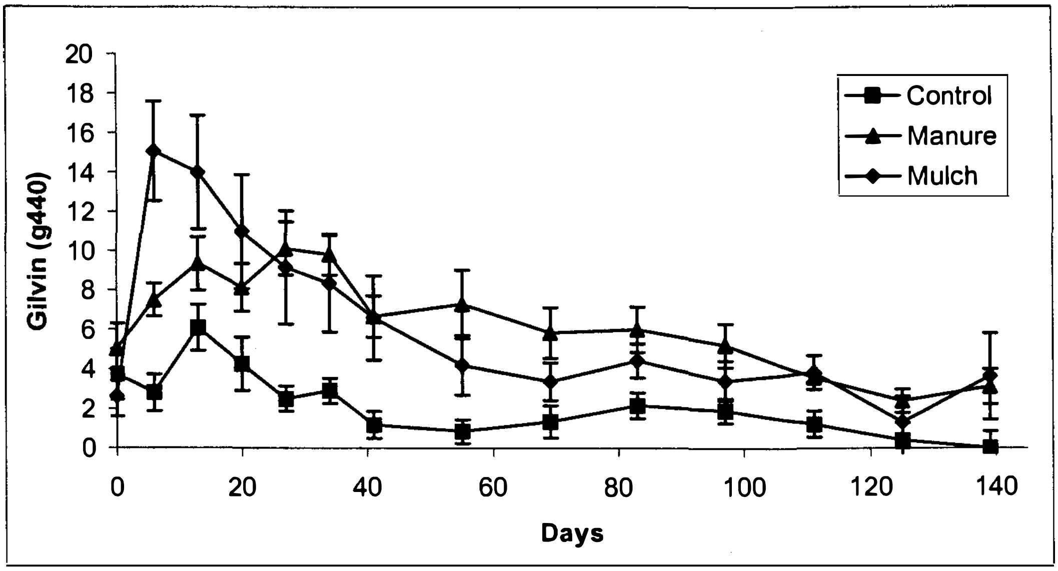


Figure 5.12 Changes in mean gilvin values for the organic matter treatments and control for the duration of the field experiment.
Standard error bars are shown for each sampling day, n = 6 for each treatment.

Chlorophyll a

The chlorophyll *a* levels in the manure treatment were significantly higher than for the control on days 111 and 153, and the manure treatment was significantly higher than the mulch treatment on day 153 {the means for days 27 to 153 are; control - (8.47 ± 1.125) μg L^{-1}; manure – 33.30 (± 4.206) μg L^{-1}; and mulch – 12.36 (± 2.117) μg L^{-1}; Figure 5.13; Table 5.3}. There was no significant change in chlorophyll *a* levels over days 27 to 153 (Table 8.23) and but there was a significant difference in chlorophyll *a* levels between the organic matter treatments and control. The control was significantly ($P < 0.5$) lower than the manure.

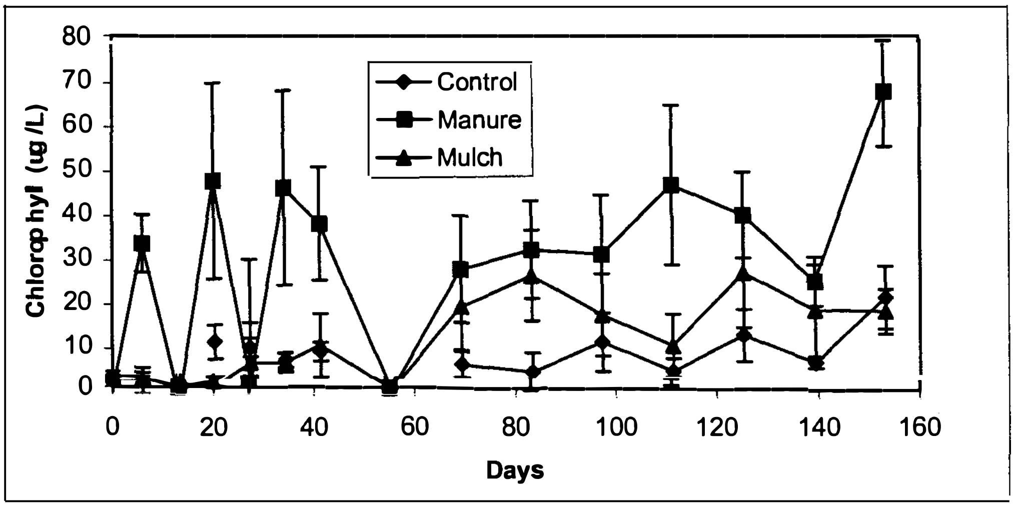


Figure 5.13 Changes in mean chlorophyll *a* levels for the treatments and the control ponds for the duration of the experiment.
Standard error bars are shown for each sampling day, n = 6 for each treatment.

Filterable reactive phosphate (FRP)

The mean FRP levels for day 153 for the two treatments and control were; control 3.67 (± 0.422) µg P L^{-1}; manure 100.17 (± 23.159) µg P L^{-1}; and mulch 3.50 (± 0.428) µg P L^{-1}. There was a significant difference in FRP among treatments (Figure 5.14; Table 5.3) and the post hoc Tukey test indicated that FRP levels in the manure treatment were higher than for the mulch and controls, but there was no difference between the mulch and the controls. The increased level of FRP found in the manure treatment probably explains why the chlorophyll *a* levels are significantly higher for the manure treatment, as the increased nutrients provides better conditions for the growth of chlorophyll *a*.

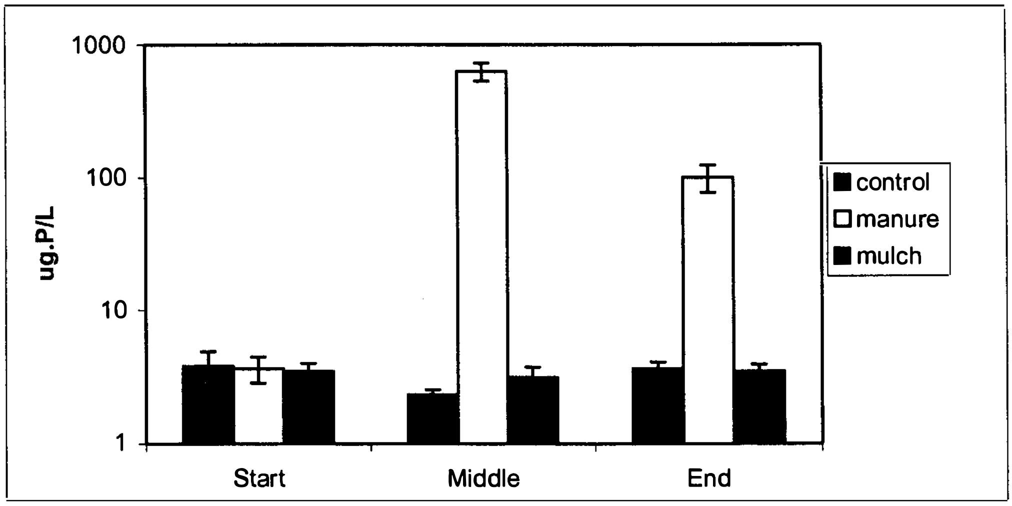


Figure 5.14 Changes in mean FRP for the treatment and control ponds for the field experiment.

Standard error bars are shown for each sampling day, n = 6 for each treatment.

Ammonia and nitrate/nitrites

There was no significant difference between the treatments and controls for ammonia or for soluble nitrate/nitrites (Table 5.2). The mean ammonia levels at the end of the field experiment were; control – 9.17 (± 1.302) µg N L $^{-1}$; manure – 12.00 (± 1.238) µg N L $^{-1}$; and mulch – 17.67 (± 7.898) µg N L $^{-1}$ and the mean nitrate/nitrite levels were; control – 1.30 (± 85.7) µg N L $^{-1}$; 1.24 ± 79.5) µg N L $^{-1}$; and mulch – 7.99 (± 98.8) µg N L $^{-1}$.

Sulphate and sulphide

There was no significant change in sulphate or sulphide levels or between the different organic matter treatments or controls over days 83 to 153 (Table 5.2). The mean sulphate levels for the field experiment were; control – 6.90 (± 0.53) mg SO_4 L^{-1}; manure – 7.64 (± 0.88) mg SO_4 L^{-1}; and mulch – 7307 (± 1.31) mg SO_4 L^{-1}. The mean sulphide levels showed a similar pattern (no significant differences) to the sulphates with the control being slightly lower than the treatments; control – 3.73 (± 3.46) ppb; manure – 7.04 (± 6.90) ppb; mulch - 9.63 (± 9.50) ppb.

Macro-invertebrates

There was considerable variability in the individual abundance and numbers of families represented among ponds (Table 8.26). There was no significant difference (ANOVA; $F_{2,15} = 2.01$, $P = 0.168$) among control and treatment ponds for the number of individual specimens caught, nor was there a significant difference (ANOVA; $F_{2,15} = 0.09$, $P = 0.911$) among control and treatment ponds for the number of Families represented. The mean number of invertebrates caught in each of the ponds was; control 54.50 (± 9.283); manure 58.17 (± 13.055); and mulch 140.50 (± 57.143), and the mean number of Families represented for each pond was; control 8.00 (± 1.00); manure 7.83 (± 0.601); and mulch 17.50 (± 0.847).

Phytoplankton

There was considerable variability between ponds for different treatments in the number of invertebrates and number of Families represented. There was no significant difference between the organic matter treatments and the control ponds. Mean phytoplankton counts for Ewington where within the 95% confidence limits for the means for the treatment and control ponds for day 153 (Table 5.3). The mean phytoplankton count for each of the ponds were; control

59.22 (± 32.555) L^{-1}; manure 393.85 (± 184.209) L^{-1}; mulch 46.55 (± 31.294) L^{-1} and Ewington 241.62 (± 116.011) L^{-1} (n = 6).

Table 5.2 Field experiment: Summary of mean and standard errors for pH, dissolved oxygen, conductivity, oxidation-reduction potential, sulphate, gilvin, ammonia, FRP and nitrate/nitrites for days 83 to 153 except for sulphate; sulphide, ammonia, FRP and nitrate/nitrites which are for the last day only.

* indicates significant values at $P \leq 0.05$;

	Treatment	$\bar{x}$ ± se	D x T *F* value	Tukey test results at last sampling day			
					Control	Manure	Mulch
pH	Control	5.16 ± 0.199	6.166	Control			
	Manure	5.95 ± 0.127		Manure	*		
	Mulch	5.75 ± 0.107		Mulch			
	Lake	4.65 ± 0.131					
DO (mg L^{-1})	Control	7.09 ± 0.879	12.789	Control			
	Manure	4.08 ± 0.915		Manure			
	Mulch	0.35 ± 0.044		Mulch	*		
	Lake	8.56 ± 0.875					
Conductivity (μs cm^{-1})	Control	1026.26 ± 138.5	1.130	Control			
	Manure	938.1 ± 129.140		Manure			
	Mulch	898.82 ± 121.33		Mulch			
	Lake	1137.00 ± 67.49					
ORP (*m*V)	Control	197.16 ± 5.563	1.481	Control			
	Manure	160.21 ± 6.636		Manure			
	Mulch	167.46 ± 13.181		Mulch			
	Lake	339.00 ± 11.86					
SO_4 (mg L^{-1})	Control	6.90 ± 0.527	0.164	Control			
	Manure	7.64 ± 0.876		Manure			
	Mulch	7.07 ± 1.314		Mulch			
Gilvin ($_{g440}$)	Control	2.02 ± 0.306	3.352	Control			
	Manure	6.27 ± 0.385		Manure			
	Mulch	6.46 ± 0.675		Mulch			
Ammonia (μg N L^{-1})	Control	9.17 ± 1.302	0.125	Control			
	Manure	12.00 ± 1.238		Manure			
	Mulch	17.67 ± 7.898		Mulch			
FRP (μg P L^{-1})	Control	3.67 ± 0.422	6.911	Control			
	Manure	100.17 ± 23.16		Manure	*		
	Mulch	3.50 ± 0.428		Mulch		*	
Nitrate/nitrite (μg NO_2/NO_3 $L^{-1)}$	Control	129.17 ± 85.69	0.628	Control			
	Manure	198.17 ± 79.48		Manure			
	Mulch	150.00 ± 98.82		Mulch			

Table 5.3 Field experiment: Summary of mean and standard errors for temperature, turbidity, chlorophyll *a*, invertebrate diversity, invertebrate abundance and phytoplankton for days 83 to 153 except for invertebrate abundance and diversity which are for the last day only.

* indicates significant values at $P \leq 0.05$;

	Treatment	$\bar{x}$ ± se	D x T *F* value	Tukey test results at last sampling day			
					Control	Manure	Mulch
Temperature	Control	14.45 ± 1.414	2.849	Control			
	Manure	14.03 ± 1.330		Manure			
	Mulch	14.60 ± 1.364		Mulch			
	Lake	16.85 ± 1.179					
Turbidity (ntu)	Control	42.85 ± 18.924	1.595	Control			
	Manure	25.75 ± 11.793		Manure			
	Mulch	45.08 ± 26.337		Mulch			
	Lake	7.68 ± 5.865					
Chlorophyll *a*	Control	8.47 ± 1.125	4.809	Control			
	Manure	33.30 ± 4.206		Manure	*		
	Mulch	12.36 ± 2.117		Mulch		*	
Invertebrate diversity	Control	8.00 ± 1.00	0.87	Control			
	Manure	7.83 ± 0.601		Manure			
	Mulch	9.83 ± 1.701		Mulch			
Invertebrate abundance	Control	54.50 ± 9.283	2.01	Control			
	Manure	58.17 ± 13.06		Manure			
	Mulch	140.50 ± 57.14		Mulch			
Phytoplankton (L^{-1})	Control	59.22 ± 32.556	2.159	Control			
	Manure	393.85 ± 184.21		Manure			
	Mulch	46.55 ± 31.294		Mulch			
	Lake	241.62 ± 116.01					

Summary and Discussion of Field Experiment

The laboratory experiments indicated that the addition of organic materials would increase the pH. Manure and mulch were better at increasing pH than hay; and as a consequence hay was not used in the field experiment. The low level of sulphate reducing bacterial activity does not adequately account for the significant increases in pH in the treated cores. Organic material introduced into the cores contained alkalising substances and was the primary contributor to the increase in pH. It was therefore expected that the addition of manure and mulch to the *in situ* ponds would result in an increase in pH. It was not known how long the effect might last nor whether dissolved oxygen and sulphate levels would decrease, and gilvin and sulphide levels would increase as they did in the cores.

The threefold aim of the field experiment was to determine a) the effect that the addition of vegetation mulch and cattle manure have on the pH of lake water in a situation similar to that in Ewington; b) the longevity of the treatment affects in a field situation; and c) whether experimental results using intact cores in a laboratory can be used to predict the outcomes of similar experiments in ponds in a field situation.

The pH values for manure and mulch ponds were generally higher (0.5 to 1.0 pH units) than for the control ponds; these values were inturn higher (< 0.25 to 0.8 pH units) than Ewington. The pH for the control ponds increased at a more rapid rate than the treatment ponds towards the end of the field experiement (day 83 to day 153). The measurement of pH values in the control and treatment ponds again in

March 2000, some 15 months after they were first established, provided evidence that the manure treatment was able to maintain a significant increase in the pH. Although the pH was 5.8, and below what might have been hoped for, it was still 1.5 pH units above the control. As the pH in both Ewington and the experimental ponds commenced increasing about the time of the winter rains (Figure 5.5). This suggests that the inflow of ground water was the primary cause for the change in pH. The reason for the sudden decline in pH for the treatment, control ponds and Ewington from day 139 to 153 is unknown, but maybe a consequence of the higher rainfall in the last weeks before the conclusion of the study that flushed a greater quantity of ground water into these voids returning the lake (and the ponds) to their more characteristic and lower pH levels. These data suggest that the addition of both manure and mulch will increase the pH of Ewington water. pH values in Ewington are likely to occilate as they did in the ponds over the study period primarily due to the inflow of surface and groundwater. The sustained higher pH values for the treated ponds compared with the controls and Ewington over a period of 15 months suggest that effects could be long term.

The dissolved oxygen levels after an initial increase slowly declined presumably due to biological oxygen demand until the winter rains commenced about day 97 from which time they increased (Figure 5.7). The sudden decline in dissolved oxygen levels at day 125 (26 May) corresponds with the first heavy rain recorded for the winter. These rains resulted in substantial runoff into the lake and presumably into some of the ponds as the sides of some of the ponds collapsed destroying the surrounding berms which were put in place to prevent surface water inflow. . The collapsed sides also released fresh organic matter into the pond water. The number of frogs and tadpoles in the ponds was observed to increase at this time although they where not counted.

For turbidity, salinity, chlorophyll *a,* sulphate and sulphide there was no significant change over time. However, significant variations within treatments for gilvin (Figure 5.12), chlorophyll *a* (Figure 5.13) and FRP may well have masked important differences and trends. These substantial differences among ponds with similar treatments were expected as minor physical changes to individual pond mesocosm environments were likely to occur (e.g., bank collapse, animal disturbance, varying ground water inflow rates) as part of the natural processes in the area.

The level of sulphate was low for the ponds in acid mine drainage areas (M. lund, pers comm. January 1999) and therefore the consequential level of sulphide was also low. These low sulphate/sulphide levels are consistent with the results for the laboratory experiments and suggest that sulphate reducing bacteria were not responsible for most of the increase in pH. For sulphate reducing bacteria to act in acidified waters an oxidation reduction potential of less than -100 *m*V is required (Lyew *et al.* 1994; Lyew and Sheppard, 1997). Only mulch had an oxidation reduction potential low enough to support sulphate reducing bacteria and it was only at this level for the first 40 days of the field experiment. In addition, the dissolved oxygen levels in the ponds were generally too high to enable sulphate reducing bacteria to exist is sufficient quantity to cause the increase in pH (Hammack and Edenborn, 1991).

The level of FRP was significantly higher in the manure treatment ponds than for the control or mulch treatments. Ponds with the manure added had the highest levels of chlorophyll *a*, with the control and mulch ponds having comparable levels. It is probable that the higher phosphate levels in conjunction with higher pH values are responsible for the increased levels of chlorophyll *a* found in the manure treatment ponds . It was also noted that for Ewington, chlorophyll *a* is lowest in winter; which is probably due to the cooler water temperatures and lower levels of sun light which would result in lower levels of photosynthesis. If the trend in chlorophyll *a* levels in Ewington parallels those in the ponds, it might be expected that as the water temperature increased in the ponds with the onset of summer, chlorophyll *a* densities would also increase.

The conductivity levels for the ponds are more irregular after the winter rains commenced (day 97). Rain and surface inflow havning less ions would reduce conductivity, whereas inflow groundwater might increase conductivty as the water would pick up ions as it moves through the soil. Inflow surface and groundwater therefore contribute to occilations not only in pH and disssolved oxygen concentration but affect the physical parameters such as conductivity, depth and turbidity.

6.0 DISCUSSION

Acid drainage is potentially the largest negative environmental impact resulting from coal mining (Harries, 1998a; Lowson, *et al.*, 1993; Robertson, 1987; Ryan and Joyce, 1991). It therefore represents a significant financial liability for mining companies. If mine site ownership is returned to the Crown then there can be a significant cost burden on the community as acidity problems can extend for many years beyond the life of the mine. This is the case for the abandoned coal mining voids in Collie.

Passive biological water treatment systems appear to represent the first real alternative to the expensive chemical neutralisation and aeration programs. Although not universally applicable, these systems have the advantage of being simple and requiring little in terms of installation, operation and maintenance (Perry and Kleinmann, 1991). Based on 25 years of data, Cambell and Lind (1969) found that acidic mine lakes with a greater input of organic matter, such as leaves and grass, exhibited a higher pH than lakes with lesser amounts of organic matter input. It was concluded from their observations, and studies by Tuttle *et al.* (1969b), and Gyure, *et al.* (1987) that the acidity of mining void lakes would decrease as a consequence of biological activity. The use of sulphate reducing bacteria and organic matter additives to increase the pH therefore represents an inexpensive biological alternative to chemical neutralisation of acidic lakes (Gyure, *et al.* 1987; Tsukamoto and Miller, 1999; Tuttle, *et al.* 1969a).

The addition of organic material significantly increased the pH of Ewington water in laboratory and field experiments. This increase in pH was of the order of 1.0 to 1.5 pH units in the laboratory experiments using manure and mulch, and 0.5 to 1.0 pH units for manure and mulch in the field experiment. However, this increase was not completely due to the activity of sulphate reducing bacteria as has been reported in other acidic mining voids (Harries, 1998a; Lowson, *et al.*, 1993; Robertson, 1987; Ryan and Joyce, 1991). In addition to the low level of sulphate reducing bacteria, organic matter introduced into the intact Ewington cores and the *in situ* ponds introduced alkalising chemicals that neutralised the acid water.

In the laboratory experiments manure, and to a less extent mulch, when added to deionised water increased the pH, however, mulch contributed more carbonates than manure. There was little difference in the alkalising affect of mulch and manure for the first 22 days of the experiment but between day 22 and the conclusion of the experiment at day 42, cores containing mulch continued to increase their pH whereas, cores containing manure produced lower levels of carbonates. Mulch may have more alkalising material available for release than manure, however, in the mulch treatment the alkalinity may not be as readily available for release under low pH compared with manure and may be more dependant on time or bacterial processes. Or alternatively, mulch has a greater surface area being composed essentially of shredded vegetation thus enabling more of the alkalsing chemicals to be exposed to the acid water. Manure on the other hand is a slurry and rapidly settles to the bottom with only the surface exposed to the water column. This might be why mulch had a greater ability to release carbonates over the duration of the experiment. If the pH in cores containing organic materials increased as a consequence of the introduction of alkalising substances, then it would be expected the pH value in the mulch would have been higher than the manure. This was not the case, as the mean pH in the manure cores was 7.08 (± 0.024) compared with the mulch cores of 6.68 (± 0.024). This result indicates that the alkalising affect contributes to the increase in pH, however, other unknown biological or chemical processes must contribute to the difference between the mulch and manure cores.

Low sulphate levels in Ewington water, intact cores and *in situ* ponds meant there was little opportunity for sulphate reducing bacterial activity to neutralise acid water. When a sulphate aliquot was added to treated cores, sulphate levels decreased and black sulphide precipitate increased indicating low levels of sulphate reducing bacterial activity, however, this activity was not sufficient to significantly increase the pH. This suggests the limiting factor for sulphate reducing bacterial activity might have been sulphate in the cores and the *in situ* ponds. Perry and Kleinmann (1991) indicate 200 – 500 m^2 of mushroom compost based wetlands are necessary to neutralise a kg/day of net acidity from acid mine drainage in a flow through system. Mushroom compost was deemed the most successful and inexpensive substrate because it supported high levels of sulphate reducing bacterial activity but it also contains limestone, which contributes additional alkalinity. The data of Perry and Kleinmann (1991) add support to the suggestion that sulphate reducing bacterial activity in the cores and ponds was inhibited by a lack of sulphate in the Ewington water and the added organic material. The addition of soluble sulphate at the time of adding the organic material might have enhanced the contribution of sulphate reducing bacteria to increase pH.

Organic matter may contribute in other ways to increasing the pH in the lakes. When organic matter is evenly spread over the sediment a capping effect may limit oxygen reaching the pyrite in the sediments reducing the oxidation of the pyrite, which reduces the amount of acidity being formed (Johnson *et al.,* 1997). Capping the substrate in the *in situ* ponds may inhibit the oxidation of any pyrite in the soil but this is not the primary mechanism operating in the ponds to increase pH. As acidic ground water was observed to enter the ponds from the side-walls during pond construction it is highly probable that it entered through the bottom as well. However. Organic matter added to the ponds covered the bottom but not the sides; thus negating a capping effect for inflow water through the side-walls. Organic matter additions could have provided a capping affect for groundwater entering through the bottom of the ponds.

The addition of some organic matter types (e.g., manure) can increase the level of soluble nutrients in the water. This in turn increases the likelihood of algal growth and chlorophyll *a* levels, which leads to the establishment of a biologically active system (Kalin and Geller, 1998). For the experimental ponds adjacent to Ewington, nutrient levels increased for FRP but not for ammonia or nitrates/nitrites when manure was added but not for mulch. This is probably the reason for the significantly higher chlorophyll *a* count in the manure ponds compared with the mulch and control ponds. Kalin and Geller (1998), in summarising the views of a number of chapter authors in *Acidic Mining Lakes*

(1998), indicate that there is a commonly held view by limnologists that autochthonous succession from young to mature lakes exists, resulting in lakes progressively accumulating sediment and developing ecosystems. During this process the emerging ecosystem neutralises the acidic content of the water. However, Kalin and Geller (1998) go on to point out this theory has yet to be tested and the underlying mechanisms are not known. Brewer and Goldman (1976) provide some indirect evidence that the hypothesis might be supported by future research, in that they demonstrated that the addition of phytoplankton can increase the alkalinity of a culture medium resulting from the NO_3^- and NH_4^+ uptake. The introduction of organic matter into Ewington mine void water may have accelerated the processes for the establishment of a sustainable ecosystem in the ponds. This hypothesis is supported by the significant increase in chlorophyll *a* and macroinvertebrates found in the ponds at the conclusion of the study.

Based on the data available it appears that the primary mechanism for increasing pH in the *in situ* ponds was the addition of alkalising substances in the manure and mulch. Low levels of sulphate reducing bacterial activity and possibly a capping affect for some of the inflowing acidic ground water might have also contributed to neutralising the pH. An increase in pH enabled the growth of micro fauna and flora in the ponds. These are the primary producers for a sustainable ecosystem (Axler, and Owen, 1994; Knox, Ladiges and Evans, 1994). The maintenance of a higher pH in the manure ponds after a period of 15 months suggest that the growth in micro flora and fauna and other associated biological processes will lead to a sustainable system capable of maintaining a pH 1 – 1.5 units above untreated ponds (Kalin and Geller, 1998). If similar biological processes operated in Ewington, the addition of a slurry of manure could increase the pH to about 5.5 – 6.0. A pH of 5.5 – 6.0 in Ewington would is sufficient to enable the lake to be used for recreational purposes and perhaps aquaculture (ARMCANZ and ANZECC, 1996; NH&MRC, 1990).

The addition of hay to mining voids has been suggested because it is relatively inexpensive, it can add phosphorus and nitrogen and provides food and shelter for macroinvertebrates (Cale and Edward, 1990, 1994b, Street and Titmus, 1982). In the laboratory experiments the addition of hay resulted a lower pH than for mulch and manure and a slimy film on the surface that would detract from the aesthetic appearance of the water voids that used this treatment. Hay treatments also had a strong and unpleasant odour. The lower increase in pH, slimy film and unpleasant odour in the hay treated cores suggests that manure and mulch are better choices than hay as rehabilitation solutions for existing coal mine voids in the Collie region.

It took about three days for the organic matter to settle to the bottom, which probably reflected the time it took the mulch and hay to become saturated. If the surface of the core was slightly agitated the mulch and hay treatments settled faster. The time taken for the mulch and hay to become saturated might be a useful feature of the organic matter treatments. If the organic matter additions floated on the surface of the water in a mining void it would be much easier to evenly spread the organic matter across the lake bottom. If the organic matter treatments sank quickly, like the manure slurry, there could be a concentration of organic matter around the point of distribution.

Is the increase in pH, as a consequence of the additions of organic matter, sustainable in an acid mine void? This question cannot be answered directly from this research as no experiments were undertaken in the lake. The laboratory experiments are too removed from the field situation and too short to provide any real indication to the permanency of any changes. The field trial results, although over a period of 21 weeks, are a series of mesocosms established in excavated ponds adjacent to Ewington. Although the physical and chemical properties of the control ponds approximated those in Ewington there were sufficient differences to indicate caution should be exercised in extrapolating the findings to the lake. However, the most interesting result is the pH values measured in the ponds in March 2000. The manure treated ponds were significantly higher (5.8) than those treated with mulch (4.5) and the control (4.3) ponds. If the pH values in Ewington in March 1998 (4.4) and March 1999 (4.2) are indicative of the values in March 2000, then the manure treated ponds were approximately 1.5 pH units above those in the lake (≈ 4.3) and the control ponds. These data suggest that the addition of manure to acidic mine void water can increase the pH significantly. Whether it would work in Ewington can only be determined by adding organic material to the lake or a similar large body of water. Alternatives to manure could also be examined. For example, if the introduction of organic matter has two effects, a) an initial increase in pH because of the introduction of alkalising substances, and b) the higher pH enables the establishment of micro flora and fauna communities based on the carbon, soluble phosphate and other nutrients introduced with the organic matter; then it might be feasible to use lime or another neutralising chemical compound to provide the initial increase in pH and the addition of phosphate and other nutrients to sustain the ecosystem, maintaining a higher pH.

The addition of organic matter also caused a decrease in dissolved oxygen and an increase in the gilvin value in the laboratory experiments. The decrease in dissolved oxygen concentrations is most probably a result of biological oxygen demand (Kim *et al.* 1999; Robinson and Robb, 1995). In the field trial, dissolved oxygen concentrations increased from day 83 corresponding to the commencement of the winter rains and the inflow of ground water into the ponds. Gilvin is an important measure in a rehabilitation situation where an aesthetic value is often placed on the final product. It is appreciated that people's opinions differ and some people will be more concerned than other about the extent of water discolouration in a rehabilitated mine site. Stockton, a neighbouring abandoned mine void, is currently used for recreational purposes and aesthetic values are important. The gilvin value is also important as it provides an indicator of the dissolved organic carbon levels in the water. Dissolved carbon is one of the primary building blocks for a functional aquatic ecosystems (Anderson and Williams, 1999) and therefore a necessary component in establishing an ecosystem in acidic mine voids as the pH increases.

Doubling the quantity of organic matter increased the levels of gilvin. The increased colouration in the water is a result of increased amounts of carbon released in to the water by the organic matter and higher quantities of organic matter enabled more humic/fulvic acids to be leached out. Gilvin values for the control ponds were lower than for the treatment ponds as might have been expected because of the addition of organic material. Gilvin values declined over the duration of the field experiment suggesting that dissolved carbon was being removed from the water column by biological activity. This aesthetically unpleasant environment could last for some time after the introduction of the organic material into the lake. Althought water colouration is an important issue in designing mine site rehabiliation management solutions the gilvin levels experienced in the cores were less than those commonly found in the Swan Coastal Plain wetlands (Wrigley *et al.*, 1988). The dissolved oxygen level of the water was not correlated with gilvin levels. Turbidity, another indicator of aesthetic appearance of the water, was not significantly different between the treatments and the controls.

If the addition of organic materal is used to accelerate the commencement of the biological succession processes (Kalin and Geller, 1998) then a combination of manure and mulch may result in a higher pH as it appears that the mechanisms manure and mulch use to increase pH could be different. Mulch was able to continue to reduce the pH by alkalising the water at a higher pH than manure, whereas similar quanitities of manure, particular at the lower pH levels, had a greater impact on increasing the pH levels. In the longer term (up to 15 months) the pH in the ponds treated with manure were significantly higher than those treated with mulch and the untreated controls. Further research is therefore required before we understand the chemical or biological processes that enable the addition of either of these two organic materials to increase pH in acidic water.

From a mine management perspective, the local availability of the organic matter types and the cost will be prime considerations when deciding the final composition of the mix. For example, in Collie, the Shire council may be willing to dispose of its shredded waste vegetation, prunings from regular street tree maintanence at the mine site instead of in its land fill program. Or alternatively, a local dairy farmer may be willing to provide the mining company with the manure that gets washed from the sheds and milking areas. The cost of transporting the organic materials to the site would be the major expense.

Future research

This study indicates that the addition of organic matter, in particular cattle faeces and mulched vegetation, may increased the pH of acidic mine void water in the vicinity of Collie, Western Australia. Both substances contributed alkalising material to the water column but there were other biological or chemical processes involved that were not understood. Low levels of sulphate reducing bacterial activity and capping pyrite in the substrate were not shown to make a significant contribution to increasing pH. These unknown process warrants further investigation.

A long term, *in situ,* lake-scale monitoring experiment, in Collie looking at the changes in pH and other chemical and physical variables should be undertaken. Laboratory trials in this situation provided a useful indication of what subsequently occurred in the *in situ* ponds with the addition of organic matter. It is unknown the extent to which laboratory and field trials will provide an indication of what will occur in Ewington. The treatment of Ewington could be used as the next experiment in the process to determine a satisfactory rehabilitation program for other voids in the area. Such as study would examine whether the organic matter treatments are useful at a depth greater than 1 m and whether they are still performing as remediation devices after a period of 12 months.

The quantity of organic material necessary to bring about a sustained long-term increase in pH is also unknown. Such a research project would also need to take into account economic considerations. Future research might also investigate the optimium mix of organic materials and quantities that could be used to determine an economic rehabilitation solution for industry.

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8.0 APPENDIX

Appendix 1

Pilot Experiment

The purpose of this pilot study was two fold:

a) to determine if the addition of hay, manure and mulch to intact cores from Ewington would result in an increase in pH,

b) to determine if the selected quantity of organic matter (250 cm^3 per core) was adequate to initiate an increase in pH.

Pilot Experiment - Effects of organic matter additions

Twenty-four intact cores were collected from Ewington (3/6/98) and transported to the laboratory on the same day. The cores consisted of approximately 25% sediment (200mm) and 75% water. Twelve cores were randomly assigned to the oxic conditions (>5mg O_2 L^{-1}) and 12 cores to the hypoxic conditions (<2mg O_2 L^{-1}). Three replicates of each treatment (hay, mulch, manure) and three controls were assigned to the oxic and hypoxic treatments. The controls were sampled under the same conditions as the treated cores except they had no organic matter added.

For each treatment 250cm^3 of either hay, manure or mulch was placed in each of the randomly selected cores. Temperature, dissolved oxygen and pH of the water columns were measured at intervals; before treatment (day –1) and then days 0, 1, 4, 6, 8, 12, 16, 26, 34 and 42. Water in the cores was sampled to measure sulphate, sulphide, gilvin and acidity on days –1, 15 and 42. Heavy metal content of the water in the cores was also measured on days –1 and 42.

Results

The addition of 250 cm^3 of mulch and manure to intact sediment cores from Ewington significantly ($P < 0.05$) increased the pH. A post hoc Tukey test indicated that the pH for the manure treatment {hypoxic; 7.83 (± 0.06) and oxic; 7.64 (± 0.04)} was significantly higher than the control and the hay treatment for days 8 to 42; and the pH for the mulch treatments {hypoxic; 6.59 (± 0.05) and oxic; 6.64 (± 0.06)} were significantly higher than the control samples for days 8 to 16 (Figure 8.1; Table 8.1). There was no significant difference between the control and hay treatment for pH.

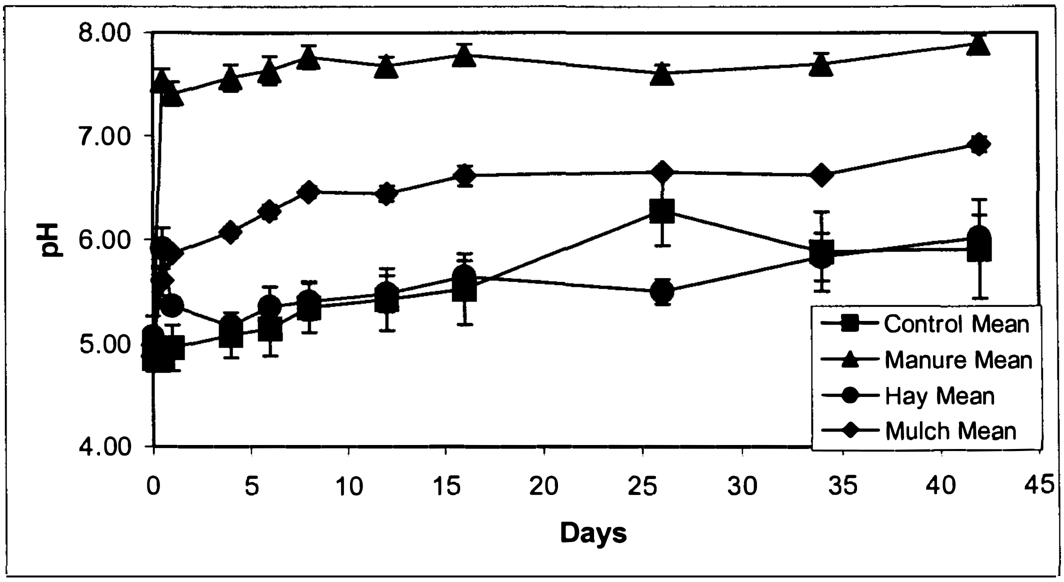
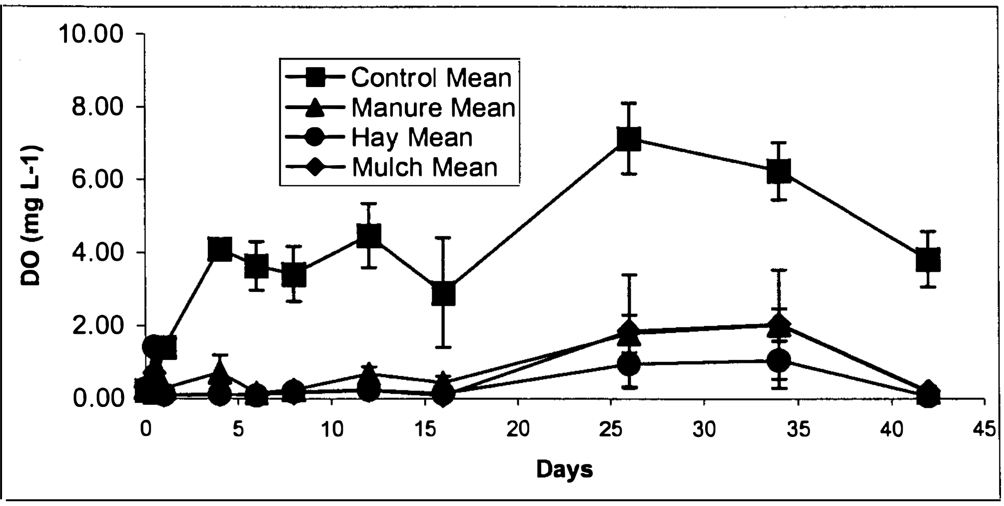


Figure 8.1 Pilot Experiment: Changes in mean pH for organic matter treatments and controls for the duration of the experiment.
Standard error bars are shown for each day, n = 3 for each treatment.

The control cores were hard to keep below the desired 2 mg O_2 L^{-1} for the hypoxic conditions, and the organic matter treatment cores were hard to keep above the 5 mg O_2 L^{-1} for the oxic conditions because of small leakages around the stopper in the cores. Mean hypoxic control core dissolved oxygen levels were: 4.57 (± 0.5) mg O_2 L^{-1} and mean oxic control core dissolved oxygen level were: 5.68 (se ± 0.4) mg O_2 L^{-1}. Despite this, a significant difference among treatments was recorded for the dissolved oxygen levels in the pilot experiment (Table 8.1). A post hoc Tukey test indicated that the manure, hay and mulch treatments all had significantly lower dissolved oxygen levels than the control (Table 8.1). There was, however, no significant difference among the manure, hay or mulch treatments for dissolved oxygen.

a)



b)

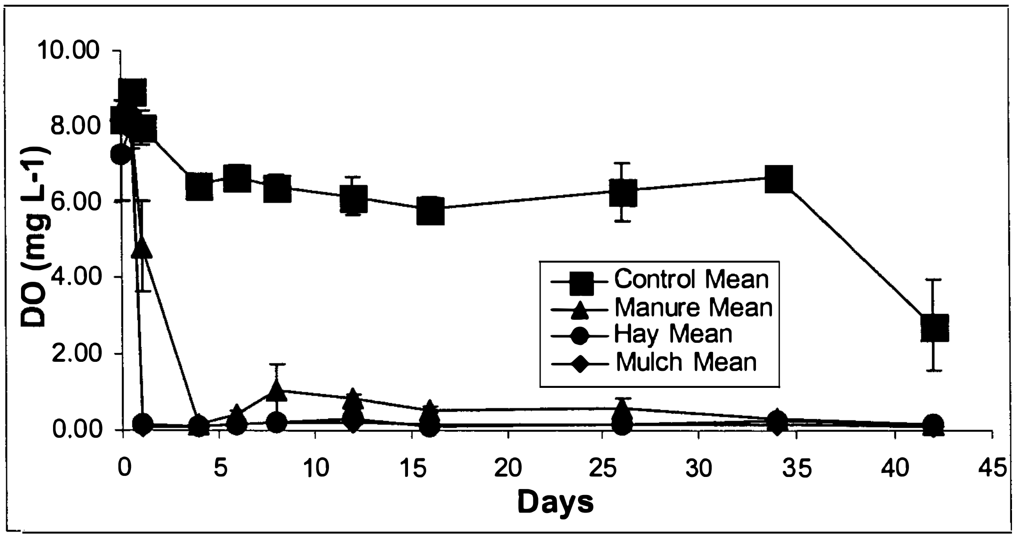


Figure 8.2 Pilot Experiment: Changes in mean dissolved oxygen (mg O_2 L^{-1}) levels for treatments and controls for a) hypoxic and b) aerobic cores for the duration of the experiment.

Standard error bars are shown for each day, n = 3 for each treatment.

When organic matter was added there was a significant increase in gilvin levels among treatments but no significant difference between aeration conditions {before 5.3 (± 2.8) $_{g440}$ m^{-1}; after - manure was 50.68 (± 0.6) $_{g440}$ m^{-1}; hay was 38.23 (± 1.1) $_{g440}$ m^{-1}; mulch was 53.10 (± 1.3) $_{g440}$ m^{-1}}. A post hoc Tukey test on day 42 results indicated that the gilvin values for the treatments were all significantly higher than the controls, and the manure and mulch treatments were significantly higher than the hay treatment (Table 8.2). The increased gilvin levels after the addition of organic matter indicates increased levels of dissolved carbon in the water under both aeration conditions.

The sulphate levels in the pilot experiment cores were low for all treatments (< 4 mg SO_4 L^{-1}). There was a significant ($P < 0.05$) difference among treatments irrespective of the aeration conditions. A post hoc Tukey test on day 42 results indicated that the mulch treatment had a significantly ($P < 0.05$) higher level of sulphate than the control, manure and hay treatments, but there were no other differences (Table 8.1).

In summary, the addition of hay, manure and mulch significantly increased pH and gilvin values, and reduced dissolved oxygen levels. Manure produced the greatest increase in pH. A quantity of 250 cm^3 or organic matter per core was sufficient to initiate a significant change in pH.

Table 8.1 Pilot Experiment: Summary of mean and standard errors for pH, dissolved oxygen, sulphate, sulphide and gilvin.

* indicates significant values at $P \leq 0.05$; SO_4 - sulphate; S^{2-} - sulphide; sulphate, sulphide and gilvin are on last day only. D - days; T - treatments; A - aeration conditions.

	Treatment	Aeration	$\bar{x} \pm$ se	D x T	D x T x A	Tukey test results at last sampling day				
				F value	*F* value		Control	Manure	Hay	Mulch
pH	Control	<2 mgL^{-1}	5.99 ± 0.201	39.17*	0.740	Control				
		>5 mgL^{-1}	5.47 ± 0.193							
	Manure	<2 mgL^{-1}	7.83 ± 0.057			Manure	*			
		>5 mgL^{-1}	7.64 ± 0.043							
	Hay	<2 mgL^{-1}	5.63 ± 0.117			Hay		*		
		>5 mgL^{-1}	5.66 ± 0.107							
	Mulch	<2 mgL^{-1}	6.59 ± 0.045			Mulch				
		>5 mgL^{-1}	6.64 ± 0.057							
DO (mg L^{-1})	Control	<2 mgL^{-1}	4.57 ± 0.499	46.10*	0.430	Control				
		>5 mgL^{-1}	5.68 ± 0.389							
	Manure	<2 mgL^{-1}	0.09 ± 0.206			Manure	*			
		>5 mgL^{-1}	0.58 ± 0.131							
	Hay	<2 mgL^{-1}	0.45 ± 0.172			Hay	*			
		>5 mgL^{-1}	0.20 ± 0.031							
	Mulch	<2 mgL^{-1}	0.77 ± 0.363			Mulch	*			
		>5 mgL^{-1}	0.17 ± 0.014							

Table 8.1 cont.

	Treatment	Aeration	$\bar{x}$ ± se	D x T	D x T x A	Tukey test results at last sampling day				
				F value	*F* value		Control	Manure	Hay	Mulch
SO_4 (mg L^{-1})	Control	<2 mgL^{-1}	1.18 ± 0.042	6.403*	0.962	Control				
		>5 mgL^{-1}	1.64 ± 0.106							
	Manure	<2 mgL^{-1}	1.54 ± 0.273			Manure				
		>5 mgL^{-1}	1.25 ± 0.127							
	Hay	<2 mgL^{-1}	0.88 ± 0.100			Hay				
		>5 mgL^{-1}	0.92 ± 0.052							
	Mulch	<2 mgL^{-1}	3.33 ± 0.113			Mulch	*	*	*	
		>5 mgL^{-1}	2.29 ± 0.110							
S^{2-} (ppb)	Control	<2 mgL^{-1}	11.37 ± 2.771	1.586	0.099	Control				
		>5 mgL^{-1}	1.59 ± 0.391							
	Manure	<2 mgL^{-1}	322.58 ± 10.760			Manure				
		>5 mgL^{-1}	403.83 ± 51.768							
	Hay	<2 mgL^{-1}	507.38 ± 138.396			Hay				
		>5 mgL^{-1}	352.43 ± 100.686							
	Mulch	<2 mgL^{-1}	97.83 ± 28.170			Mulch				
		>5 mgL^{-1}	124.00 ± 35.507							
Gilvin (g_{440} m^{-1})	Control	<2 mgL^{-1}	4.00 ± 0.465	90.316*	3.979*	Control				
		>5 mgL^{-1}	2.10 ± 0.459							
	Manure	<2 mgL^{-1}	49.32 ± 2.282			Manure	*			
		>5 mgL^{-1}	49.91 ± 2.888							
	Hay	<2 mgL^{-1}	46.98 ± 5.788			Hay	*	*		
		>5 mgL^{-1}	29.04 ± 4.877							
	Mulch	<2 mgL^{-1}	48.84 ± 3.927			Mulch	*		*	
		>5 mgL^{-1}	57.44±6.000							

Appendix 2

Table 8.2 Experiment 1: Means and standard errors for pH.

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Conditions		Days from start of Experiment 1											*Grand mean* ± Standard Error
		0	0.5	1	4	6	8	12	16	26	34	42	
Hypoxic	Control	4.99 ± 0.229	4.99 ± 0.229	5.24 ± 0.393	5.37 ± 0.384	5.42 ± 0.518	**5.65** **± 0.447**	**5.71** **± 0.555**	**5.91** **± 0.607**	**6.49** **± 0.062**	**5.93** **± 0.610**	**6.26** **± 0.740**	5.99 ± 0.201
	Manure	5.07 ± 0.225	7.46 ± 0.240	7.47 ± 0.233	7.68 ± 0.245	7.74 ± 0.280	**7.88** **± 0.214**	**7.80** **± 0.117**	**7.92** **± 0.139**	**7.71** **± 0.101**	**7.66** **± 0.162**	**8.04** **± 0.031**	7.83 ± 0.057
	Hay	5.24 ± 0.408	6.13 ± 0.148	5.37 ± 0.076	5.07 ± 0.059	5.04 ± 0.070	**5.15** **± 0.075**	**5.36** **± 0.214**	**5.58** **± 0.195**	**5.56** **± 0.225**	**6.11** **± 0.354**	**6.04** **± 0.267**	5.63 ± 0.117
	Mulch	5.05 ± 0.193	5.62 ± 0.303	5.82 ± 0.028	5.98 ± 0.042	6.14 ± 0.025	**6.41** **± 0.042**	**6.41** **± 0.076**	**6.62** **± 0.108**	**6.64** **± 0.059**	**6.64** **± 0.019**	**6.82** **± 0.131**	6.59 ± 0.045
Oxic	Control	4.71 ± 0.037	4.71 ± 0.037	4.67 ± 0.081	4.80 ± 0.106	4.88 ± 0.077	**5.05** **± 0.087**	**5.14** **± 0.222**	**5.14** **± 0.250**	**6.07** **± 0.720**	**5.83** **± 0.592**	**5.56** **± 0.682**	5.47 ± 0.193
	Manure	4.88 ± 0.135	7.61 ± 0.020	7.35 ± 0.069	7.45 ± 0.078	7.53 ± 0.058	**7.64** **± 0.013**	**7.56** **± 0.087**	**7.65** **± 0.129**	**7.51** **± 0.110**	**7.74** **± 0.169**	**7.75** **± 0.090**	7.64 ± 0.043
	Hay	4.88 ± 0.064	5.70 ± 0.352	5.35 ± 0.111	5.27 ± 0.122	5.67 ± 0.292	**5.67** **± 0.289**	**5.60** **± 0.306**	**5.71** **± 0.269**	**5.45** **± 0.139**	**5.55** **± 0.247**	**6.00** **± 0.403**	5.66 ± 0.107
	Mulch	4.92 ± 0.012	5.58 ± 0.072	5.91 ± 0.045	6.16 ± 0.065	6.40 ± 0.072	**6.51** **± 0.110**	**6.47** **± 0.150**	**6.60** **± 0.191**	**6.66** **± 0.030**	**6.59** **± 0.015**	**7.01** **± 0.037**	6.64 ± 0.057

Table 8.3 Experiment 1: Means and standard errors for dissolved oxygen (mg L^{-1}).

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Conditions		Days from start of Experiment 1											*Grand mean* ± Standard Error
		0	0.5	1	4	6	8	12	16	26	34	42	
Hypoxic	Control	0.24 ± 0.058	0.18 ± 0.049	1.41 ± 0.056	4.10 ± 0.183	3.64 ± 0.662	**3.42 ± 0.746**	**4.47 ± 0.872**	**2.91 ± 1.501**	**7.13 ± 0.971**	**6.25 ± 0.788**	**3.82 ± 0.764**	4.57 ± 0.513
	Manure	0.31 ± 0.152	0.59 ± 0.279	0.29 ± 0.090	0.73 ± 0.472	0.16 ± 0.012	**0.24 ± 0.051**	**0.69 ± 0.198**	**0.44 ± 0.172**	**1.78 ± 0.509**	**2.04 ± 0.444**	**0.18 ± 0.065**	0.90 ± 0.206
	Hay	0.27 ± 0.102	1.41 ± 0.069	0.10 ± 0.013	0.12 ± 0.003	0.10 ± 0.009	**0.19 ± 0.055**	**0.25 ± 0.090**	**0.13 ± 0.003**	**0.95 ± 0.660**	**1.07 ± 0.760**	**0.07 ± 0.024**	0.45 ± 0.172
	Mulch	0.32 ± 0.107	0.70 ± 0.092	0.09 ± 0.015	0.11 ± 0.009	0.12 ± 0.019	**0.16 ± 0.049**	**0.23 ± 0.036**	**0.09 ± 0.007**	**1.86 ± 1.531**	**2.05 ± 1.495**	**0.22 ± 0.140**	0.77 ± 0.363
Oxic	Control	8.21 ± 0.233	8.95 ± 0.082	7.98 ± 0.455	6.44 ± 0.299	6.69 ± 0.295	**6.43 ± 0.312**	**6.16 ± 0.502**	**5.80 ± 0.347**	**6.27 ± 0.760**	**6.65 ± 0.094**	**2.78 ± 1.178**	5.68 ± 0.389
	Manure	8.42 ± 0.254	8.23 ± 0.328	4.83 ± 1.191	0.17 ± 0.018	0.41 ± 0.123	**1.06 ± 0.684**	**0.86 ± 0.114**	**0.53 ± 0.079**	**0.56 ± 0.302**	**0.30 ± 0.032**	**0.16 ± 0.127**	0.58 ± 0.131
	Hay	7.25 ± 1.208	8.01 ± 0.624	0.18 ± 0.033	0.11 ± 0.012	0.16 ± 0.026	**0.19 ± 0.025**	**0.31 ± 0.090**	**0.10 ± 0.015**	**0.16 ± 0.032**	**0.28 ± 0.121**	**0.17 ± 0.089**	0.20 ± 0.031
	Mulch	8.13 ± 0.317	8.34 ± 0.268	0.11 ± 0.015	0.12 ± 0.018	0.13 ± 0.026	**0.24 ± 0.062**	**0.21 ± 0.021**	**0.16 ± 0.003**	**0.15 ± 0.006**	**0.16 ± 0.012**	**0.11 ± 0.026**	0.17 ± 0.014

Table 8.4 Experiment 1: Mean and standard errors for sulphate (mg L^{-1}), sulphide (ppb) and gilvin values ($_{g440}$ m^{-1}).

n = 1 for day 0, n = 6 for days 15 and 42.

Variable	Condition	Treatment	Days from start of Experiment		
			0	15	42
Sulphate	Hypoxic	Control	1.20	4.09 ± 1.229	1.18 ± 0.147
		Manure	1.20	1.13 ± 0.353	1.54 ± 0.946
		Hay	1.20	2.35 ± 0.466	0.88 ± 0.346
		Mulch	1.20	3.64 ± 0.214	3.33 ± 0.391
	Oxic	Control	1.20	2.75 ± 0.175	1.64 ± 0.369
		Manure	1.20	1.40 ± 0.522	1.25 ± 0.439
		Hay	1.20	0.68 ± 0.179	0.92 ± 0.178
		Mulch	1.20	4.69 ± 0.181	2.29 ± 0.380
Sulphide	Hypoxic	Control	6.25	0.00 ± 0.000	11.37 ± 9.601
		Manure	6.25	120.50 ± 34.324	322.58 ± 37.273
		Hay	6.25	93.00 ± 20.664	507.38 ± 479.419
		Mulch	6.25	41.90 ± 37.133	97.83 ± 97.583
	Oxic	Control	6.25	56.17 ± 50.990	1.59 ± 1.354
		Manure	6.25	129.83 ± 129.83	403.83 ± 179.328
		Hay	6.25	350.78 ± 298.88	352.43 ± 348.786
		Mulch	6.25	3.97 ± 3.967	124.00 ± 123.000
Gilvin	Hypoxic	Control	5.33 ± 2.822	3.77 ± 0.826	4.00 ± 0.465
		Manure	5.33 ± 2.822	22.76 ± 1.109	49.32 ± 2.282
		Hay	5.33 ± 2.822	41.01 ± 2.474	46.98 ± 5.788
		Mulch	5.33 ± 2.822	64.15 ± 8.837	48.84 ± 3.927
	Oxic	Control	5.33 ± 2.822	8.57 ± 4.996	2.10 ± 0.459
		Manure	5.33 ± 2.822	23.09 ± 1.712	49.91 ± 2.888
		Hay	5.33 ± 2.822	38.59 ± 2.319	29.04 ± 4.877
		Mulch	5.33 ± 2.822	59.43 ± 4.882	57.44 ± 6.000

Table 8.5 Experiment 2: Means and standard errors for pH.

Figures in bold indicate data from days used in MANOVA analysis, n = 5 for each treatment.

Cond.	Quantity of organic matter		Days from start of Experiment 2													*Grand mean ±* Standard Error
			0	1	2	4	6	9	11	13	17	23	29	37	43	
Hypoxic	50 cm³	Control	4.46 ± 0.080	4.79 ± 0.184	4.32 ± 0.114	4.44 ± 0.110	4.52 ± 0.123	**4.26** **± 0.106**	**4.65** **± 0.121**	**5.31** **± 0.167**	**3.70** **± 0.271**	**4.60** **± 0.175**	**4.54** **± 0.142**	**4.95** **± 0.160**	**4.96** **± 0.138**	4.62 ± 0.243
		Manure	4.40 ± 0.042	6.88 ± 0.107	7.13 ± 0.132	7.18 ± 0.116	7.33 ± 0.124	**7.29** **± 0.077**	**7.32** **± 0.054**	**7.30** **± 0.069**	**7.44** **± 0.109**	**7.16** **± 0.066**	**7.22** **± 0.106**	**7.15** **± 0.178**	**7.12** **± 0.039**	7.25 ± 0.035
		Hay	4.58 ± 0.144	6.47 ± 0.245	5.12 ± 0.144	5.32 ± 0.117	5.77 ± 0.153	**5.52** **± 0.144**	**5.77** **± 0.111**	**5.90** **± 0.074**	**4.79** **± 0.145**	**5.45** **± 0.141**	**5.62** **± 0.096**	**6.38** **± 0.213**	**6.20** **± 0.112**	5.70 ± 0.080
		Mulch	4.46 ± 0.052	5.56 ± 0.193	5.29 ± 0.063	5.87 ± 0.061	6.26 ± 0.058	**6.23** **± 0.045**	**6.45** **± 0.065**	**6.56** **± 0.043**	**6.16** **± 0.054**	**6.49** **± 0.065**	**6.61** **± 0.058**	**7.10** **± 0.167**	**6.74** **± 0.053**	6.54 ± 0.048
	100 cm³	Control	4.38 ± 0.051	4.56 ± 0.028	4.02 ± 0.056	4.21 ± 0.086	4.53 ± 0.077	**4.40** **± 0.13**	**4.69** **± 0.106**	**5.38** **± 0.157**	**3.83** **± 0.347**	**5.03** **± 0.296**	**4.88** **± 0.331**	**5.49** **± 0.322**	**5.24** **± 0.292**	4.87 ± 0.114
		Manure	4.49 ± 0.038	7.17 ± 0.057	7.27 ± 0.082	7.34 ± 0.104	7.47 ± 0.053	**7.48** **± 0.051**	**7.62** **± 0.117**	**7.67** **± 0.122**	**7.74** **± 0.120**	**7.43** **± 0.070**	**7.47** **± 0.086**	**7.37** **± 0.052**	**7.38** **± 0.058**	7.52 ± 0.036
		Hay	4.72 ± 0.203	7.17 ± 0.064	5.07 ± 0.090	5.11 ± 0.174	5.71 ± 0.105	**5.46** **± 0.142**	**5.62** **± 0.127**	**5.78** **± 0.089**	**4.32** **± 0.225**	**5.27** **± 0.040**	**5.31** **± 0.100**	**5.96** **± 0.090**	**6.06** **± 0.032**	5.47 ± 0.090
		Mulch	4.39 ± 0.041	6.12 ± 0.094	5.24 ± 0.029	5.41 ± 0.067	6.20 ± 0.026	**6.33** **± 0.029**	**6.49** **± 0.059**	**6.57** **± 0.055**	**6.33** **± 0.048**	**6.64** **± 0.024**	**6.64** **± 0.019**	**6.73** **± 0.025**	**6.78** **± 0.028**	6.57 ± 0.028
Oxic	50 cm³	Control	4.37 ± 0.127	3.39 ± 0.231	3.92 ± 0.208	4.60 ± 0.243	4.56 ± 0.297	**5.11** **± 0.254**	**4.84** **± 0.324**	**5.19** **± 0.199**	**4.81** **± 0.341**	**4.68** **± 0.353**	**4.90** **± 0.352**	**5.13** **± 0.356**	**5.11** **± 0.376**	4.97 ± 0.109
		Manure	4.29 ± 0.087	6.54 ± 0.240	6.76 ± 0.143	6.88 ± 0.179	6.64 ± 0.133	**7.03** **± 0.116**	**7.12** **± 0.036**	**7.15** **± 0.060**	**7.04** **± 0.023**	**6.72** **± 0.141**	**6.90** **± 0.040**	**6.90** **± 0.036**	**6.88** **± 0.028**	6.97 ± 0.031
		Hay	4.39 ± 0.038	6.12 ± 0.348	5.99 ± 0.131	5.66 ± 0.178	5.61 ± 0.240	**6.05** **± 0.075**	**6.12** **± 0.060**	**6.21** **± 0.066**	**5.91** **± 0.124**	**5.71** **± 0.121**	**6.02** **± 0.111**	**6.24** **± 0.094**	**6.40** **± 0.102**	6.08 ± 0.043
		Mulch	4.31 ± 0.062	4.04 ± 0.064	4.95 ± 0.110	5.73 ± 0.074	5.91 ± 0.120	**6.30** **± 0.046**	**6.40** **± 0.012**	**6.54** **± 0.030**	**6.46** **± 0.041**	**6.24** **± 0.175**	**6.57** **± 0.049**	**6.65** **± 0.028**	**6.72** **± 0.031**	6.48 ± 0.032
	100 cm³	Control	4.33 ± 0.072	3.34 ± 0.044	4.02 ± 0.056	4.24 ± 0.188	4.55 ± 0.056	**5.17** **± 0.121**	**4.76** **± 0.115**	**5.09** **± 0.147**	**4.70** **± 0.215**	**4.76** **± 0.199**	**4.83** **± 0.160**	**5.38** **± 0.196**	**5.26** **± 0.259**	4.19 ± 0.092
		Manure	4.44 ± 0.044	7.25 ± 0.134	7.20 ± 0.084	7.15 ± 0.055	7.07 ± 0.074	**7.00** **± 0.037**	**7.38** **± 0.050**	**7.27** **± 0.072**	**6.98** **± 0.133**	**6.99** **± 0.193**	**7.04** **± 0.135**	**7.16** **± 0.105**	**7.19** **± 0.117**	7.13 ± 0.043
		Hay	4.35 ± 0.034	7.29 ± 0.128	6.51 ± 0.114	6.18 ± 0.083	6.23 ± 0.127	**5.99** **± 0.054**	**5.96** **± 0.045**	**5.92** **± 0.072**	**5.54** **± 0.092**	**5.66** **± 0.088**	**5.90** **± 0.060**	**6.26** **± 0.058**	**6.43** **± 0.042**	5.96 ± 0.048
		Mulch	4.42 ± 0.036	5.03 ± 0.167	5.60 ± 0.032	6.08 ± 0.039	6.23 ± 0.074	**6.35** **± 0.027**	**6.53** **± 0.025**	**6.53** **± 0.039**	**6.56** **± 0.072**	**6.36** **± 0.202**	**6.66** **± 0.028**	**6.75** **± 0.160**	**6.89** **± 0.054**	6.58 ± 0.042

Table 8.6 Experiment 2: Means and standard errors for dissolved oxygen (mg L^{-1}).

Figures in bold indicate data from days used in MANOVA analysis. n = 5 for each treatment.

Cond.	Quantity of organic matter		Days from start of Experiment 2													*Grand mean ±* Standard Error
			0	1	2	4	6	9	11	13	17	23	29	37	43	
Hypoxic	50 cm^3	Control	0.98 ± 0.299	0.37 ± 0.205	0.88 ± 0.210	1.96 ± 0.198	1.87 ± 0.122	**2.49** **± 0.218**	**1.98** **± 0.221**	**1.73** **± 0.176**	**2.24** **± 0.085**	**1.92** **± 0.215**	**1.64** **± 0.213**	**2.69** **± 0.187**	**2.67** **± 0.213**	2.17 ± 0.085
		Manure	0.41 ± 0.127	0.18 ± 0.072	0.42 ± 0.115	0.67 ± 0.310	0.10 ± 0.019	**0.48** **± 0.167**	**0.67** **± 0.219**	**0.63** **± 0.195**	**1.09** **± 0.146**	**0.48** **± 0.176**	**0.37** **± 0.150**	**0.96** **± 0.328**	**0.59** **± 0.117**	0.66 ± 0.072
		Hay	0.35 ± 0.194	0.22 ± 0.122	0.05 ± 0.020	0.03 ± 0.005	0.04 ± 0.002	**0.05** **± 0.012**	**0.07** **± 0.020**	**0.06** **± 0.006**	**0.07** **± 0.013**	**0.12** **± 0.015**	**0.14** **± 0.011**	**0.21** **± 0.094**	**0.12** **± 0.014**	0.11 ± 0.014
		Mulch	0.73 ± 0.238	0.27 ± 0.114	0.04 ± 0.012	0.07 ± 0.017	0.05 ± 0.008	**0.02** **± 0.002**	**0.05** **± 0.008**	**0.04** **± 0.002**	**0.07** **± 0.006**	**0.09** **± 0.012**	**0.14** **± 0.009**	**0.10** **± 0.012**	**0.14** **± 0.034**	0.08 ± 0.007
	100 cm^3	Control	0.93 ± 0.529	0.67 ± 0.299	1.14 ± 0.182	2.03 ± 0.157	2.26 ± 0.181	**2.93** **± 0.232**	**2.43** **± 0.296**	**2.02** **± 0.149**	**2.43** **± 0.132**	**2.06** **± 0.262**	**1.71** **± 0.215**	**2.77** **± 0.240**	**2.83** **± 0.293**	2.40 ± 0.097
		Manure	0.20 ± 0.078	0.14 ± 0.033	0.19 ± 0.014	0.19 ± 0.089	0.08 ± 0.022	**0.23** **± 0.178**	**0.23** **± 0.088**	**0.37** **± 0.073**	**0.50** **± 0.108**	**0.29** **± 0.057**	**0.23** **± 0.010**	**0.29** **± 0.019**	**0.44** **± 0.073**	0.32 ± 0.033
		Hay	0.32 ± 0.254	0.22 ± 0.109	0.08 ± 0.026	0.04 ± 0.007	0.04 ± 0.002	**0.02** **± 0.002**	**0.05** **± 0.003**	**0.07** **± 0.009**	**0.07** **± 0.000**	**0.46** **± 0.360**	**0.20** **± 0.012**	**0.14** **± 0.011**	**0.17** **± 0.036**	0.15 ± 0.046
		Mulch	0.11 ± 0.031	0.22 ± 0.064	0.03 ± 0.006	0.04 ± 0.010	0.04 ± 0.002	**0.02** **± 0.002**	**0.04** **± 0.002**	**0.05** **± 0.002**	**0.06** **± 0.009**	**0.09** **± 0.002**	**0.19** **± 0.015**	**0.08** **± 0.010**	**0.15** **± 0.040**	0.09 ± 0.010
Oxic	50 cm^3	Control	4.89 ± 0.110	4.75 ± 0.031	4.46 ± 0.085	5.40 ± 0.263	5.44 ± 0.361	**5.61** **± 0.365**	**5.28** **± 0.316**	**4.59** **± 0.175**	**3.87** **± 0.244**	**3.34** **± 0.164**	**2.91** **± 0.183**	**3.91** **± 0.331**	**4.17** **± 0.212**	4.21 ± 0.151
		Manure	4.79 ± 0.082	4.77 ± 0.166	4.52 ± 0.191	4.14 ± 0.305	1.43 ± 0.697	**2.27** **± 0.237**	**3.81** **± 0.261**	**3.21** **± 0.257**	**1.51** **± 0.311**	**0.43** **± 0.116**	**0.65** **± 0.137**	**1.10** **± 0.346**	**1.56** **± 0.332**	1.82 ± 0.185
		Hay	4.96 ± 0.056	4.97 ± 0.187	0.65 ± 0.392	0.13 ± 0.025	0.06 ± 0.011	**0.04** **± 0.013**	**0.15** **± 0.029**	**0.11** **± 0.024**	**0.12** **± 0.012**	**0.13** **± 0.016**	**0.20** **± 0.017**	**0.20** **± 0.043**	**0.20** **± 0.035**	0.15 ± 0.011
		Mulch	4.82 ± 0.106	4.79 ± 0.128	3.53 ± 0.185	0.93 ± 0.254	1.77 ± 0.291	**1.55** **± 0.468**	**1.13** **± 0.404**	**1.51** **± 0.481**	**0.30** **± 0.163**	**0.23** **± 0.117**	**0.18** **± 0.007**	**0.23** **± 0.065**	**0.21** **± 0.073**	0.67 ± 0.126
	100 cm^3	Control	4.91 ± 0.033	5.21 ± 0.145	4.66 ± 0.105	5.06 ± 0.080	5.73 ± 0.133	**4.73** **± 0.180**	**4.90** **± 0.326**	**4.79** **± 0.230**	**3.55** **± 0.065**	**3.27** **± 0.168**	**2.72** **± 0.068**	**3.99** **± 0.137**	**4.55** **± 0.223**	4.06 ± 0.127
		Manure	4.88 ± 0.112	4.91 ± 0.147	3.88 ± 0.143	2.58 ± 0.443	0.19 ± 0.069	**1.07** **± 0.667**	**2.28** **± 0.632**	**1.77** **± 0.525**	**0.56** **± 0.219**	**0.44** **± 0.143**	**0.39** **± 0.057**	**0.50** **± 0.066**	**0.83** **± 0.417**	0.98 ± 0.169
		Hay	4.95 ± 0.114	4.43 ± 0.109	0.15 ± 0.042	0.07 ± 0.012	0.06 ± 0.004	**0.08** **± 0.030**	**0.11** **± 0.029**	**0.10** **± 0.002**	**0.12** **± 0.020**	**0.17** **± 0.009**	**0.22** **± 0.009**	**0.21** **± 0.036**	**0.14** **± 0.017**	0.14 ± 0.010
		Mulch	4.85 ± 0.116	5.11 ± 0.171	0.35 ± 0.131	0.09 ± 0.007	0.08 ± 0.010	**0.07** **± 0.007**	**0.07** **± 0.007**	**0.08** **± 0.002**	**0.08** **± 0.013**	**0.15** **± 0.016**	**0.22** **± 0.014**	**0.12** **± 0.006**	**0.12** **± 0.013**	0.11 ± 0.008

Table 8.7 Experiment 2: Mean and standard errors for sulphate (mg L^{-1}), sulphide (ppb) and gilvin values ($_{g440}$ m^{-1}).

n = 10 for days 0 and 43.

	Aeration conditions	Quantity of organic matter		Days from start of Experiment	
				0	43
Sulphate	Hypoxic	50 cm^3	Control	5.45 ± 1.232	0.88 ± 0.222
			Manure	9.71 ± 3.069	2.40 ± 0.526
			Hay	8.78 ± 4.054	2.51 ± 0.674
			Mulch	5.13 ± 1.909	0.59 ± 0.107
		100 cm^3	Control	4.13 ± 1.706	1.14 ± 0.497
			Manure	6.42 ± 3.867	3.02 ± 0.590
			Hay	5.21 ± 1.452	4.56 ± 1.595
			Mulch	3.89 ± 1.042	0.67 ± 0.049
	Oxic	50 cm^3	Control	3.65 ± 0.833	0.65 ± 0.118
			Manure	11.68 ± 8.588	3.53 ± 1.962
			Hay	5.71 ± 1.391	2.58 ± 0.518
			Mulch	17.61 ± 8.998	3.60 ± 2.896
		100 cm^3	Control	3.29 ± 0.845	0.57 ± 0.115
			Manure	6.38 ± 2.360	4.25 ± 0.908
			Hay	3.71 ± 0.623	3.91 ± 0.501
			Mulch	3.62 ± 0.648	0.70 ± 0.211
Sulphide	Hypoxic	50 cm^3	Control	0.30 ± 0.073	60.33 ± 18.724
			Manure	69.18 ± 68.763	444.23 ± 114.728
			Hay	0.35 ± 0.043	114.02 ± 14.943
			Mulch	62.20 ± 61.760	96.33 ± 27.532
		100 cm^3	Control	8.22 ± 4.877	119.25 ± 39.635
			Manure	0.48 ± 0.206	471.67 ± 76.210
			Hay	102.77 ± 102.247	371.33 ± 76.812
			Mulch	0.82 ± 0.276	204.90 ± 66.141
	Oxic	50 cm^3	Control	0.70 ± 0.219	175.50 ± 29.208
			Manure	42.24 ± 41.191	664.80 ± 59.170
			Hay	134.12 ± 60.981	540.60 ± 94.955
			Mulch	117.96 ± 49.723	410.40 ± 184.409
		100 cm^3	Control	0.58 ± 0.277	175.95 ± 90.811
			Manure	0.44 ± 0.196	418.70 ± 276.123
			Hay	29.72 ± 29.320	693.70 ± 502.643
			Mulch	45.54 ± 45.365	180.98 ± 67.507

Table 8.7 continued

Gilvin	Hypoxic	50 cm^3	Control	1.46 ± 0.448	0.54 ± 0.571
			Manure	0.75 ± 0.569	15.07 ± 1.772
			Hay	0.34 ± 0.371	25.86 ± 3.947
			Mulch	0.38 ± 0.468	15.07 ± 3.491
		100 cm^3	Control	0.71 ± 0.519	3.71 ± 0.392
			Manure	1.00 ± 0.805	12.60 ± 1.057
			Hay	1.07 ± 0.660	35.78 ± 8.280
			Mulch	0.25 ± 0.560	15.86 ± 1.441
	Oxic	50 cm^3	Control	1.24 ± 1.071	0.00 ± 0.140
			Manure	0.51 ± 0.386	19.08 ± 1.012
			Hay	0.64 ± 0.855	38.79 ± 4.078
			Mulch	1.42 ± 0.379	49.84 ± 5.203
		100 cm^3	Control	0.44 ± 0.548	4.67 ± 1.948
			Manure	0.66 ± 0.891	21.08 ± 3.512
			Hay	0.57 ± 0.708	79.74 ± 6.615
			Mulch	0.00 ± 0.356	35.75 ± 4.028

Table 8.8 Experiment 3: Means and standard errors for pH.

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Cond.		Days from start of Experiment 3										*Grand mean* ± Standard Error
		0	2	8	14	20	23	28	34	41	48	
No Bacteria	Control	4.05	4.18	3.10	**4.88**	**5.30**	**5.38**	**5.63**	**5.63**	**5.65**	**5.72**	5.45
		± 0.120	± 0.101	± 0.313	**± 0.216**	**± 0.194**	**± 0.230**	**± 0.213**	**± 0.232**	**± 0.280**	**± 0.267**	± 0.093
	Manure	4.02	5.88	5.29	**6.49**	**6.70**	**6.81**	**6.86**	**7.02**	**7.02**	**7.01**	6.84
		± 0.071	± 0.131	± 0.121	**± 0.090**	**± 0.061**	**± 0.143**	**± 0.135**	**± 0.066**	**± 0.048**	**± 0.123**	± 0.046
	Mulch	3.98	6.24	5.40	**6.56**	**6.51**	**6.67**	**6.75**	**6.90**	**6.97**	**7.00**	6.76
		± 0.029	± 0.097	± 0.117	**± 0.070**	**± 0.071**	**± 0.122**	**± 0.125**	**± 0.039**	**± 0.027**	**± 0.052**	± 0.040
Bacteria	Control	4.04	4.20	2.93	**5.06**	**5.26**	**5.26**	**5.51**	**5.61**	**5.60**	**5.66**	5.42
		± 0.076	± 0.051	± 0.363	**± 0.378**	**± 0.336**	**± 0.470**	**± 0.391**	**± 0.373**	**± 0.376**	**± 0.366**	± 0.139
	Manure	4.15	5.97	5.19	**6.50**	**6.57**	**6.68**	**6.75**	**6.90**	**6.90**	**6.94**	6.75
		± 0.237	± 0.079	± 0.097	**± 0.089**	**± 0.084**	**± 0.124**	**± 0.117**	**± 0.092**	**± 0.085**	**± 0.084**	± 0.042
	Mulch	3.93	6.11	5.37	**6.56**	**6.48**	**6.61**	**6.75**	**6.92**	**7.00**	**6.96**	6.75
		± 0.005	± 0.067	± 0.088	**± 0.045**	**± 0.092**	**± 0.130**	**± 0.115**	**± 0.069**	**± 0.090**	**± 0.056**	± 0.044

Table 8.9 Experiment 3: Means and standard errors for dissolved oxygen (mg L^{-1}).

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Cond.		Days from start of Experiment 3										*Grand mean* ± Standard Error
		0	2	8	14	20	23	28	34	41	48	
No Bacteria	Control	0.62	1.53	2.35	**1.64**	**1.96**	**1.66**	**1.70**	**1.81**	**1.76**	**1.99**	1.79
		± 0.285	± 0.201	± 0.290	**± 0.323**	**± 0.412**	**± 0.230**	**± 0.192**	**± 0.215**	**± 0.326**	**± 0.239**	± 0.102
	Manure	0.43	1.20	1.00	**0.73**	**0.70**	**0.63**	**0.62**	**1.12**	**0.72**	**0.89**	0.77
		± 0.141	± 0.248	± 0.361	**± 0.357**	**± 0.253**	**± 0.276**	**± 0.187**	**± 0.346**	**± 0.321**	**± 0.315**	± 0.107
	Mulch	1.20	0.25	0.45	**0.08**	**0.08**	**0.18**	**0.42**	**0.67**	**0.76**	**0.80**	0.43
		± 0.258	± 0.171	± 0.159	**± 0.021**	**± 0.021**	**± 0.059**	**± 0.279**	**± 0.388**	**± 0.465**	**± 0.367**	± 0.111
Bacteria	Control Mean	0.23	1.25	1.92	**1.02**	**1.28**	**1.27**	**1.07**	**1.56**	**1.28**	**1.43**	1.27
		± 0.071	± 0.177	± 0.101	**± 0.293**	**± 0.376**	**± 0.275**	**± 0.180**	**± 0.363**	**± 0.368**	**± 0.276**	± 0.112
	Manure	0.63	1.13	0.95	**1.26**	**1.23**	**1.06**	**0.73**	**0.97**	**0.51**	**0.71**	0.92
		± 0.232	± 0.196	± 0.279	**± 0.509**	**± 0.333**	**± 0.284**	**± 0.230**	**± 0.471**	**± 0.268**	**± 0.258**	± 0.129
	Mulch	0.57	0.25	0.15	**0.23**	**0.17**	**0.53**	**0.65**	**1.18**	**1.04**	**0.96**	0.68
		± 0.247	± 0.152	± 0.034	**± 0.147**	**± 0.060**	**± 0.260**	**± 0.343**	**± 0.568**	**± 0.589**	**± 0.393**	± 0.146

Table 8.10. Experiment 3: Mean and standard errors for sulphate (mg L^{-1}), sulphide (ppb) and gilvin (g_{440} m^{-1}).

n = 6 for each treatment in days 0 and 43.

Conditions			Mean ± standard error
Sulphate	No Bacteria	Control	2156.22 ± 287.851
		Manure	14.79 ± 5.54
		Mulch	42.06 ± 16.07
	Bacteria	Control	2044.24 ± 176.95
		Manure	23.25 ± 11.82
		Mulch	39.80 ± 20.89
Sulphide	No Bacteria	Control	0.03 ± 0.033
		Manure	2.08 ± 1.110
		Mulch	8.30 ± 4.567
	Bacteria	Control	2.23 ± 2.233
		Manure	0.90 ± 0.682
		Mulch	1.95 ± 1.254
Gilvin	No Bacteria	Control	2.56 ± 0.761
		Manure	18.95 ± 2.051
		Mulch	18.93 ± 1.699
	Bacteria	Control	7.52 ± 3.999
		Manure	14.70 ± 3.364
		Mulch	16.30 ± 1.173

Table 8.11 Experiment 3: Means and standard errors for pH.

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Conditions		Days from start of Experiment 4										*Grand mean* ± Standard Error
		0	1	3	7	10	15	22	28	35	42	
Hypoxic	Control	7.13	7.08	7.02	**6.44**	**6.70**	**6.59**	**6.49**	**6.57**	**6.79**	**6.82**	6.63
		± 0.165	± 0.081	± 0.142	**± 0.026**	**± 0.042**	**± 0.042**	**± 0.043**	**± 0.070**	**± 0.079**	**± 0.080**	± 0.029
	Manure	7.27	7.72	7.34	**6.98**	**7.11**	**7.14**	**7.02**	**7.29**	**7.33**	**7.38**	7.18
		± 0.189	± 0.052	± 0.069	**± 0.014**	**± 0.034**	**± 0.037**	**± 0.025**	**± 0.038**	**± 0.057**	**± 0.042**	± 0.026
	Mulch	7.10	7.29	6.59	**6.49**	**6.60**	**6.64**	**6.54**	**6.86**	**6.91**	**6.92**	6.71
		± 0.167	± 0.096	± 0.027	**± 0.046**	**± 0.029**	**± 0.032**	**± 0.027**	**± 0.029**	**± 0.037**	**± 0.031**	± 0.029
Oxic	Control	6.60	6.58	6.48	**6.44**	**6.40**	**6.35**	**6.32**	**6.54**	**6.65**	**6.65**	6.48
		± 0.045	± 0.039	± 0.027	**± 0.020**	**± 0.024**	**± 0.043**	**± 0.068**	**± 0.051**	**± 0.046**	**± 0.051**	± 0.025
	Manure	6.57	7.59	7.08	**6.81**	**6.75**	**6.74**	**6.95**	**7.20**	**7.28**	**7.19**	6.99
		± 0.028	± 0.042	± 0.031	**± 0.029**	**± 0.027**	**± 0.041**	**± 0.042**	**± 0.038**	**± 0.036**	**± 0.036**	± 0.036
	Mulch	6.58	6.91	6.22	**6.39**	**6.42**	**6.43**	**6.59**	**6.84**	**7.01**	**6.91**	6.65
		± 0.026	± 0.041	± 0.037	**± 0.040**	**± 0.024**	**± 0.030**	**± 0.044**	**± 0.024**	**± 0.034**	**± 0.026**	± 0.039

Table 8.12 Experiment 3: Means and standard errors for dissolved oxygen (mg L^{-1}).
Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Conditions		Days from start of Experiment 4										*Grand mean* ± Standard Error
		0	1	3	7	10	15	22	28	35	42	
Hypoxic	Control	0.68	1.41	1.63	**2.58**	**2.31**	**1.78**	**4.81**	**3.39**	**3.17**	**3.17**	3.03
		± 0.358	± 0.308	± 0.090	**± 0.145**	**± 0.147**	**± 0.053**	**± 0.069**	**± 0.167**	**± 0.185**	**± 0.189**	± 0.149
	Manure	0.40	1.00	0.85	**0.79**	**0.82**	**1.04**	**3.35**	**2.17**	**2.22**	**2.20**	1.80
		± 0.156	± 0.097	± 0.151	**± 0.119**	**± 0.203**	**± 0.066**	**± 0.226**	**± 0.161**	**± 0.080**	**± 0.059**	± 0.146
	Mulch	0.70	1.54	0.13	**0.30**	**0.34**	**0.21**	**0.56**	**0.13**	**0.08**	**0.13**	0.25
		± 0.359	± 0.313	± 0.021	**± 0.038**	**± 0.074**	**± 0.028**	**± 0.027**	**± 0.034**	**± 0.011**	**± 0.045**	± 0.028
Oxic	Control	5.37	5.44	5.77	**4.85**	**6.25**	**3.06**	**6.22**	**4.21**	**4.19**	**4.06**	4.69
		± 0.063	± 0.087	± 0.114	**± 0.122**	**± 0.151**	**± 0.065**	**± 0.140**	**± 0.097**	**± 0.109**	**± 0.190**	± 0.176
	Manure	5.62	5.00	4.86	**3.26**	**3.49**	**1.85**	**5.83**	**2.95**	**3.01**	**2.70**	3.30
		± 0.076	± 0.065	± 0.219	**± 0.185**	**± 0.238**	**± 0.126**	**± 0.312**	**± 0.262**	**± 0.219**	**± 0.272**	± 0.197
	Mulch	5.66	5.42	1.85	**2.19**	**2.23**	**0.97**	**1.94**	**0.29**	**1.10**	**0.15**	1.27
		± 0.089	± 0.114	± 0.466	**± 0.493**	**± 0.651**	**± 0.214**	**± 0.148**	**± 0.026**	**± 0.483**	**± 0.050**	± 0.181

Table 8.13 Experiment 4: Mean and standard errors for sulphate (mg L^{-1}), sulphide (ppb) and alkalinity.

n = 6 for each treatment in days 15 and 42.

			Days from start of Experiment		
			0	22	42
Sulphate	Hypoxic	Control	4.49	3.46 ± 0.647	7.33 ± 1.787
		Manure	4.49	5.07 ± 1.359	6.25 ± 1.650
		Mulch	4.49	4.88 ± 1.191	9.80 ± 5.310
	Oxic	Control	4.49	4.56 ± 0.757	4.51 ± 1.148
		Manure	4.49	4.12 ± 0.708	8.93 ± 2.673
		Mulch	4.49	3.13 ± 0.619	7.76 ± 1.901
Sulphide	Hypoxic	Control	0.00	0.03 ± 0.033	0.00 ± 0.000
		Manure	0.00	0.33 ± 0.333	0.00 ± 0.000
		Mulch	0.00	0.02 ± 0.017	0.00 ± 0.000
	Oxic	Control	0.00	0.00 ± 0.000	0.00 ± 0.000
		Manure	0.00	0.07 ± 0.067	0.00 ± 0.000
		Mulch	0.00	1.03 ± 0.935	0.00 ± 0.000
Alkalinity	Hypoxic	Control	1.74	10.60 ± 2.205	19.05 ± 5.942
		Manure	1.74	62.31 ± 10.98	90.90 ± 5.512
		Mulch	1.74	79.65 ± 7.041	146.16 ± 7.614
	Oxic	Control	1.74	11.70 ± 3.226	21.00 ± 3.737
		Manure	1.74	78.59 ± 3.228	95.90 ± 3.658
		Mulch	1.74	84.78 ± 8.308	156.50 ± 9.971

Table 8.14 Field Experiment: Means and standard errors for pH.

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Cond.		Days from start of field experiment															*Grand Mean ±* Standard Error
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Top	Control	5.74 ± 0.113	5.01 ± 0.326	6.11 ± 0.250	6.55 ± 0.308	6.42 ± 0.246	5.55 ± 0.257	4.39 ± 0.154	5.96 ± 0.169	4.46 ± 0.111	**4.53** **± 0.168**	**4.69** **± 0.159**	**5.02** **± 0.175**	**5.47** **± 0.155**	**5.75** **± 0.178**	**5.58** **± 0.273**	5.17 ± 0.105
	Manure	5.94 ± 0.116	6.43 ± 0.215	6.97 ± 0.098	7.03 ± 0.133	7.23 ± 0.080	5.91 ± 0.090	5.52 ± 0.198	6.73 ± 0.103	5.69 ± 0.202	**5.63** **± 0.201**	**5.56** **± 0.205**	**5.88** **± 0.209**	**6.15** **± 0.171**	**6.45** **± 0.146**	**5.86** **± 0.177**	5.92 ± 0.087
	Mulch	5.60 ± 0.074	5.90 ± 0.165	6.79 ± 0.115	7.30 ± 0.090	7.58 ± 0.122	6.31 ± 0.088	5.89 ± 0.170	7.04 ± 0.254	5.83 ± 0.231	**5.57** **± 0.226**	**5.46** **± 0.248**	**5.74** **± 0.213**	**5.96** **± 0.188**	**6.16** **± 0.200**	**5.64** **± 0.221**	5.76 ± 0.091
Bottom	Control	5.77 ± 0.128	4.77 ± 0.341	6.07 ± 0.252	6.46 ± 0.325	6.42 ± 0.270	5.32 ± 0.197	4.37 ± 0.156	5.87 ± 0.153	4.46 ± 0.105	**4.50** **± 0.158**	**4.66** **± 0.154**	**5.14** **± 0.230**	**5.43** **± 0.141**	**5.68** **± 0.171**	**5.49** **± 0.251**	5.15 ± 0.102
	Manure	5.95 ± 0.122	6.57 ± 0.280	6.97 ± 0.116	7.07 ± 0.133	7.24 ± 0.086	5.98 ± 0.082	5.52 ± 0.162	6.87 ± 0.113	5.69 ± 0.188	**5.70** **± 0.167**	**5.71** **± 0.141**	**5.95** **± 0.207**	**6.18** **± 0.145**	**6.45** **± 0.148**	**5.91** **± 0.190**	5.98 ± 0.078
	Mulch	5.59 ± 0.084	5.70 ± 0.094	6.93 ± 0.071	7.31 ± 0.082	7.65 ± 0.112	6.40 ± 0.084	5.91 ± 0.150	7.20 ± 0.241	5.79 ± 0.218	**5.56** **± 0.223**	**5.46** **± 0.222**	**5.77** **± 0.236**	**5.93** **± 0.202**	**6.16** **± 0.213**	**5.59** **± 0.220**	5.75 ± 0.092
Lake		*	4.37	5.35	5.76	5.68	5.08	4.02	5.51	4.24	**4.26**	**4.34**	**4.70**	**4.87**	**5.11**	**4.60**	4.65 ± 0.131

Table 8.15 Field experiment: Means and standard errors for dissolved oxygen (mg L^{-1}).
Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Cond.		Days from start of field experiment															*Grand Mean ± Standard Error*
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Top	Control	1.32 ± 0.164	3.57 ± 0.497	5.97 ± 0.381	5.68 ± 0.585	6.43 ± 0.488	5.98 ± 0.365	5.35 ± 0.392	4.83 ± 1.060	4.90 ± 0.411	**4.02** **± 0.522**	**5.55** **± 0.267**	**8.35** **± 0.203**	**7.68** **± 0.481**	**9.47** **± 0.260**	**8.67** **± 0.282**	7.29 ± 0.348
	Manure	1.52 ± 0.257	6.80 ± 0.349	7.35 ± 0.555	5.72 ± 0.201	6.05 ± 0.697	4.65 ± 0.823	4.02 ± 0.769	3.93 ± 0.350	4.45 ± 0.560	**3.38** **± 0.461**	**5.10** **± 0.213**	**8.12** **± 0.541**	**5.58** **± 0.851**	**8.03** **± 0.563**	**7.48** **± 0.474**	6.28 ± 0.361
	Mulch	2.53 ± 0.636	0.35 ± 0.106	0.98 ± 0.257	0.43 ± 0.199	0.37 ± 0.201	0.53 ± 0.251	1.36 ± 0.317	2.18 ± 0.901	1.83 ± 0.493	**1.62** **± 0.442**	**2.41** **± 0.785**	**4.33** **± 1.003**	**3.70** **± 0.864**	**5.90** **± 0.920**	**7.47** **± 0.438**	4.24 ± 0.446
Bottom	Control	1.17 ± 0.282	2.57 ± 0.401	5.77 ± 0.382	6.05 ± 0.468	6.63 ± 0.635	6.15 ± 0.440	5.05 ± 0.257	5.00 ± 0.970	4.96 ± 0.324	**3.26** **± 0.208**	**5.67** **± 0.306**	**8.37** **± 0.308**	**6.32** **± 0.483**	**9.35** **± 0.322**	**8.40** **± 0.270**	6.89 ± 0.370
	Manure	0.92 ± 0.151	6.32 ± 0.590	5.97 ± 0.476	4.83 ± 0.482	5.08 ± 0.704	4.07 ± 0.626	4.45 ± 0.586	3.97 ± 0.563	4.26 ± 0.505	**3.09** **± 0.465**	**4.70** **± 0.249**	**8.05** **± 0.547**	**4.33** **± 0.453**	**7.65** **± 0.593**	**7.07** **± 0.503**	5.81 ± 0.364
	Mulch	1.60 ± 0.465	0.17 ± 0.033	0.00 ± 0.000	0.05 ± 0.034	0.32 ± 0.117	0.30 ± 0.118	0.61 ± 0.170	0.78 ± 0.135	1.37 ± 0.567	**1.16** **± 0.437**	**2.15** **± 0.748**	**3.97** **± 0.941**	**3.12** **± 0.777**	**5.72** **± 0.922**	**7.38** **± 0.485**	3.92 ± 0.454
Lake		*	4.60	8.10	8.60	10.10	10.80	3.62	*	6.48	**5.24**	**6.60**	**9.70**	**9.60**	**10.80**	**9.40**	8.56 ± 0.875

Table 8.16 Field experiment: Means and standard errors for dissolved oxygen (% saturation).
Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment, * no data collected.

Cond.		Days from start of field experiment															*Grand Mean* ± Standard Error
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Top	Control	16.05 ± 2.685	*	68.60 ± 4.041	62.40 ± 6.618	72.47 ± 5.714	67.67 ± 4.560	58.85 ± 3.624	57.30 ± 11.15	52.18 ± 4.519	**45.58** **± 5.707**	**57.00** **± 3.296**	**82.03** **± 2.081**	**73.42** **± 4.530**	**87.53** **± 2.277**	**80.08** **± 2.580**	70.94 ± 2.867
	Manure	17.00 ± 2.901	*	83.65 ± 5.853	61.48 ± 2.265	66.40 ± 7.729	51.35 ± 9.170	47.76 ± 9.712	42.88 ± 3.392	47.80 ± 5.877	**36.65** **± 4.969**	**50.83** **± 2.023**	**79.87** **± 5.148**	**53.08** **± 8.018**	**73.70** **± 5.255**	**68.82** **± 4.326**	60.49 ± 3.211
	Mulch	19.75 ± 5.884	*	12.08 ± 3.136	5.08 ± 2.268	5.04 ± 1.978	5.72 ± 2.137	15.78 ± 4.286	14.08 ± 3.920	18.47 ± 5.270	**12.21** **± 3.025**	**25.00** **± 8.398**	**44.27** **± 9.454**	**35.08** **± 8.309**	**49.57** **± 12.14**	**68.97** **± 4.060**	39.18 ± 4.351
Bottom	Control	13.33 ± 3.119	*	64.72 ± 4.070	65.50 ± 5.373	74.37 ± 7.389	67.53 ± 4.531	54.07 ± 1.921	57.18 ± 12.68	50.08 ± 3.522	**38.63** **± 2.867**	**54.83** **± 1.759**	**81.92** **± 2.919**	**60.00** **± 4.529**	**85.83** **± 2.764**	**76.80** **± 2.671**	66.34 ± 3.046
	Manure	10.15 ± 1.771	*	65.80 ± 4.999	51.45 ± 5.210	54.92 ± 7.765	43.10 ± 7.363	42.53 ± 9.656	43.50 ± 6.084	43.47 ± 6.286	**35.78** **± 5.384**	**45.83** **± 1.195**	**79.08** **± 5.075**	**41.52** **± 4.042**	**68.60** **± 5.830**	**64.35** **± 4.490**	55.86 ± 3.175
	Mulch	17.65 ± 5.947	*	0.20 ± 0.077	0.40 ± 0.137	3.25 ± 1.416	3.50 ± 1.431	6.00 ± 1.498	8.75 ± 1.479	15.85 ± 6.319	**13.40** **± 4.652**	**21.17** **± 7.803**	**40.00** **± 9.301**	**29.85** **± 7.412**	**52.97** **± 8.552**	**68.22** **± 4.351**	37.60 ± 4.183
Lake		*	*	98.5	105	125.4	135	47.7	*	14	**63.4**	**80**	**100**	**97**	**105.2**	**90.5**	89.35 ± 6.286

Table 8.17 Field experiment: Means and standard errors for temperature (°C).

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment, * no data collected.

Cond.		Days from start of field experiment															*Grand Mean* ± Standard Error
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Top	Control	23.35 ± 1.156	29.73 ± 0.709	22.01 ± 0.594	19.87 ± 0.519	20.82 ± 0.432	21.37 ± 0.382	22.25 ± 0.577	21.31 ± 0.305	18.13 ± 0.381	**21.23 ± 0.284**	**15.45 ± 0.467**	**14.57 ± 0.192**	**13.19 ± 0.131**	**11.74 ± 0.264**	**11.72 ± 0.203**	14.65 ± 0.558
	Manure	22.10 ± 0.298	28.87 ± 0.495	21.92 ± 0.444	19.09 ± 0.269	19.48 ± 0.189	20.61 ± 0.164	21.18 ± 0.361	20.54 ± 0.101	17.28 ± 0.282	**20.23 ± 0.232**	**14.33 ± 0.176**	**14.39 ± 0.112**	**12.98 ± 0.128**	**11.37 ± 0.170**	**11.47 ± 0.109**	14.13 ± 0.508
	Mulch	23.25 ± 1.170	30.63 ± 0.846	23.42 ± 0.649	20.27 ± 0.472	20.48 ± 0.610	21.27 ± 0.529	22.62 ± 0.756	21.36 ± 0.576	18.18 ± 0.468	**20.95 ± 0.401**	**15.67 ± 0.418**	**15.07 ± 0.081**	**13.26 ± 0.119**	**11.99 ± 0.234**	**11.65 ± 0.129**	14.76 ± 0.539
Bottom	Control	21.26 ± 0.578	25.72 ± 0.980	20.61 ± 0.958	19.51 ± 0.584	20.17 ± 0.599	20.80 ± 0.409	21.18 ± 0.728	20.53 ± 0.445	17.97 ± 0.558	**20.55 ± 0.384**	**14.52 ± 0.538**	**14.35 ± 0.240**	**12.93 ± 0.207**	**11.46 ± 0.243**	**11.48 ± 0.157**	14.21 ± 0.535
	Manure	20.50 ± 0.198	24.03 ± 0.339	20.07 ± 0.172	18.41 ± 0.164	18.98 ± 0.166	19.80 ± 0.171	19.72 ± 0.316	19.63 ± 0.155	16.72 ± 0.274	**19.78 ± 0.194**	**13.58 ± 0.217**	**14.25 ± 0.148**	**12.83 ± 0.145**	**11.08 ± 0.144**	**11.19 ± 0.130**	13.79 ± 0.498
	Mulch	21.03 ± 0.456	23.03 ± 0.596	19.74 ± 0.222	19.21 ± 0.232	19.61 ± 0.407	20.52 ± 0.329	21.13 ± 0.601	20.30 ± 0.368	17.78 ± 0.494	**20.33 ± 0.431**	**15.03 ± 0.362**	**14.91 ± 0.083**	**13.17 ± 0.126**	**11.73 ± 0.177**	**11.48 ± 0.106**	14.44 ± 0.512
Lake		*	25	25.3	24.56	24.58	24.32	25.6	23.06	20.6	**21.1**	**19.4**	**16.93**	**15.42**	**14.45**	**13.8**	16.85 ± 1.179

Table 8.18 Field experiment: Means and standard errors for conductivity (μs cm^{-1}).

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Cond.		Days from start of field experiment															*Grand Mean ±* Standard Error
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Top	Control	1166 ± 59.874	1182 ± 60.874	377 ± 14.011	1221 ± 40.055	1246 ± 34.027	1255 ± 25.377	1275 ± 26.476	1300 ± 8.370	1304 ± 7.619	**1298** **± 33.442**	**1285** **± 13.230**	**830** **± 197.55**	**1219** **± 38.319**	**967** **± 32.804**	**422** **± 94.439**	1003 ± 63.292
	Manure	1183 ± 30.508	1215 ± 35.215	539 ± 154.00	1071 ± 174.83	1285 ± 13.455	1264 ± 22.027	1261 ± 8.755	1251 ± 14.178	1288 ± 9. 787	**1293** **± 8.631**	**1156** **± 108.33**	**507** **± 137.00**	**995** **± 129.44**	**885** **± 22.960**	**581** **± 91.544**	903 ± 60.402
	Mulch	1139 ± 28.246	1171 ± 117.51	558 ± 161.15	1259 ± 38.330	1284 ± 34.532	1286 ± 0.981	1245 ± 27.787	1240 ± 31.005	1265 ± 22.677	**1261** **± 20.933**	**1222** **± 22.572**	**771** **± 179.40**	**847** **± 159.32**	**847** **± 32.258**	**517** **± 82.738**	911 ± 59.150
Bottom	Control	915 ± 117.43	1171 ± 48.826	380 ± 16.828	1207 ± 41.146	1245 ± 34.685	1255 ± 25.056	1256 ± 23.243	1280 ± 14.151	1301 ± 13.963	**1322** **± 10.056**	**1293** **± 10.129**	**902** **± 196.80**	**1340** **± 98.595**	**966** **± 32.903**	**480** **± 95.986**	1054 ± 63.546
	Manure	1128 ± 75.859	1327 ± 124.32	390 ± 7.968	1255 ± 11.509	1279 ± 12.564	1280 ± 13.656	1260 ± 8.356	1259 ± 13.455	1295 ± 11.114	**1297** **± 7.250**	**1264** **± 4.660**	**657** **± 179.85**	**1021** **± 131.04**	**892** **± 22.577**	**461** **± 111.64**	932 ± 64.090
	Mulch	931 ± 92.153	1269 ± 31.860	399 ± 11.325	1260 ± 35.755	1278 ± 35.755	1275 ± 32.603	1249 ± 27.425	1252 ± 32.277	12633 ± 22.817	**1257** **± 20.482**	**1233** **± 18.881**	**641** **± 170.88**	**867** **± 163.96**	**848** **± 31.805**	**476** **± 114.45**	887 ± 63.369
Lake		*	1195	368	1186	1199	1209	1208	1239	1265	**1278**	**1257**	**1216**	**1219**	**939**	**913**	1137 ± 67.485

Table 8.19 Field experiment: Means and standard errors for turbidity (ntu).

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment, * no data collected.

Cond.		Days from start of field experiment															*Grand Mean* ± Standard Error
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Top	Control	361.18 ± 65.999	*	42.05 ± 10.410	21.37 ± 2.366	21.28 ± 4.174	22.32 ± 4.052	*	22.80 ± 3.360	*	*	*	**0.00** **± 0.000**	**7.48** **± 3.264**	**10.97** **± 6.324**	**76.42** **± 28.797**	23.72 ± 9.421
	Manure	270.32 ± 58.252	*	32.75 ± 2.755	30.77 ± 5.753	42.78 ± 18.887	47.27 ± 14.689	*	40.55 ± 7.061	*	*	*	**10.20** **± 8.878**	**7.92** **± 5.992**	**14.80** **± 9.226**	**74.87** **± 33.386**	26.95 ± 10.246
	Mulch	220.00 ± 41.689	*	48.47 ± 10.583	55.20 ± 10.102	42.28 ± 8.799	40.47 ± 7.655	*	32.30 ± 6.149	*	*	*	**25.57** **± 21.428**	**6.47** **± 2.797**	**7.73** **± 4.923**	**121.90** **± 47.632**	40.42 ± 15.770
Bottom	Control	554.60 ± 45.320	*	161.57 ± 89.199	41.75 ± 5.567	48.17 ± 14.373	30.58 ± 14.386	*	44.80 ± 7.585	*	*	*	**39.87** **± 18.601**	**16.85** **± 7.776**	**44.18** **± 18.888**	**147.07** **± 91.554**	61.99 ± 24.630
	Manure	447.88 ± 83.061	*	216.18 ± 116.729	38.75 ± 6.044	65.27 ± 18.905	53.10 ± 18.651	*	61.27 ± 13.381	*	*	*	**17.43** **± 10.849**	**8.67** **± 6.464**	**21.02** **± 15.775**	**81.90** **± 21.173**	32.25 ± 9.119
	Mulch	399.42 ± 95.166	*	57.98 ± 11.460	63.80 ± 8.996	49.38 ± 9.956	41.07 ± 6.610	*	33.55 ± 9.361	*	*	*	**0.88** **± 0.883**	**16.78** **± 7.911**	**19.53** **± 5.458**	**161.78** **± 88.317**	49.75 ± 24.761
Lake		*	*	600	59.6	37.7	198.1	*	17.1	*	*	*	**1.5**	**0**	**0**	**29.2**	7.68 ± 5.865

Table 8.20 Field experiment: Means and standard errors for oxidation reduction potential (ORP).

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment, * no data collected.

Cond.		Days from start of field experiment															*Grand Mean ±* Standard Error
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Top	Control	192.00 ± 16.831	*	142.33 ± 30.791	88.33 ± 46.463	44.33 ±22.494	185.67 ± 23.831	*	198.50 ± 30.129	*	*	*	**182.83 ± 19.805**	**207.00 ± 30.100**	**208.33 ± 25.252**	**177.50 ± 19.099**	193.92 ± 11.551
	Manure	171.50 ± 9.248	*	23.67 ± 24.300	4.67 ± 37.502	-3.83 ± 23.582	71.15 ± 31.212	*	95.67 ± 19.532	*	*	*	**141.83 ± 28.634**	**169.50 ± 20.683**	**147.17 ± 18.207**	**161.50 ± 12.439**	155.00 ± 9.976
	Mulch	198.50 ± 7.442	*	-34.00 ± 49.652	-126.33 ± 47.198	-157.67 ± 47.731	-11.00 ± 38.092	*	23.83 ± 53.385	*	*	*	**126.67 ± 40.209**	**163.67 ± 30.928**	**208.50 ± 21.444**	**176.17 ± 18.431**	168.75 ± 14.853
Bottom	Control	166.17 ± 19.668	*	160.67 ± 33.164	104.83 ± 48.926	72.67 ± 26.929	218.33 ± 34.448	*	228.00 ± 29.095	*	*	*	**198.17 ± 22.912**	**193.95 ± 43.588**	**220.83 ± 25.392**	**188.67 ± 21.904**	200.40 ± 14.122
	Manure	157.00 ± 19.470	*	26.50 ± 14.646	11.00 ± 27.064	-7.00 ± 12.712	82.33 ± 22.242	*	73.17 ± 12.422	*	*	*	**116.17 ± 26.363**	**149.17 ± 14.910**	**148.00 ± 15.388**	**150.83 ± 10.852**	141.04 ± 8.839
	Mulch	198.00 ± 6.429	*	-133.50 ± 20.003	-161.50 ± 37.119	-197.83 ± 37.296	-34.50 ± 34.470	*	33.00 ± 95.464	*	*	*	**129.67 ± 42.420**	**154.67 ± 34.529**	**201.67 ± 21.677**	**178.67 ± 19.419**	166.17 ± 15.490
Lake		*	*	246	245	153	285	*	279	*	*	*	**311**	**364**	**352**	**329**	339 ± 11.825

Table 8.21 Field experiment: Means and standard errors for salinity (ppm).

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment, * no data collected.

Cond.		Days from start of field experiment															*Grand mean ±* Standard Error
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Top	Control	*	*	*	*	*	*	*	*	*	*	*	**0.40 ± 0.115**	**0.62 ± 0.023**	**0.64 ± 0.023**	**0.32 ± 0.041**	0.49 ± 0.041
	Manure	*	*	*	*	*	*	*	*	*	*	*	**0.24 ± 0.078**	**0.50 ± 0.068**	**0.58 ± 0.015**	**0.37 ± 0.063**	0.42 ± 0.039
	Mulch	*	*	*	*	*	*	*	*	*	*	*	**0.39 ± 0.101**	**0.43 ± 0.086**	**0.55 ± 0.023**	**0.32 ± 0.056**	0.42 ± 0.038
Bottom	Control	*	*	*	*	*	*	*	*	*	*	*	**0.42 ± 0.108**	**0.68 ± 0.059**	**0.63 ± 0.023**	**0.29 ± 0.070**	0.51 ± 0.047
	Manure	*	*	*	*	*	*	*	*	*	*	*	**0.33 ± 0.098**	**0.51 ± 0.067**	**0.58 ± 0.016**	**0.29 ± 0.084**	0.43 ± 0.043
	Mulch	*	*	*	*	*	*	*	*	*	*	*	**0.31 ± 0.094**	**0.43 ± 0.088**	**0.54 ± 0.022**	**0.30 ± 0.078**	0.40 ± 0.041
Lake		*	*	*	*	*	*	*	*	*	*	*	**0.63**	**0.6**	**0.62**	**0.56**	0.60 ± 0.015

Table 8.22 Field experiment: Means and standard errors for depth (m).

Figures in bold indicate data from days used in MANOVA analysis, n = 6 for each treatment.

Cond.		Days from start of field experiment															*Grand mean ±* Standard Error
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Top	Control	0.75 ± 0.066	0.75 ± 0.066	0.74 ± 0.045	0.72 ± 0.060	0.69 ± 0.044	0.67 ± 0.049	0.63 ± 0.051	0.60 ± 0.065	0.58 ± 0.040	**0.61** **± 0.042**	**0.65** **± 0.052**	**0.65** **± 0.045**	**0.83** **± 0.036**	**0.93** **± 0.040**	**0.98** **± 0.070**	0.77 ± 0.031
	Manure	0.78 ± 0.031	0.78 ± 0.031	0.67 ± 0.031	0.64 ± 0.024	0.59 ± 0.024	0.58 ± 0.044	0.57 ± 0.031	0.53 ± 0.036	0.57 ± 0.061	**0.53** **± 0.031**	**0.51** **± 0.027**	**0.67** **± 0.049**	**0.80** **± 0.037**	**0.88** **± 0.044**	**0.95** **± 0.034**	0.72 ± 0.032
	Mulch	0.96 ± 0.064	0.96 ± 0.064	0.81 ± 0.033	0.83 ± 0.044	0.80 ± 0.050	0.82 ± 0.060	0.77 ± 0.056	0.73 ± 0.040	0.73 ± 0.042	**0.71** **± 0.045**	**0.72** **± 0.088**	**0.79** **± 0.057**	**0.97** **± 0.071**	**0.99** **± 0.055**	**0.98** **± 0.060**	0.86 ± 0.032

Table 8.23 Field Experiment: Mean and standard errors for sulphate (mg L^{-1}), sulphide (ppm), gilvin($_{g440}$ m^{-1}) and chlorophyll *a* values.

n = 6 for each treatment for days 0 to 153, * no data collected.

Cond.		Days from start of field experiment															*Grand mean* ± Standard Error
		0	6	13	20	27	34	41	55	69	83	97	111	125	139	153	
Sulphate	Control	7.28 ± 2.110	1.72 ± 0.218	1.88 ± 0.437	1.34 ± 0.209	1.60 ± 0.246	3.41 ± 0.867	2.78 ± 0.390	2.84 ± 1.169	4.47 ± 0.544	**5.49 ± 0.781**	**6.70 ± 1.815**	**8.74 ± 2.797**	**8.07 ± 1.678**	**6.71 ± 1.385**	**5.66 ± 1.560**	6.90 ± 0.527
	Manure	17.35 ± 5.311	2.86 ± 0.379	3.38 ± 1.055	2.33 ± 0.576	4.68 ± 1.695	6.58 ± 1.760	4.22 ± 1.023	3.06 ± 0.717	4.18 ± 1.194	**9.11 ± 3.099**	**4.93 ± 1.524**	**10.85 ± 3.904**	**6.12 ± 0.751**	**8.06 ± 1.612**	**6.79 ± 2.050**	7.64 ± 0.876
	Mulch	16.55 ± 4.815	2.93 ± 1.262	1.05 ± 0.179	1.37 ± 0.439	1.19 ± 0.101	4.27 ± 0.811	2.61 ± 0.535	3.86 ± 1.276	3.63 ± 0.568	**5.50 ± 0.777**	**4.57 ± 1.128**	**5.35 ± 0.795**	**12.20 ± 5.345**	**10.02 ± 3.649**	**4.81 ± 0.753**	7.07 ± 1.314
Sulphide	Control	12.27 ± 7.864	13.70 ± 12.212	0.00 ± 0.000	60.40 ± 41.361	173.17 ± 67.032	0.00 ± 0.000	0.00 ± 0.000	339.50 ± 69.88	0.78 ± 0.189	**21.03 ± 12.883**	**0.45 ± 0.062**	**0.92 ± 0.628**	**0.00 ± 0.000**	**0.00 ± 0.000**	**0.00 ± 0.000**	3.73 ± 3.463
	Manure	13.22 ± 2.952	0.00 ± 0.000	0.10 ± 0.082	65.07 ± 64.987	90.90 ± 56.623	0.02 ± 0.017	0.00 ± 0.000	306.83 ± 97.06	1.03 ± 0.176	**41.52 ± 40.697**	**0.57 ± 0.112**	**0.13 ± 0.080**	**0.00 ± 0.000**	**0.00 ± 0.000**	**0.00 ± 0.000**	7.04 ± 6.897
	Mulch	28.00 ± 5.854	250.87 ± 120.05	1.57 ± 1.351	72.98 ± 71.010	104.05 ± 57.863	0.08 ± 0.065	0.00 ± 0.000	196.83 ± 88.688	6.03 ± 5.155	**57.13 ± 46.853**	**0.53 ± 0.071**	**0.10 ± 0.063**	**0.00 ± 0.000**	**0.00 ± 0.000**	**0.00 ± 0.000**	9.63 ± 9.499
Gilvin	Control	3.73 ± 1.283	2.81 ± 0.925	6.08 ± 1.157	4.26 ± 1.360	2.50 ± 0.649	2.90 ± 0.646	1.17 ± 0.0.689	0.85 ± 0.609	1.35 ± 0.812	**2.17 ± 0.657**	**1.90 ± 0.634**	**1.22 ± 0.663**	**0.39 ± 0.895**	**0.00 ± 0.854**	**0.88 ± 2.356**	1.07 ± 0.002
	Manure	5.01 ± 1.274	7.48 ± 0.843	9.34 ± 1.360	8.13 ± 1.218	10.10 ± 1.362	9.80 ± 1.055	6.67 ± 1.048	7.28 ± 1.724	5.84 ± 1.274	**6.00 ± 1.143**	**5.20 ± 1.100**	**3.58 ± 0.226**	**2.40 ± 0.579**	**3.12 ± 0.896**	**4.03 ± 1.508**	4.05 ± 0.002
	Mulch	2.62 ± 1.019	15.06 ± 2.530	13.97 ± 2.874	10.97 ± 2.900	9.15 ± 2.891	8.32 ± 2.471	6.59 ± 2.127	4.19 ± 1.508	3.39 ± 0.946	**4.43 ± 0.862**	**3.42 ± 0.994**	**3.84 ± 0.856**	**1.31 ± 1.300**	**3.63 ± 2.178**	**6.07 ± 3.026**	3.79 ± 0.003
Chl *a*	Control	2.29 ± 1.618	2.66 ± 1.959	*	11.45 ± 3.880	10.13 ± 2.130	7.19 ± 1.677	8.99 ± 2.140	*	5.71 ± 2.930	**3.89 ± 4.411**	**10.56 ± 6.676**	**3.73 ± 2.922**	**11.77 ± 5.925**	**5.39 ± 1.238**	**20.10 ± 7.158**	9.24 ± 0.010
	Manure	0.00 ± 4.780	33.86 ± 6.494	*	47.87 ± 22.079	2.05 ± 28.079	46.17 ± 21.895	38.05 ± 12.739	*	27.33 ± 12.111	**31.66 ± 11.018**	**30.46 ± 13.333**	**45.95 ± 17.977**	**39.07 ± 9.668**	**23.98 ± 5.571**	**66.56 ± 12.372**	39.61 ± 0.024
	Mulch	2.55 ± 1.229	2.06 ± 3.691	*	1.20 ± 0.925	6.57 ± 9.212	6.56 ± 2.150	10.47 ± 7.213	*	18.91 ± 9.927	**25.82 ± 10.260**	**16.74 ± 9.321**	**9.59 ± 7.164**	**26.00 ± 12.485**	**17.34 ± 10.448**	**16.91 ± 5.077**	18.73 ± 0.017

Table 8.24 Field Experiment: Mean and standard errors for ammonia (µg N L $^{-1}$), FRP (µg P L $^{-1}$) and nitrate/nitrite (µg NO_2/NO_3 L $^{-1}$).

n = 6 for each treatment in days 1, 83 and 153.

		Days from start of field experiment		
		0	83	153
Ammonia	Control	112.67 ± 36.914	7.83 ± 0.792	9.17 ± 1.302
	Manure	111.83 ± 26.461	12.50 ± 3.314	12.00 ± 1.238
	Mulch	175.67 ± 38.332	8.33 ± 1.926	17.67 ± 7.898
FRP	Control	3.83 ± 1.078	2.33 ± 0.211	3.67 ± 0.422
	Manure	3.67 ± 0.803	630.00 ± 98.975	100.17 ± 23.159
	Mulch	3.50 ± 0.500	3.17 ± 0.601	3.50 ± 0.428
Nitrate/Nitrite	Control	105.17 ± 24.674	5.50 ± 1.176	129.17 ± 85.686
	Manure	178.83 ± 40.930	3.83 ± 1.078	198.17 ± 79.479
	Mulch	186.00 ± 100.79	3.67 ± 0.715	150.00 ± 98.821

Table 8.25 Field Experiment: Mean weekly and cumulative rainfall (mm).
Figures in bold indicate data from which the *grand mean* is calculated.

Cond.		Days from start of field experiment															***Grand mean***
		0	6	13	20	27	34	41	**55**	**69**	**83**	**97**	**111**	**125**	**139**	**153**	
Rainfall	Weekly	0	1.83	0	0	0	0	0	**0.09**	**0.89**	**0.03**	**0.09**	**4.32**	**4.84**	**3.75**	**7.35**	3.40
	Cumulative	0.00	11.00	11.00	11.00	11.00	11.00	11.00	12.20	24.70	25.10	26.40	86.90	154.60	207.10	310.00	

Table 8.26 Field Experiment: Mean invertebrate numbers and family at the end of week 153.

Individual Abundance	Control	54.50 ± 9.283
	Manure	58.17 ± 13.055
	Mulch	140.50 ± 57.143

Family Richness	Control	8.00 ± 1.00
	Manure	7.83 ± 0.601
	Mulch	9.83 ± 1.701

Table 8.27 Field Experiment: MANOVA, Wilks' Lambda and Tukey test results.
DO – Dissolved oxygen, D – Days, T – Treatments, Temp – Temperature °C, % sat – percentage saturation, Cond – Conductivity (us cm^{-1}), ORP – Organic Redox Potential, 1- Control; 2 – Manure, 3 – Hay, 4 – Mulch.

		MANOVA					Wilks' Lambda				
		F value	Hyp./error Df	Sig.	Obs Power		Value	Hyp./error df	Sig.	Obs Power	
					alpha = 0.05	alpha = 0.1				alpha = 0.05	alpha = 0.1
pH	D * T	6.166	2/15	0.011	0.816	0.902	0.275	10/22	0.086	0.713	0.831
Temp	D * T	8.268	2/15	0.075	0.508	0.651	0.175	10/22	0.014	0.907	0.959
DO (%sat)	D * T	13.451	2/15	0.000	0.991	0.997	0.160	10/22	0.009	0.930	0.971
DO (mg L^{-1})	D * T	12.789	2/15	0.001	0.988	0.996	0.183	10/22	0.017	0.894	0.951
Conductivity	D * T	1.130	2/15	0.235	0.285	0.416	0.337	10/22	0.176	0.592	0.773
Turbidity (ntu)	D * T	1.595	2/15	0.661	0.106	0.186	0.887	6/26	0.947	0.108	0.189
ORP	D * T	1.481	2/15	0.259	0.267	0.395	0.541	6/26	0.199	0.496	0.639
Chl *a*	D * T	4.809	2/15	0.024	0.708	0.823	0.340	10/22	0.180	0.587	0.728
Sulphate (mg L^{-1})	D * T	0.164	2/15	0.850	0.071	0.133	0.480	10/22	0.492	0.367	0.515
Sulphide (ppb)	D * T	0.235	2/15	0.793	0.080	0.147	0.742	6/26	0.654	0.227	0.348
Gilvin ($_{g440}$ m^{-1})	D * T	3.352	2/15	0.063	0.543	0.684	0.573	10/22	0.708	0.265	0.398
Ammonia	D * T	0.125	2/33	0.883	0.068	0.127					
FRP	D * T	6.911	2/33	0.003	0.899	0.948					
Nitrate/Nitrite	D * T	0.628	2/33	0.540	0.146	0.238					
Invertebrate Diversity		0.87	2/15	0.440							
Invertebrate Abundance		2.01	2/15	0.168							
Phytoplankton (L^{-1})		2.159	3/17	0.130							

Table 8.27 continued, Tukey test results represented as *P* values.

		Day 83		Day 97		Day 111		Day 125		Day 139		Day 153	
		1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
pH	**2**	0.002		0.008		0.034		0.022		0.027		0.520	
	4	0.004	0.931	0.027	0.801	0.092	0.859	0.126	0.632	0.211	0.501	0.960	0.682
DO (mg L^{-1})	**2**	0.747		0.565		0.953		0.075		0.232		0.112	
	4	0.003	0.013	0.001	0.004	0.001	0.002	0.002	0.201	0.004	0.099	0.174	0.964
DO (% sat)	**2**	0.534		0.539		0.958		0.075		0.268		0.099	
	4	0.000	0.002	0.001	0.007	0.001	0.002	0.002	0.197	0.006	0.129	0.188	0.925
Conductivity	**2**	0.830		0.257		0.571		0.304		0.185		0.829	
	4	0.144	0.353	0.431	0.926	0.869	0.862	0.074	0.676	0.031	0.592	0.924	0.977
Temp (°C)	**2**	0.156		0.165		0.785		0.696		0.439		0.378	
	4	0.845	0.363	0.773	0.048	0.059	0.016	0.714	0.271	0.653	0.113	0.985	0.467
Chlorophyll *a*	**2**	0.110		0.373		0.046		0.151		0.170		0.005	
	4	0.233	0.893	0.904	0.615	0.929	0.091	0.568	0.619	0.455	0.777	0.964	0.003
ORP	**2**					0.347		0.574		0.101		0.522	
	4					0.338	1.000	0.571	1.000	0.947	0.173	0.970	0.662
Turbidity	**2**					0.878		0.809		0.671		0.884	
	4					0.855	0.999	0.996	0.854	0.446	0.923	0.904	0.646
Sulphate (mg L^{-1})	**2**	0.392		0.693		0.858		0.906		0.920		0.866	
	4	1.000	0.394	0.592	0.985	0.677	0.374	0.652	0.407	0.612	0.839	0.920	0.647
Sulphide (ppb)	**2**	0.918		0.602		0.316		1.000		1.000		1.000	
	4	0.769	0.951	0.768	0.958	0.288	0.998	1.000	1.000	1.000	1.000	1.000	1.000
Gilvin (g_{440} m^{-1})	**2**	0.024		0.059		0.049		0.337		0.275		0.626	
	4	0.216	0.463	0.494	0.391	0.028	0.953	0.786	0.712	0.187	0.967	0.299	0.818
Ammonia (µg N L^{-1})	**2**											0.902	
	4											0.880	1.000
Phosphate (µg P L^{-1})	**2**											0.039	
	4											0.996	0.003
Nitrate/Nitrite (µg N L^{-1})	**2**											0.934	
	4											0.582	0.709

Table 8.28 Field Experiment: Mean phytoplankton counts at the end of week 153.

Phytoplankton (L^{-1})	Control	59.22 ± 32.555
	Manure	393.85 ± 184.209
	Mulch	46.55 ± 31.294
	Ewington	241.62 ± 116.011