Effects of organic amendments and plants on the chemistry of acid sulfate soils under aerobic and anaerobic conditions

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Table of contents

Abstract

Acid sulfate soils with sulfuric horizons (sulfuric soils) can exert a range of negative impacts on the ecology and productivity of soils. The primary treatment for these soils is to raise the pH using lime. Although often effective, this treatment can be expensive and not well suited to large areas. In this research, the possible use of plant organic matter to ameliorate sulfuric soils or to prevent acid sulfate soils with sulfidic materials (sulfidic soils) from acidifying was investigated. The advantage of this approach is that organic matter is readily available, inexpensive and environmentally friendly, especially in Ramsar listed wetlands where lime cannot be used. The experimental treatments used ground leaves of *Phragmites*, lucerne hay, pea straw and wheat straw as sources of organic matter with varying nitrogen, which were either incorporated into or overlaid on the surface of the soils. After 6 months of incubation under either aerobic or anaerobic soil conditions, pH, Eh and sulfate content were measured. Incorporation of complex organic matter significantly increased the pH of both sulfuric and sulfidic soils. These changes were correlated with reductions in soil redox and sulfate content. The magnitude of the changes depended on the nitrogen content of the complex organic matter.

The relative importance of carbon and nitrogen in ameliorating acid sulfate soils was further investigated respectively using glucose, sodium acetate and molasses as simple carbon sources, and urea, nitrate and ammonium as simple nitrogen compounds. It was found that compounds containing inorganic nitrogen alone without carbon were ineffective, while urea significantly increased pH and reduced Eh, but did not affect the sulfate content of the soil. Glucose had no significant effect on sulfuric soils, either at low (catalytic) or high concentrations, while acetate significantly increased pH. Molasses (which may contain small amounts of nitrogen) caused moderate changes in pH, Eh and sulfate content. On sulfidic soils, acetate prevented oxidation but glucose strongly acidified the soil, most probably by fermentation to butyric acid.

The effects of live roots on sulfidic and sulfuric soil chemistry under either aerobic or anaerobic soil conditions were investigated using *Typha*, *Phragmites* and *Melaleuca*. *Typha* and *Melaleuca* are respectively common wetland and inland plants, whereas *Phragmites* grows under both wetland and inland soil conditions. The study was extended to investigate the combined effects of incorporated ground *Phragmites* leaves as organic matter and *Phragmites* plants together. Generally, a great deal of variability was found in

the changes in pH, redox and sulfate content, the overall effects being dependent on plant type, whether there was incorporated organic matter, the type of soil and the moisture conditions. However, in all cases the growth of the live plants resulted in greater acidity than in the unplanted control soils. In the case of *Typha* and *Phragmites,* which have aerenchymatous tissues, the acidification under anaerobic conditions was attributed to the transport of oxygen in these tissues into the soil. Under non-flooded conditions, the acidification was most likely due to increased oxygen penetration as a result of loosening of the soil by the plant roots.

Synopsis

Acid sulfate soils are naturally occurring soils formed under reducing soil conditions. These soils either contain sulfuric acid or have the potential to form it, in an amount that can have detrimental impacts on other soil characteristics and the environment (Melville and White, 2012). The principle strategy to manage sulfuric soil is to neutralize the actual acidity and minimize its by-product discharge by application of an alkaline or neutral material such as agricultural lime while for a sulfidic soil is to minimize oxidation. In some localities such as in the tropics, however, availability of mineral lime is an issue and in most situations considered impractical because of excessive costs and the need for large quantities (Hue, 1992). In addition, lime cannot be applied under certain sensitive soil conditions such as in Ramsar-listed wetland environments. As a result, other more feasible management strategies need to be studied and established to effectively manage acid sulfate soils.

What follows are studies on understanding the effects of organic amendments and plants on the chemistry of acid sulfate soils. Firstly, the effects of addition of complex organic matter on acid sulfate soil chemistry under aerobic and anaerobic soil conditions were assessed. In Chapter 4, the changes induced by organic matter in sulfuric soils are investigated and in Chapter 5 the ability of organic matter to prevent oxidation of sulfidic soils is examined. The relative importance of carbon and nitrogen for ameliorating acid soils is also studied in these chapters through the addition of simple carbon and nitrogen compounds.

The second major component of this research assessed the impacts of live plants on acid sulfate soil chemistry and this is presented in Chapter 6. Chapter 7 also describes effects of live plants on soil pH, but using “neutralised sulfuric soil” as the substrate.

The final Discussion in Chapter 8 brings together the results from the various studies to evaluate the benefits and drawbacks of plants in treating acid sulfate soils, and attempts to give some insight into the mechanisms that underlie the changes induced in soil chemistry by the addition of organic matter or by live plants.

As part of the description of changes in the chemistry of acid sulfate soils in response to addition of organic matter, it was originally intended to attempt to identify the types of bacteria that contributed to these changes, at least to confirm a major involvement of sulfur reducing bacteria (SRBs) in the changes. Some good progress was

made in this area, but not sufficient to justify a chapter of its own in the thesis, so it has been included in a separate appendix (A1).

Publications arising from this thesis

The University of Adelaide encourages the publication of papers during candidature and permits theses to be presented as either a collection of published papers or a combination of papers and conventional chapters. This thesis incorporates two journal papers based on some of the data from Chapters 4 and 5. One of these papers is published and the other has been submitted. Additionally, a peer reviewed conference paper based on early data from Chapter 7 is appended to that chapter.

1. Michael, P. S., Fitzpatrick, R., Reid, R., 2015. The role of organic matter in ameliorating acid sulfate soils with sulfuric horizons. Geoderma 225, 42-49.

2. Michael, P. S., Fitzpatrick, R., Reid, R., 2015. The importance of soil carbon and nitrogen for amelioration of acid sulphate soils. Soil Use and Management (submitted).

3. Michael, P.S., Reid, R., Fitzpatrick, R.W., 2012. Amelioration of slowly permeable hypersaline peaty-clayey sulfuric and sulfidic materials in acid sulfate soils by mixing with friable sandy loam soil. In: L.L. Burkitt, L.A. Sparrow (Eds.), Proceedings of the 5th Joint Australian and New Zealand Soil Science Conference: Soil solutions for diverse landscapes, pp. 146-149.

Signed declaration for thesis submission

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Patrick S. Michael

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Chapter 1

Thesis Scope and Outline

1.0 Introduction

Acid sulfate soils (ASS) are naturally occurring soils, sediments or substrates formed under waterlogged (reduced) conditions (Dent and Pons, 1995; Fitzpatrick et al., 2009a; Pons, 1973). These soils either contain sulfuric acid (H_2SO_4) or have the potential to form it, in an amount that can have detrimental impacts on soil properties (Fitzpatrick et al., 2009b; Ljung et al., 2009)). In general, ASS with sulfuric material (pH <4; Isbell, 2002) and that have acidified through the oxidation of pyrite are referred to as "*Sulfuric* soils" in accordance with the Australian ASS classification key (Fitzpatrick et al. 2008; Fitzpatrick 2013). ASS with sulfidic material (pH > 4; Isbell, 2002) that are unoxidized and contain pyrite and have the potential to acidify when exposed to air are referred to as "*Sulfidic soils* "(Fitzpatrick et al. 2008; Fitzpatrick 2013). Throughout the thesis, both soil types are referred to as ASS.

Sulfidic soils are formed through bacterially-induced formation of iron sulfides, mostly pyrite (FeS_2) in coastal (Fitzpatrick et al., 2009c) and inland environments (Sammut, 2004). In an undisturbed state below the water table, the sulfidic soils are benign unless exposed due to various natural processes and man-induced activities (Dent, 1986; Österholm and Åström, 2004). These processes and activities allow the sulfides present in the sulfidic soils to react with oxygen and form H_2SO_4, which in turn acidifies the surrounding environments (Fitzpatrick et al., 2009c; Nordmyr et al., 2008).

Release of the H_2SO_4 in turn solubilizes the soil matrix releasing metals such as iron (Fe^{2+}, Fe^{3+}), aluminium (Al^{3+}) and other toxic elements, making them readily available to be dispersed into surrounding soil and water systems (Ljung et al., 2009; Nordmyr et al., 2008; Poch et al., 2009; Roos and Astron, 2005; Wilson et al., 1999). The major ecological impacts associated with the release of the H_2SO_4 and toxic metals, metalloids or elements are loss of coastal and inland habitats, reduced soil productivity, degradation of civic infrastructure and deoxygenation of water bodies (Macdonald et al., 2004; Sammut et al., 1996). Because of these impacts, ASS have been described as the "nastiest soils on earth" (Dent, 1992; Dent and Pons, 1995; Pons, 1973).

It is therefore, important to mitigate these impacts by putting in place effective management strategies, especially from an agricultural soil productivity and environmental perspective. Under Sections 1.1.1, 1.1.2 and 1.1.3, literature on ecological impacts, impact management strategies and the approaches to understanding soil biochemical changes have been reviewed and are presented as background to the research questions in Section 1.2. The study aim and objectives are highlighted in Section 1.3. The significance of the studies undertaken and their original contributions, and outline of the thesis are given in Sections 1.4, and 1.5, respectively.

1.1.1 Ecological impacts

Acid sulfate soils have destructive ecological impacts on both the natural and the built environments (Roziere et al., 2009; Sullivan et al., 2009), soil and water systems (Buschmann et al., 2008), and on agricultural (Desmond, 2000) and aquaculture (Sammut, 2004) productivities. The literature reviewed shows that most of the studies carried out have concentrated on hydrological and geological features of wetlands and only a few studies have focused on agricultural impacts of ASS (Buschmann et al., 2008; Kochian et al., 2004).

Research on the impacts of acidic soils on cereal crops have been carried out but the constraints caused by the presence of sulfidic soil materials have not been explicitly considered (Kochian et al., 2004), in particular, the impacts on plant biomass production and the soil chemical (reduction-oxidation status, pH and sulfate concentration) changes that occur in the presence of plants. There are numerous ways in which plants could influence the soil oxidation and reduction processes. These include increased aeration of soil via root penetration, pumping of air via rhizomes and alteration of the moisture content as a result of transpiration.

The reduction processes may be stimulated by organic residues excreted by plant roots or by decomposition of dead plant tissues. The basic understanding of the biochemical processes taking place under such conditions need to be established for developing alternative management strategies. Furthermore, the ASS literature shows that no study has ever been conducted to investigate the effects of plants, organic matter or both on ASS chemistry, especially on redox potential (Eh) and pH as the two major factors influencing soil biochemical changes, mobilisation and immobilisation of nutrients,

and availability under varying moisture regimes. The literature also lacks data on the resultant changes on sulfate concentration, in response to the changes in pH and Eh.

Therefore, the studies presented in Chapters 4 and 5 investigated the effects of organic matter (effectively dead plants) while those in Chapter 6 investigated the effects of live plants on ASS chemistry, under varying levels of moisture.

1.1.2 Management strategies

Various management strategies addressing the adverse impacts of ASS have been proposed. These include proper surface and ground water management, maintenance of the acid neutralizing capacity (Hinwood et al., 2006), neutralisation of the actual acidity (Ljung et al., 2009) and use of acid-tolerant plants to extract contaminants (Haling et al., 2011). The use of plants to stabilise contaminants within the soil and minimising the use of chemical fertilizers have also received considerable attention (Babula et al., 2008; Haling et al., 2010).

The understanding of the effects of soil moisture content on acid production, the effects of plant organic matter amendments, and the effects of plant roots on ASS chemistry as alternative management strategies are however limited (Powell and Martens, 2005). Studies on the effects of plant residues on pH are available but the data are contradictory, with conflicting results coming from differences in organic matter composition, type of plant residues, characteristics of the soil types or the experimental conditions between studies (Xu et al., 2006). Recently, the effect of plants on soil pH in the root zones of various plant types grown on heavy textured ASS has been investigated (Reid and Butcher, 2011). This study found dominant effects of soil moisture on acid production but the influence of plants varied considerably, depending on the root structure. A strong ameliorative effect was also observed when dead plant material was added as surface mulch but the effects when the mulch was incorporated under varying moisture regimes from an agricultural soil perspective has not been investigated.

Tang and Yu (1999) concluded that chemical reactions and oxidation of organic anions during residue decomposition are responsible for the ameliorative effects on acidic soil pH. Many other researchers however reported that plant organic matter causes soil acidification by release of H^+, nitrification or increase in cation exchange capacity (Bolan et al., 1991; Dolling, 1995; Williams, 1980). Another study using different plant organic

matter and soils indicated that addition of plant organic matter increased, decreased or did not affect the soil pH (Pocknee and Sumner, 1997). Such contradicting results call for additional work to be done to assess the effects of organic matter.

In an effort to establish ASS ecological impact management strategies in general, the studies presented in Chapter 4 evaluated the ameliorative effects of organic matter, simple carbon and nitrogen compound addition on sulfuric soil whereas studies presented in Chapter 5 assessed the effects of amendments on acid production by sulfidic soil under aerobic (exposed) and anaerobic (flooded) conditions, as management strategies. The studies presented in Chapters 6 and 7 respectively assessed the effects of organic matter and live plants on the chemistry of acid sulfate soils and sulfuric soil neutralised with alkaline sandy loam.

1.1.3 Bacterial sulfate reduction

The extent to which biotic as opposed to abiotic factors influence oxidation and reduction in ASS is poorly understood. Despite the huge body of knowledge generated around ASS, most studies have concentrated on understanding the soil chemical and physical processes with little effort to understand the biochemical processes involved. Chemical oxidation of sulfidic materials can be easily measured, but the reduction process is much more complicated and seems to be principally mediated by microbial metabolism, predominantly under anaerobic conditions. Depending on the ASS environment, microorganisms can affect both sulfate reduction and oxidation.

Dürr (2008) used molecular techniques to characterize bacterial community structure and diversity, functional abundance and species identification under natural ASS conditions. While this study was able to identify the types of microorganisms present, it did not address the soil physical and chemical properties that determined microbial abundance or the biotic capacity to alter pH through biochemical processes. Modern molecular techniques have the capacity to measure not only the abundance of sulfate reducing bacteria present, but also to measure the expression of key enzymes involved in sulfate reduction. In this research, sulfate content was quantified as described under Section 3.6 and compared against the initial values to measure the microbial reduction of sulfate that occurred under different treatment conditions.

An attempt to identify the types of bacteria that contributed to the changes in soil chemistry, at least to confirm a major involvement of sulfur reducing bacteria (SRBs) is in addition presented under Appendix 1.

1.2 Research Questions

On the basis of the knowledge gaps identified in the literature, the general research questions on whether different plant-based systems would be useful as alternative management strategies to address the ecological impacts of ASS are as follows:

(i) Would soil amendments under different soil conditions have an effect on ASS chemistry?

(ii) Would plants grown under varying soil moisture conditions have an effect on ASS chemistry?

(iii) If questions 1 and 2 are verified, what are the chemical and biochemical mechanisms for the altered chemistry?

1.3 Research Aims and Objectives

The principal aim of the studies presented in this thesis is to investigate the effects of plants, both alive and dead, on key soil chemical properties, most notably, pH. The project-specific objectives are:

(a) To investigate the effects of amendments on Eh, pH and SO_4 under varying moisture regimes.

(b) To investigate the effects of plants on Eh, pH and SO_4 concentration under varying moisture regimes.

(c) To investigate the chemical and biochemical mechanisms for the altered soil chemistry.

1.4 Contextual Statement

The studies conducted provide important insights on various aspects of ASS chemistry and the interactions between plant-based systems as a background to understanding the ecological impacts. As mentioned previously in this chapter, there is very little information regarding these phenomena that could be used in strategies to manage the ecological impacts. While there is some knowledge on the influence of fluctuating water levels on ASS chemistry, there is little understanding of the effects of plants on soil chemical changes, acid neutralising effects of organic matter, and acid production as a function of soil moisture content. Additionally, there needs to be greater clarity concerning the importance of microbial activity in determining soil chemical properties under different soil treatment regimes.

1.5 Thesis Structure and Chapters

There are four primary inter-related experimental chapters that address the principal research questions:

a) Chapter 4 – Effects of amendments on sulfuric soil chemistry
b) Chapter 5 – Effects of amendments on sulfidic soil chemistry
c) Chapter 6 – Effects of plants on ASS chemistry
d) Chapter 7 – Neutralisation of sulfuric soil acidity with alkaline sandy loam and plants

Under each experimental chapter, specific background to the experiments conducted is included. The rationale for the studies has been presented above, and the theoretical background in the form of a general literature review, and a summary highlighting knowledge gaps on ecological impacts and management of ASS are given in Chapter 2. Chapter 3 provides the general description of the methods applied in all the experiments to avoid repetition under each experimental chapter. Chapter 8 presents the key findings of the experiments as a general discussion and answers to the research questions raised in Section 1.2, and highlights future studies.

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Chapter 2

Literature Review

2.1 Acid Sulfate Soils

Acid sulfate soils are naturally occurring soils or sediments formed under reducing conditions with sulfide minerals, predominantly pyrite (FeS_2) (Connell and Patrick, 1968; Dent and Pons, 1995; Fitzpatrick et al., 2008b; Pons, 1973; Wilson, 2005). These soils either contain sulfuric acid (H_2SO_4) or have the potential to form it, in an amount that can have adverse impacts on other soil properties, water and living things (Dent, 1986; Dent and Pons, 1995; Fitzpatrick et al., 2009e; Pons, 1973).

The global distribution of ASS is shown in Fig. 2.1. Of the estimated 17-24 million ha of ASS (Ljung et al., 2009; Poch et al., 2009), 6.5 million occur in Asia, 4.5 million in Africa, 3 million in Australia, 3 million in Latin America, 200 000 in Finland, 235 000 in Finland and 100 000 in North America, respectively (Faltmarsch et al., 2009; Simpson and Pedini, 1985). The impacts of ASS are therefore widespread and are of global significance. However, ASS under different conditions can have quite different impacts and therefore require tailored management strategies (Thomas, 2010).

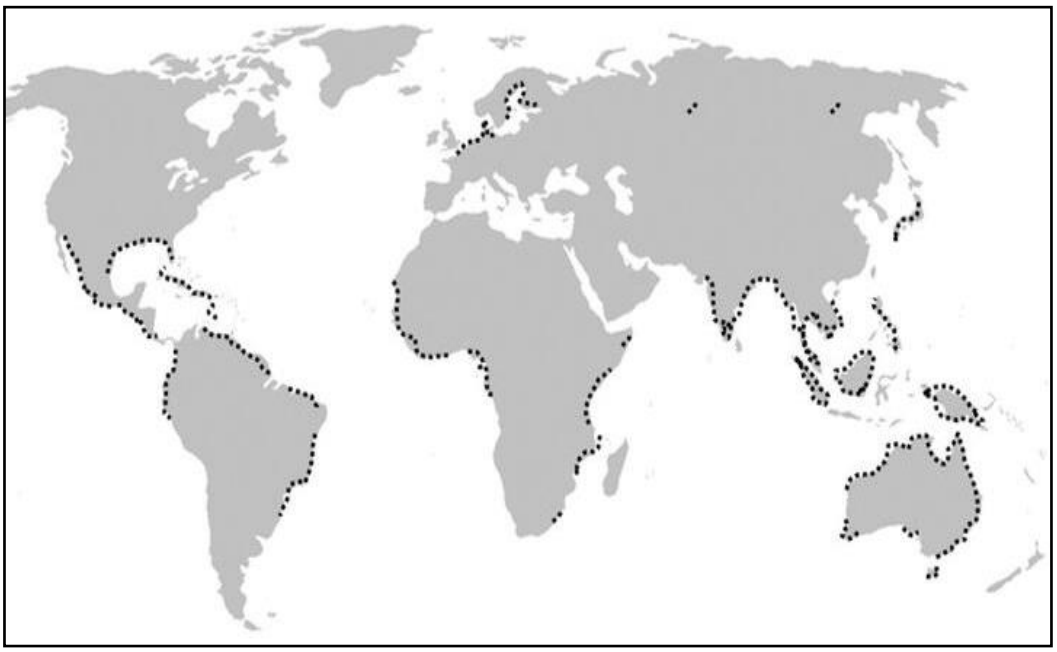

Figure 2.1. Global distribution of ASS (Fitzpatrick et al., 2009b; Simpson and Pedini, 1985).

Under a natural water table, ASS pose no problems unless the FeS_2 is exposed, whereupon it reacts with oxygen to form H_2SO_4 (Fitzpatrick et al., 2010b; Nordmyr et al., 2008; Ward et al., 2004a). Release of the H_2SO_4 in turn dissolves the soil matrices in which iron species (Fe^{2+}, Fe^{3+}), aluminium (Al^{3+}) and other potential toxic contaminants (elements, metals or metalloids) are held, which are released into the soil and water systems (Ljung et al., 2010; Ljung et al., 2009; Nordmyr et al., 2008; Poch et al., 2009; Roos and Astrom, 2005). Production and propagation of H_2SO_4, and mobilisation and transportation of toxic metals are major processes through which ASS pose ecological impacts on the environment. ASS have generally been classified under three generic names. These are:

- **Sulfuric material** (Isbell, 2002) and **sulfidic soil horizon** (Soil Survey Staff, 2010)- These soil materials contain H_2SO_4 and may also contain FeS_2, previously referred to as 'actual' ASS.
- **Sulfidic material** - This is soil material containing FeS_2 (Isbell, 2002; Soil Survey Staff, 2010), and previously referred to as 'potential' ASS.
- **Monosulfidic material** - This soil contains iron monosulfide (FeS) minerals that are still waterlogged, also known as monosulfidic black ooze (MBO) (Ward et al., 2004a).

However, a revised classification of ASS materials is presented in section 2.3.

2.2 Formation of Acid Sulfate Soils

2.2.1 Sulfide-bearing minerals

Acid sulfate soils have formed within the last 10, 000 years after the last sea level rise (Joukainen and Yli-Halla, 2003; Wilson, 2005). When the sea level rose and inundated the land, sulfate in sea water mixed with iron oxides in sediments and decomposable organic matter, allowing sulfate reducing microbes to form iron sulfide minerals (FeS_2) under anaerobic soil conditions (Canfield et al., 2006). This process led to formation of FeS_2 according to equation (Eqn.) 2-1.

$$Fe_2O_{3(s)} + 4SO^{2-}{}_{(aq)} + 8CH_2O + \tfrac{1}{2}O_{2\,(g)} \rightarrow 2FeS_{2(s)} + 8CHO_3^{-}{}_{(aq)} + 4H_2O_{(aq)} \qquad \text{Eqn. 2-1}$$

2.2.2 Oxidation of sulfide- bearing minerals

Oxidation of FeS_2 formed (Eqn. 2-1) occurs as a result of several processes: iso-static land uplift (Cook et al., 2000; Joukainen and Yli-Halla, 2003; Wilson, 2005), lowering of water table due to drought and artificial drainage (Reid and Butcher, 2011; Shand et al., 2008), and changes in soil moisture caused by land use and climate (Brown and Jurinak, 1989; Kawahigashi et al., 2008). In the tropics for example, distinct dry-wet season cycles cause seasonal variations in acid production, with more acid being produced during dry seasons and subsequent reduction during the wet season (Husson et al., 2000; Minh et al., 1998). Figure 2.2 shows an oxidised ASS surface of a wetland bed on the Finniss River, South Australia after a prolonged drought.

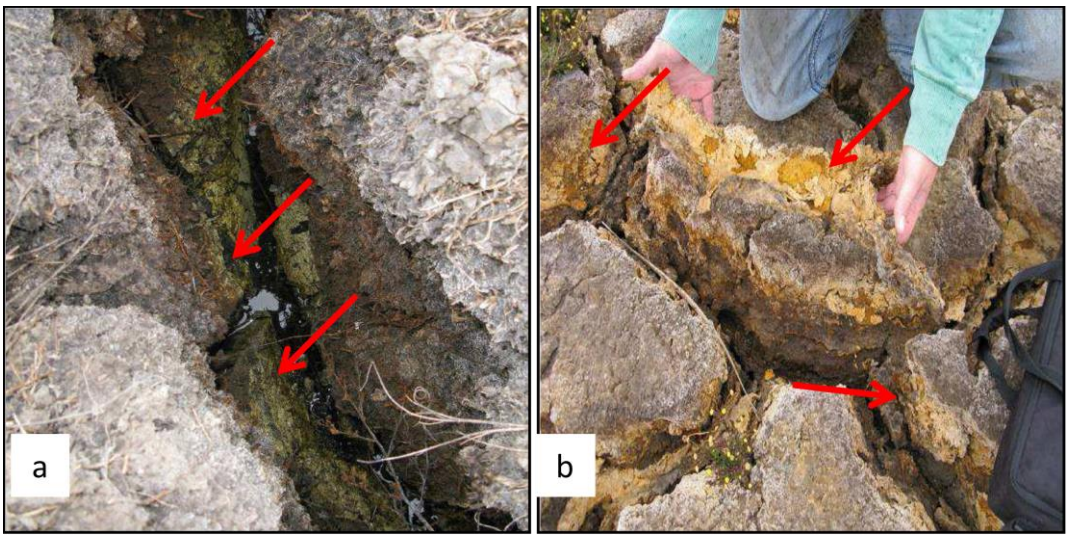

Figure 2.2. Sulfuric cracking clay soil in a dry wetland bed of the Finniss River showing (a) thick layers (pale yellow mottles/precipitate) of jarosites in cracks and (b) thick precipitates and layers of Schwertmannite. The pH values are 3-4 (Fitzpatrick et al., 2009a).

Land use changes such as construction of ditches, drains, raised beds, excavated soil surfaces and destruction of impermeable layers of soils are potent sources of sulfidic mineral exposure (Minh et al., 1997; Nordstrom, 1982). Ponded pastures, aquaculture ponds, gravel extraction and roads contribute to sulfidic mineral oxidation (Powell and Martens, 2005). The oxidation processes and the biochemical pathways that initially convert FeS_2 to ferrous iron (Fe^{2+}) and sulfate when sulfidic minerals are oxidised generally proceed in 4 steps (Lin et al., 2000; Nordstrom, 1982; Shand and Thomas, 2008)

as shown in Eqns. 2-2 to 2-5. Equation 2-6 summarizes the oxidation processes that take place when sulfidic minerals are oxidised.

Step 1 – Eqn. 2-2

Initially, the FeS_2 formed under anaerobic conditions (Eqn. 2-1) reacts with oxygen and water to liberate Fe^{2+}, sulfate and acid (H^+). In this reaction, every mole of FeS_2 consumed yields 2 moles of acidity.

$$FeS_{2(s)} + 3½O_{2\,(g,\,aq)} + H_2O \rightarrow Fe^{2+}{}_{(aq)} + 2H^+{}_{(aq)} + 2SO_4{}^{2-}{}_{(aq)} \qquad \text{Eqn. 2-2}$$

Step 2 – Eqn. 2-3

Secondly, the Fe^{2+} formed in the first reaction is hydrolysed to Fe^{3+} in a slow, FeS_2 oxidation rate-determining reaction (Singer and Stumm, 1970). However, when soil pH drops below 4, Fe^{2+} is oxidised at a faster rate catalysed by Fe oxidising bacteria (*Acidothiobacillus ferroxidans*) to Fe^{3+}. This reaction consumes oxygen and is responsible for de-oxygenation of water systems (Bush et al., 2004b).

$$Fe^{2+}{}_{(aq)} + H^+{}_{(aq)} + ¼\ O_2 \rightarrow Fe^{3+} + ½\ H_2O \qquad \text{Eqn. 2-3}$$

Step 3 – Eqn. 2-4

Thirdly, hydrolysis of Fe^{3+} from Eqn. 2-3 with water occurs to form solid ferric hydroxide (ferrihydrite). This process is soil pH dependent and is rapid at circumneutral pH (Morel and Hering, 1993). As the pH drops below 4, solid minerals do not form and, Fe^{3+} remains in solution. At higher pH, precipitates are formed instead (Thomas, 2010).

$$Fe^{3+}{}_{(aq)} + 3H_2O \rightarrow Fe(OH)_{3\,(s,\,aq)} + 3H^+{}_{(aq)} \qquad \text{Eqn. 2-4}$$

Step 4 – Eqn. 2-5

Fourthly, oxidation of the remaining FeS_2 generated by Fe^{3+} hydrolysis occurs. Oxygen is not required in this reaction as it is biochemically mediated (van Mensvoort and Dent, 1998). Until the supply of either FeS_2 or Fe^{3+} is exhausted, the reaction is cyclic and continuous (Thomas, 2010).

$$FeS_{2(s)} + 14Fe^{3+}{}_{(ag)} + 8H_2O \rightarrow 15Fe^{2+}{}_{(aq)} + 2SO_4^{2-} + 16H^{+}{}_{(aq)} \quad \text{Eqn. 2-5}$$

Eqn. 2.6 shows the overall oxidation processes which yields 4 moles of H^+ for every mole of FeS_2 consumed. The Fe^{2+} produced in this reaction is transported back up the soil profile (van Breemen, 1975) by capillary rise (Lin et al., 1998) or diffusion (Patrick and Delaune, 1972). This allows Eqn. 2-3 to recur if O_2 is present. If Fe^{3+} is transported down the profile to FeS_2, Fe^{2+} generated in the Eqns. 2-2 and 2-3 propagate oxidation further, even if the FeS_2 is submerged (Cook et al., 2000).

$$FeS_{2(s)} + 15/4\ O_{2\ (g,\ ag)} + 7/2H_2O \rightarrow Fe(OH)_3 + 2SO_4^{2-} + 4H^{+}{}_{(aq)} \quad \text{Eqn. 2-6}$$

The oxidation products also undergo hydrolysis reactions and form secondary minerals (iron-oxyhydroxides and iron-oxyhydroxysulfates): jarosite or natrojarosite (pale yellow mottles and coatings), Schwertmannite (orange-yellowish coatings), sideronatrite (bright-yellow green coatings) and metavoltine (distinct golden yellowish or greenish crystals) that act as a store of acidity (Fitzpatrick et al., 2009c). Figure 2.2 shows pale yellow mottles of jarosite and yellowish coating of Schwertmannite of a sulfuric clay soil of a dry wetland bed of Finniss River, South Australia.

2.3 Classification of Acid Sulfate Soils

The presence of sulfide-bearing minerals sufficient to cause severe acidity when oxidised or contain severe acidity has been used to classify ASS (Isbell, 2002; Pons, 1973; Soil survey Staff 2014; van Breemen, 1973). It was seen, however, not all sulfide-bearing soil materials have the capacity to acidify and therefore some do not pose sulfide-related problems. To accommodate these soil materials, changes to the classification system given in Section 2.1 have been proposed and five descriptive terminology and classification definitions have been introduced, and agreed to by the ASS Working Group of the International Union of Soil Science (Sullivan et al., 2010).

The proposed definitions replaced ***sulfidic*** with ***hypersulfidic*** and introduced a new term ***hyposulfidic***. This was included to account for a sulfidic soil material that is not capable of severe acidification. The proposed changes also included another term ***monosulfidic*** to distinguish other sulfidic materials from soil materials bearing detectable

monosulfides. The definitions presented in Section 2.1 have been redefined to accommodate the following changes currently being used in Australia and recently adopted in the 2nd Edition of the Australian Soil Classification (Isbell and the National Committee for Soils and Terrain, 2015):

(i) **Sulfuric soil material** – This is a soil material that contains H_2SO_4. The acidity is measured by various methods including pH in water. This definition has been extended to mean a soil material that has pH<4 (1:1 w/w in water).

(ii) **Sulfidic soil material** – This is a soil material that mainly contains FeS_2 and has the potential to form severe acidity upon oxidation. This material has been defined previously as soil materials containing detectable sulfide minerals of more than 0.01% sulfidic sulfur.

(iii) **Hypersulfidic soil material** – This is a sulfidic soil material that has a field pH of 4 or more and is identified based on a substantial decrease in pH after incubating aerobically a 2-10 mm of sulfidic soil material at field capacity. The substantial drop in pH is at least 0.5 pH units to 4 or less, measured 1:1 in water (w/w). The duration of incubation could be until the soil pH has changed by at least 0.5 pH units to below 4 or until a stable pH is reached, at least after 8 weeks of incubation. A stable pH is said to be achieved at least after 8 weeks of incubation and decrease in pH is <0.1 pH unit over at least 14 days, or when the pH starts to increase.

(iv) Hyposulfidic material – This is a sulfidic material that has field pH of 4 or more but does not experience a substantial drop in pH to 4 or less, when a 2-10 mm thick layer of soil material is incubated aerobically. The period of incubation is until a stable pH has been reached, at least after 8 weeks of incubation.

(v) Monosulfidic material – These are soil materials that have an acid volatile sulfur (AVS) content of 0.01% S or more. These soils are similar to MBOs except that they encompass a wider array of soil textures and consistencies. These materials include monosulfidic sands, which are not included as MBOs, based on consistency.

2.3.1 Characterisation of acid sulfate soils

Due to many devoted studies (Ahern et al., 2004; Bloomfield and Coulter, 1973; Lin et al., 2000; Sullivan and Bush, 2000; Sullivan et al., 2002b; Sullivan et al., 2009; Ward et al., 2002a), a diverse range of field and laboratory techniques to measure the different classes of ASS have been developed (Fitzpatrick et al., 2009c). The measurements undertaken and the techniques used depend either on pH decrease from the initial pH as an evidence of acid generation (i.e. H^+ in a solution correlates directly to the amount of FeS_2 dissolved) or concentration of sulfate (Vegas-Vilarrubia et al., 2008). Based on such studies, four major approaches are commonly used to measure the presence of actual acidity or the presence of sulfidic minerals (Creeper et al., 2012; Fitzpatrick et al., 2008b; Shand and Thomas, 2008), which were adapted and used in this research:

(a) **Total potential acidity (TPA) and total actual acidity (TAA)** – This method determines existing acidity after oxidation using hydrogen peroxide (H_2O_2). The difference between acidity and base is considered to be the measure of acidity after oxidation.
(b) **Sulfidic-sulfur determination** – This is a quantitative analysis of total sulfur using methods such as induction furnace combustion and chromium inducible sulfur that measure surplus presence of sulfidic minerals.
(c) **Acid-base accounting (ABA) methods** – These methods separately estimate acidity and acid neutralising capacity (ANC) of soils to identify sulfidic materials.
(d) **Incubation method** – This is a qualitative method used to identify whether a soil material is sulfidic. The soil material is incubated at room temperature and at field capacity by rewetting until a required duration (at least 2 months) and a critical pH target has been reached.

The two main measures of acidity based on H_2O_2 oxidation are TPA and total sulfidic acidity (TSA). Total potential acidity measures maximum amount of acidity that partly or totally reduced ASS contains after complete oxidation. Total sulfidic acidity is acidity attributed to complete oxidation of all remaining sulfidic compounds by H_2O_2. TAA is acidity existing prior to H_2O_2 oxidation (Ahern et al., 2004; Sullivan et al., 2002a; Ward et al., 2002b). The ABA method is used to determine potential hazards as an alternative approach to the H_2O_2 method (Ahern et al., 2004; Shand and Thomas, 2008; Sullivan et

al., 2009). The ABA is similar to TPA in that it determines the maximum amount of acidity, which a partially or a totally reduced acid sulfate soil contains after it has been completely oxidised. This has been referred to as 'net acidity' (Ward *et al*., 2002), and is shown in Eqn. 2-7.

Net acidity = sulfidic acidity + actual acidity – ANC Eqn. 2-7

The H_2O_2 method aims to provide an estimate of net acidity and the TSA method aims to provide an estimate of sulfidic acidity (Ahern et al., 2004; Ward et al., 2002b). The net acidity derived from the ABA approach is determined by gaining separate estimations of each component on the right hand side of Eqn. 2-7. The sulfidic acidity is determined using pyritic S, assuming that 1 mole of FeS_2 consumed produces 4 moles of H^+ (sulfidic acidity = pyrite (%) x 0.6237mol H^+kg^{-1}). Actual acidity is estimated by the TAA method of (Lin et al., 2000).

Oxidation of sulfides with H_2O_2 and measurement of acidity produced is commonly used to determine environmental impacts of pyritic materials (Jennings et al., 2000). The underlying assumption is that there is complete oxidation of any FeS_2 present. This chemical reaction happens when pure pyritic minerals are oxidised. Oxidation of complex mixtures of sulfidic and silicate minerals are complicated by clay dissociation, organic matter, sulfate minerals (e.g. gypsum) and loss of sulfur dioxide (Sullivan *et al*., 2000; Ward *et al*., 2004; Ward *et al*., 2002).

2.3.2 Acid neutralising capacity

The acid neutralisation process is summarized in Eqn. 2-8. Acidification is severe when ANC is low and acid production exceeds the environment's capacity to neutralize it (Fitzpatrick et al., 2010). When sources of carbonate such as shell fragments are present, acidity is neutralized and oxidation of Fe^{2+} to Fe^{3+} proceeds slowly (Fitzpatrick et al., 2008). In soils containing H_2SO4, product of FeS_2 oxidation can form sulfate-rich salts (e.g. epsomite and hexahydrite) due to evaporation (Fitzpatrick *et al*., 2009); and many methods for measuring ANC have been developed as highlighted by Ahern *et al*. (2004).

In general, ANC is equivalent to the maximum amount of acid that can be neutralized by a material to maintain a $pH_{KCl} > 5.5$. A $pH_{KCl} > 5.5$ for assessing TAA and ANC

has been chosen for biological and chemical reasons. By the use of this definition, ASS with pH_{KCl} <5.5 are thought to have TAA but no capacity to maintain a pH_{KCl} >5.5. The ANC of these materials is therefore zero, even though jarosite formation and clay mineral decomposition can buffer added acidity at lower pH (Fitzpatrick et al., 2008a).

$$Fe^{3+}_{(ag)} + 2SO_4^{2-}_{(aq)} + H^+_{(aq)} + 2CaCO_{3\,(s)} + H_2O \rightarrow Fe(OH)_{3\,(s)} + 2CaSO_{4(s)} + 2CO_{2\,(g)} \quad \text{Eqn. 2-8}$$

2.4 Ecological Impacts

Concern over the ecological impacts of ASS began in the 1970s (Pons, 1973) and since then, increasing numbers of studies have been conducted (Buschmann et al., 2008; Ljung et al., 2009; Simpson and Pedini, 1985; Tang and Yu, 1999). More recent studies have investigated impacts on the built (e.g. farms and irrigation systems) and natural (hydrological and biological) (Joukainen and Yli-Halla, 2003; Nordmyr et al., 2008; Poch et al., 2009; Powell and Martens, 2005; Roziere et al., 2009; Sullivan et al., 2009) components of the environments.

Other studies have examined the impacts on specific components of the environment: soil, water and different types of life forms (Buschmann et al., 2008; Desmond, 2000; Haling et al., 2010; Haling et al., 2011; Hanhart et al., 1997; Hinwood et al., 2006; Joukainen and Yli-Halla, 2003; Meda et al., 2001; Nordmyr et al., 2008; Powell and Martens, 2005; Robarge and Johnson, 1992; Roziere et al., 2009; Sammut et al., 1996; Simpson and Pedini, 1985; Tang and Yu, 1999; White et al., 1997). These studies strongly emphasized the environmental and economical importance of understanding the nature of ASS (Boylen, 1996; Starr, 1996).

In Australia, White *et al*. (1997) estimated that 2.2-23 million dollars are lost by the NSW fishery sector while 7-12 million dollars are lost by the Sydney rock lobster production annually. In Queensland, 189 million dollars are spent annually to manage an estimated 2.3 million ha of ASS along its coastline (Sutherland and Powell, 2000). Acidity discharge into farmland drains in Australia is estimated to be 400-3400 kg of H_2SO_4 ha^{-1} annually (Cook et al., 2001).

2.4.1 Impacts on soil

Two of the main problems associated with ASS are acidification and accumulation of potentially toxic contaminants (Bessho and Bell, 1992; Desmond, 2000; Faltmarsch et al., 2009; Fältmarsch et al., 2010). Under severe conditions, the acidity produced lowers the soil pH to values <4 if ANC is absent (Fanning et al., 2002; Nordmyr et al., 2008). This in turn dissolves the soil matrix in which major constituents of the soil (e.g. aluminium), as well as trace elements (e.g. arsenic) are present, which them mobilize them. In ASS, enrichment of leached elements affects microbial communities (Bingham et al., 1975; Duncan, 1999; McGrath et al., 1995; Oliveira and Pampulha, 2006), indigenous flora and fauna (Moore and Patrick, 1991), and destroys natural habitats (Toan and Debergh, 2004).

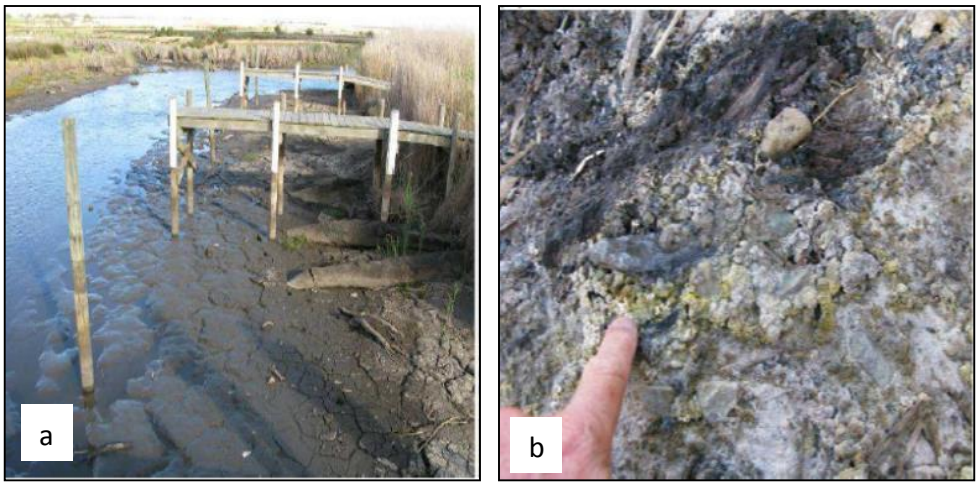

Figure 2.3. Changes in water level in Finniss River at Wally's Landing where sulfidic clayey soil was sampled for the current research, (a) a November 2008 photo showing substantial lowering of water level and dry clayey river bed and (b) white salt efflorescences of Mg-sulfate minerals (hexahydrate $MgSO_4.6H_2O$, epsomite $MgSO_4.7H_2O$ and gypsum) and bright yellow coloured iron oxyhydrosulfate minerals comprising sideronitrate $Na_2Fe(SO_4)_2.OH.H_2O$, tamarugite $Na_2Al(SO_4)_2.6H_2O$. The pH values ranged 1.6–2.5 (Fitzpatrick *et al*., 2009a, Poch *et al*., 2009.

2.4.2 Impacts on water

Disturbed ASS are potent sources of acidity in different aquatic ecosystems (Baldwin and Fraser, 2009; Cook et al., 2000; Sammut et al., 1996; Wilson et al., 1999). In addition to the reduction in pH, contaminants released from soil and transported in groundwater affect food production and stock care, water for drinking, personal hygiene, washing and cooking (Ljung et al., 2009). Aquaculture and fisheries industries are also known to be badly affected (Fitzpatrick et al., 2010; White et al., 1997). Where developments disturb

ASS, human exposure is prevalent (Buschmann et al., 2008; Hinwood et al., 2008; Hinwood et al., 2006). In the Swan Coastal Plain in Western Australia, for example, tests have shown elevated concentrations of toxic metals (Appleyard et al., 2004). Similarly, high concentrations, well in excess of drinking and recreational water quality guidelines and, in some cases, irrigation guidelines, have been reported (Hinwood et al., 2006; Sammut et al., 1996).

An important ecological impact associated with oxidation of sulfidic minerals is deoxygenation, which results from bacterial oxidation of Fe^{2+} to Fe^{3+} (Bush et al., 2004b; Fitzpatrick et al., 2008a). Acidified water conditions coupled with low levels of oxygen are undesirable for most forms of aquatic life (Cook et al., 2000; Moore and Patrick, 1991). Drainage containing high levels of acidity and low levels of oxygen threaten most aquatic plants, inshore fisheries and breeding grounds of marine organisms (Fitzpatrick et al., 2009a; Sammut et al., 1996). Recent studies have indicated secondary accumulation of MBOs, comprising largely of AVS in drains leading to reef waterways, have similar effects (Bush et al., 2002; Sullivan and Bush, 2000). Examples of farmland drains polluted with iron flocculation in ASS in South Australia are shown in Fig. 2-4. Aquatic organisms are sensitive to water quality and freshwater fish, aquaculture and other important aquatic services (Baker and Schofield, 1982; Baldwin and Mitchell, 2012; Burton et al., 2006; Ljung et al., 2009). The adverse consequences for aquatic life of large drainage projects in ASS regions in Venezuela, Sierra Leone, Vietnam, Senegal and Malaysia have been documented (Sammut *et al*., 2004).

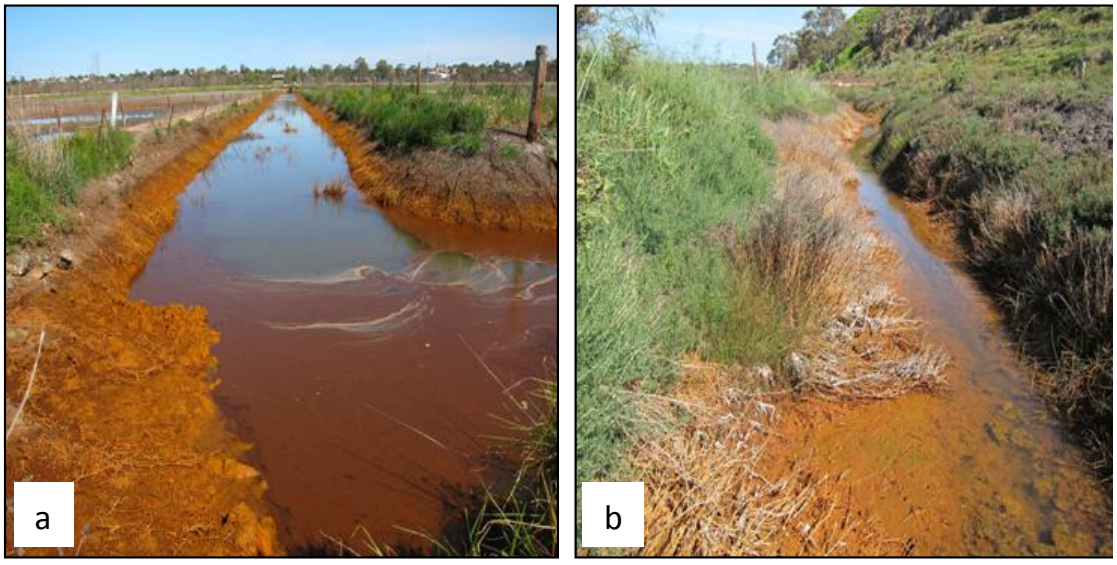

Figure 2.4. Polluted farmland drains near Murray Bridge (a) and Toora (b), South Australia with iron flocs containing dominantly Schwertmannite, which is formed because of the presence of ASS with sulfuric materials adjacent to the drains (Fitzpatrick et al., 2012).

2.4.3 Agricultural impacts

Subsistence farming is common in the developing countries and people depend entirely on what they produce. Where fertile land is limited, acidification of soil can have severe impacts on crop productivity, food security and even employment. This is due to the fact that acidification leads to impoverished soil (Desmond, 2000), enhances nutrient depletion, damages soil structure and reduces soil buffering capacity (Simpson and Pedini, 1985). This leads to poor seed germination, and inhibition of root elongation, coleoptile and hypocotyl growth (Munzuroglu and Geckil, 2002), as well as economically damaging chronic scalds (White et al., 1999). An example of a farmland scalded by ASS in South Australia is shown in Fig. 2.5.

There is an extensive literature on the impacts of acid soil on agriculture in general (Faltmarsch et al., 2009; Fältmarsch et al., 2010; Yli-Halla and Palko, 1987) but data on specific impacts of ASS are scarce (Fältmarsch et al., 2008). This is a serious concern as agriculture is the backbone of many economies, more so in the developing countries. Falling agricultural production as a consequence of acidity has already been reported in Thailand (Charoenchamratcheep et al., 1987; van Breemen, 1975), Malaysia (Shamshuddin et al., 2004), Vietnam (Dent and Pons, 1995), Florida (Thomas et al., 1995),

Netherland (Dent, 1986), Finland (Astrom and Spiro, 2000) and India (Ponnamperuma, 1972).

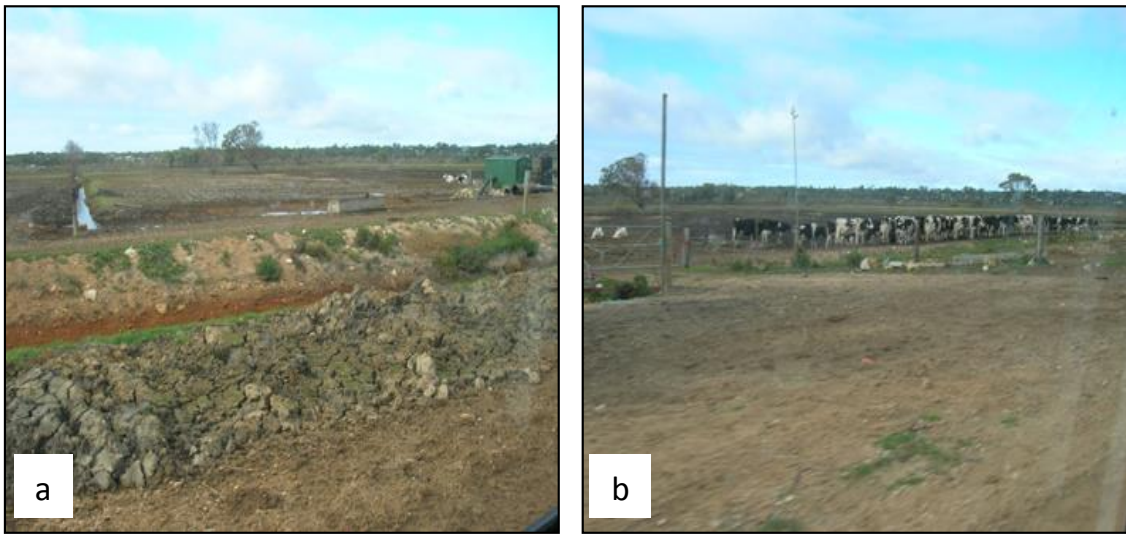

Figure 2.5. Paddocks on ASS at Toora, Murray River Basin, South Australia. (a) An abandoned paddock and (b) cattle scavenging on paddock scalded by ASS.

It is also seen that over half of the world's productive agricultural land is affected by changes in soil chemistry (Reddy and Patrick, 1977), yet there is limited understanding of these potential stresses in ASS. The effects of plant roots on pH, Eh and other chemical components of ASS under varying moisture conditions are limited, despite a few plant-based studies using other acidic soil types (Haling et al., 2010; Hindwood et al., 2006; Kochian et al., 2004). More so, crops such as rice (*Oryza sativa*) and taro (*Colocasia esculenta*) are often cultivated on ASS amended with organic matter (e.g. mulch). Under such conditions, either the plant or the amendment would alter the soil chemistry; however, no study has ever investigated the effects of such amendments. This knowledge could potentially benefit many communities in places where such practices are common, e.g. in Papua New Guinea.

Similarly, accumulation of toxic elements in edible parts of agricultural crops serve as direct conduits for entry into human and stock food chains (Clemente et al., 2003; Fältmarsch et al., 2010; Fältmarsch et al., 2008; Hinwood et al., 2008). This was confirmed by Ljung *et al*. (2009) who concluded that movement of toxic elements from crops grown on ASS needs thorough investigation due to the potential for chronic exposure to toxic metals that affect the health of humans and agricultural stock (Fältmarsch et al., 2008; Michael, 2013). A study by (Yli-Halla and Palko, 1987) showed that mobile elements

(manganese, cobalt and nickel) were elevated in oat and timothy plants grown on ASS. The chromium concentration in plants depends on the soluble content of the element but it is immobile in ASS. However, Cr^{3+} can easily be oxidised by manganese-oxides to Cr^{6+}, the most plant available and mobile form of Cr (Kabata-Pendias, 2000).

Metal accumulation in dairy cattle fed on forage and pasture established on ASS can also be a problem. A study in Finland which investigated the concentration of metals in cow milk originating from ASS found relatively high levels of iron, zinc and aluminium, compared to other dairies on non-ASS soils (Varo et al., 1980). Aluminium is highly mobile in ASS and its enrichment in plants and subsequent elevated levels in milk is not surprising. The health effects of high concentrations of Al in the human diet are still being debated (Fältmarsch et al., 2008).

2.5 Impact Management

The impacts of acidified soil and water systems, and toxic element leachates have become an important issue (Powell and Ahern, 1999; Powell and Waite, 2000; Thomas, 2010; Vegas-Vilarrubia et al., 2008), and various management strategies have been proposed and tested (Bloomfield and Coulter, 1973; David, 1986; Dent, 1992). These include 1) minimising the disturbance of sulfidic soil and maintenance of the natural water table management to prevent and slow the extent of pyrite oxidation (Ward et al., 2004b), and 2) neutralisation of actual (sulfuric) acidity by application of an alkaline material such as agricultural lime, and management of acidic water discharge and toxic by-product leachate (Dear et al., 2002; Fitzpatrick et al., 2010; Thomas, 2010). Mitigation and rehabilitation, retaining existing acidity and discharge management on a broader sense have received considerable attention (Cook and Gardner, 2001; Cook et al., 2000; Dear et al., 2002; Thomas, 2010).

Whilst the principle management strategies proposed and established are for large-scale impacts and involve large inputs (Dear et al., 2002), management of impacts on a smaller-scale requires more tailored strategies, especially in poorer economies where the cost of soil remediation or maintenance by these established methods may be too high. For this, more research needs to be done to find locally acceptable solutions.

2.6 Redox Potential and pH

In simple terms, oxidation-reduction potential (redox) is a measure of a chemical compound's tendency to acquire or donate electrons (Delaune and Reddy, 2005), or to oxidise and reduce other compounds (Ponnamperuma, 1972), and pH is a measure of the concentration of hydrogen ions (protons) expressed on a logarithmic scale.

Redox and pH in a given soil condition (basic, neutral or acidic) are not uniform, and are regulated by various factors including microbial activity, soil oxygen, organic matter, soil water status, solubility of various compounds and ion bonding exchange sites of chemical species (McLean, 1982). Redox and pH are important because they affect oxidation and reduction of minerals, release and mobility of metals, and stability and availability of nutrients (Delaune and Reddy, 2005; McLean, 1982; Thomas, 2010).

2.6.1 Redox potential

Redox potential is widely measured to characterize reduction-oxidation status of surface (soil, water and marine) environments (Delaune and Patrick, 1991; DeLaune et al., 1998). The measurement undertaken is in fact a measure of electrochemical potentials for electrons, which are important to all organic and inorganic chemical reactions (Ponnamperuma, 1972). This is also used in characterisation of the degree of reduction, predicting stability of various compounds that regulate nutrients and important biochemical reactions (Delaune and Patrick, 1991; Fiedler et al., 2007; Ponnamperuma, 1972). Determination of redox is based on the concentration of inorganic oxidants (O_2, NO_3, NO_2, Mn^{4+}, Fe^{3+}, SO_4 and CO_2) and reductants (various organic substrates and reduced inorganic compounds; NH_4^+, Mn^{2+}, Fe^{2+}, S^{-2}, CH_4, and H_2) (Delaune and Reddy, 2005; Wang et al., 1993). Molecular O_2 is the preferred electron acceptor, however, when the supply becomes limited, alternative electron acceptors are used in a descending order with well-defined Eh ranges (Fig. 2.7).

The relationship between oxidation reactions (removal of electrons from reductants) and reduction (addition of electrons to an oxidant) can involve transfer of electrons from one compound to another under the control of microbes utilizing an energy source (Ponnamperuma, 1972; Wang et al., 1993).

Changes in redox potential are measured potentiometrically using an inert indicator electrode and a suitable reference electrode. The inert electrode functions as a source or sink of electrons without itself undergoing chemical changes; adopting a potential that is determined by available electrons at its interface with the surrounding solution (Teasdale et al., 1998). Commonly, Eh is measured using platinum (Pt) electrodes and a calomel or Ag/AgCl reference electrode, either manually or automatically using a data logger (Mueller et al., 1985).

The voltage difference between the electrodes measured is then corrected for the reference electrode's standard voltage relative to standard hydrogen electrode (SHE, E = 0 V) and kept as Eh (Rabenhorst et al., 2009). In general, a positive Eh value corresponds to an oxidised soil condition and a negative to a reduced condition (Chao et al., 1962; Ponnamperuma, 1972; Ponnamperuma, 1984). The measured Eh value is therefore the ratio of oxidised to reduced forms within a soil, than concentration of redox-active species, which are important for determining stability 'poise' of measurements and retards any changes in Eh; and is equivalent to buffering capacity concept of pH (Teasdale et al., 1998).

2.6.2 Redox couples

In soils, several redox couples function. Upon flooding, O_2 is initially reduced (Eqn. 2-9), followed by NO_3 (Eqn. 2-10), and then oxidised Mn^{4+} (Eqn. 2-11). After Mn^{4+} reduction, Fe^{3+} is reduced (Eqn. 2-12) followed by SO_4 (Eqn. 2-13), and finally CO_2 (Eqn. 2-14) (Delaune and Reddy, 2005; Teasdale et al., 1998; Wang et al., 1993).

(i) $O_2 + 4e^- + 4H^+ \longleftrightarrow 2H_2O$ Eqn. 2-9

(ii) $2NO_3^- + 10e^- + 12H^+ \longleftrightarrow N_2 + 6H_2O$ Eqn. 2-10

(iii) $MnO_2 + 2e^- + 4H^+ \longleftrightarrow Mn^{2+} + 2H_2O$ Eqn. 2-11

(iv) $Fe(OH)_3 + e^- + 3H^+ \longleftrightarrow Fe^{2+} + 3H_2O$ Eqn. 2-12

(v) $SO_4^{-2} + 8e^- + 8H^+ \longleftrightarrow S^{2-} + 4H_2O$ Eqn. 2-13

(vi) $CO_2 + 8e^- + 8H^+ \longleftrightarrow CH_4 + 2H_2O$ Eqn. 2-14

Thermodynamically, a redox couple with stronger affinity for electrons is reduced and the one with lesser affinity oxidised. The energy released when two or more redox couples are in contact react is termed 'Gibbs free energy' (ΔG), which is related to Eh of each couple. In principle, the measured Eh values are corrected to the potential of a standard hydrogen electrode (SHE) (Fiedler et al., 2007). When corrected, redox couples that are more reducing than the SHE have negative Eh, and the more oxidising have positive values (Fiedler et al., 2007).

Table 2.1. Microbial metabolism and electron acceptors at given soil Eh range.

Sediment condition	Aerobic			Anaerobic		
Redox condition	Oxidised	Moderately reduced		Reduced		Highly reduced
Electron acceptor	O_2	NO_3^-	Mn^{4+}	Fe^{3+}	SO_4^{2-}	CO_2
Microbial metabolism	Aerobic	Facultative				Anaerobic
Redox potential	700 - 400	300	200	100	0 -100	-200 -300

An Eh of 0 mV indicates the absence of O_2 and NO_3 but presence of bio-reducible M^{4+}, Fe^{3+} and stable SO_4 while a +400 mV indicates the presence of O_2, even if excess H_2O is in the soil (Delaune and Reddy, 2005). When O_2 supply is terminated, microbes switch from aerobic to facultative, and eventually to anaerobic respiration (Fiedler et al., 2007; Patrick and DeLaune, 1977). Positive Eh values correspond to an oxidising (oxic) condition and negative values to a reducing (anoxic) condition, respectively (Teasdale et al., 1998).

2.6.4 Normal pH and Eh limits

In nature, O_2 is the strongest oxidising agent and an agent stronger than O_2 cannot persist to react with H_2O and liberate O_2. Therefore, this reaction of H_2O defines the upper limit of Eh. The lower Eh limit is that of hydrogen reaction. pH in ASS can be as low as -0.6 to 3.2 due to the oxidation of pyrite and marcasite with an Eh of +860 mV (Becking et al., 1960). The upper pH limit in soils is usually associated with CO_2 free water in contact with carbonate rocks and silicates, which generate pH up to 10 and 11 respectively.

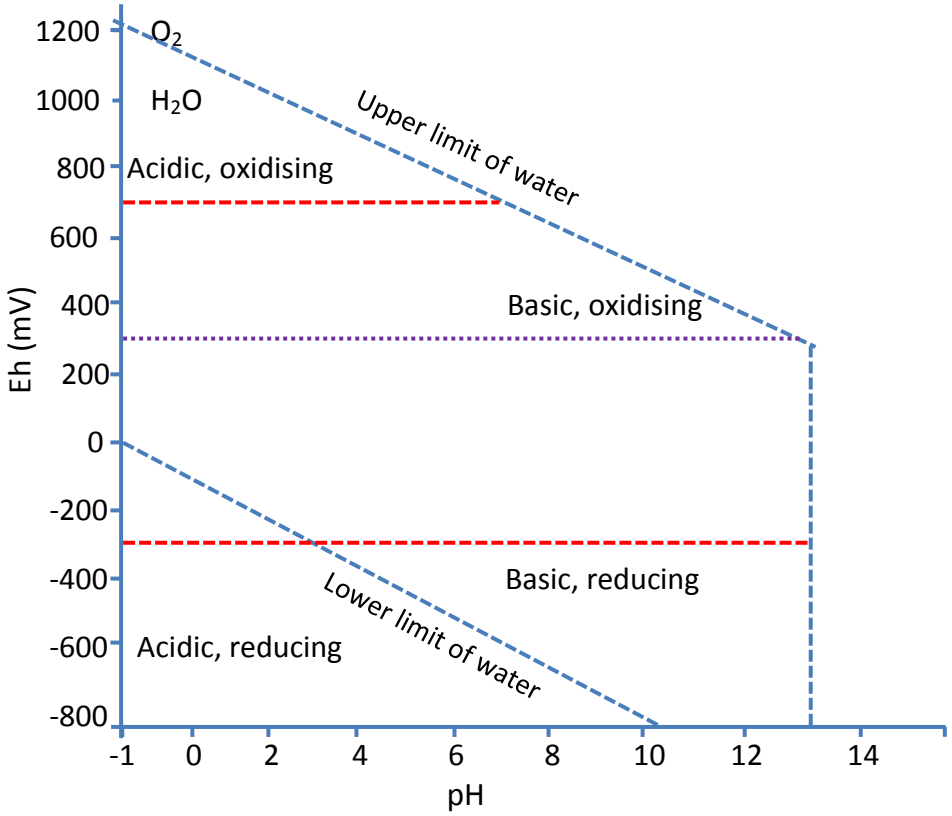

Figure 2.6. The Eh-pH range in surface environments showing four redox classes: (i) acidic-oxidising, (ii) basic-oxidising, (iii) acidic-reducing and, (iv) basic-reducing (adapted with slight modifications from (Krauskopf, 1967) as per (Delaune and Reddy, 2005; Poch et al., 2009). The lower and upper Eh limits are shown by the red dotted lines. The purple dotted line shows the break between an aerobic and anaerobic condition (Fiedler et al., 2007).

2.7 Bacterial Sulfate Reduction

Microbial sulfate respiration is a metabolic pathway for energy conservation (Meyer and Kuevert, 2007). In all the prokaryotes known to reduce sulfate, three main enzymes are believed to be involved in the dissimilatory process. ATP sulfurylase (Sat) is involved in activating chemically inert sulfate to adenosine-5'-phosphosulfate (APS), which is then converted to adenosine monophosphate (AMP) and sulfite by APS *reductase* (Apr). Finally, sulfite is reduced to sulfide by sulfite *reductase* (Dsr)(Rabus et al., 2004).

In anaerobic sulfate reducing bacteria (SRB), ATP sulfurylase forms APS solely to serve as a terminal electron acceptor of heterotrophic metabolism. In certain chemo- and photolithotropic bacteria, ATP sulfurylase catalyses the final reaction in the oxidation of reduced inorganic sulfur compounds to sulfate and plays a key role in the sulfur cycle in an anaerobic ecosystem (Gavel et al., 1998). This process can be utilised for the biogenic neutralisation of sulfuric acidity in ASS.

2.7.1 Sulfate reduction inhibition

There is evidence that sulfate reduction at pH<5 is possible, however, metabolites (H_2S), organic acids, metal sulfide precipitates and iron-reducing bacteria are likely to inhibit sulfate reduction (Koschorreck, 2008). At low pH, biological macromolecules are destabilized and proteins denature (Schleper et al., 1995). Therefore, to survive the low pH, acidophilic organisms maintain an elevated cytosolic pH, a process which requires a significant fraction of the metabolic energy (Lowe et al., 1993). The SRB, having low metabolic energy yields, are susceptible (Hamilton, 1998).

Sulfide reacts with metal ions and functional groups of electron carriers (Hao et al., 1996), amino acids, metabolic coenzymes and is an important inhibitory factor to sulfate reduction (Koschorreck, 2008). The literature show too that oxygen, temperature and organic matter availability that control the rate of microbial reduction have not been well characterised (Holmer and Storkholm, 2001).

2.8 Review Summary

Acid sulfate soils are naturally occurring soils or sediments formed under reducing conditions with bacterially formed sulfide (pyrite, FeS_2) minerals. The FeS_2 is benign under reduced conditions unless exposed and oxidised. The oxidation processes then leads to formation of H_2SO_4, which in turn solubilizes soil matrices and mobilizes potentially toxic elements, which become readily available in solutions. Release of H_2SO4 and toxic elements coupled with de-oxygenated water and soil systems are major causes of widespread ecological impacts. The main impacts are loss of agricultural productivity due to soil acidity and leaching of essential nutrients, loss of terrestrial and aquatic habitats, impacts on aquaculture and fisheries, and degradation of civic infrastructure.

Ecological impacts of ASS associated with propagation and release of the acidity and toxic elements have been well researched and understood, and various strategic management principles developed. Widely established strategies include protecting the exposure of FeS_2, neutralisation of actual acidity, and management of the discharge of toxic by-products and acidic water. In contrast, low cost approaches suitable for poorer economies have not been adequately researched. In this thesis, the value of organic and plant-based remediation of ASS is investigated, including the chemical and biological processes that drive pH change in soil in the presence of living or dead plant matter.

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Chapter Three

Descriptions of Studies and Methodologies

3.0 Introduction

The aim of this chapter is to present the general descriptions of the studies and methodologies used in this research. Experiment-specific methods are described under each experimental chapter. As numerous studies were conducted, each study described in this chapter was allocated a unique number for references to be made from the experimental chapters. There are seven sections focussing on: (i) collection and preparation of soil and organic matter - Sections 3.1 and 3.2, (ii) general descriptions of the studies - Section 3.3, (iii) redox potential and pH measurements - Sections 3.4 and 3.5, (iv) quantification of sulfate content - Section 3.6, and (v) statistical analysis of data - Section 3.7.

3.1 Soil Collection and Characterisation

The acid generating potential and existing acidity of ASS are normally assessed using a range of methods, taking into consideration mineral composition and Acid Neutralization Capacity (ANC). However, the most realistic methodology to estimate whether a soil will acidify is still debated (Shand et al., 2009). In this research, the following three of the most generally accepted methodologies have been used: (i) pH measurement in water and after peroxide treatment, (ii) acid-base accounting, and (iii) incubation test (Creeper et al., 2012; Fitzpatrick et al., 2009a; Fitzpatrick et al., 2009b; Fitzpatrick et al., 2008b; Shand et al., 2008).

Soil pH measurement in solution (1:5 soil: water, *w/w*) is a widely used method for testing soil acidity (Fitzpatrick et al., 2010b; Fitzpatrick et al., 2008b; Reid and Butcher, 2011). Soil pH measurement after peroxide treatment is considered as a screening tool for assessment of sulfidic soils (Ahern et al., 2004). The principle is that peroxide oxidises sulfide minerals (especially pyrite), providing an estimate of the maximum acidity that can be produced when oxidised, which occurs in the absence of carbonate minerals (e.g. calcite) (Thomas, 2010).

Figure 3.1. Soil pH measurements (a) in water 1:5 (pH_w), (b) and (c) sulfidic materials and soils treated with peroxide prior to pH_{ox} measurements, and (d) chip-tray being filled with samples of sulfuric, sulfidic and monosulfidic materials for incubation prior to $pH_{incubation}$ measurement. Note the nature of reactions in (b) forming froth / effervescence and (c) vigorous effervescence reaction, indicating acid generating potential of the sulfidic materials and soils.

The incubation ($pH_{incubation}$) or aging test is a standard method used in Australian Soil Classification (Isbell, 2002) and Soil Taxonomy (Soil Survey Staff, 2010). It is considered to be realistic as it resembles a field scenario where sulfide minerals are allowed to oxidise slowly under moist soil conditions, at least over a period of 8 weeks (Sullivan et al., 2009). This process allows the soil material to closely mimic what happens in nature, and lets the soil "speak for itself" (Dent, 1986), compared to pH_{ox}, which forces sulfide minerals to react and produce acidity (Thomas, 2010).

Throughout the thesis, pH measured in water is referred to as pH_w, measurement after peroxide (1:5 w/w) treatment as pH_{ox} and after incubation, at least after 8 weeks as $pH_{incubation}$. If pH_{ox} was measured in the field, pH_{Fox} is used instead.

The ASS profiles used in this research were collected from the following two sites:

- Gillman in Barker Inlet (34°82'92.3"S, 138°54'05.0"E) and
- Finniss River at Wally's Landing (35˚24'28.28''S; 138˚49'54.37''E).

The localities of these two sites in South Australia are shown in Fig. 3.2.

All the ASS material used were classified as: (i) sulfuric material ($pH_w < 4$), (ii) sulfidic material ($pH_w > 4$) to be consistent with current ASS classification terminologies used in the Australian Classification (Sullivan et al., 2002; Sullivan et al., 2010) or Soil Taxonomy (Soil Survey Staff 2010) or (iii) hypersulfidic material ($pH_w > 4$) to be consistent with current ASS classification terminologies used in Sullivan *et al*. (2010).

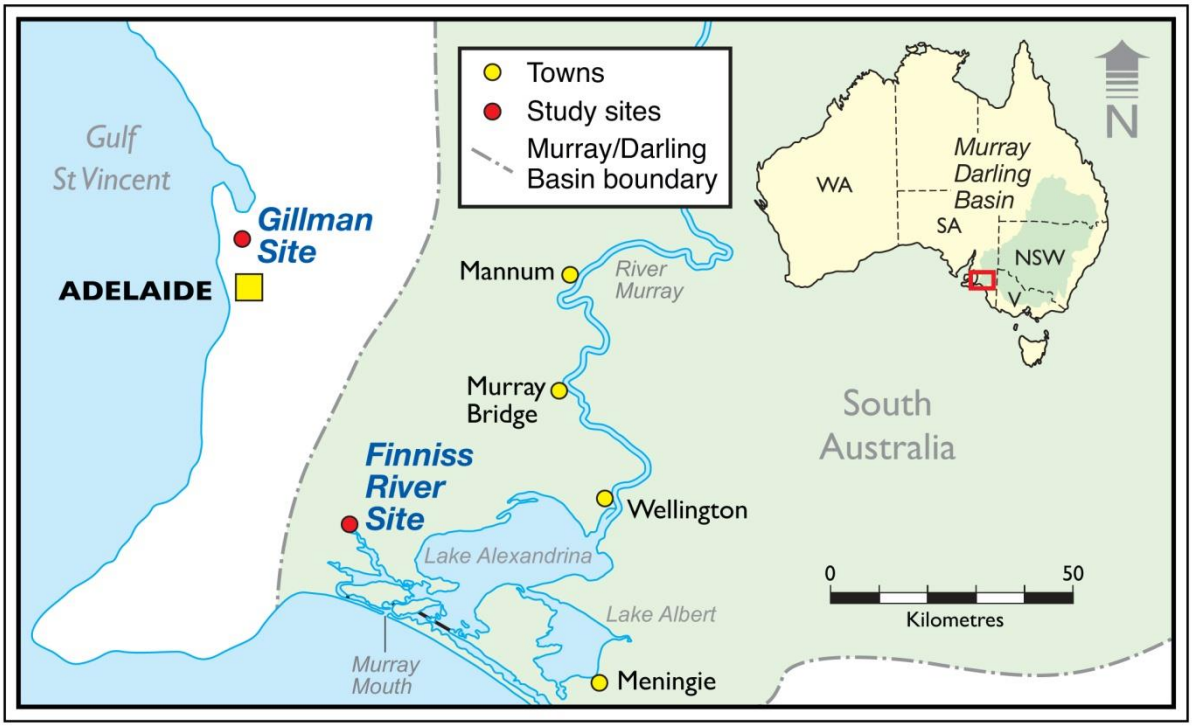

Figure 3.2. Locality of samples from the Gillman site in Barker Inlet and Finniss River site at Wally's Landing.

3.1.1 Soil collection at Gillman

The Gillman soil was collected from a trench as shown in Fig. 3.3b. On average, the soil sample collected between 0.5-1.5 m "was classified" as sulfuric material (pH_w ranged from 2.4–2.8 and pH_{ox} 1.2-2.0, with 79% field "capacity". The soil sample collected between 1.6-3.5 m classified as hypersulfidic material (pH_w ranged from 5.6-6.7 units and pH_{ox} 1.9-2.3, with 48% field capacity. The residual organic matter content, estimated by weight loss-on-ignition (Schulte and Hopkins, 1996) was 3.7%. The initial sulfate content of the sulfuric soil ranged between 31.6 to 22.3 µmol g^{-1} dry soil and in the sulfidic soil, 14.6 to 23.8 µmol g^{-1} dry soil, (sulfate quantification is described in Section 3.6).

Results of soil samples incubated for 12 weeks further indicated that soil samples between 0.5-1.5 m classified as sulfuric material ($pH_{incubation}$ ranging from 2.4-2.5), and those samples taken at a depth of 1.6-3.5 m classified as hypersulfidic material ($pH_{incubation}$ ranging from 2.1-3.1). The sulfidic soils of lower depths were greyish (Fig. 3.3d) and the sulfuric soil of the upper depths were brown with yellow mottles (Fig. 3.3e). Further details of this site can be found in Thomas (2010) who also conducted research at this location. The site of collection for this research is shown in Fig. 3.2 and in Thomas (2010) as 'Gillman Focus area D'. Thomas further highlighted that the sulfuric material has very high existing acidity and the underlying hypersulfidic material contains high sulfidic acidity (reduced inorganic and chromium reducible sulfur with a minor amount of monosulfidic "material".

An ASS with sulfidic material was also collected in a mangrove swamp area between the Gillman site and Kilda but was not used due to its high shell content, which would have tended to buffer any oxidation of the sulfides. The "sulfuric soil" obtained following oxidation of "sulfidic soil", or neutralised soil was prepared by mixing the Gillman "sulfuric soil" with alkaline sandy loam, was classified based on the initial pH and on the changes in pH measured after exposure (Thomas, 2010; Vegas-Vilarrubia et al., 2008).

Figure 3.3. Collecting ASS materials under the watchful eyes of (a) Assoc. Prof. Rob Reid at St Kilda mangrove site and (b) Prof. Rob Fitzpatrick at Gillman, and (c) collecting sulfidic soil material under water at Wally's Landing in Finniss River. The sulfidic soil collected from Gillman was brown (d) to (e) light brown due to presence of low residual organic matter and (f) the Finniss River sulfidic soil black due to the presence of higher residual organic matter. The St Kilda soil was not used due to high content of shells.

3.1.2 Soil collection in the Finniss River at Wally's Landing

A study undertaken in 2007 identified the presence of a "Hypersulfidic subaqueous clayey soil" with hypersulfidic material in the Finniss River at Wally's Landing (Fig. 3.2), which became exposed following a prolonged drought between 2007 and 2010 (Fitzpatrick *et al*., 2009c). Detailed geochemistry, major element concentrations and reduced sulfur content of the soils at this site sampled in 2007, identified as AA26.3 and FIN26 are given in Fitzpatrick *et al*. (2009b).

In this study, the ASS at this same location was re-sampled in 2012. However, because this soil was sampled three (3) years after the drought ended (i.e. post rewetting in 2010 as indicated in Table 3.1) the profile had been subjected to: (i) drying during the drought and formation of a "Sulfuric clayey soil" and (ii) reflooding in 2010 when the river returned to its normal level with the formation of a "Sulfuric subaqueous clayey soil" with hypersulfidic material at depth (Table 3.1). The sample collected below approximately 1 m of water (see Fig. 3.3c) and below the sulfuric material comprised "sulfidic clayey material (Table 3.1). It was blackish in colour (Fig. 3.3f) due to the presence of high residual organic matter content. The average pH_w of the soil was 6.7, pH_{ox} was 1.4 and the field capacity was 49%. The initial sulfate content ranged between 12.3 and 16 µmol g^{-1} soil.

Currently no subgroup exists in Soil Taxonomy (Soil Survey Staff, 2014) that adequately describes these Finniss River soils following their rewetting. They are best described as subaqueous soils with sulfuric horizons or "Sulfuric subaqueous clayey soils" in accordance with the Australian ASS classification key (Fitzpatrick *et al*., 2008; Fitzpatrick 2013). This presents little issue if these soils exist in this transient state for a short period of time (e.g. during transformation from Hydraquentic Sulfaquept to Sulfic Hydraquent). However, in some instances it is expected that these soils will persist for a number of years. In these cases, such as at Walley's Landing in the Finniss River it would be appropriate to have the ability to classify these soils accurately within Soil Taxonomy. Fitzpatrick and Grealish (personal communication) have proposed the subgroups Hydraquentic Sulfowassepts and Typic Sulfowassepts to describe the active subaqueous ASS in the Finniss River (Table 3.1). This also involves the creation of the Inceptisol sub order, Wassepts, and the great group Sulfowassepts. These proposals are currently being

drafted by Fitzpatrick and Grealish (personal communication) for USDA-NRCS for consideration to be included in revised versions of the US Keys to Soil Taxonomy.

Throughout this thesis and in all submitted and published journal papers the terms "**Sulfuric soil**" or "**Sulfidic soil**" will be used when describing soils used in the experiments (e.g. Table 3.2). The justification for this is twofold:

(i) the use of "**soil**" instead of the terms "material or horizon" is because in all experiments the original ASS materials were often changed by further oxidation or pre-treated with an ameliorant such as neutralisation with alkaline sandy loam before being used in experiments "as a soil" (e.g. adding complex organic matter).

(ii) terms s**ulfuric**" or "**sulfidic** to simplify "soil labelling" but also in Table 3.1 providing an explanation for labelling / classifying these soils as a "Sulfuric Soil or Sulfidic Soil and their equivalent soil classifications using Soil Taxonomy (Soil Survey Staff, 2014) and the Australian ASS classification key (Fitzpatrick et al., 2008; Fitzpatrick, 2013).

A so-called "Sulfuric soil" was manufactured by oxidising the hypersulfidic material or sulfidic material sampled in the Finniss River (FR). This was achieved by spreading the sulfidic material in a thin layer (approximately 2 cm thick), and maintaining it in a moist state until it became a "sulfuric soil" (pH_w <4). The sulfate content of the sulfuric soil ranged between 20.6 to 32 μmol g^{-1} soil.

Table 3.1. Sampling location labels and equivalent soil classifications of the two acid sulfate soils used in this thesis from the Finniss River and Gillman shown in Fig. 3.2.

Sampling locations[1]	Previous sampling location reference	Depth (cm bgl)	**Sulfuric horizon**[5]/*Sulfidic material*[6]	Soil Class[7]	Australian ASS classification key[8]	**Sulfuric horizon**[5]/*Sulfidic material*[6]	Soil Class[7]	Australian ASS identification key[8]
Finniss River			Finniss River: Prior to rewetting (2009)			Finniss River: Post rewetting (post 2010)		
Sulfidic soil	FIN26 M3-4[2] FC10740[3] LF01-B[4]	0 - 5 5 - 17 17 - 40 40 - 60 60 – 150	**Sulfuric** **Sulfuric** **Sulfuric** **Sulfuric** *Sulfidic*	Hydaquentic Sulfaquept	Sulfuric cracking clay soil	*Sulfidic* *Sulfidic* **Sulfuric** **Sulfuric** *Sulfidic*	Hydraquentic Sulfowassept	Sulfuric subaqueous clayey soil
Gillman	BG 15[9]		Gillman Prior to draining (2003)			Post draining (post 2003)		
Sulfuric soil		0-25 25-110 110-125	**Sulfuric** **Sulfuric** *Sulfidic*	Typic Sulfaquept	Sulfuric clayey peat soil	**Sulfuric** **Sulfuric** *Sulfidic*	Typic Sulfaquept	Sulfuric clayey peat soil

[1.] Sampling location label used in this thesis
[2.] Sampling location label used in (Fitzpatrick et al., 2009a; Fitzpatrick et al., 2009b)
[3.] Sampling location label used in (Fitzpatrick et al., 2011)
[4.] Sampling location label used in (Baker et al., 2010; Baker et al., 2011)
[5.] Acid sulfate soil horizon (Soil Survey Staff, 2014)
[6.] Acid sulfate soil material (Soil Survey Staff, 2014)
[7.] The following new proposals are currently being submitted by (Fitzpatrick et al., 2015) to USDA-NRCS to consider for inclusion in revised versions of the US Keys to Soil Taxonomy: (i) Inceptisol Suborder: Sulfowassepts, (ii) Inceptisol Great Groups & Subgroups for Hydraquentic Sulfowassept and Typic Sulfowassept.
[8.] Australian acid sulfate soil classification (Fitzpatrick, 2013; Fitzpatrick et al., 2008a)
[9] Thomas (2010)

3.1.3 Sandy loam

Early trials indicated that plant-based remediation of the collection sites, particularly Gillman, would be difficult because of the dense clayey texture of these soils, which prevented oxygen penetration to plant roots. Several experiments were conducted with mixtures of sulfuric or sulfidic soils and sand. A commercially available alkaline sandy loam (pH_w 9.4 and pH_{ox} 7.2) from Langhorne Creek in South Australia, and washed sand was used. The alkaline sandy loam soil was used in the neutralisation of sulfuric soil and the sand for mixing into the ASS used in the plant-based studies. The sand was washed in an acid solution (pH_w <3) made by mixing 750 ml of HCl in 10 litres of water and soaked overnight to remove bicarbonates and rinsed with tap water until the final pH_w was 6.8. The acid washed sand (henceforth referred to as sand) was dried under glasshouse conditions. Similarly, the sandy loam soil was air-dried for three days and sieved to remove coarse materials. Both of these were stored dry until use.

3.1.4 Processing of soils

Due to high salinity (ranging from 13.0 to 19.6 $mScm^{-1}$ (sulfidic) and 18.7 $mScm^{-1}$ (sulfuric) and presence of coarse materials (small stones and pebbles), the Gillman soils were filtered using a coarse cloth and collected in buckets. These soils were rinsed in tubs using water and salinity level monitored until the final concentration was 7.2 (0.072 x100) $mScm^{-1}$ and 4.7 (0.047 x 100) $mScm^{-1}$, respectively. pH_w and pH_{ox} were monitored using a field pH meter at the same time to ensure sulfides were not lost during the washing process. The processed soils were filtered through a cloth overnight and collected dry. Processed sulfidic soils were kept under water to minimise exposure and oxidation.

In order to investigate the stability of the sulfuric soil that was neutralised with alkaline sandy loam soil following the addition of amendment and establishment of plants (Chapter Seven), some of the Gillman sulfuric soil (pH_w < 4) from the surface 0.5 to 1.5 m and sandy loam were mixed (henceforth referred to as neutralised soil), initially in 3:1 (sandy loam: sulfuric soil, *w/w*) using a cement mixer (Fig. 3.4c) until an adequate amount of the neutralised soil (pH_w 3.7 and pH_{ox} 2.5) was obtained. The proportion of sandy loam added to the sulfuric soil to obtain the neutralised soil of final pH_w of 3.8, pH_{ox} of 2.7 and

field capacity of 69%, and pH_w of 6.7, pH_{ox} of 2.8 and field capacity of 28% is appended in Table B1. For reference only, the neutralised soil of pH_w 3.8 was kept as sulfuric (Fig. 4.3e) and pH 6.7 as sulfidic soil (Fig. 4.3f), respectively. The initial sulfate content of the neutralised soil of pH_w 3.8 was 23.8 and pH_w of 6.7 was 23 µmol g^{-1} soil. The neutralised soil of pH_w 6.7 was used in [I] – [[III] (Table 3.2) and in studies [PI] – [PII] (Table 3.4).

The stability of the neutralised soil when used in the long-term studies was assessed using separately prepared neutralised soil of pH_w 5.6 and pH_{ox} 2.9 and pH_w 7.8 and pH_{ox} 1.9 in two incubation studies lasting two weeks. The data obtained showed the changes in pH were relatively stable throughout in both studies, strongly indicating that changes in soil chemical properties measured in the long-term studies would be due to treatment effects (Michael et al., 2012). Based on these results, the neutralised soil was used in the studies pointed out above.

Figure 3.4. Processing of soils: (a) oxidation of Finniss River and (b) Gillman sulfidic soils, (c) a cement mixer for mixing, (d) mixing soils using the cement mixer, (e) mixed sulfuric soil with alkaline sandy loam of pH_w 3.8 (1:3) and (f) neutralised soil of pH_w 6.7 (1:10). Note the colour differences between (e) and (f) due to the differences in the amounts of sandy loam used, and the final pH of the neutralised soil was obtained by continuous mixing of sample soils from the 1:3 mix.

3.1.5 Estimation of water holding capacity, moisture content and field capacity

The water holding capacity (Whc) was estimated by setting soil samples at 100% field capacity after soaking in water and draining through a filter overnight. These soils were weighed to obtain the wet weight (Ww), and oven dried for 3 h, then microwaved for 30 seconds to ensure removal of any residual moisture and reweighed to obtain a final dry weight (Fdw). Based on the Ww and Fdw, Whc was estimated using Eqn. 3-1 and expressed as percentage.

- $Whc = ([(Ww-Fdw)/Fdw) \times 100])$ Eqn. 3-1
- $Mc = ([(Ww-Fdw)/Dw)] \times 100)$ Eqn. 3-2
- $Fc = ([(Mc/Whc)] \times 100)$ Eqn. 3-3
- $Fw = [(Fc \times Mc \times Dw) + Dw \pm OM + Tw]$ Eqn. 3-4

To estimate the soil moisture content (Mc), wet soil samples were weighed (Ww) then oven dried at 60^{o}C overnight and reweighed for the dry weight. The dry soil was microwaved for 30 seconds and reweighed to obtain the final dry weight (Fdw). The Ww and Fdw were used to estimate Mc using Eqn. 3-2. The Whc and Mc in turn were used to estimate the field capacity (Fc) using Eqn. 3-3. Moisture content for the different experiments is expressed as a percentage of field capacity, and was maintained by regularly weighing and addition of water as required.

3.2 Organic Matter

Four different types of plant material were used as amendments, and will be referred to as complex organic matter to distinguish them from simple organic compounds such as glucose that were also used in some studies.

Leaves of the common reed *Phragmites australis* were harvested from the banks of the Torrens River in Adelaide. Sample photos showing *Phragmites* plants established in the current research are shown in Fig. C2. Bales of lucerne (*Medicago sativa*) hay, wheat (*Triticum* sp.) and pea (*Pisum sativum*) straws were purchased from a commercial supplier. All of the plant material was chopped into pieces, air-dried overnight and then oven dried at 60^{o}C for three days, after which it was finely chopped using an electric blender and sieved through a 0.5 mm mesh.

The nitrogen content of the complex organic matter analysed by ICP-OES using a 0.5 g samples (n=3) are shown in Fig. 3.5. The carbon content can be approximated from the data in (Kamp et al., 1992), with the *Phragmites* leaf being similar to grass (leaf) clipping.

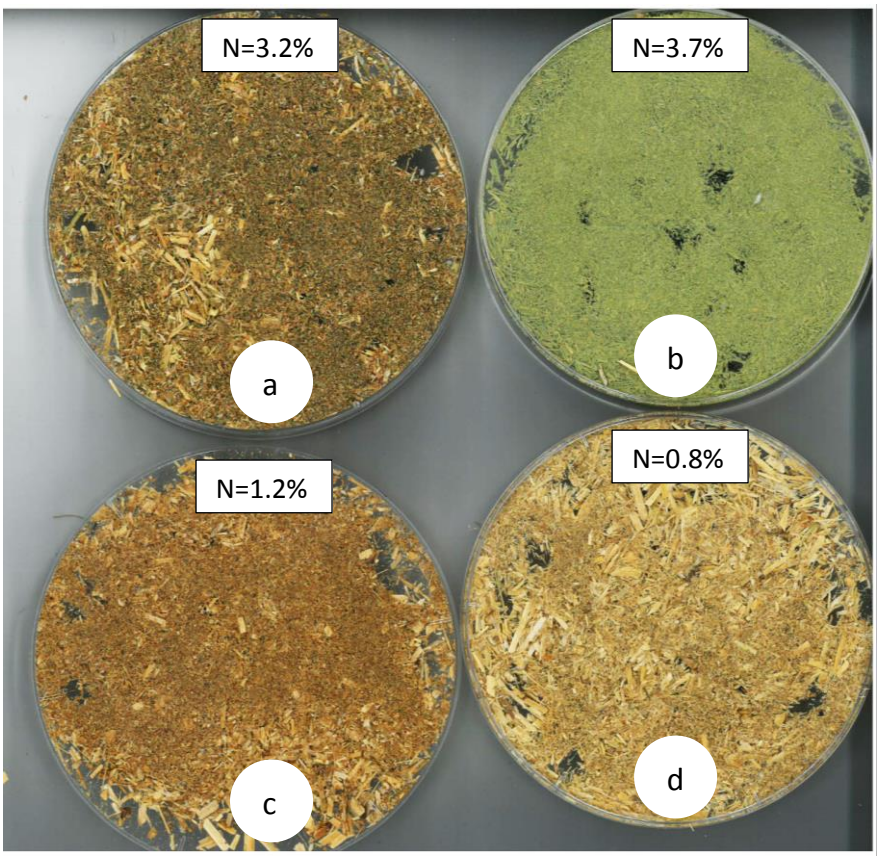

Figure 3.5. Ground organic matter: (a) lucerne hay (b) *Phragmites* leaf, (c) pea straw, and (d) wheat straw. The images were taken prior to sieving.

3.3 Descriptions of Studies

The studies aimed at establishing knowledge of soil chemical changes that ASS undergoes in the presence of: (i) organic matter when applied as surface mulch or incorporated in soil, (ii) live plants, and (iii) soil moisture. The combined effects of organic matter and live plants were also investigated as it is practically relevant under certain soil use and management conditions.

3.3.1 Amendments

To investigate the effects of amendments, a total of 20 studies lasting 6 months were conducted, numbered consecutively in Roman numerals as presented under Sections 3.3.1a, 3.3.1b and 3.3.1c, respectively. The amendments included simple carbon and nitrogen compounds, and complex organic matter with varying nitrogen, as well as alkaline sandy loam.

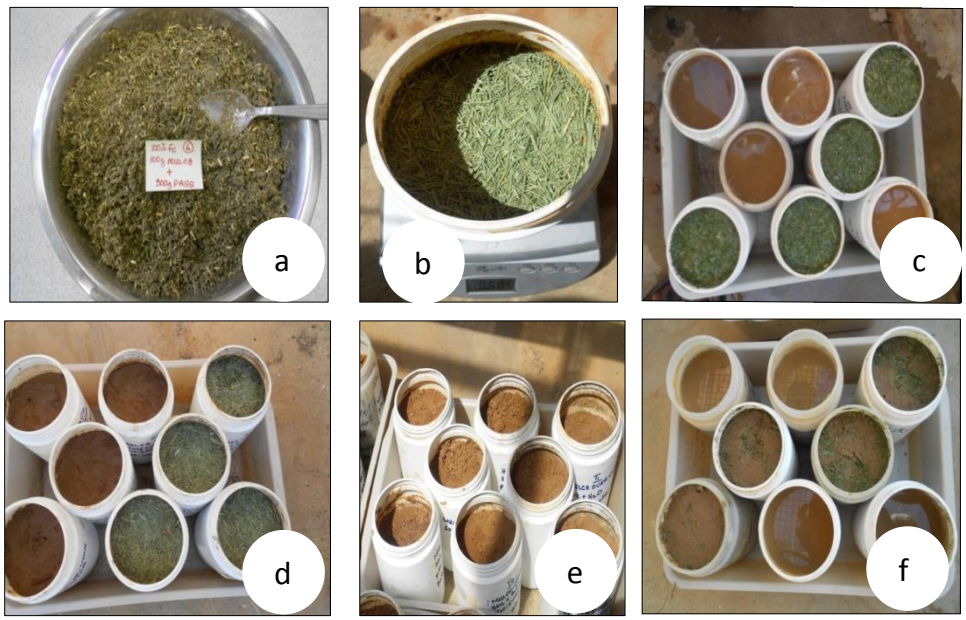

Figure 3.6. Processes of organic matter addition in small pots (140 mm high, capacity 1.1 L): (a) incorporation by bulk mixing, (b) overlaying as surface mulch, (c) organic matter overlaid under anaerobic (flooded) and (d) under aerobic (75% field capacity) conditions, (e) organic matter incorporated under aerobic and (f) under anaerobic conditions.

3.3.1a Effects of complex organic matter

To investigate the effects of complex organic matter on ASS chemistry, 12 studies were conducted (Table 3.2). In studies [I], [II] and [III], the neutralised sulfuric soil (described in Section 3.1.4) was used, whereas in [IV] sulfidic soil from Finniss was used instead. Both the neutralised soil and Finniss soils' chemistry (initial pH_w and pH_{ox}) were similar (see Table B1). Studies [V] to [XII] were conducted using Finniss ASS. In studies ([I] to [VIII], *Phragmites* leaf was used as the complex organic matter source.

Table 3.2. Investigating the effects of complex organic matter. Organic matter was either overlaid on the surface or incorporated in the soil.

Study	**Descriptions of treatments**							
	[1]Soil	**Origin**	**pH_w**	**pH_{ox}**	**Composition**		**Mc**	**Ap**
[I]	NS	NS	6.7	2.8	Soil: *Phragmites* leaf	90:1	Ae	Ol
[II]	NS		6.7	2.8	Soil: *Phragmites* leaf	90:1		In
[III]	NS		6.7	2.8	Soil: *Phragmites* leaf	90:1	An	Ol
[IV]	Sulfidic	FR	6.6	1.4	Soil: *Phragmites* leaf	80:1		In
[V]	Sulfuric		3.8	2.7	Soil: *Phragmites* leaf	80:1		In
[VI]	Sulfuric		3.8	2.7	Soil: *Phragmites* leaf	80:1		Ol
[VII]	Sulfuric		3.8	2.7	Soil: *Phragmites* leaf	80:1	Ae	In
[VIII]	Sulfuric		3.8	2.7	Soil: *Phragmites* leaf	80:1		Ol
[IX] & [X]	Sulfuric		3.8	2.7	Soil: Lucerne hay Soil: Pea straw Soil: Wheat straw	80:1	Ae & An	In
[XI] & [XII]	Sulfidic		6.7	1.5	Soil: Lucerne hay Soil: Pea straw Soil: Wheat straw	80:1		

Explanation: Soils are of the neutralised sulfuric soil (NS) or Finniss River (FR). Soil: organic matter (composition, g w/w), moisture content (Mc) at 75% field capacity as aerobic (Ae) and flooded as anaerobic (An), organic matter application techniques (Ap) as overlying (Ol) or incorporated (In). Studies [I] to [III] were set in small pots (see Fig. 3.6) and the rest in 70 ml Falcon (110 mm high, capacity 70 mls) tubes. Note: The slight differences in amount of soil were dictated by size of the small pots or Falcon tubes.

[1]Table 3.1 provides an explanation for labelling / classifying these soils as a "Sulfuric Soil or Sulfidic Soil and their equivalent soil classifications using Soil Taxonomy (Soil Survey Staff, 2014) and the Australian ASS classification key (Fitzpatrick et al., 2008; Fitzpatrick, 2013).

In addition, studies [IX], [X], [XI] and [XII] investigated the effects of lucerne hay, pea straw and wheat straw incorporated in both soil types. In all the studies, soil: organic matter ratio was obtained by weighing the exact amounts of each and incorporated by bulk mixing (Fig. 3.6a) or overlying (Fig. 3.6b). Studies undertaken under aerobic conditions (75% field capacity) in Falcon tubes or small pots and were maintained on weight basis using Eqn. 3.4 by adding water. The anaerobic conditions were obtained by allowing adequate amount of water to pond (flood) over the soil (Fig. 3.6c).

3.3.1b Effects of simple carbon and nitrogen compounds

To investigate the effects of carbon and nitrogen, a total of 6 studies were conducted (Table 3.3). The treatments comprising simple carbon and nitrogen compounds were weighed and mixed into the soil. A complex organic matter with high nitrogen content was included in studies using sulfuric soil to compare the results. The effect of the simple nitrogen compounds on sulfuric soil was investigated by studies [XIII] and [XIV] and on sulfidic soil by [XV] and [XVI].

Studies [XVII] and [XVIII] investigated the effects of simple carbon compounds on sulfuric soil alone under aerobic and anaerobic soil conditions. All the studies were conducted under both aerobic and anaerobic conditions. In addition, two studies were conducted to investigate the time-course effect of simple carbon sources and complex organic matter. The first study was set under aerobic conditions and the second study under anaerobic conditions respectively. These two studies are separately described in Chapter 4, Section 4.2.3.

Table 3.3. Investigating the effects of simple carbon and nitrogen compounds. A complex organic matter source with high nitrogen was included to compare the results.

Study	Descriptions of treatments							
	[1]Soil	Origin	pH_w	pH_{ox}	Composition (g)		Mc	Ap
[XIII]	Sulfuric	FR	3.7	2.2	Soil: Lucerne hay	80:1	Ae & An	In
&					Soil: $NaNO_3$	40:0.303		
[XIV]					Soil: Urea	40:0.214		
					Soil:NH_4Cl	40:0.193		
[XV]	Sulfidic	FR	6.7	2.3	Soil: Urea	40:0.214	Ae	
&					Soil: $NaNO_3$	40:0.303	&	
[XVI]					Soil: NH_4Cl	40:0.193	An	
[XVII]	Sulfuric	FR	3.7	2.2	Soil: *Phragmites* leaf	50:5		
&					Soil: Glucose	50:4	Ae	
[XVIII]					Soil: $C_2H_3NaO_2$	50:4	&	
					Soil: Molasses	50:4	An	

Explanation: Urea contains both C and N and molasses may contain small amount of nitrogen (Professor Emeritus M. J. Goss, University of Guelf, Canada, 2014 email communication). Explanations of descriptions not given are in Table 3.2.

[1]Table 3.1 provides an explanation for labelling / classifying these soils as a "Sulfuric Soil or Sulfidic Soil" and their equivalent soil classifications using Soil Taxonomy (Soil Survey Staff, 2014) and the Australian ASS classification key (Fitzpatrick et al., 2008; Fitzpatrick, 2013).

3.3.2 Studies investigating the effects of live plants

As summarized in Table 3.4, a total of 13 studies lasting 11 months were conducted to investigate the long-term effects of live plants on ASS chemistry, in the absence of added organic matter, except studies [PIV] and [PXI] as described below. Plants were harvested after 12 months. In all the studies, plants were established in 50 cm high stormwater tubes with caps tightly screwed at the bottom end. The bottom 22 cm of the tubes was filled with sand and the top 22 cm with 1300 g of ASS (either sulfidic or sulfuric) except in [PI] and [PII] where the whole tubes were filled. The top end of 5 cm was left unfilled for watering and maintenance.

The procedures established for the plant trials are presented in Fig. 3.7 and the types of plant population established shown in Fig. 3.9. The plants chosen were common

inland (upland) and wetland (coastal) species. The inland plants included lucerne as forage, barley and wheat as crop and four tree species (Table 3.4). *Phragmites* and *Typha* are common wetland plants. Studies [PI] and [PII] investigated the effects of plants on the neutralised sulfuric soil chemistry (Chapter 7). Studies [PIII]*, [PIV], [PV] and [PVI] investigated the effects of plants on sulfidic while [PVII], [PVIII] and [PIX] on sulfuric soils respectively under aerobic conditions. Studies [PX]* and [PXI] investigated the effects of plants on both ASS types under anaerobic soil conditions. The results of studies [PIII] to [PXI] are presented in Chapter 6.

Table 3.4. Studies investigating the effects of selected common forage, crop, inland and wetland plants on ASS chemistry.

Study	**Descriptions of treatments**				
	Soil	**pH_w**	**pH_{ox}**	**Plant species**	**Mc**
[PI]	NS	6.7	2.8	Lucerne	Ae
				Wheat	
[PII]		6.7	2.8	Allocasurina	
				Eucalyptus	
				Melaleuca	
[PIII]*	Sulfidic	6.6	2.7	*Phragmites*	
[PIV]		6.1	2.0	Melaleuca	
[PV]		6.1	2.0	Typha	
[PVI]	Sulfuric	3.9	2.1	*Phragmites*	
[PVII]		3.6	1.6	Melaleuca	
[PVIII]		3.6	1.6	Typha	
[PIX]		3.9	2.1	*Phragmites*	An
[PX]*		4.2	2.4		
[PXI]	Sulfidic	6.8	3.3		

Explanation: The studies were set using Finniss soil. Note: Explanations of descriptions not given are in Table 3.2. The studies highlighted with an asterisk are further explained below.

Only the plants in study [PI] were fertilized with 25 mls of 2 x Hoagland Solution once every month to boost plant growth. To ascertain whether fertilizing had an effect on soil pH alone, non-planted treatments receiving the same amount of nutrient solution were included in study [DIII]* (Table 3.5).

Studies [PIII]* and [PX]* assessed the combined effect of organic matter and plants following the addition (incorporated) of chopped *Phragmites* leaves (80:1, soil: organic matter *w/w*) under both aerobic and anaerobic soils conditions. To achieve this, two sets of treatments of each study were prepared. The first set investigated an inland scenario where organic matter is incorporated and plants established under aerobic conditions. The second set was kept under flooded condition in a pond (Fig. 3.8g) to investigate a wetland scenario.

In the studies shown in Table 3.4, lucerne and barley were established by seeds and the trees from seedlings (e.g. Fig. 3.7f). *Phragmites* and *Typha* were raised by rooting parent stocks in a medium (compost: sandy loam 2:1) and germinating young shoots (Fig. 3.7d, e). The parent stocks of both *Phragmites* and *Typha* were collected along the Torrens River near Adelaide, South Australia. Figure 3.10k-l describes how root biomass was quantified to assess the effects of live roots on ASS chemistry.

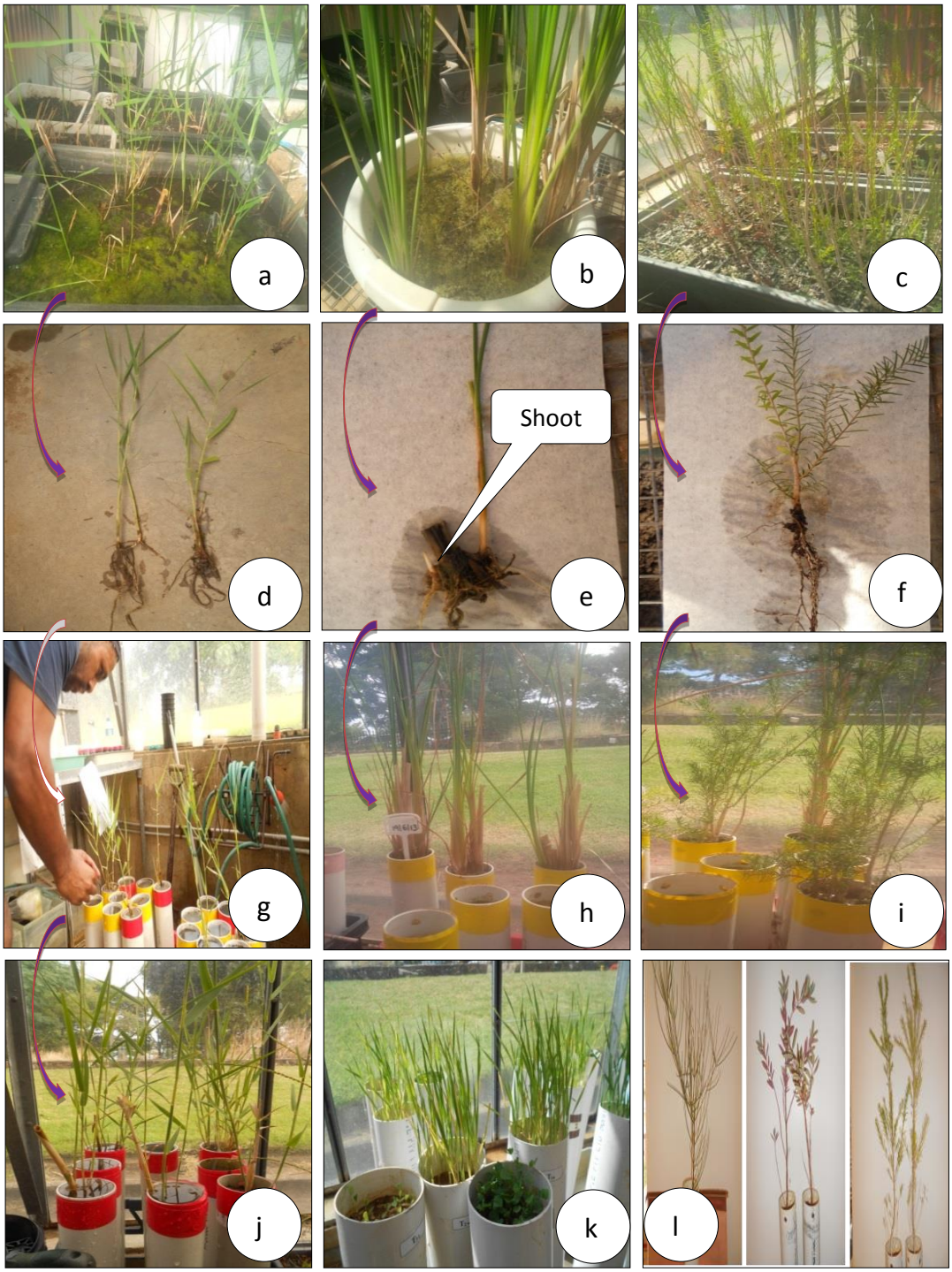

Figure 3.7. The procedure developed for the establishment of seedlings and plantlets for the plant-based studies (Table 3.4). Rooting (a) *Phragmites* and (b) *Typha* from parent root stocks, (c) seedlings raised in a seedbed, (d, e and f) well rooted *Phragmites, Typha* and *Melaleuca* plantlets isolated prior to transplanting, (g) transplanting in tubes; (h) *Typha*, (i) *Melaleuca*, (j) *Phragmites*, (k) wheat and lucerne and (l) from left to right *Allocasurina*, *E. calycogona* and *M. amillaris* plants growing on treatment soils.

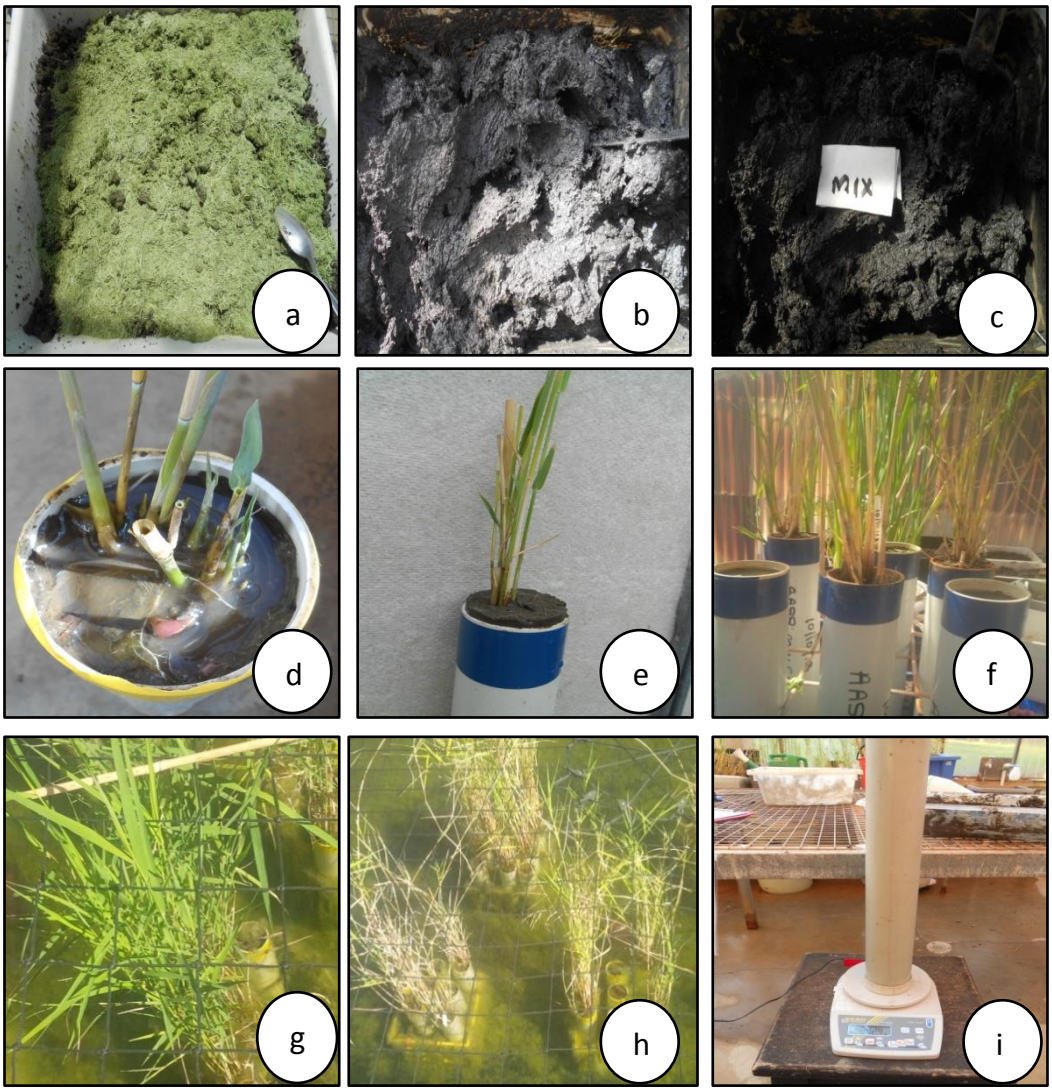

Figure 3.8. Investigating the combined effect of organic matter and plants under aerobic and anaerobic (flooded) conditions: (a) bulk mixing of organic matter, (b) sulfuric soil mixed with organic matter, (c) sulfidic soil mixed with organic matter, (d) well rooted *Phragmites* parent stocks with young shoots transplanted, (e) a replicate of a treatment with plants under aerobic condition showing the soil being pushed up by an unidentified gas (methane or hydrogen sulfide) two weeks after transplanting, (f) and (g) fully grown plants growing under aerobic and flooded conditions, respectively and (h) plants under flooded condition at the time of harvest. Notes: (1) The sulfuric soil after mixing with organic matter was brown (b) and the sulfidic soil completely black (c), and (2), equal amounts of the unamended (control) and mixed soils were placed in each tube by weighing (i). All the soils are from Finniss River.

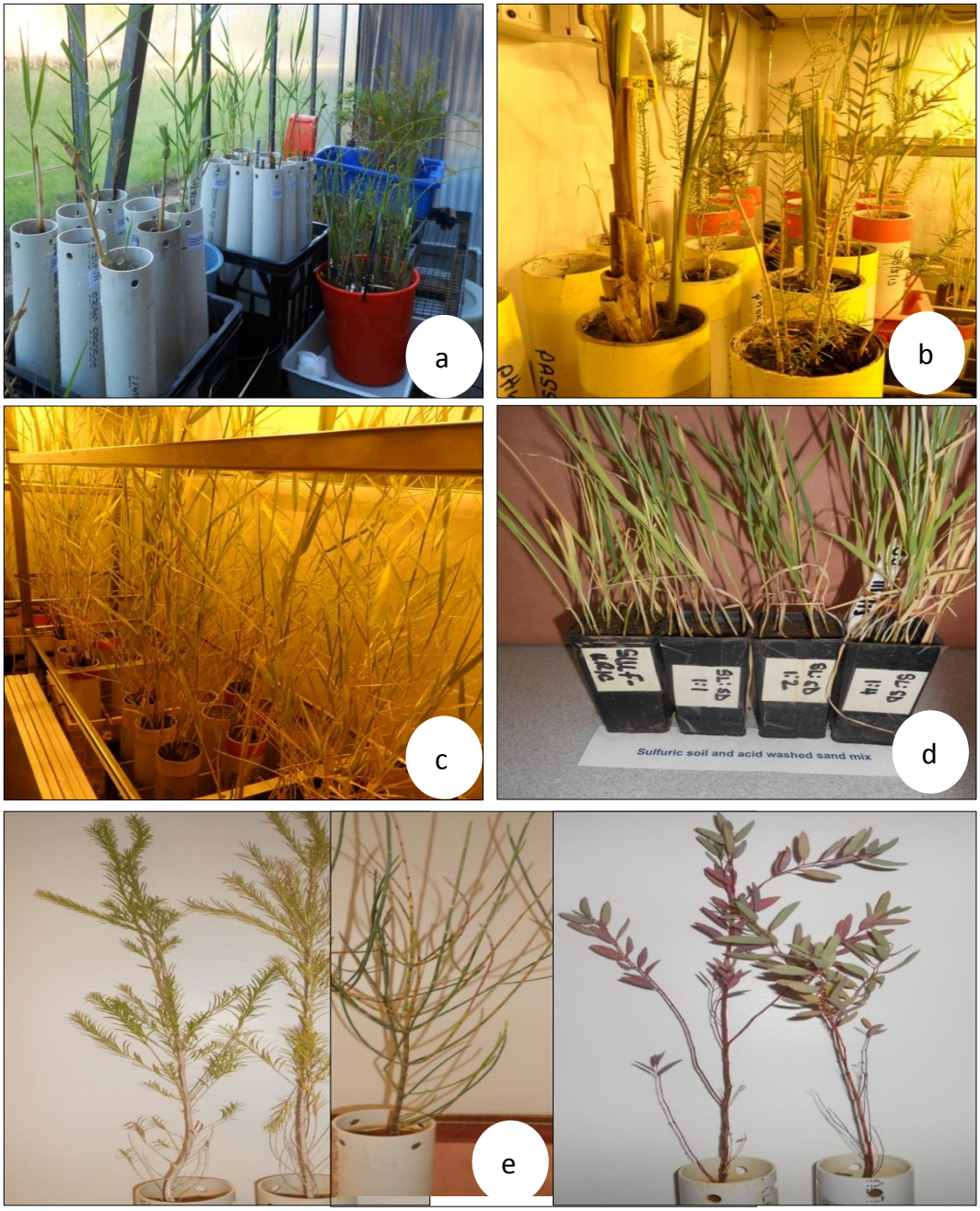

Figure 3.9. Photos showing the growth of the different plants established in the plant-based studies: (a) *Phragmites* growing in a glasshouse, (b) *Typha* and *Melaleuca*, and (c) *Phragmites* plants maintained in a growth room during winter, (d) barley on ASS: sand mix, and (e) *M. armillaris* (left), *Allocasurina* sp. (middle) and *E. calycogona* (right) plants. Plants in (a) were approximately 4 weeks old, (b) 6 weeks, (c) 6 months, (d) 6 weeks and (e) 12 months.

3.3.3 Effects of dead roots

Since annual plants will have two different phases in soil, i.e. live roots while growing and dead roots at the end of the season, further experiments were conducted with pots in which the plant residue remained after the death of the plant. The studies presented in Table 3.5 investigated the effects of dead roots on ASS chemistry. In each treatment, a total of 40 seeds were sown. Sample photos showing the type of plant population established and how live root biomass of the plant-based studies was quantified are shown in Fig. 3.10. To ensure the changes in soil chemistry measured were caused by the dead roots, dead plant materials that have fallen on the surface were regularly removed. To ensure the dead roots were complete decomposed, measurements were made after 12 months.

Table 3.5. Investigating the effects of dead root on ASS chemistry.

Study	**Descriptions of treatments**						
	Soil	**Composition (g)**		**pH_w**	**pH_{ox}**	**Plant**	**# of plants**
[DI]	Sulfuric	Sulfuric: sand	1:0	3.9	2.1	Barley	33
			1:1	4.0	2.2		37
			1:2	4.1	2.1		35
			1:4	4.1	2.2		40
[DII]	Sulfidic	Sulfidic: sand	1:0	6.8	2.3		39
			1:1	7.0			40
			1:2	7.0			38
			1:4	7.0	2.2		40
[DIII*]	Sulfuric	Sulfuric: sand	2:1	4.0	2.1	Unplanted and fertilised	
	Sulfidic	Sulfidic: sand		6.9	2.5		

Explanation: Total number of plants (population) was counted at maturity (after 4 months). Explanations of descriptions not given are in Table 3.2. As shown in Fig. 3.10, the studies were conducted in small pots. Study [DIII*] was described under Section 3.3.2 using Gillman ASS.

Figure 3.10. Investigating the effects of dead and live roots on ASS chemistry. Photos showing (a) sulfuric control without plants, barley plants of [I] growing on (b) sulfuric and (c) sulfidic soil 3 months after germination, (d) wheat and (e) lucerne plants of [II] (Table 3.6), (f) dead barley plants of [I] after 6 months, (g) and (h) show the early stages of barley growth in sulfuric and sulfidic soil, respectively. To assess the effects of live roots, roots in different profiles were carefully: (i) collected, (j) stumps removed and (k) washed and placed in weighing boats, oven dried overnight at 60˚C and (l) weighed to quantify the biomass produced on dry weight basis. Note that the stormwater tubes were cut at specific profiles (i.e. 20 mm at the surface then at every 50 mm thereafter to 200 mm) to collect the roots. Photo Fig. 3.13a shows how the cutting was done, either to obtain the roots or soil samples.

3.3.4 Effects of soil moisture content on oxidation of sulfidic soil

The effect of moisture on sulfidic soil oxidation and resultant production of sulfuric soil (acidity) was investigated using Gillman sulfidic soil (pH_w 6.0 and pH_{ox} 2.6) set at different field capacities (Table 3.6). Initially, 100 g soil (80 g soil and 20 g water) previously set at 100% field capacity were placed in square petri dishes (10 mm x 10 mm) by weighing, forming a thickness of 10 mm.

Table 3.6. Investigating the effects of moisture on sulfidic soil oxidation.

Treatments	Descriptions of the treatments and moisture conditions				
	Fc	Initial weight	*Water	Water	Final weight
	(%)	(g)	(ml)	(%)	(g)
(i)	0	100	-20	0	80
(ii)	25	100	-15	5	85
(iii)	50	100	-10	10	90
(iv)	75	100	-5	15	95
(v)	100	100	0	20	100
(vi)	150	100	+10	30	110
(vii)	200	100	+20	40	120

*All treatments were initially at 100 g field capacity (Fc). Lower water contents were achieved by natural evaporation, and higher water contents by addition. The notation – or + means water was either removed by drying down or increased by adding water.

To attain the field capacities, treatments (i) to (iv) were dried down to final weight of 80, 85, 90 and 95 g, leaving the soils at 0%, 25%, 50% and 75% field capacity. Treatment (v) was left at 100%, while (vi) and (vii) were rewetted by adding 10 and 20 mls of water, making the soils to be effectively at 100%, 150%, and 200% field capacity. When the desired water content was achieved, dishes were sealed with insulation tape.

To assess the effect of temperature variation on sulfidic soil oxidation and acid production, such as during warm summer and cooler winter, the treatments were prepared in two sets. The first set was incubated at 25˚C (room temperature) and the second at 4˚C in a cold room. To measure the changes in pH as evidence of acid production, a 2 g soil was sampled at random transects of equidistant (3 mm apart) and pH_w measured.

In addition to the studies described in Tables 3.3 and 3.6, effects of moisture on sulfidic soil chemistry in the presence of appropriate organic substrates investigated using simple carbon compounds are presented in Table 3.7. Study [MI] was conducted to assess the long-term effects of simple carbon compounds under anaerobic conditions. Study [MII] was designed to investigate the time-course effects on ASS chemistry following the addition of simple carbon compounds and complex organic matter after 3, 6, and 12 weeks under both aerobic and anaerobic conditions.

Table 3.7. Investigating the effect of moisture and amendments on sulfidic soil oxidation.

	Descriptions of the treatments				
Study	**Origin**	**Treatment**	**Composition**	**Total (g)**	**Moisture**
[MI]	FR	Glucose	40:4	44	An
		Acetate	40:4	44	
		Sodium sulfate	40:4	44	
		Molasses	40:4	44	
[MII]	FR	Glucose	50:5	55	Ae & An
		Sodium acetate	50:5	55	
		Lucerne hay	50:5	55	

Explanations: Composition is soil: simple carbon sources or soil: organic matter. “FR” is Finniss River.

3.4 Redox Potential Measurement

In practice, reduction-oxidation potential (Eh) is measured by installing electrodes such that O_2 penetration from the soil surface to the electrode tip is minimised and the wire is in direct contact with the natural soil by the way of direct insertion, rigid rod or slurry-seal method using working and reference electrode, connected to a voltmeter or pH meter (Pearsall and Mortimer, 1939).

In this research, however, Eh was measured using an Ag/AgCl reference and Platinum (Pt) electrode combination (shown in Fig. 3.11), whose accuracy was tested using standard test solutions as per Fiedler *et al*. (2007). For more details on the construction of the Pt electrodes and data logger used in all experiments, see Thomas (2010), Dowley et al. (1998) and Merry et al. (2002).

Prior to measurements, the Pt electrode was marked on the frame at intervals from 10 to 100 mm (tip to end). During measurements, the reference electrode was securely inserted into the surface of the soil and allowed to remain inserted throughout. The Pt electrode, however, was inserted variably using the marks as guides. A small amount of deionised water was periodically added on the soil surface to maintain closed circuits during measurements. Contaminants and surface coatings on the tip (wire) in contact with soil were cleaned (removed) using a scouring pad and a steel wool pad by rubbing, and rinsed several times in deionised water, after each measurement (Delaune and Reddy, 2005). Alternatively, the tip was immerged in 0.5M HCl for 1 min and subsequently rinsed in deionized water as per Ponnamperuma (1972).

The reliability of the electrodes was additionally tested in ZoBell and saturated quinhydrone (pH 4.0 and 7.0) solutions as a standard redox electrode test at the beginning of each measurement. Tests in running tap water and 4 M KCl were also done regularly to confirm the accuracy of the electrodes. Eh values were compared to standard solution values as a function of daily temperature (Fiedler et al., 2007). The variations recorded were < 5 mV, indicating the electrodes were accurately working during measurements (Delaune and Reddy, 2005).

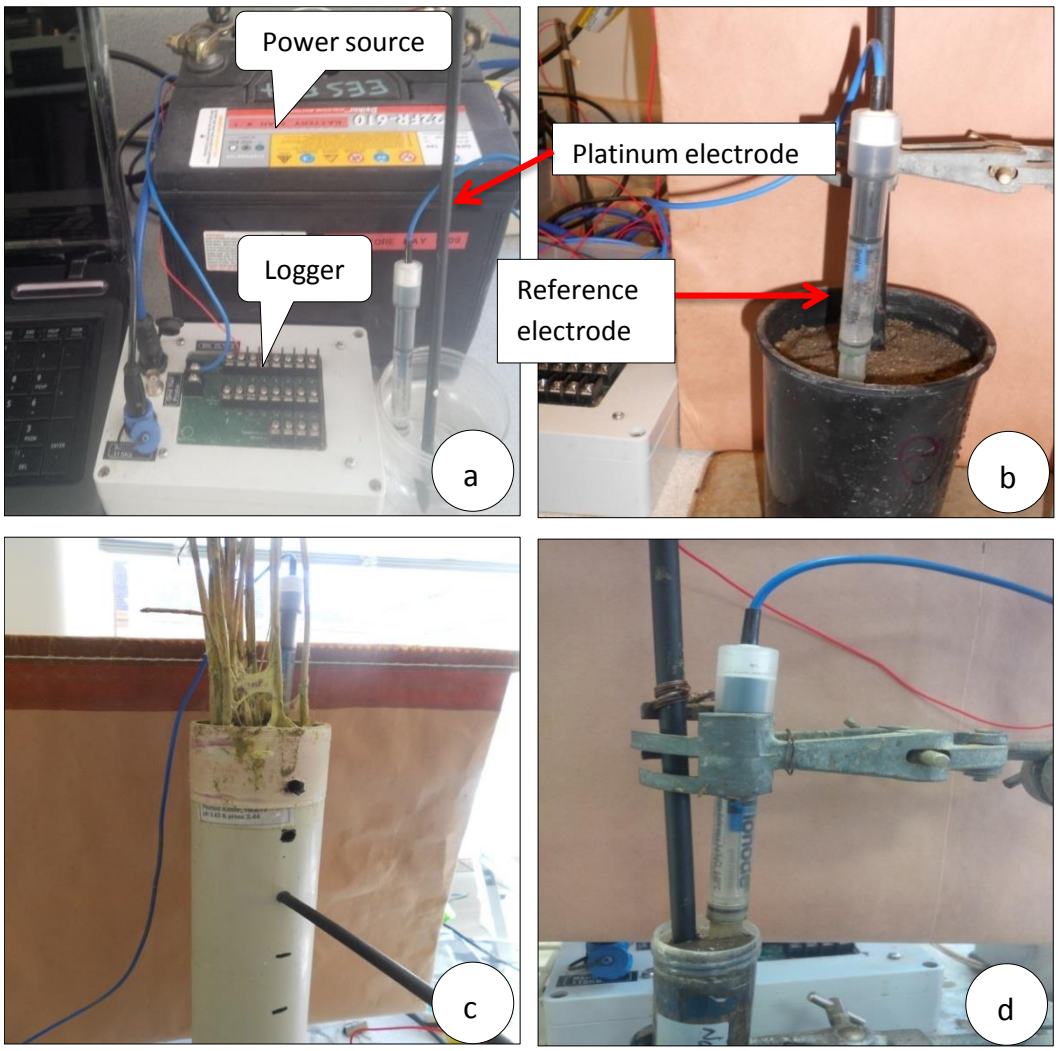

Figure 3.11. Setup of the Ag/AgCl reference and Platinum electrode combination used in redox measurement: (a) a 12 V car battery as a power source, the electrode combinations and the data logger, measurement in (b) a small pot, (c) stormwater tube and (d) Falcon tube, respectively. Note the Pt electrode was inserted from the sides in (c) while in (b) and (d) from the top down.

Prior to the measurements, the electrodes were connected to a data logger (SN: CLW 3, baud rate 115 kb), which was connected to a 12 volt car battery (Delkor, Delkor Corporation), and a laptop, preinstalled with the logger's program (Fig. 3.11a). As a standard procedure, the electrode tip was placed in the soil and allowed to equilibrate for 10 min (Rabenhorst et al., 2009) and Eh measured in the next 10 min. The data uploaded were saved as Microsoft Excel files.

In this research, a single redox electrode combination was used, based on the fact that the soils were pre-processed (homogenized by mixing and washed to remove coarse materials to create uniformity, received similar kinds of treatments and experimental

conditions under which the soils were subjected to were fairly uniform throughout, minimising significant variability to exist within profiles). The use of the same electrode on replicates and different treatments ensured that relative changes in Eh were accurate. The effects of daily temperature on Eh measured were avoided by comparing the 'Eh of the ZoBell's solution as a function of temperature' and confirmed with the daily temperature reading of a laboratory based handheld thermometer. For that reason, temperature effect on the Pt electrode, and hence on the Eh, is considered negligible (Delaune and Reddy, 2005).

During measurements, slight variations existed between studies due to the type of materials (stormwater tubes, Falcon tubes or small pots) in which trials were set. Treatments set in Falcon tubes or small pots were measured from the top soil surface by inserting the marked platinum tip into the soil profiles as described (Fig. 3.11 b and d). In order to measure Eh in stormwater tubes (Fig. 3.11c), the tubes were marked out from top to bottom at 20, 50, 100, 150, 200, 250 and 300 mm (Fig. 3.13a). A handheld electric drill, with a drill bit head the size of the Pt electrode was used to make holes through the tubes (Fig. 3.13a) with care taken to avoid disturbing the soil. To measure Eh, about 2 cm of the Pt frame towards the tip was wrapped several times using parafilm as to form a knot. During measurements, the Pt electrode was inserted through the holes (Fig. 3.11c), the knot forming a tight seal and minimising O_2 entry, while the tip (wire) was directly inserted into the undisturbed natural soil.

In all the studies, Eh was measured from the 0-10, 10-20, 20-40, 40-60 and 60-80 mm profiles of the studies conducted in Falcon tubes and small pots. For the plant-based studies conducted in stormwater tubes presented in Chapter 6, measurements were made from the 0-20, 20-50, 50-100, 100-150 and 150-200 mm profiles. Of the plant-based studies conducted in the neutralised soil presented in Chapter 7, measurements were made up to the 250-300 mm profiles. The data collected from the 0-20, 50-100 and 150-200 mm are used in Chapter 6 and to 250-300 mm in Chapter 7, respectively.

Based on the Eh values measured, redox conditions of the treatments in this research were categorized as: (i) oxidized (≥ +300 mV), (ii) moderately reduced (+300 to 0 mV), (iii) reduced (0 to -100 mV), or (iv) highly reduced (-100 to -400 mV) as per Fiedler *et al*. (2007).

3.5 Soil pH measurement

A range of methods have been developed in order to identify and measure acid generation potential of ASS but the most realistic method to estimate whether a soil will acidify is still debated (Thomas, 2010). In addition, methods considered suitable vary depending on soil types and associated minerals (e.g. jarosite, sideronatrate, and Schwertmannite). In ASS, the widely acceptable methods are pH_w, pH_{ox}, acid-base accounting, and $pH_{incubation}$ (aging) using chip-tray (Fitzpatrick et al., 2010) as described previously.

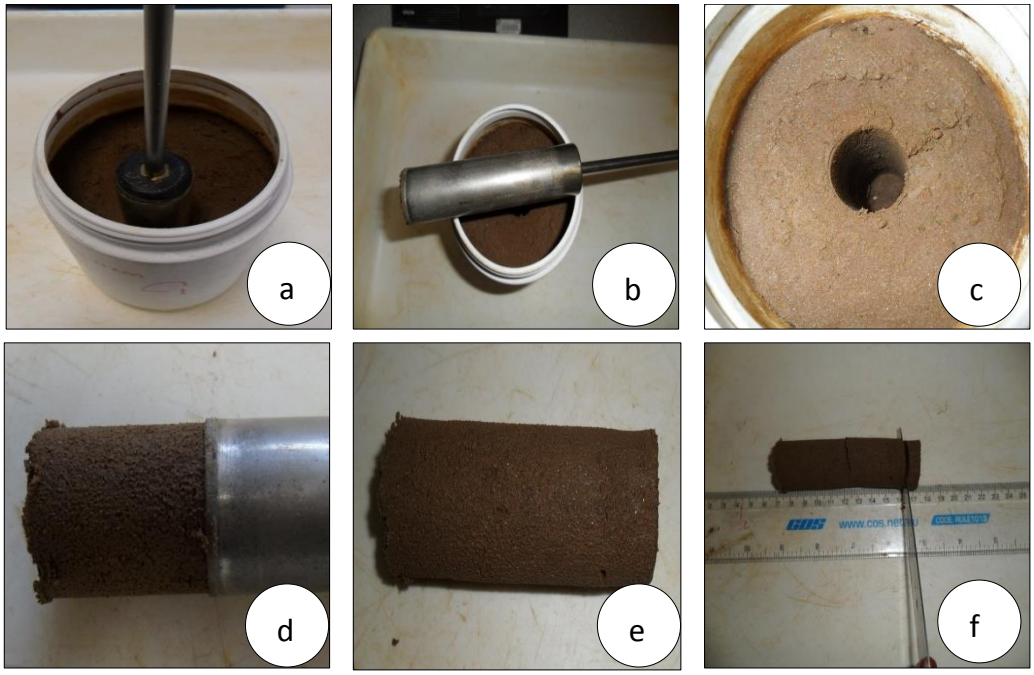

Figure 3.12. Sampling soil core from small pots: (a) inserting a core sampler, (b) core sampler with intact core, (c) surface hole showing size of the core sampled, (d) taking a soil core out of the sampler by gently pushing it at one end, (e) an intact core, and (f) the sampled core placed against a ruler to be cut at depths Eh have been measured.

In this research, pH_w measurement by suspending soil in water (1:5; w/w) (Ahern et al., 2004; Lin et al., 2000; Reid and Butcher, 2011; Sullivan et al., 2002; Sullivan et al., 2009) and pH_{ox} measurement after peroxide treatment (Ahern et al., 2004) were used as the standard methods to identify ASS and the potential of sulfidic soil to generate acidity. The chip-tray method (Fitzpatrick *et al.*, 2009a) was used to assess acid generation potential of the Gillman sulfidic soils after 12 weeks of incubation ($pH_{incubation}$). As a standard procedure, all solutions were stirred to dissolve soil constituents and allowed to

settle for 30 min prior to the measurements. Both pH_w and pH_{ox} were measured using an electrode of a pre-calibrated (lower and upper limits set at 4.1 and 7.0) Orion pH meter (720SA model).

Figure 3.13. Preparation of soil for pH_w measurement: (a) a stormwater tube containing treatment soil marked at profiles to measure Eh, (b) cut Falcon tubes and (c) stormwater tubes cut into sections as per the depths at which Eh was measured for soil sample collection for pH measurement, and (d) homogenising soil sample prior to pH measurements. The diameter of a Falcon tube and the core sampler (Fig. 3.12) are the same size (approx. 25 mm and 28 mm, respectively). Note that the stormwater tubes (c) were only used in the plant trials (Section 3.3.2).

As shown in Figs. 3.12 and 3.13, pH was measured from the same profiles at which Eh was measured. To measure pH of soil in small pots (Fig. 3.12), a metallic core sampler was manually driven into the bottom end of the soil and an entire core with the intact soil was carefully taken out. The sampled core was laid out on a flat surface along a 300 mm

ruler and cut into small sections as per the profiles at which Eh had been measured. To measure pH of soils in stormwater tubes or Falcon tubes (Fig. 3.13), the tubes were marked out as described previously and carefully cut into small sections. Finally, 2 g soil (*n*=3) from the 0-10, 10-20, 20-40, 40-60 and 60-80 mm profiles of studies conducted in Falcon tubes and smaller pots, and from 0-20, 20-50, 50-100, 100-150 and 150-200 mm of the plant-based studies established in the stormwater tubes was taken one at a time, gently mixed (Fig. 3.13d) so as to homogenize and pH_w measured. Data from the same profiles as for the Eh are presented in Chapters Six and Seven.

3.6 Quantification of Sulfate Content

To assess bacterial reduction of sulfate in studies where the redox was sufficiently reduced, it became necessary that sulfate content at various profiles be quantified. Soil was sampled from the surface (0-10 mm), middle (20-40 mm) and depth (60-80 mm) of selected studies conducted in Falcon tubes and small pots, and from the surface (0-20 mm), middle (50-100 mm) and depth (150-200 mm) of the plant-based studies established in stormwater tubes. These were dried at 60^0C overnight and sulfate was extracted according to the methods of Hoeft *et al*. (1973) for soluble soil sulfate. Replicate samples of (0.5 g dried soil each) were placed in tubes with 1.5 ml of an extraction solution (made of 0.2 g $Ca(H_2PO_4)_2$ in 88.5 g of deionised water and 12 ml of glacial acetic acid). The soil samples were mixed by vortexing then left for 30 min. The solution was vortexed again to mix and centrifuged at maximum speed for 5 min on an Eppendorf Microfuge. Duplicate 0.5 ml aliquots of each extracts were transferred into 4 ml cuvettes then diluted with 1.5 ml of the extraction solution.

To quantify the sulfate extracted, a standard curve (e.g. shown in Fig. 3.14) in the range of 0 to 2 mM SO_4 was constructed by adding between 0 and 2 ml of 2 mM Na_2SO_4 and the final volume made to 2 ml with extraction solution. The sample extracts and the standards were diluted with 0.7 ml of 0.5 M HCl and 0.7 ml of barium chloride-polyethylene glycol reagent, mixed between additions and left for 10 min, mixed again and absorbance read at 600 nm using a spectrophotometer.

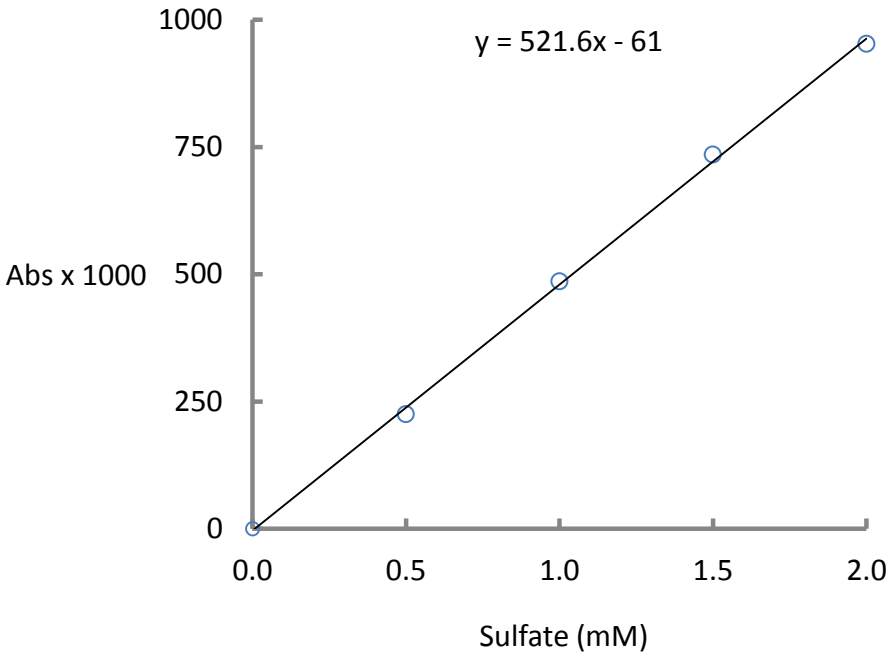

Figure 3.14. Standard curve for estimating sulfate contents of soils.

3.7 Presentation of Data and Statistical Analysis

Unless otherwise described, all the treatments of the studies were replicated three times and set in a completely randomised design (CRD), with soils either 'without amendments or plants' as control and with 'amendments or plants' as treatments, respectively. To clearly show the changes in soil chemistry of studies conducted in Falcon tubes or small pots, data collected from the surface (10 mm), middle (40 mm) and depth (80 mm) of the aerobic studies and only the surface and depth of the anaerobic studies are presented rather than whole data collected throughout the profiles (10, 20, 40, 60 and 80 mm). The reason for presenting the surface and depth soil data is that if there was oxygen penetration under the flooded soil conditions, then only the surface soil would get oxidised than at depth, clearly showing the trends the changes in soil chemistry. For the studies conducted in 45 mm stormwater tubes, data collected from the surface (20 mm), middle (40 or 100 mm) and depth (200 or 300 mm) profiles.

The Eh values obtained over the 10 min were averaged and a treatment average obtained by taking the mean of the three replicates (n=3). These values were corrected for the reference offset to be relative to the potential of a standard hydrogen electrode by adding 200 mV (Fiedler et al., 2007). Similarly, treatment average pH and sulfate was

obtained by taking the mean of the three replicates.

In order to compare the treatment means, significant differences ($p<0.05$) between treatments means of each profile was compared by two-way ANOVA using statistical software JMPIN, AS Institute Inc., SAS Campus Drive, Cary, NC, USA 27513. If an interaction between the treatments and profile depths was found, one-way ANOVA with all combination was performed using Tukey's HSD (honest significant difference) and pairwise comparisons.

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The importance of soil carbon and nitrogen for amelioration of acid sulphate soils

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Abstract
When exposed to air and adequate moisture, soils containing sulphides (Sulphidic soils with pH >4) become oxidised and generate sulphuric acid to form "Sulphuric soils" (ph <4). Treatment of this sever acidity (pH <4) is commonly done by addition of lime. In this study we investigated the effectiveness of adding plant organic matter, and simple carbon and nitrogen compounds, as alternatives to lime to Sulphuric and Sulphidic soils. In Sulphuric soils under aerobic conditions, plant organic matter increased pH, the extent depending on the nitrogen content. Lucerne hay, which had the highest nitrogen content, increased the pH from 3.7 to 8.0 while pea straw and wheat straw effected smaller changes, in proportion to their respective nitrogen contents. Lucerne hay also caused the greatest reductions in soil redox potential and sulphate content, consistent with the action of sulphate reducing bacteria. The individual effects of carbon and nitrogen compounds were then examined and compared to plant organic material. Glucose was ineffective at both low and high concentrations while molasses increased the pH slightly to 4.6 and acetate to 5.9. None of these simple carbon compounds was as effective as complex organic matter. Nitrogen added alone as nitrate or ammonia had little or no effect on pH whereas organic nitrogen in the form of urea caused the pH to rise to 6.3, reduced the redox to less than 0 mV but had no significant effect on sulphate content. Incorporation of plant organic matter to Sulphidic soils under aerobic conditions was also shown to be effective in preventing the acidification.

Keywords: ASS, simple carbon and nitrogen, complex organic matter, pH, redox

Introduction

Acid sulphate soils (ASS) are naturally occurring soils formed under anaerobic conditions (Pons, 1973). On estimation, ASS occupy 17-24 million hectares of the global soil of which 6.5 million occurs in Asia, 4.5 million in Africa, 3 million in Australia, 3 million in Latin America, 200 000 in Finland, 235 000 in Sweden and 100 000 in North America (Simpson & Pedini, 1985). ASS pose no threat unless exposed to atmospheric oxygen, which causes oxidation of sulphidic minerals (predominately pyrite, FeS_2) to form sulphuric acid. The sulphuric acid then lowers soil pH to < 4 (Fitzpatrick *et al.*, 2010), which in turn solubilises soil matrices. The solubilisation process leads to mobilisation and release of potentially toxic soil constituents which accumulate in soils and cause a range of adverse impacts, as summarized in recent reviews (e.g. Melville and White 2012, Michael 2013)

In places where ASS are present, appropriate management practices need to be employed to minimise soil oxidation and acid production (Melville and White 2012; Vegas-Vilarrubia *et al.* 2008). Strategies focusing on two key principles have been proposed, tested and established (e.g. Baldwin & Fraser, 2009; Fitzpatrick *et al.* 2011). The first principle is use of an alkaline material such as lime to neutralize the actual acidity and manage the by-products. The second is to prevent sulphidic soil containing oxidisable sulphidic minerals from exposure (Fitzpatrick et al., 2010), which can be achieved by inundation to restrict access to oxygen (Sullivan *et*

al., 1999). However, the latter process reduces the options for using the land for agricultural production.

While liming is effective, it is also expensive and therefore impractical in some situations such as: (i) in Ramsar wetlands even if lime is readily available, lime application is often not recommended because of the potential harm it may cause to such protected environments (e.g. benthic invertebrates) and (ii) in farming communities with poor economies (Hue, 1992; Shamshuddin *et al.*, 2004). As a consequence, other more feasible management options need to be investigated. One such option is the application of organic matter, which is relatively cheap and readily available. Farmers, for thousands of years have used plant organic matter to improve soil fertility, recycle nutrients, control weeds and pests and maintain moisture content. Several studies have also reported moderate increases in pH of mildly acidic soil following the addition of plant organic matter (Barrow, 1960; Yan *et al.*, 1996b; Reid & Butcher, 2011).

The alkalising effect of the added organic matter has been related to the total content of cations (Pocknee & Sumner, 1997). However, other studies found that addition of inorganic salts (e.g. Ca_2SO_4) had no effect, while organic compounds such as sodium malate, sodium citrate, calcium oxalate or calcium gluconate caused soil pH to rise (Yan et al., 1996b). Pocknee and Summer (1997) proposed biochemical decarboxylation of carboxylic groups of organic anions as the mechanism responsible, due to the consumption of protons in the decarboxylation process. However, other researchers (Ritchie & Dolling, 1985; Tang & Yu, 1999) pointed out that organic matter acidifies soil due to the release of protons associated with the organic anions.

Although ameliorative effects on acidic soils of addition of crude organic matter and organic salts have been observed, there is little understanding of the mechanism by which this occurs or of the most effective material to use. We have demonstrated in a recent study that incorporation of organic mulch in the form of *Phragmites* leaves, which has a high nitrogen content, effectively ameliorates acid sulphate soil, while leaf material applied only to the surface is partially effective (Michael *et al.*, 2014). 2015 In this study, we extend our investigation of this phenomenon by considering the independent roles of carbon and nitrogen compounds in the alkalinisation of ASS with sulfuric horizons (Sulfuric Soils) .

Materials and methods

Soils

Sulfidic material was collected from a "sulfuric subaqueous clayey soil" (Fitzpatrick 2013) at a depth of approximately 1 m in the Finniss River in South Australia (35˚24'28.28''S; 138˚49'54.37''E). Detailed information on the soil classification of this soil profile using the Australian Acid sulfate soil Identification key (Fitzpatrick et al., 2008; Fitzpatrick, 2013) and Soil Taxonomy (Soil Survey Staff, 2014) is given in Table 1. In addition, a list of comprehensive references, which contain further information on the soil morphology and geochemistry prior to rewetting (i.e. sites AA26.3 and FIN26 in Fitzpatrick et al. 2009a) and after reflooding can be found in Table 1. When the sample of sulfidic material was freshly collected in 2012, the pH measured in water 1:5 (pH_w) was 6.7. The water holding capacity of the sulfidic material when freshly collected was 49.2%. The residual organic matter (ROM) content, identified using weight loss-on-ignition (Schulte & Hopkins, 1996) was 10.6%. After peroxide treatment (pH_{ox}) the pH decreased to 1.4. The sampled sulfidic material was spread thinly on plastic sheets and kept moist in order to oxidise sulphides to produce sulphuric acid so as to manufacture "sulfuric horizon material". Henceforth this sample will be referred to as a "Sulphuric soil" (pH_w <4) or "Sulphidic soil (pH_w >4) when used to conduct soil organic matter experiments in 70 ml Falcon tubes. The initial pH measured in water (pH_w 1:5 w/w), and after hydrogen peroxide treatment (pH_{ox}), as well as the initial sulphate content range for each experiment is shown in Table 2.

Treatments

The composition of the three soil treatments is shown in Table 3. The amendments were uniformly mixed into the soil, which was then placed into 70 ml Falcon tubes. The dried plant material was chopped in an electric blender and passed through a 0.5 mm sieve before use. The soil was maintained at a moisture content of 75% field capacity for 6 months. However, because the tubes were not free draining, pH and Eh values measured near the bottom of the Falcon tubes were variable and so measurements in this paper

Table 1. Sampling location label and equivalent soil classifications of the acid sulfate soil used in this paper from the Finniss River

Soil Type[1]	Previous sampling location reference	Depth (cm bgl)	**Sulfuric horizon**[5]/*Sulfidic material*[6]	Soil Class[7]	Australian ASS classification key[8]	**Sulfuric horizon**[5]/*Sulfidic material*[6]	Soil Class[7]	Australian ASS identification key[8]
			Finniss River: Prior to rewetting (2009)			Finniss River: Post rewetting (post 2010)		
Sulphidic soil	FIN26 M3-4[2] FC10740[3] LF01-B[4]	0 - 5	**Sulfuric**	Hydaquentic Salfaquept	Sulfuric cracking clay soil	*Sulfidic*	Hydraquentic Sulfowassept	Sulfuric subaqueous clayey soil
		5 - 17	**Sulfuric**			*Sulfidic*		
		17 - 40	**Sulfuric**			**Sulfuric**		
		40 - 60	**Sulfuric**			**Sulfuric**		
		60 – 150	*Sulfidic*			*Sulfidic*		

1. Soil type label used in this paper when this layer of sulfidic material is used to conduct soil organic matter experiments in 70 ml Falcon tubes
2. Sampling location label used in (Fitzpatrick *et al.* 2009a; Fitzpatrick *et al.* 2009b)
3. Sampling location label used in (Fitzpatrick *et al.* 2011)
4. Sampling location label used in (Baker *et al.* 2013;)
5. Acid sulfate soil horizon (Soil Survey Staff 2014)
6. Acid sulfate soil material (Soil Survey Staff 2014)
7. Currently no subgroup exists in Soil Taxonomy (Soil Survey Staff, 2014) that adequately describes these Finniss River soils following their rewetting. They are best described as subaqueous soils with sulfuric horizons or "Sulfuric subaqueous clayey soils" in accordance with the Australian ASS classification key (Fitzpatrick et al. 2008; Fitzpatrick 2013). Consequently, the following new proposal is currently being submitted by Fitzpatrick and Grealish (personal communication) to USDA-NRCS to consider for inclusion in revised versions of the US Keys to Soil Taxonomy: (i) Inceptisol Suborder: Sulfowassepts, (ii) Inceptisol Great Group & Subgroup for Hydraquentic Sulfowassept .
8. Australian acid sulfate soil classification (Fitzpatrick *et al.* 2008; Fitzpatrick 2013)

are restricted to the surface layers of the soil the tubes. Each treatment was conducted in triplicate and the tubes arranged in a completely randomized design (CRD).

The following four experiments were conducted to examine changes in soil chemical parameters (pH, Eh and sulphate content) induced when:

1. Sulfuric soil was mixed with with plant material containing different nitrogen contents. Soil was incubated under aerobic (75% field capacity) for 6 months
2. Sulfuric soil was was mixed with nitrogen compounds or chopped of lucerne hay following incubation under aerobic conditions with a moisture content of 75% field capacity for 6 months plant material containing different nitrogen contents. Soil was incubated under aerobic (75% field capacity) for 6 months.
3. Sulfuric soil was was mixed with simple carbon compounds or chopped leaves of *Phragmites* following incubation under aerobic conditions at a moisture content of 75% field capacity for 6 months.
4. Sulfidic soil was was mixed with with plant material containing different nitrogen contents. Soil was incubated under aerobic conditions for 6 months (75% field capacity)

Measurements

Soil pH and Eh and sulphate content were measured at a depth of 10 mm where it could reasonably be assured that the soil was in free contact with air. Redox was measured using a single Ag/AgCl reference and platinum (Pt) electrode combination using an automated data logger (Dowley et al. 1998; Merry et al. 2002). The Pt electrode and reference were inserted into the soil and allowed to equilibrate for 10 min and then Eh measured at 1 min intervals for the next 10 min and averaged (Rabenhorst *et al.*, 2009). These values were corrected for the reference offset to be relative to the potential of a standard hydrogen electrode by adding 200 mV (Fiedler *et al.*, 2007).

The stability and accuracy of the electrodes were maintained as per (Fiedler *et al.* 2007). Redox conditions of the soils are categorized as: (i) oxidised (≥ +300 mV), (ii) moderately reduced (+300 to 0 mV), (iii) reduced (0 to -100 mV) and (iv) highly reduced (-100 to -400 mV) based on the Eh values measured as per (Fiedler et al., 2007). pH was measured using 2 g soil (1:5 water) with a pre-calibrated Orion pH meter (720SA model).

For sulphate estimation, replicate samples (0.5 g each) were placed in tubes with 1.5 mls of an extraction solution (0.2 g CaH_2PO_4, 12 g glacial acidic acid, and 88.5 g deionised water). After 30 min soil was sedimented by centrifugation for 5 min and duplicate aliquots from the three replicates were transferred into 4 ml cuvettes and diluted with 1.5 ml of the extraction solution. The samples were mixed with 0.7 ml of 0.5 M HCl and 0.7 ml of 0.1 M barium chloride-polyethylene glycol reagent was added and mixed again. After 10 min, the samples were mixed again and the absorbance read at 600 nm using a spectrophotometer. The readings were compared to a standard solution of 0-2 mM Na_2SO_4.

Statistical analyses

In order to compare the treatment effects, significant ($P < 0.05$) differences between treatments means were analysed (ANOVA) using CRD of Statistix 10 Analytical Software Tallassee, FL 32317, USA.

Results

Effects of complex organic matter with varying nitrogen content on ASS chemistry

Experiment 1: The first experiment examined the changes in soil parameters induced by complex organic carbon in the form of common agricultural crop material added as a mulch throughout a sulfuric soil. The mulches were selected so as to contain different levels of nitrogen. Two legume species, lucerne (3.2% N) and pea straw (1.2% N) were compared with wheat straw (0.8% N).

Under the aerobic conditions, the unamended Sulphuric control soil remained strongly acidic at pH 4 (Figure 1). Among the organic matter, lucerne hay and pea straw with higher nitrogen content increased the pH to near 8.0 and 6.5, respectively. Wheat straw with a lower nitrogen content increased the pH to approximately 6.

Soil redox potential inversely correlated to soil pH. The control soil remained aerobic while each of the organic amendments reduced Eh to less than 0 mV, the value roughly in proportion to the changes in pH (Figure 1). Changes in soil sulphate content also correlated with the changes in redox and pH. Lucerne hay induced a large

reduction in sulphate content. Pea straw and wheat straw treatments also showed significant changes in sulphate content compared to the control (Figure 1).

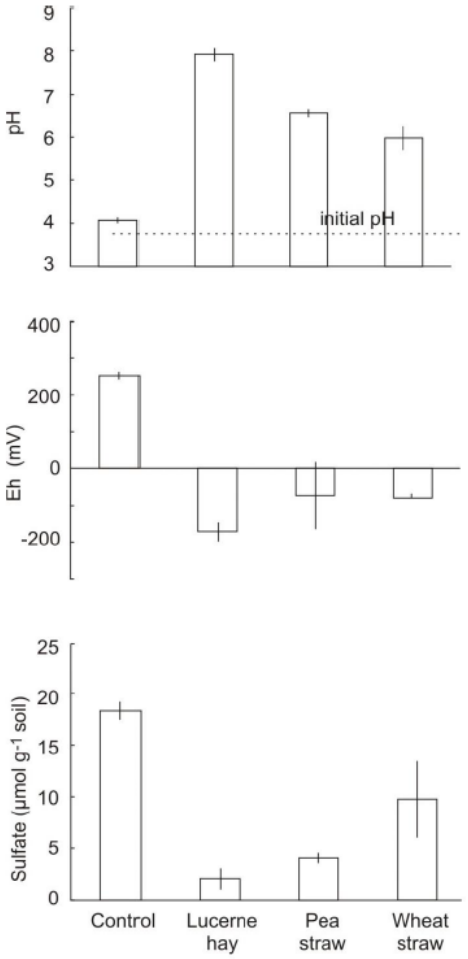

Figure 1. Changes in pH, Eh and sulfate content of sulfuric soil mixed with plant material containing different nitrogen contents. Soil was incubated under aerobic (75% field capacity) for 6 months. Each value is the mean ± s.e. of 3 measurements.

Effects of simple nitrogen and carbon compounds on ASS chemistry

Experiment 2: The previous experiment demonstrated the importance of both carbon and nitrogen in neutralising Sulphuric soil. In the following two experiments, the individual effects of carbon and nitrogen on ASS chemistry were examined. Neither nitrate nor ammonia added alone was effective in increasing soil pH (Figure 2). In contrast urea, which contains both carbon and nitrogen, raised the pH almost to the same extent as lucerne hay. However, all treatments reduced Eh but only urea and lucerne hay lowered Eh to below 0 mV. None of the simple nitrogen compounds had a significant effect on sulphate content of the Sulfuric soil (Figure 2).

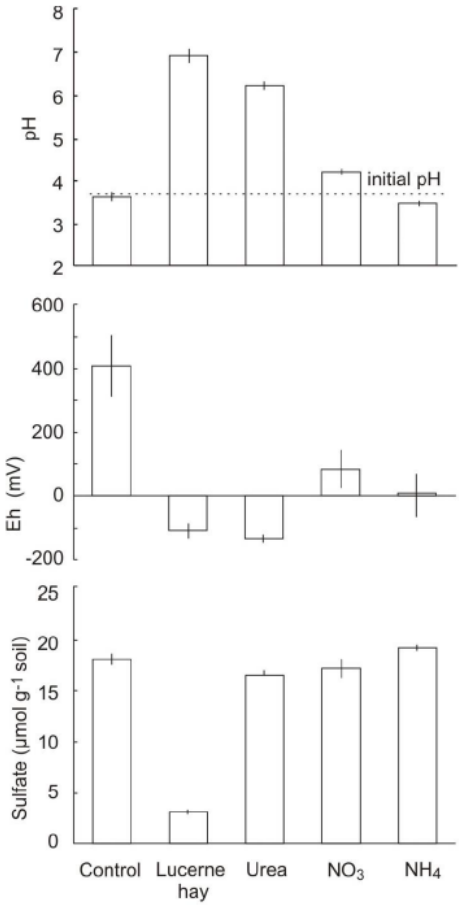

Figure 2. Changes in pH, Eh and sulfate content of sulfuric soil mixed with simple nitrogen compounds or chopped of lucerne hay following incubation under aerobic conditions with a moisture content of 75% field capacity for 6 months. Each value is the mean ± s.e. of 3 measurements.

Experiment 3: Glucose did not have any effect on soil pH. In the experiment shown in Figure 3, glucose was added at a high concentration (4g/80g soil) but in an earlier experiment when added at a low concentration (0.1g/80g soil) the result was the same (data not shown). Molasses increased the pH slightly but this may have been partly due to small amounts of nitrogen in the syrup. Sodium acetate was more effective and increased the pH by almost two units. In this study, *Phragmites*

australis (a common reed), was used as the reference material. It has a similar nitrogen content to lucerne hay and increased the pH almost to the same extent. None of the simple carbon compounds lowered the soil redox potential, whereas in the *Phragmites* treatment, the Eh fell to nearly -200 mV. Molasses and acetate produced minor reductions in sulphate content, but much less than that produced by *Phragmites*.

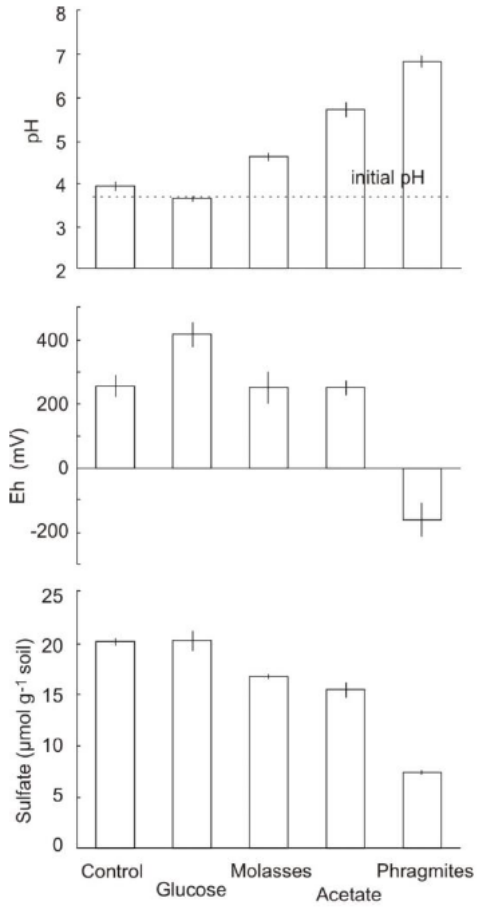

Figure 3. Changes in pH, Eh and sulfate content of sulfuric soil mixed with simple carbon compounds or chopped leaves of *Phragmites* following incubation under aerobic conditions at a moisture content of 75% field capacity for 6 months. Each value is the mean ± s.e. of 3 measurements.

Effects of complex organic carbon on a Sulphidic soil

Experiment 4: The experiment shown in Figure 1 clearly demonstrated the effectiveness of organic matter in neutralising ASS that has become acidic, namely in a Sulfuric soil. A problem that commonly arises when a submerged sulphidic soil is exposed by falling water levels is how to prevent its oxidation and the production of sulphuric acid. We found that when organic mulches were incorporated into Sulphidic soil, acidification was prevented. In the case of lucerne hay and pea straw, the pH actually increased (Figure 4). Acidification was observed with wheat straw, but not to the extent seen in the unamended control. As with the Sulphuric soil, the pH was correlated with both Eh and sulphate content. However, Eh values were much higher.

Longer term incubation indicated that when the organic matter is exhausted by microbial breakdown, the protective effect is lost. After 12 months, the pH in all treatments became very acid (see grey bars in Figure 4).

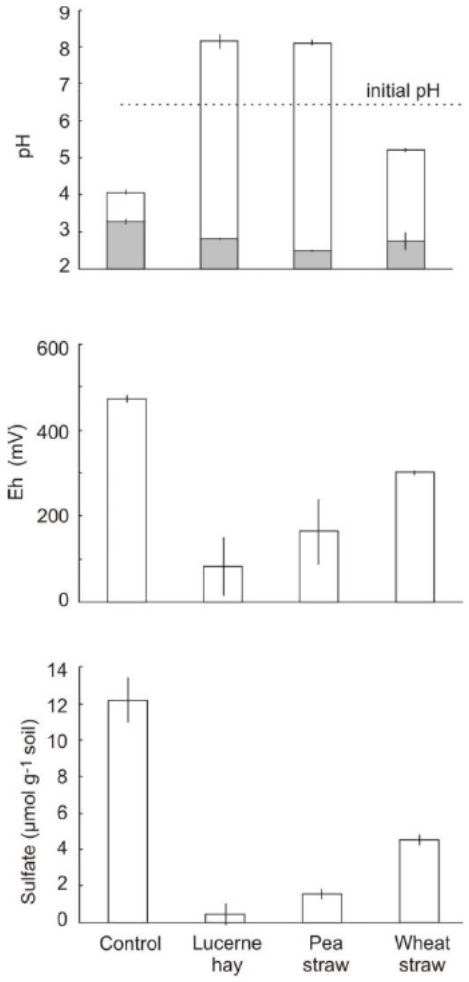

Figure 4. Changes in pH, Eh and sulfate content of sulfidic soil mixed with plant material containing different nitrogen contents. Soil was incubated under aerobic (75% field capacity) for 6 months. The grey bars in the pH plot show the pH after 12 months. Each value is the mean ± s.e. of 3 measurements.

Discussion

Plant organic matter can neutralise acidic soil

Acidification of soils has a range of adverse effects on the local environment. For plants, the high proton concentration makes it difficult to regulate intracellular pH, and the solubilisation of toxic elements such as Al and Mn, which causes inhibition of root growth. Low pH also results in the release of various heavy metals and metalloids such as Cd and As, which may then be transported into water bodies (wetlands, streams, rivers and lakes). Incorporation of lime has been shown to be effective in raising soil pH to counter these problems but it is expensive to apply and its effectiveness is dependent on the particle size of the lime and the method of incorporation. In addition, lime cannot be applied in environmentally sensitive areas, such as Ramsar wetlands. Here we have examined the practicalities of using organic matter as a replacement for lime for the amelioration of ASS. The results clearly demonstrate that complex organic matter can restore extremely acidic soils such as Sulfuric soils to neutrality. For example, lucerne hay was able to increase the soil pH from less than 4 to nearly 8 (Figure 1). Barrow (1960) found much smaller effects of lucerne hay on pH of a mildly acidic soil at higher dose rates and only 3 months incubation. After 12 months of incubation, we found soils become strongly acidic because added organic matter gets completely broken down by soil microbes (Figure 4, grey bars), indicating that microbial activities in generating biogenic alkalinity cease as metabolic substrates become limited in the soil.

It was also clear that the effectiveness of the complex organic matter was dependent on the nitrogen content. Pea straw and wheat straw produced smaller increases in pH, in proportion to their nitrogen content. However, these fodder crops may also be too expensive as soil pH treatments, although the cost may be offset by additional benefits such as increasing fertility and better water retention, benefits which are not conferred by lime. *Phragmites* offers a more economical alternative. It grows abundantly in wetlands and along watercourses and has a broad global distribution. The local *Phragmites* used in these studies was found to have a high nitrogen content, similar to lucerne, and was almost as effective in raising soil pH.

Chemical and biological processes involved in pH increases

For all of the treatments using complex organic matter, increase in pH was closely correlated with reduction in Eh and the consumption of soil sulphate, consistent with the involvement of sulphur-reducing bacteria (SRBs). These bacteria are unable to function under aerobic conditions so the observed reductions in Eh to below 0 mV must have initially occurred due to oxygen depletion by aerobic bacteria capable of using the organic matter as substrates. It is significant that refined carbon sources such as glucose, molasses and acetate did not generate these reducing conditions. It is hypothesised that the mulching effect of the complex organic matter plays an important role in restricting oxygen diffusion into the soil, thereby allowing the creation of a microenvironment for the lowering of Eh to a level that is suitable for SRBs.

The ASS used in this study already contained appreciable amounts of organic carbon, as judged by loss on ignition, yet remained acidic. It is known that some soil carbon pools are 'recalcitrant' and difficult for microbes to break down (Marschner *et al.*, 2008). Various studies have shown that addition of simple compounds such as glucose, amino acids or organic matter can enhance the breakdown of this resistant carbon, implying that supply of essential substrates for microbial activity is the main limitation (Kuzyakov *et al.*, 2000; Neff *et al.*, 2002). Of the simple carbon sources used in the current study, glucose had no effect, molasses slightly increased pH while acetate raised the pH by 2 units. Under aerobic conditions some groups of bacteria are able to grow on acetate and nitrate as sole energy sources, with the net consumption of protons (Thauer *et al.*, 1989). Other groups such as sulphate reducing and iron reducing bacteria can use acetate as a carbon source but do so only under anaerobic conditions (Thauer et al., 1989). The measured Eh in the acetate treatment remained in the aerobic zone making these latter reactions less likely.

Addition of N as nitrate or ammonia had no effect on soil pH, but urea increased the pH by more than two units without changing the sulphate content, even though the Eh in this treatment was low enough to support SRBs. Most likely, urea was broken down by urease secreted by soil bacteria producing CO_2 and NH_3 which would have an alkalising effect (Lloyd & Sheaffe, 1973).

It was also demonstrated that plant organic matter can protect Sulphidic soils from

oxidation, and the mechanism appears to be the same as that for Sulphuric soils; soil pH was correlated with reductions in Eh and sulphate content and was related to the nitrogen content of the organic matter. Further studies are needed to ascertain the time frames over which the beneficial effects of organic matter occur, how long they persist, and how much organic matter is required to achieve useful pH changes.

Conclusions

Plant organic matter added to Sulphuric soils has the capacity to neutralise acidity. This appears to be achieved by a combination of a complex microbial ecology and the ability of incorporated organic matter to create a redox environment capable of supporting anaerobic microbial metabolism. Nitrogen appears to be a limiting resource, as indicated by the larger increases in soil pH induced by organic matter with high nitrogen contents. Incorporation of plant organic matter into Sulphidic soils may also be an effective strategy for preventing the sulphide oxidation that is responsible for acidification of ASS.

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Chapter Four

Effects of Amendments on Sulfuric Soil Chemistry

4.0 Introduction

Sulfuric soil acidity management is based on two principles: (i) neutralisation of the actual acidity by addition of mineral lime and (ii), management of the by-products of sulfidic soil oxidation (Baldwin and Fraser, 2009; Sullivan et al., 2011; Thomas, 2010). Management of sulfuric soil acidity by addition of lime in poor economies (e.g. in the tropics), however, is expensive and availability is a major constraint (Moore et al., 1990; Powell and Martens, 2005; Xu and Coventry, 2003). Several studies have shown that the addition of organic matter can be as effective as lime in ameliorating acidic soils (Pocknee and Sumner, 1997; Tang and Yu, 1999; Williams, 1980; Williams and Donald, 1957; Xu et al., 2006a; Xu and Coventry, 2003; Xu et al., 2002). These findings, however, cannot be easily extrapolated as the studies were conducted in "acidic soils of non-ASS origin" and the available accounts are contradictory (Xu et al., 2006b).

In addition to the buffering effect, organic matter addition would act as metabolic substrates for microbes to generate biogenic alkalinity and regulate other soil chemical properties that influence pH. Reid and Butcher (2011) examined the effects of live plants on sulfuric and sulfidic soils and found that different plants could either increase or reduce pH. However, the mechanisms behind these changes were not investigated and only pH was measured. In this chapter, the role of organic matter derived from dead plant material in altering the pH of sulfuric soil is examined. Some insight into the processes that are responsible for changes in pH was obtained by measuring the following two other key soil parameters: (i) redox potential and sulfate content, and (ii) by treatments that examined the individual effects of carbon and nitrogen.

4.1 Methodology

All the studies presented were conducted in either small pots or Falcon tubes using Finniss River sulfuric soil. Individual studies as described in Chapter 3 are pointed out clearly in this chapter. Redox potential and pH were measured as described under

Sections 3.4 and 3.5 from the 10, 20, 40, 60 and 80 mm profiles. Descriptions of individual studies not described in Chapter 3 are described separately below.

Similarly, pH was measured using a 2 g soil sample (n=3) from each of the profile. The sulfate content was quantified using soil samples obtained from the 10 mm (surface), 40 mm (middle) and 80 mm (depth) profiles as described under Section 3.6. Due to the large amount of data, throughout the chapter, only data collected from the surface, middle and bottom of the profiles of studies conducted under aerobic conditions, and from the surface and bottom of the profile under anaerobic conditions are presented.

To investigate the effects of organic matter addition, four studies were conducted. In the first study, two organic matter application techniques were tested, one in which organic matter was uniformly incorporated into the soil, and another in which the organic matter was simply placed on top of the soil (designated 'overlying'). There were a total of eight experiments as described under Section 3.3.1 and in Table 3.1 as [I] to [VIII] using chopped leaves of *Phragmites australis* as the source of organic matter. *Phragmites* was chosen based on the fact that it is freely available and in abundance in many areas, in contrast to other sources such as lucerne hay or pea straw which have economic value as fodder crops. These latter materials were used in the second study to investigate the long-term effects of organic matter with varying nitrogen content, described as [IX] and [X] under Section 3.3.1a and in Table 3.2.

In the third study, the effects of addition of simple carbon compounds in the form of glucose, molasses or acetate were examined and compared with the changes caused by addition of lucerne hay. Treatments were replicated three times and incubated under either aerobic or anaerobic (flooded) conditions. Measurements were collected at various depths down the profile after 3, 6 and 12 weeks and after 6 months (detailed in Table 3.3). In the fourth study, the effects of addition of simple nitrogen compounds; nitrate, ammonium and urea were investigated (detailed in Table 3.3).

4.2 Results

4.2.1 Effects of complex organic matter

The changes in sulfuric soil properties as a function of time were initially measured over 5 weeks in 80 g of flooded soil with or without incorporation of 1 g of lucerne hay.

Soil pH and Eh were logged simultaneously from probes inserted in the top 20 mm below the surface of the soil.

An immediate increase in pH from 4.4 to 5.5 and a decrease in Eh was observed within the first 3 days in the lucerne hay treatment (Fig. 4.1). Changes in the control treatment were slower. Over the next 14 days, pH continued to increase (Fig. 4.1a) and Eh declined (Fig. 4.1b). Between 18 and 38 days, the pH of the control soil fell, corresponding to an increase in Eh, while the pH of the lucerne hay treatment continued to rise and Eh was relatively stable.

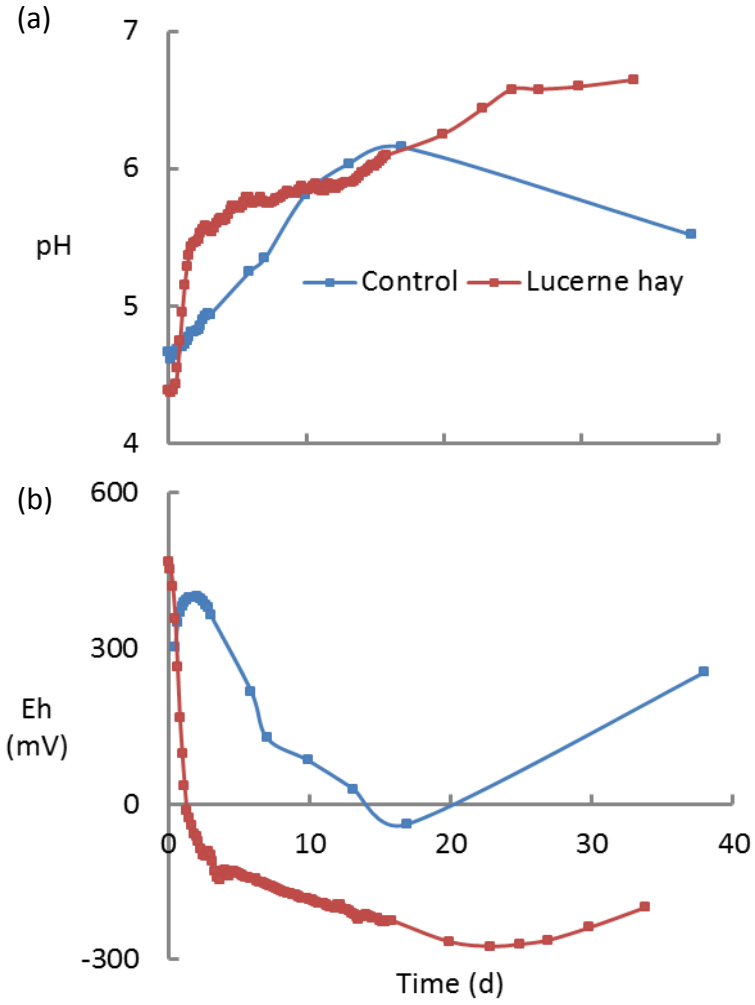

Figure 4.1. Short-term changes of (a) pH and (b) redox of sulfuric soil maintained under anaerobic conditions with or without addition of organic matter (lucerne hay). The initial pH was 4.4.

Longer-term (6 month) changes in sulfuric soil chemistry measured following either incorporation or overlaying of organic matter (studies [V] – [VIII], Table 3.2) are shown in Figs. 4.2 – 4.5. When organic matter (*Phragmites*) was incorporated and maintained

under aerobic conditions, changes in pH closely correlated with changes in Eh. The control soil remained highly oxidised, but the soil amended with *Phragmites* became highly reduced to between -136 mV to -219 mV (Fig. 4.2b). Similarly, treatment with *Phragmites* resulted in large reductions in soil sulfate content (Fig. 4.2c).

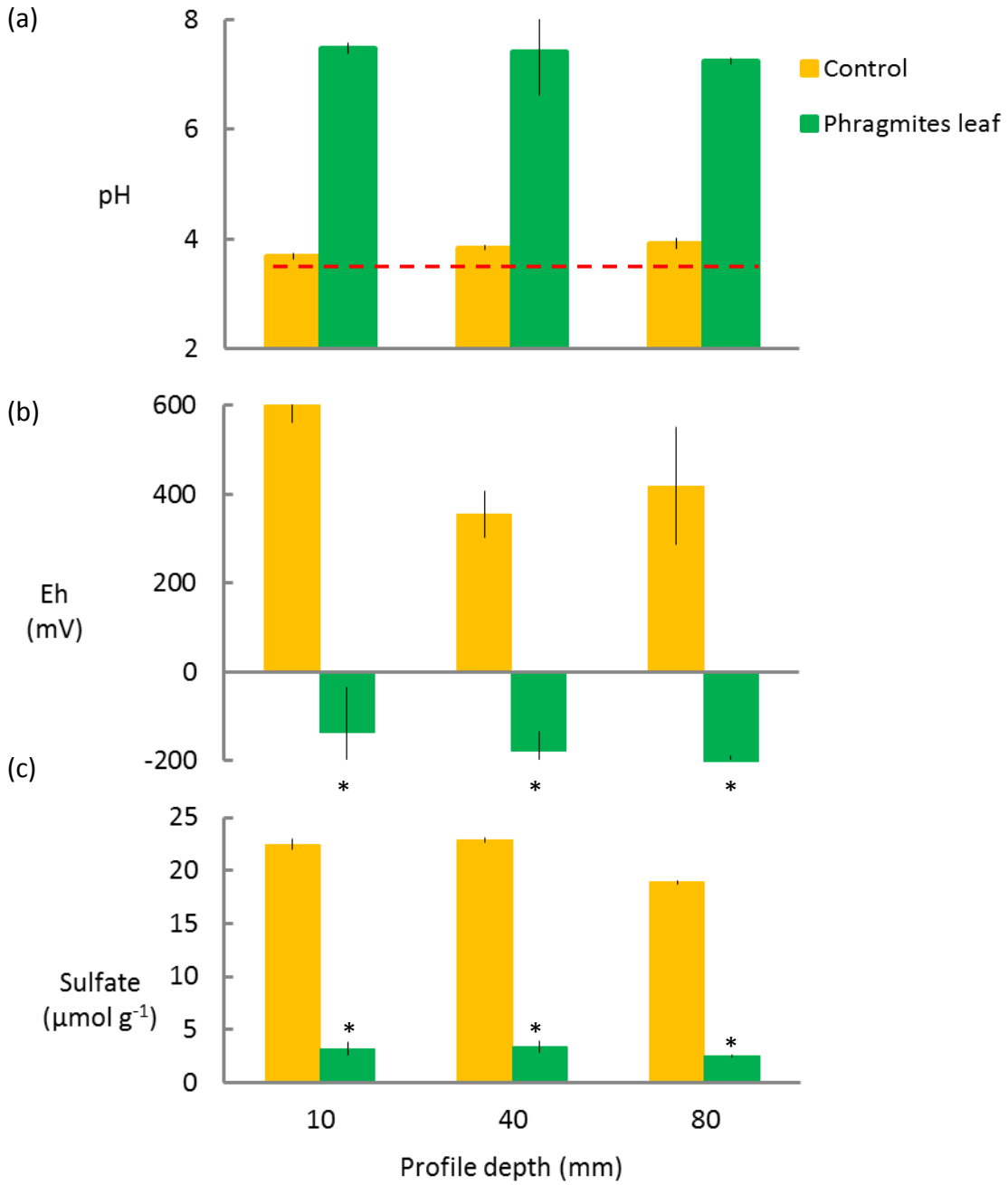

Figure 4.2. Effects of incorporated organic matter (*Phragmites*) on (a) pH, (b) redox and (c) sulfate content of sulfuric soil maintained under aerobic conditions for 6 months. The red dotted line is the initial pH. Values are means ± s.e. of three measurements (n=3). The initial sulfate content ranged between 21-32 µmol g^{-1} soil. Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

The changes in soil chemistry caused by incorporation of organic matter under anaerobic conditions are shown in Fig. 4.3. The surface of the control soil remained oxidised, presumably by oxygen diffusion through the surface water, but decrease to an Eh of 0 mV at depth. Corresponding to Eh, the control soil remained strongly acidic at the surface but increased moderately at depth (Fig. 4.3a). In contrast, the pH in the soil amended with *Phragmites* rose to 7.6 at both the surface and at depth, with concurrent reductions in Eh and sulfate content.

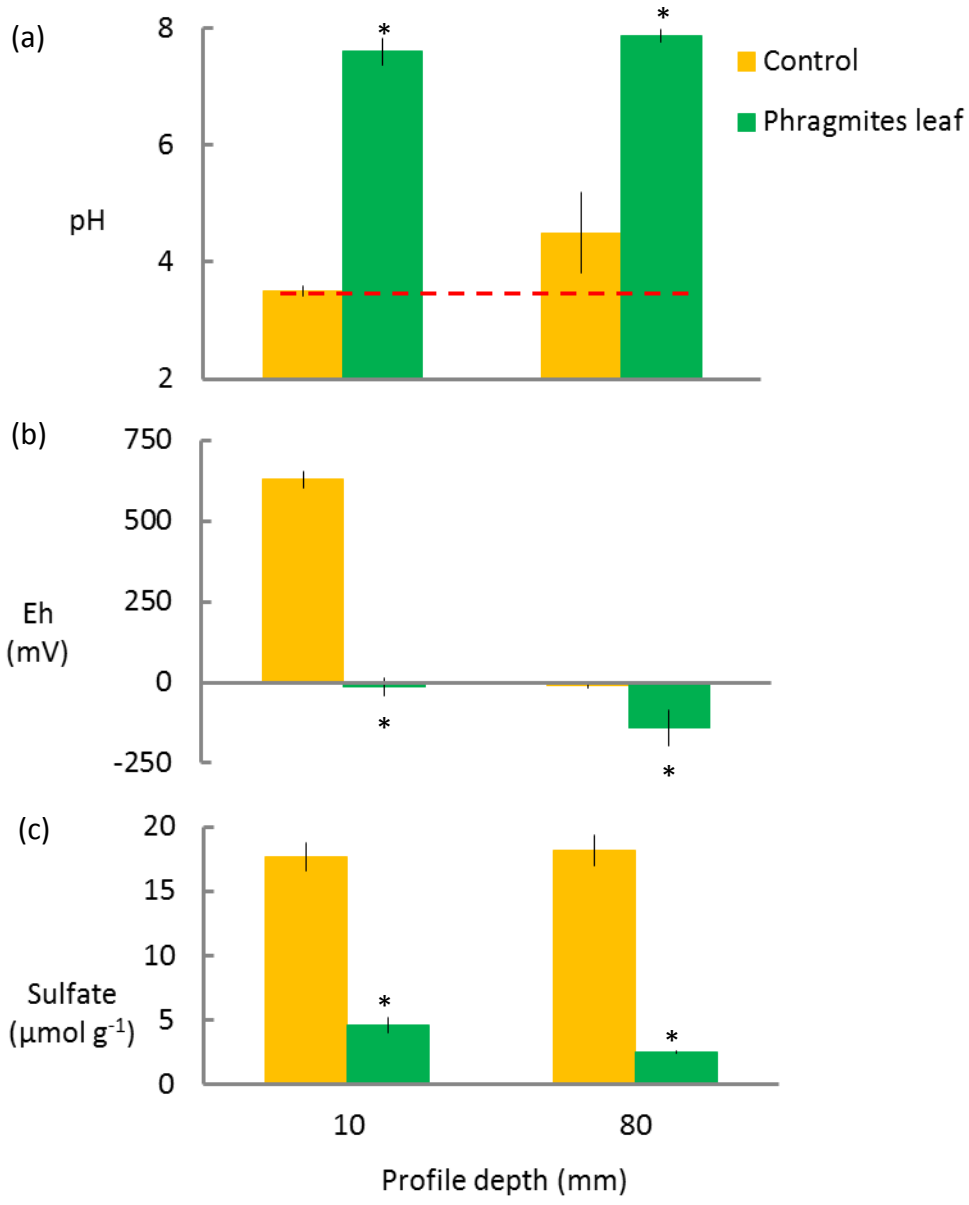

Figure 4.3. Effects of incorporated organic matter on (a) pH, (b) redox and (c) sulfate content of sulfuric soil maintained under anaerobic conditions for 6 months. The red dotted line is the initial pH. The values are means ± s.e. of three measurements (n=3). The initial sulfate content ranged between 21-32 µmol g^{-1} soil. Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

In the preceding experiments, organic matter was uniformly incorporated into the soil. A simpler strategy would be to just apply organic matter to the surface as mulch. The changes in soil chemistry measured when organic matter was overlaid and maintained under aerobic conditions are shown in Fig. 4.4. The control soil remained highly acidic compared to the amended soil whose pH increased moderately to near 5 throughout (Fig. 4.4a).

The Eh of the control soil remained high and the amended soil moderately reduced (Fig. 4.4b). Comparatively, the control soil Eh declined from 451 mV at the surface to 311 mV at depth, whereas in the *Phragmites* treatment, the changes in Eh ranged from 88 mV at the surface to 54 mV at depth. Changes in sulfate content were small (Fig. 4.4c), consistent with the moderate changes in pH and Eh, but much smaller than when organic matter was incorporated throughout the profile (Figs. 4.2 & 4.3).

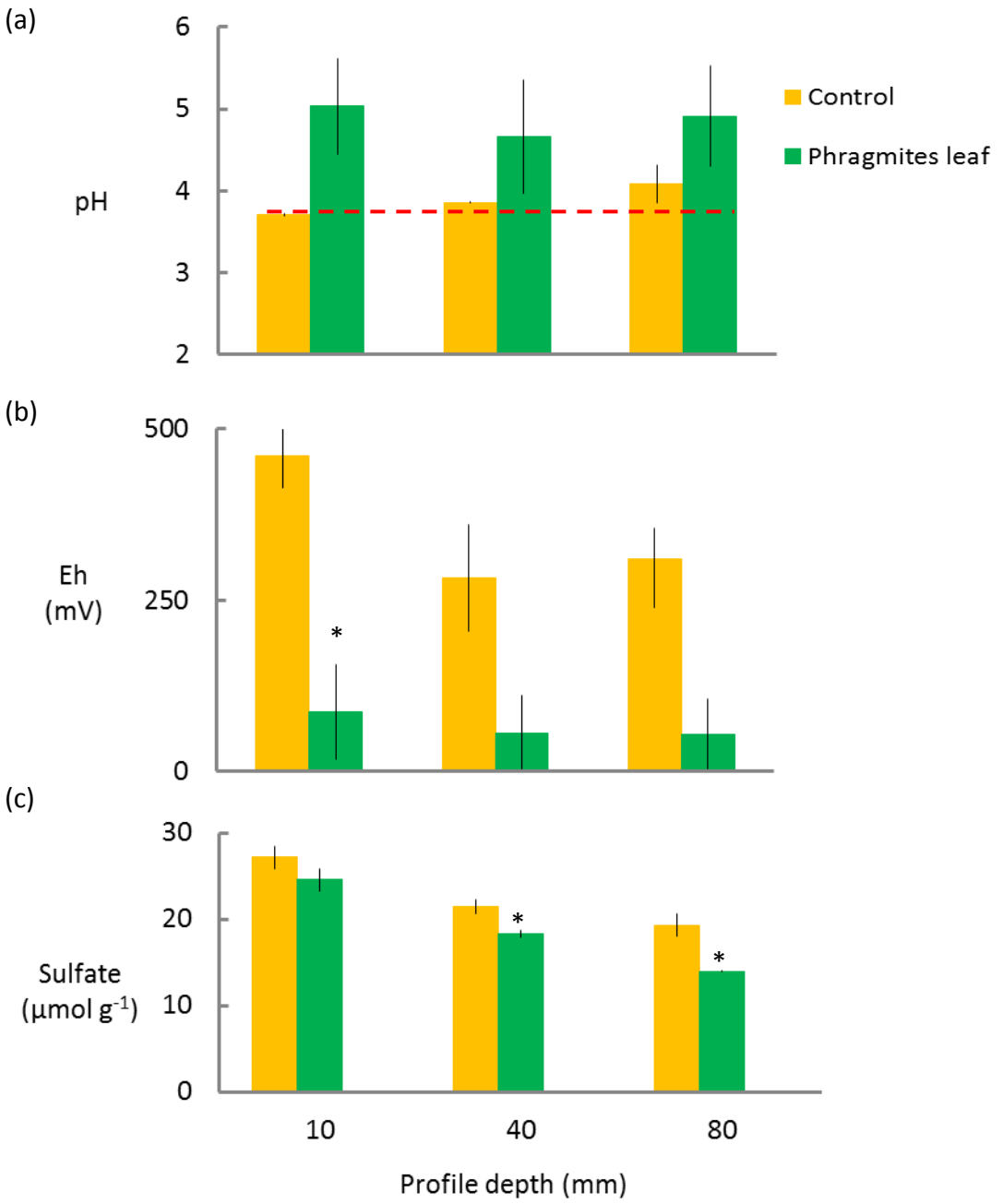

Figure 4.4. Effects of overlaid organic matter on (a) pH, (b) redox and (c) sulfate content of sulfuric soil maintained under aerobic conditions for 6 months. The red dotted line is the initial pH. Values are means ± s.e. of three measurements (n=3). The initial sulfate content ranged between 21-32 µmol g^{-1} soil. Asterisks indicate significant differences ($p < 0.05$) between treatment and control at the same depth.

Figure 4.5 shows the changes measured when organic matter was overlaid and maintained under anaerobic conditions. The control soil again remained acidic at the surface but sharply increased to near 6 (Fig. 4.5a), similar to that shown in Fig. 4.2a but slightly higher at depth. The amended soil pH increased to 7 at the surface but was

slightly lower (0.6 units) at depth and similar to the control soil. Despite the flooded conditions, the control soil remained oxidised at the surface, but became reduced to near 12 mV at depth (Fig. 4.5b); the amended soil was highly reduced throughout. As shown in Fig. 4.5c, sulfate reduction correlated with changes in Eh; the content of the amended soil decreased to 9.6 µmol g^{-1} compared to 21 µmol g^{-1} in the control soil.

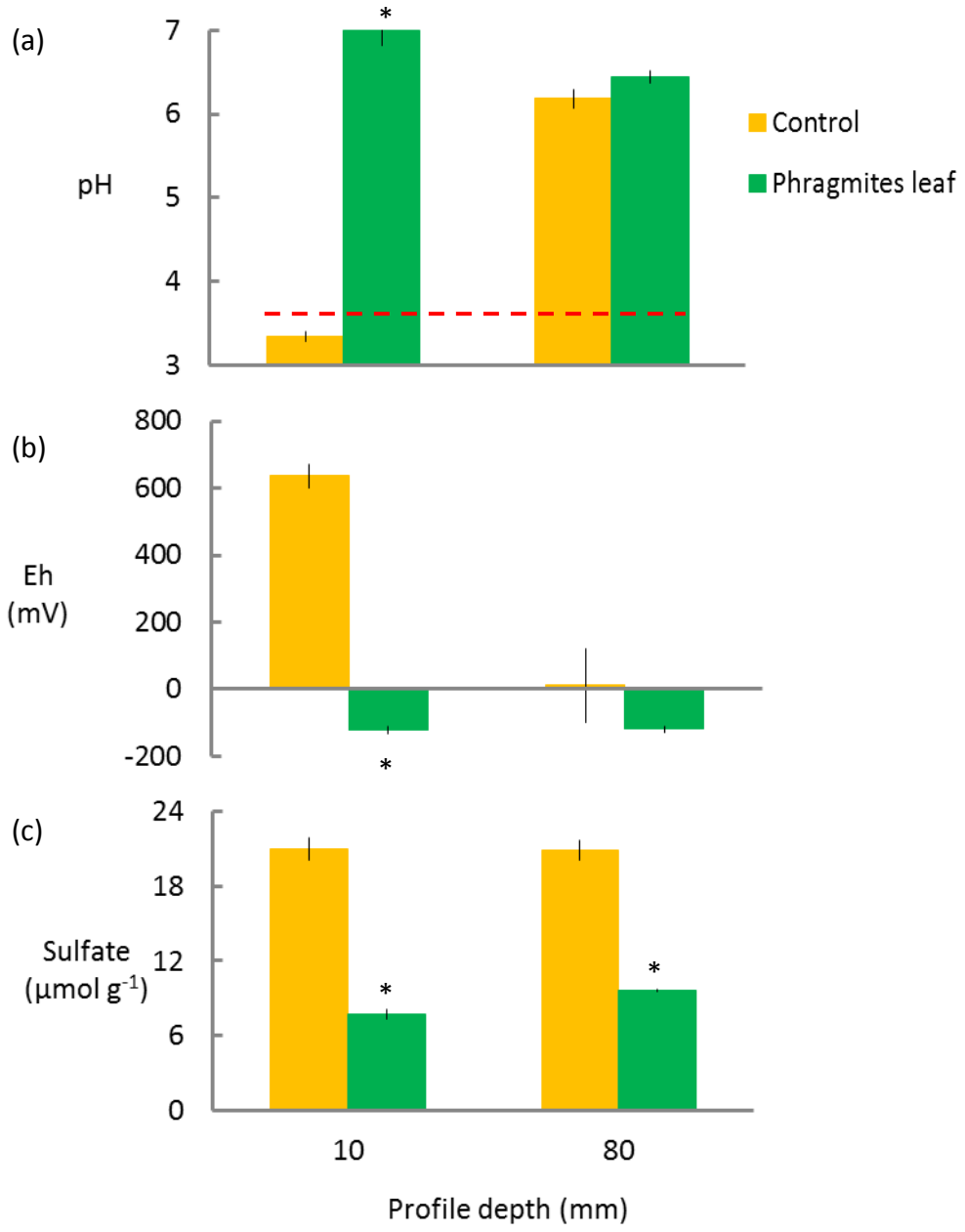

Figure 4.5. Effects of overlaid organic matter on (a) pH, (b) redox and (c) sulfate content of sulfuric soil maintained under anaerobic conditions for 6 months. The red dotted line is the initial pH. The values are means ± s.e. of three measurements (n=3). The initial sulfate content ranged between 21-32 µmol g^{-1} soil. Asterisks indicate significant differences ($p < 0.05$) between treatment and control at the same depth.

The ameliorative effects of complex organic matter have been clearly demonstrated but the relative contributions of its constituent carbon and nitrogen to the changes in pH needed further investigation. To test this, organic mulches with varying nitrogen content were selected and used in studies [IX] & [X] (Table 3.2). Lucerne hay had a measured nitrogen content of 3.2%, similar to *Phragmites* (3.7%), and higher than pea straw (1.2%) and wheat straw (0.8%). The effects on pH, Eh and sulfate content of these treatments are shown in Fig. 4.6. Under non-flooded conditions (75% field capacity), the pH in the top of the profile remained around 4 in the control soil, but rose to 8 in the lucerne treatment (Fig. 4.6a). This was accompanied by a large reduction in Eh to around -170 mV (Fig. 4.6b) and total depletion of the sulfate content. The changes in pH, Eh and sulfate content of the pea straw and wheat straw treatments were intermediate between the control and the lucerne treatments.

In the control treatment, the pH rose with increasing depth and the Eh declined. It was not clear whether this was due to the anaerobic conditions created by higher water content towards the bottom, or to microbial respiration of residual organic matter in the soil. The profiles of pH, Eh and sulfate content of the added organic matter treatments did not vary greatly with depth (Fig. 4.6).

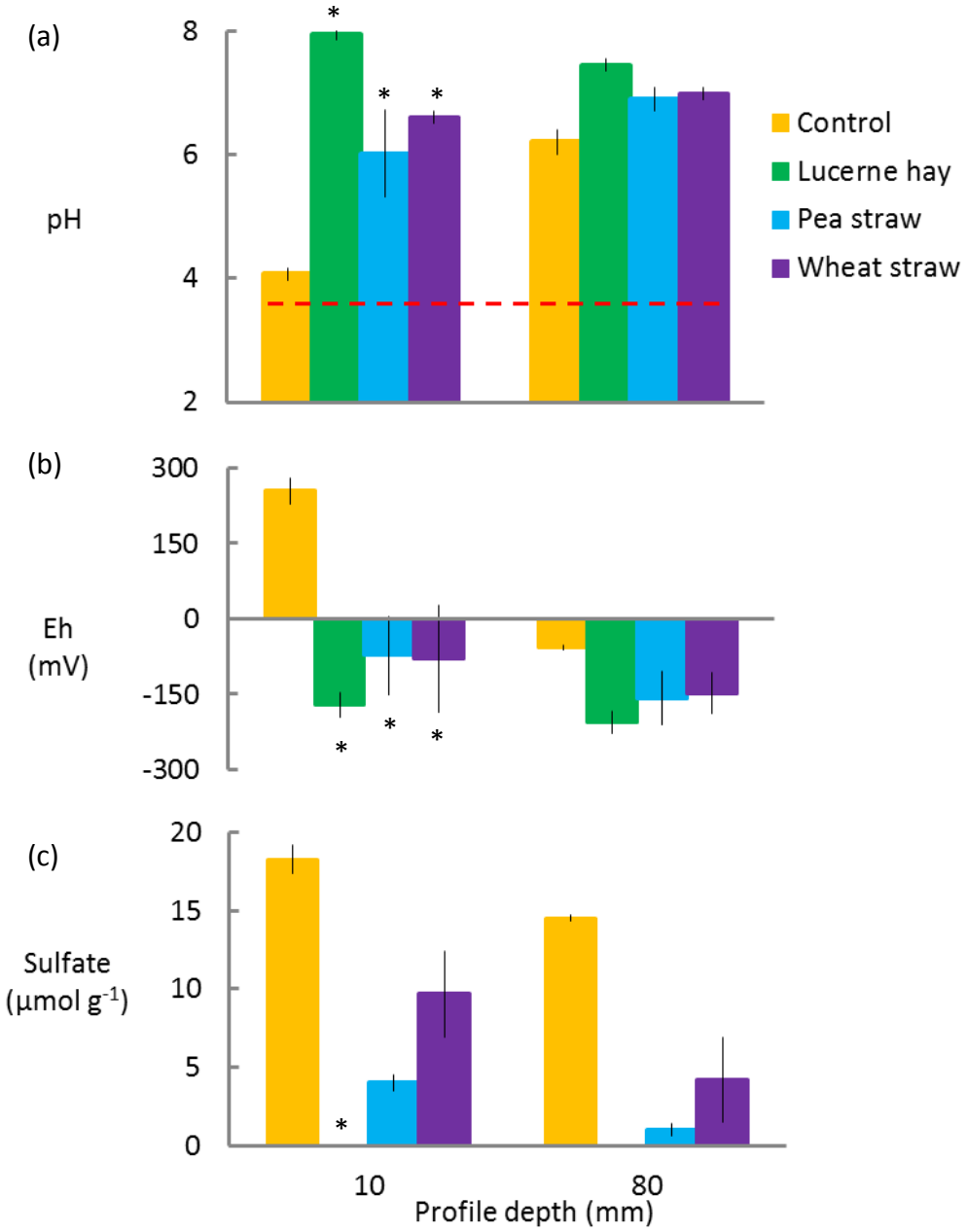

Figure 4.6. Effects of incorporated organic matter with varying nitrogen content on (a) pH, (b) redox and (c) sulfate content of sulfuric soil maintained under aerobic conditions for 6 months. The values are means ± s.e. of three measurements (n=3). The initial sulfate content ranged between 21-32 µmol g^{-1} soil. Asterisks indicate significant differences ($p < 0.05$) between treatment and control at the same depth.

Under flooded conditions, the changes in pH and Eh were similar to those under aerobic conditions except the changes were generally smaller (Fig. 4.7). In this experiment there was a clear trend in the size of the responses according to the nitrogen content with lucerne > pea straw > wheat straw. Although sulfate content under anaerobic conditions was not quantified, it is highly likely that the changes would be similar to those previously

described for *Phragmites* (Fig. 4.3) under similar conditions, since *Phragmites* has a similar nitrogen content to lucerne.

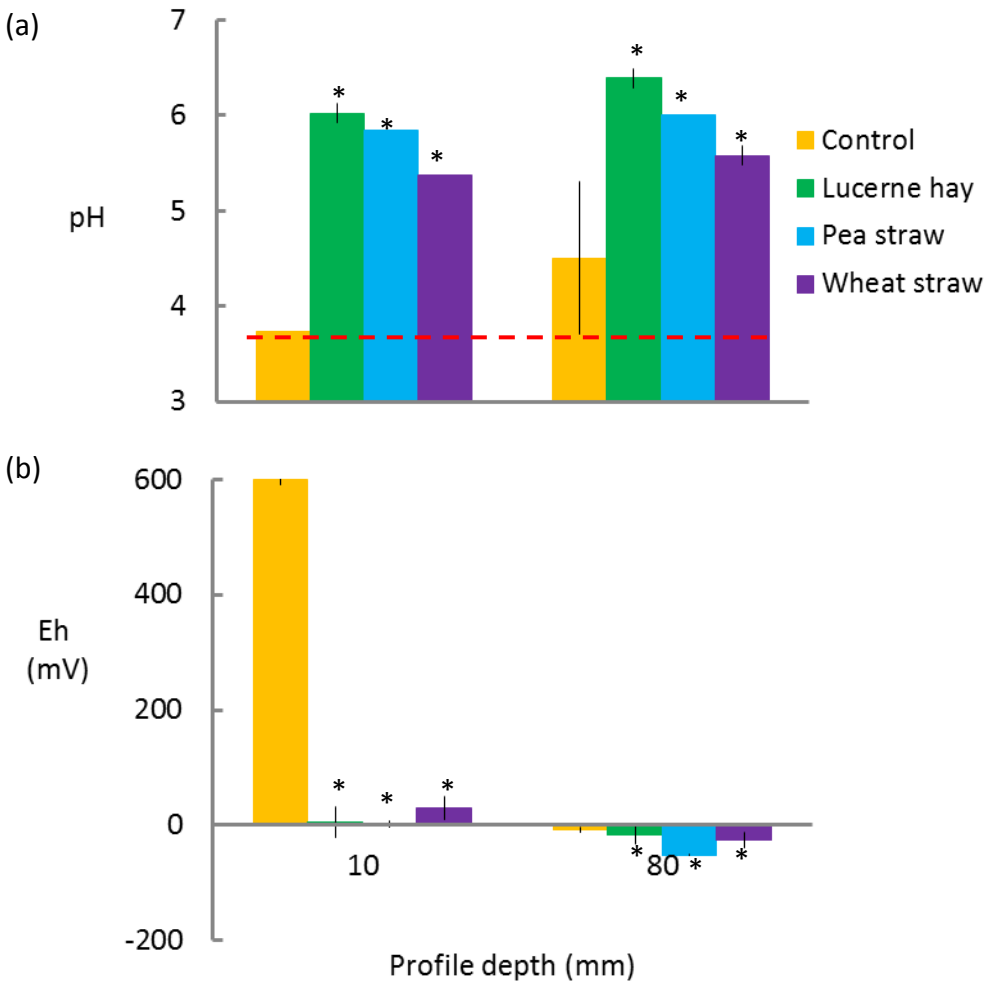

Figure 4.7. Effects of incorporated organic matter with varying nitrogen content on (a) pH and (b) redox of sulfuric soil maintained under anaerobic conditions for 6 months. The red dotted line is the initial pH. Values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

4.2.2 Effects of simple carbon compounds

To test the hypothesis that carbon in the organic matter was mainly responsible for the effects on sulfuric soil chemistry, a series of experiments were conducted with simple carbon compounds, and the changes compared to those observed with complex organic matter.

Initially, glucose and acetate were added at low (catalytic) concentrations but no changes were observed relative to the control soil (results not shown). The amounts were therefore increased to be similar to those of the complex organic matter (see Table 3.3,

study [XVII]). After 6 months under aerobic conditions, glucose was found to have no effect on pH while acetate and molasses increased it moderately (Fig. 4.8a). The changes were similar at the top and bottom of the profile, except for the control pH which increased at depth. As in previous studies, increase in pH was associated with decreases in Eh and sulfate content (Fig. 4.8b, c). The effects of the simple carbon sources on pH under anaerobic conditions were similar to the treatments under aerobic conditions (Fig. 4.9a). In both experiments, the changes induced by complex organic matter were much greater than those of the simple carbon compounds.

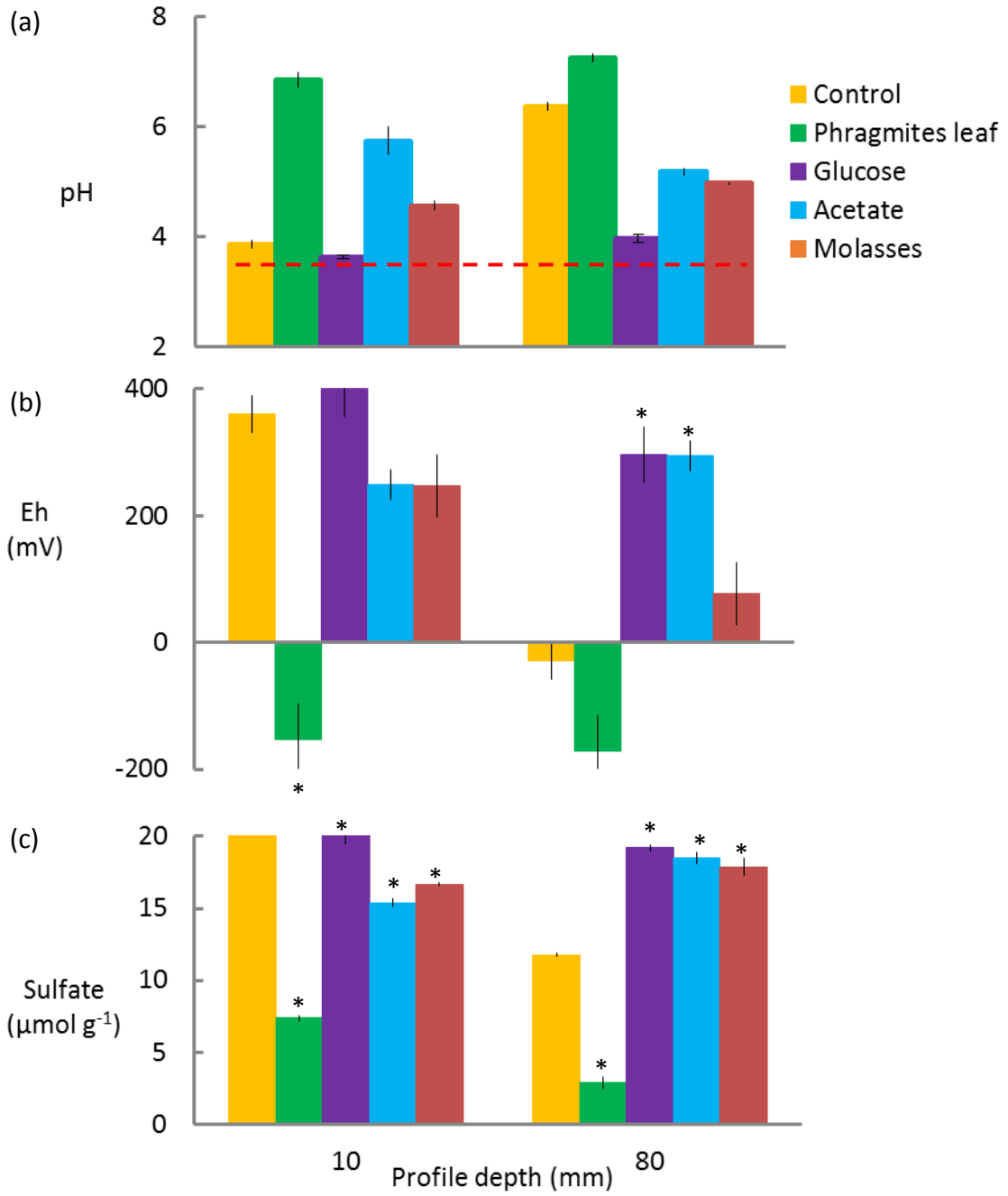

Figure 4.8. Effects of simple carbon sources on (a) pH, (b) redox and (c) sulfate content of sulfuric soil maintained under aerobic conditions for 6 months. The red dotted line is the initial pH. Values are means ± s.e. of three measurements (n=3). The initial sulfate content ranged between 21-32 µmol g^{-1} soil. Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

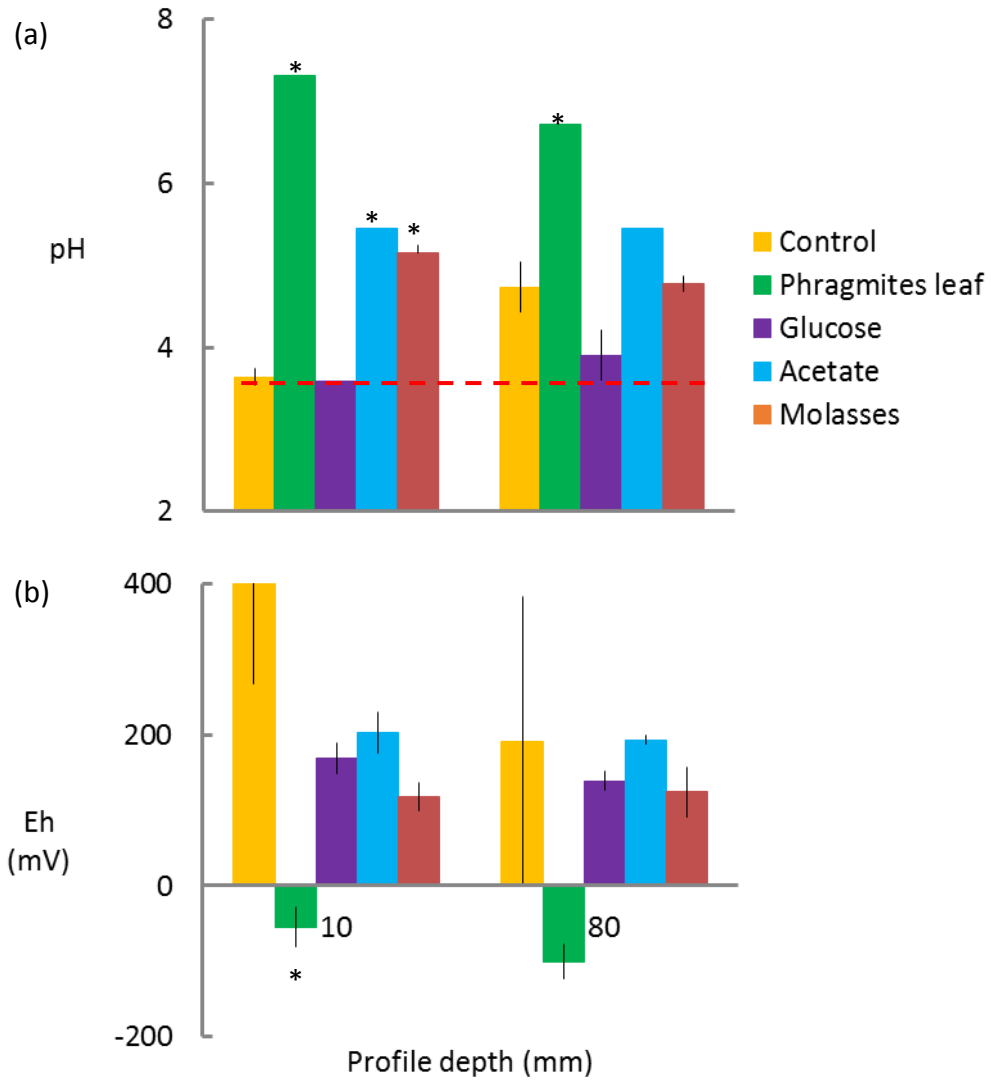

Figure 4.9. Effects of simple carbon sources on (a) pH and (b) redox of sulfuric soil maintained under anaerobic conditions after 6 months. The red dotted line is the initial pH. Values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

4.2.3 Time-course of changes in pH and Eh by simple carbon compounds

The time-course of changes in pH and Eh following the addition of complex organic matter (Fig. 4.1) showed that the values obtained by these treatments are highly dependent on treatment time. Therefore, the effects of simple carbon compounds were monitored after 3, 6 and 12 weeks, for comparison with the changes described in Figs. 4.8 and 4.9 after 24 weeks.

The changes in pH and Eh measured under aerobic conditions in the time-course study are shown in Fig. 4.10. Throughout the 12 weeks, the control soil pH remained

strongly acidic (Fig. 4.10a). Among the amended treatments, increase in pH from the highest to the lowest was: lucerne hay > acetate > glucose. The changes elicited by the various treatments were rapid and essentially complete by 3 weeks, although lucerne hay continued to increase pH between 3 and 12 weeks. The increases in pH were strongly correlated with reductions in Eh, with large changes observed in the lucerne and acetate treatments.

Under anaerobic conditions, the effect of acetate was immediate and organic matter with high nitrogen content was time-dependent (Fig. 4.11). Within the first 6 weeks (Fig. 4.11a, b), acetate strongly increased the pH to 6.3 throughout, with lesser changes by lucerne hay, but no response in the control and glucose treatments. After 12 weeks, the pH of all the treatments remained similar to 6 weeks, except that lucerne hay increased in pH higher than 7 at the surface and 6.5 throughout the profile (Fig. 4.11c). In the glucose amended soil, pH remained lower than 5, close to the initial pH. The overall effects of the amendments on pH after 12 weeks were: lucerne hay > acetate > control > glucose.

A major difference between the aerobic and anaerobic treatments was in the response of Eh. As shown in Fig. 4.10d-f, the unamended control soils, the soil amended with glucose remained highly oxidised at the end of three weeks, while the soils amended with acetate and lucerne hay were moderately reduced (150 – 300 mV) throughout (Fig. 4.10d). Under anaerobic conditions, the control and the glucose amended soils remained highly oxidised throughout the 12 weeks (Fig. 4.11d, e, f) but the Eh of the lucerne hay and acetate amended soils were 200 to 400 mV lower, and remained below 0 mV throughout.

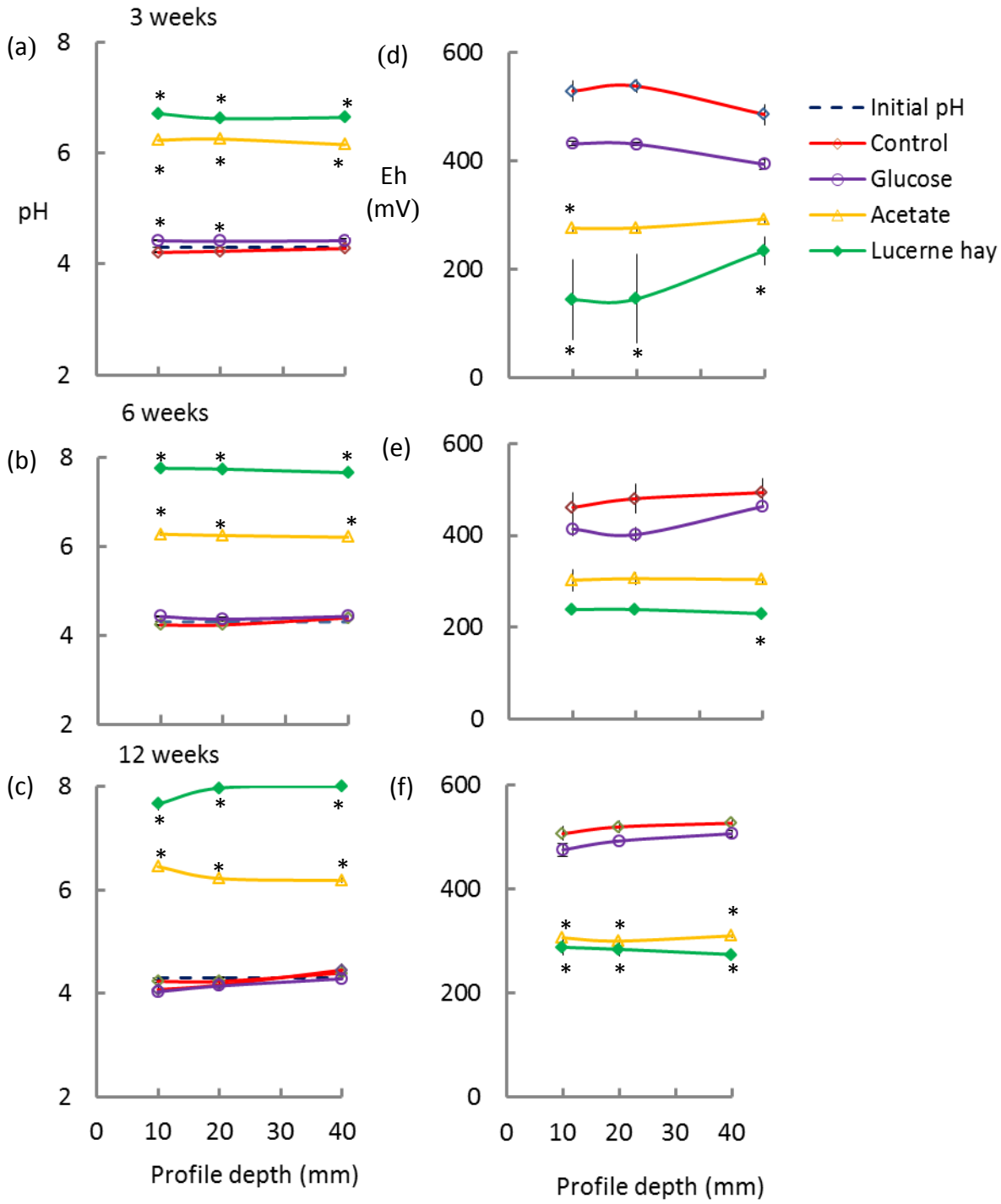

Figure 4.10. Time-course of the effects of organic matter and simple carbon sources on (a-c) pH and (d-f) redox of sulfuric soil maintained under aerobic conditions for 3, 6 and 12 weeks. The values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

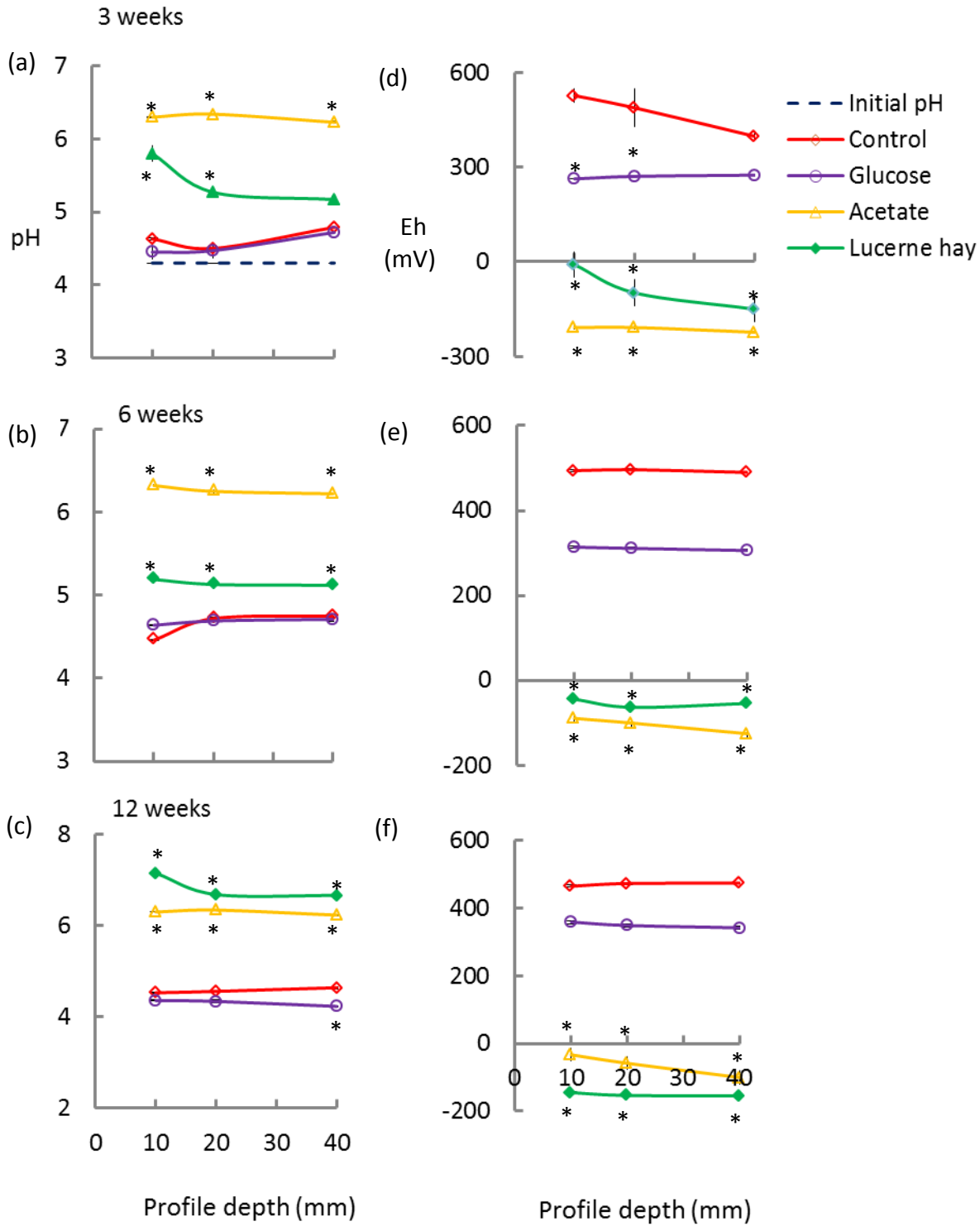

Figure 4.11. Time-course of the effects of organic matter and simple carbon sources on (a-c) pH and (d-f) redox of sulfuric soil maintained under anaerobic conditions for 3, 6 and 12 weeks. The values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

4.2.4 Effects of simple nitrogen compounds

To investigate the long-term effects of simple nitrogen compounds, two studies described as [XIII] and [XIV] under Section 3.3.1b and in Table 3.2 were conducted. In addition, a lucerne hay treatment was included as a source of complex organic matter with high nitrogen to compare the results. The amounts of nitrogen added were adjusted to be the same (as actual N) as that contributed by lucerne (3.2% N). The trend shown by the changes in soil chemistry measured in both studies are shown in Figs. 4.12 and 4.13.

As expected under aerobic conditions, the control soil pH remained acidic at 3.6 at the surface and lucerne hay increased it to near 7 (Fig. 4.12a). Neither ammonium nor nitrate significantly affected pH, but urea caused it to rise above 6. Despite not affecting pH, ammonium and nitrate caused large reductions in Eh. Both lucerne hay and urea reduced Eh to less than zero, but oddly, only lucerne hay caused a decline in the sulfate content (Fig. 4.12c).

Under anaerobic conditions, changes in pH by lucerne hay and urea were similar to those under aerobic conditions, but ammonium and nitrate behaved differently. Ammonium increased the pH near the surface but less so at depth, whereas nitrate displayed the opposite trend (Fig. 4.13).

One consistent feature of all of the treatments was that increases in pH corresponded to decreases in Eh (Fig. 4.13c).

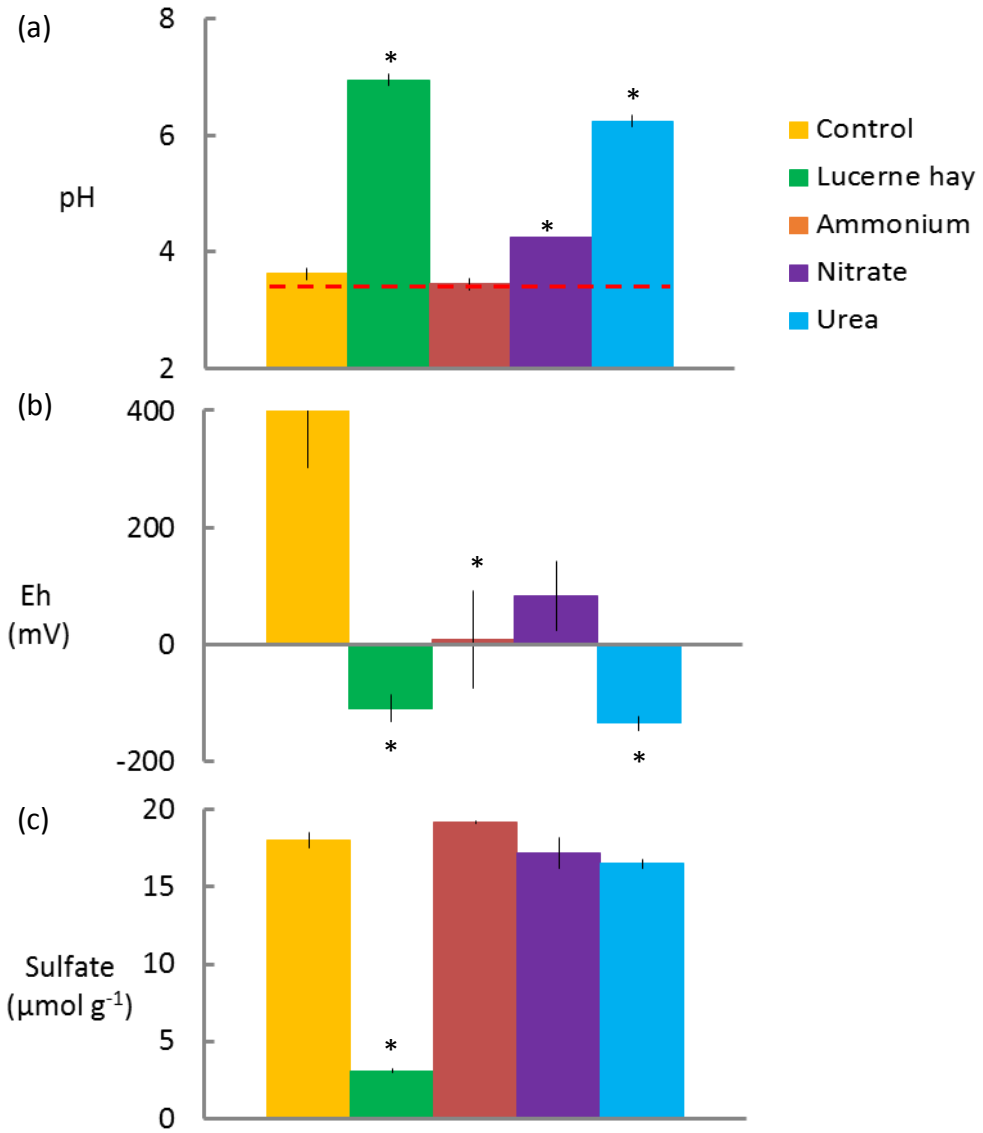

Figure 4.12. Effects of simple nitrogen sources on (a) pH, (b) redox and (c) sulfate content of sulfuric soil maintained under aerobic conditions for 6 months. The red dotted line is the initial pH. Data from the top 10 mm of the soil profile are shown. Values are means ± s.e. of three measurements (n=3). The initial sulfate content ranged between 21-32 µmol g^{-1} soil. Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

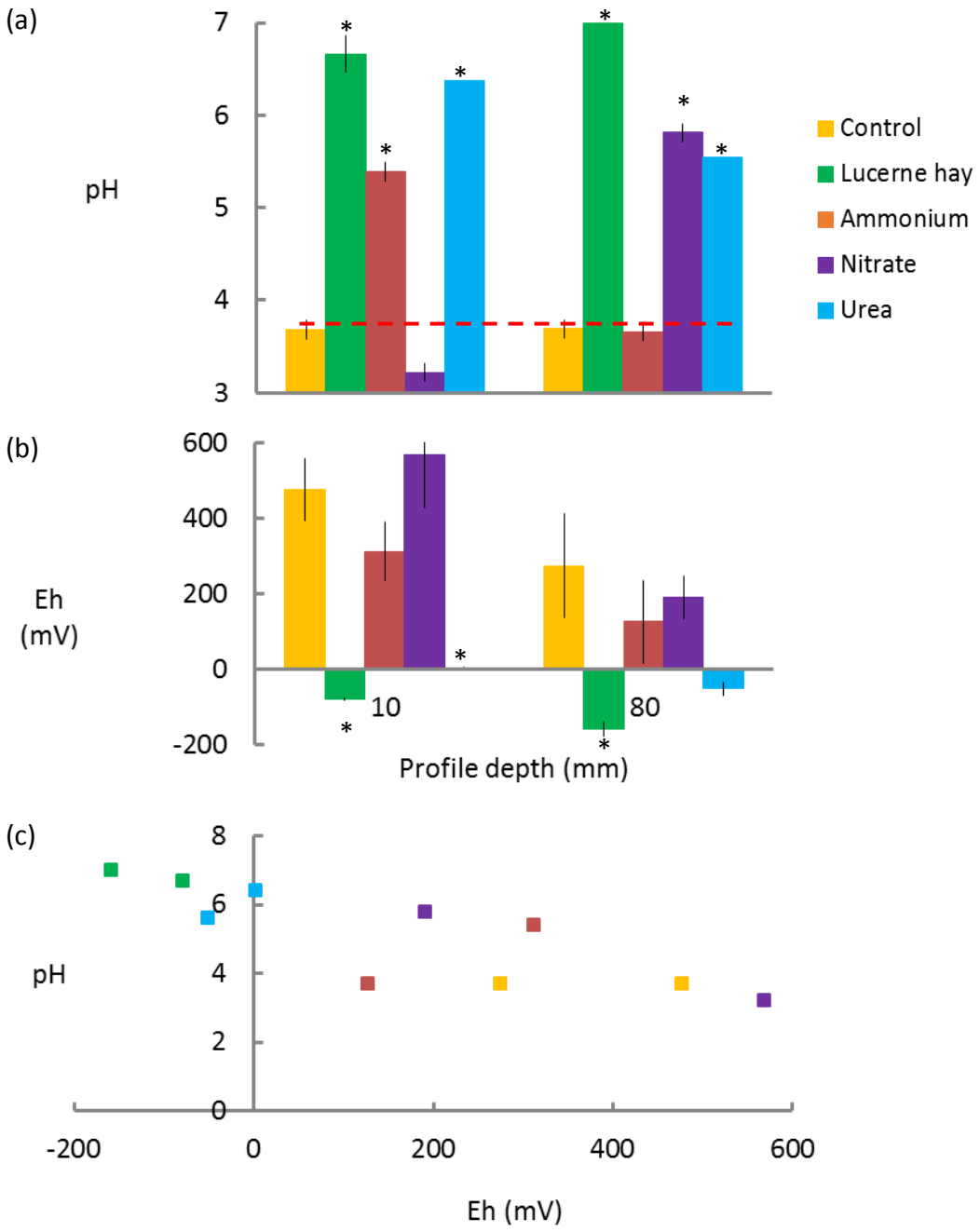

Figure 4.13. Effects of simple nitrogen sources on (a) pH and (b) redox of sulfuric soil maintained under anaerobic conditions for 6 months. The scatter plot (c) shows the relationship of redox and pH. The red dotted line is the initial pH. Values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

4.3 Discussion

4.3.1 Complex organic matter

Incorporation of complex organic matter significantly increased the pH of sulfuric soil, reduced the Eh and lowered the sulfate content, even under aerobic conditions (see Figs. 4.2–4.7). On the other hand, organic matter applied to the surface was much less effective under aerobic conditions but caused significant increases in surface pH under flooded conditions, although not to the same extent as when the organic matter was uniformly incorporated (Figs. 4.4 and 4.5).

The close correlation between the generation of alkalinity and the decrease in soil sulfate concentration is a strong indication that the pH changes resulted from the action of sulfate-reducing bacteria using the organic matter as a food source. The rapid changes in pH and Eh immediately following flooding (Fig. 4.1) suggested that initially aerobic microbial activity was involved and that the oxygen demand generated by these organisms drove the Eh into the range that was more suitable for anaerobic bacteria such as sulfate reducers. The pH of the control soil also responded to flooding and increased over the first 18 days but then began to decline. The changes in Eh mirrored the changes in pH. The differences between the control soil and the lucerne treatment were in the speed and magnitude of the changes, being much slower in the control and reversing after approximately 3 weeks (Fig. 4.1), possibly due to the exhaustion of the residual carbon in the soil. Apart from the alkalinising effects found, complex organic matter contains other essential metabolic substrates in addition to carbon and nitrogen (Jarvis and Robson, 1983; Jarvis et al., 1996; Marschner and Noble, 2000; Mengel, 1994; Noble et al., 1996; Pocknee and Sumner, 1997) cellulosic materials that are beneficial to the sulfuric soil and the microbes in holding water and creating soil microenvironments.

Under certain treatment conditions, the changes in pH did not correspond to the changes in sulfate content (e.g. Fig. 4.5), suggesting that other processes were involved in regulating the changes measured. Sulfuric soil is produced as a result of oxidation of sulfides and is expected to contain oxidised acidic minerals (Al^{3+}, Fe^{3+} and Mn^{4+}). The reducing conditions created by addition of organic matter might also lead to transformation of these minerals to their reduced forms (Johnson and Hallberg, 2005; Lin et al., 2003), thereby contributing to the increase in pH. For reference, the Eh range in

which reduction of inorganic substrates is possible (Fiedler et al., 2007) is shown in Fig. 4.14.

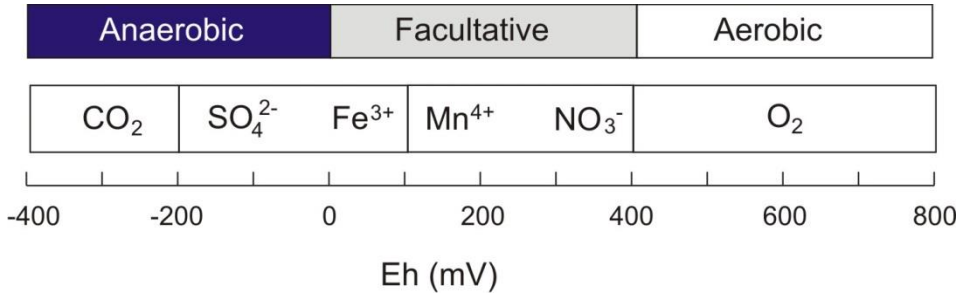

Figure 4.14. Approximate redox ranges for microbial energy metabolism for different electron acceptors.

4.3.2 Simple carbon compounds

The hypothesis that microbial alkalinisation of acidic soil is limited by organic carbon was further tested using simple metabolic substrates. Glucose had little effect on pH, except perhaps to counter increases in pH (compare the control and glucose treatments at depth in Fig. 4.8). During the measurements, an unpleasant smell of butyric acidic was detected in the treatments amended with glucose which suggests fermentative metabolism rather than aerobic degradation. However, the Eh of the glucose treatment was only partially reduced, but perhaps enough for microbes to switch to fermentation. Under anaerobic conditions fermentation of glucose generates hydrogen and CO_2, which should not affect the pH of sulfuric soil, even under reducing conditions (Fang and Liu, 2002; Lin and Chang, 1999; Roychowdhury et al., 1988). In Chapter 5, it will be seen that glucose had a strong acidifying effect on sulfidic soil, which was tentatively attributed to production of butyric acid (based on odour). Such an acidifying affect would be masked in sulfuric soils.

Molasses behaved differently to glucose, possibly due to the presence of small amounts of nitrogen (C:N approximately 27:1) that favoured the metabolism of different microbes than glucose. Acetate also increased the pH, but in this case the involvement of nitrogen can be discounted. However, the changes induced by molasses and acetate were much smaller than those due to incorporated *Phragmites* leaf. There was also little evidence that the changes caused by the simple carbon compounds were associated with changes in sulfate content of the soil. This may have been due to the activation of

different microbial populations than sulfate reducing bacteria by the different carbon sources (Hoyle et al., 2008), leading to the reduction of substrates other than sulfate.

4.3.3 Simple nitrogen compounds

Like carbon, it is well known that nitrogen in the form of amines and amino acids are needed by soil microbes for general growth and regulation of various soil factors. The hypothesis that microbial activity was limited by nitrogen was tested using both inorganic and organic nitrogen sources. The effects of ammonium addition are difficult to interpret since under aerobic conditions (Fig. 4.12) the soil became quite reduced without changing the pH, while under flooded conditions the Eh was higher and the pH increased by 1.5 units at the surface. Oxidation of ammonium can be coupled to the reduction of iron, with the consumption of protons according to the following processes:

$$3Fe(OH)_3 + 5H^+ + NH_4^+ \rightarrow 3Fe^{2+} + 9H_2O + 0.5N_2$$

or

$$6Fe(OH)_3 + 10H^+ + NH_4^+ \rightarrow 6Fe^{2+} + 16H_2O + NO_2^-$$

Nitrate also elicited variable responses, the only consistency being that pH increased if the Eh was below 200 mV and acidified if the pH was in the aerobic range. On the basis of the redox range expected for nitrate reduction (Table 2.1 and Fig. 4.14), the following reaction is possible:

$$2NO_3^- + 10\,e^- + 12H^+ \rightarrow N_2 + 6H_2O$$

Urea was found to be very effective in increasing the pH of sulfuric soil (see Figs. 4.12 & 4.13) and lowered Eh to 0 mV or below, without significant changes in sulfate concentration. Urea can generate alkalinity when it is broken down by the enzyme urease according to the following reaction sequence:

$$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2$$

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$$

4.4 Conclusions

⇒ Following the flooding of sulfuric soil manufactured from the sulfidic material extracted from the Finniss River, changes in pH and Eh were measurable within 10h. Addition of organic matter resulted in a rise in pH of approximately 1 unit within the first 24 h and a simultaneous fall in Eh towards 0 mV.

⇒ The magnitude of pH increases due to incorporation of plant material was dependent on the nitrogen content. Higher nitrogen resulted in larger changes in pH and more negative Eh.

⇒ Complex organic matter was more effective in increasing the pH, reducing the Eh and sulfate content even under aerobic condition when incorporated into the sulfuric soil than when overlaid.

⇒ Addition of carbon without nitrogen produced variable results. Glucose appeared to maintain acidity through the production of butyric acid, while acetate increased pH. The acetate results demonstrate that nitrogen may not be absolutely necessary for alkalinisation of sulfuric soils.

⇒ Addition of nitrogen without added carbon produced variable results.

⇒ Simultaneous addition of carbon and nitrogen in the form of urea and molasses significantly increased pH. Urea, which has a much higher N content was more effective than molasses.

⇒ Not all treatments that increased pH caused reductions in soil sulfate content, suggesting that a range of microbial processes other than sulfate reduction can contribute to the generation of alkalinity in sulfuric soils.

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Chapter 5

Effects of Amendments on Sulfidic Soil Chemistry

5.0 Introduction

Acid sulfate soil ecological impacts create serious concerns when strongly acidic sulfuric soils are produced following oxidation of reduced inorganic sulfur compounds (predominately pyrite) (Sullivan et al., 2009), especially when the acidification potential has exceeded the acid neutralisation capacity (ANC) of the soil environments (i.e. net positive acidity) (Nordmyr et al., 2008). In broad acre management, the most cost effective strategy to prevent this from happening is to minimise disturbance and exposure and to curtail oxidation by maintaining an anoxic environment by flooding or water table management (White et al., 1999; White et al., 1997). This would exclude oxygen and prevent pyrite oxidation even though in the presence of ferric iron, oxidation may still continue (van Breemen, 1973; White et al., 1997), especially in strongly acidic soils of pH<3.5 (Evangelou, 1995).

While some knowledge on management of sulfidic soil oxidation, especially through water table management (e.g. Wilson et al. 1999) and land use management is available, an understanding of the combined roles of organic matter and moisture content on oxidation processes in sulfidic soils is lacking. In the preceding chapter, it was clear that soil carbon and nitrogen were important factors in ameliorating “sulfuric soil”, and that the effects of these compounds on “sulfidic soil” needs to be evaluated.

In this chapter, the findings of several studies conducted to investigate the effects of complex organic matter, simple carbon and nitrogen compounds as well as sulfate on sulfidic soil oxidation as a function of soil moisture are presented.

5.1 Methodology

The general methodologies are the same as those used with sulfuric soils described in Chapter 4, and detailed descriptions of the studies are given in Chapter 3. The sulfidic soils used in these studies are from the Finniss River. Data collected are presented as described under Section 3.7 (Chapter 3).

5.2 Results

5.2.1 Effects of moisture on oxidation of sulfidic soil

The overall changes in pH of soils set at different field capacities under two different soil temperature conditions are shown in Table 5.1. The initial pH of the soil used was 6.0. Under cool conditions (4^0C), the pH of the dry soil (0% field capacity) declined to 5.6, whereas under warmer soil condition (25^0C), the pH fell to 4.7. With increasing moisture content, the pH progressively declined to 2.6 at both 4^oC and at 25^0C. When fully flooded (200% field capacity), the pH did not decrease as much, presumably due to the restriction of oxygen penetration due to waterlogging.

Table 5.1. Effects of soil moisture content of sulfidic soil on pH at different field capacities (Fc).

Treatment conditions				
	4^0C		25^0C	
Fc (%)	pH	s.e.	pH	s.e.
Initial pH	6.0	0.0	6.0	0.0
0	5.6	0.1	4.7	0.3
25	4.4	0.6	3.7	0.3
50	2.7	0.0	3.0	0.1
75	2.7	0.0	2.8	0.0
100	2.6	0.0	2.7	0.0
150	2.9	0.0	2.6	0.0
200	3.4	0.1	3.0	0.1

The values are means of three measurements (n=3) ± standard error.

5.2.2 Effects of complex organic matter on oxidation of sulfidic soil

The long-term (6 months) effects on sulfidic soil chemistry measured following the addition of complex organic matter with varying nitrogen content under the two moisture conditions of studies [XI] and [XII] (Table 3.2) are shown in Figs. 5.1 and 5.2. Under aerobic conditions, the unamended sulfidic soil strongly acidified, the pH declining to near 4 at depth (Fig. 5.1a). In the amended treatments under aerobic conditions, lucerne hay and pea straw significantly prevented the soil from acidifying and increased the pH to well over 8, whereas with wheat straw, the pH fell but not as much as in the control. The pH

changes were again correlated with changes in Eh (Fig. 5.1b), and also with changes in soil sulfate content (Fig. 5.1c). With lucerne hay, sulfate was completely depleted.

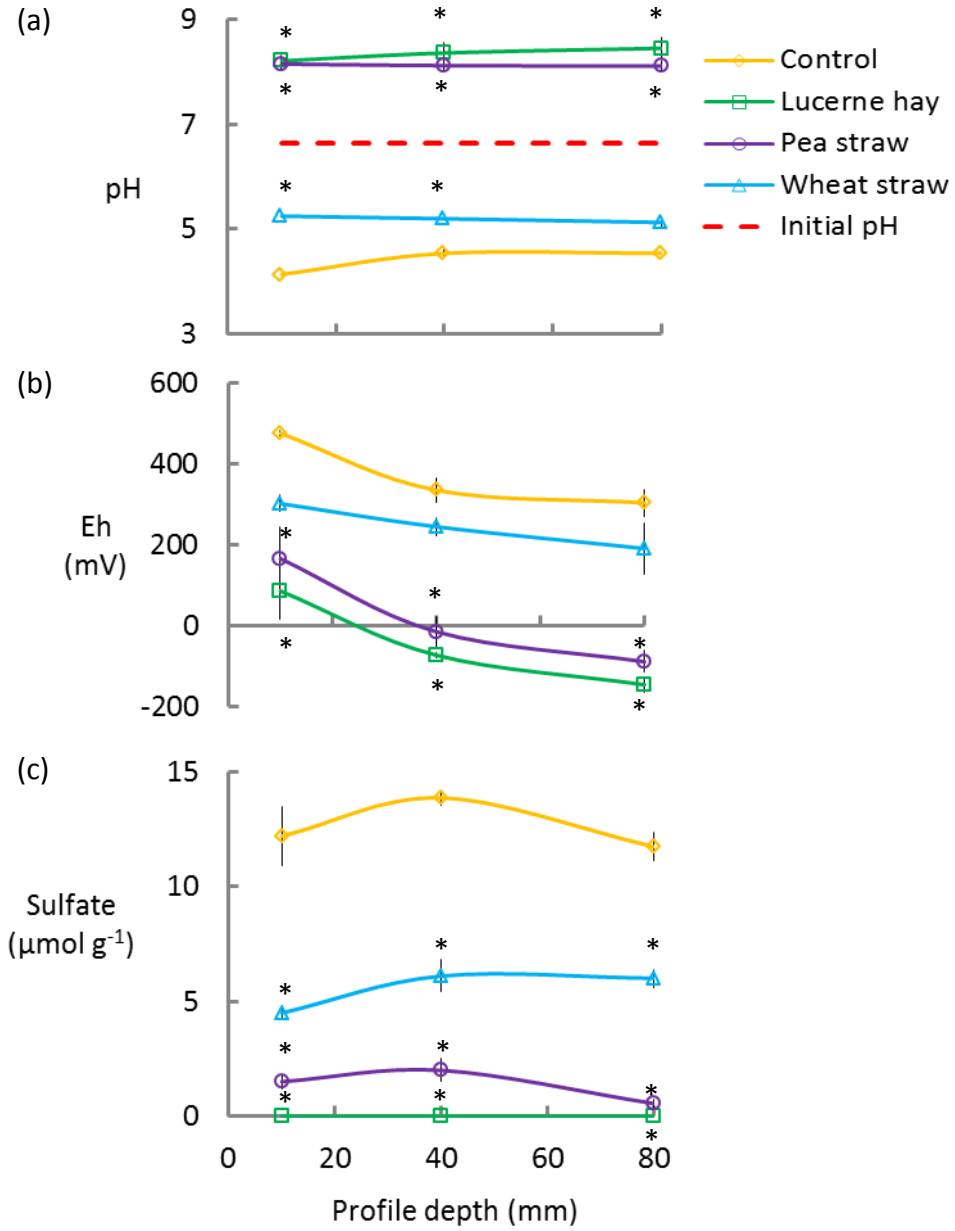

Figure 5.1. Effects of incorporated organic matter on (a) pH, (b) redox and (c) sulfate content of sulfidic soil maintained under aerobic conditions for 6 months. The initial sulfate content of the soil ranged from between 12 to 16 µmol g^{-1} soil. The values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

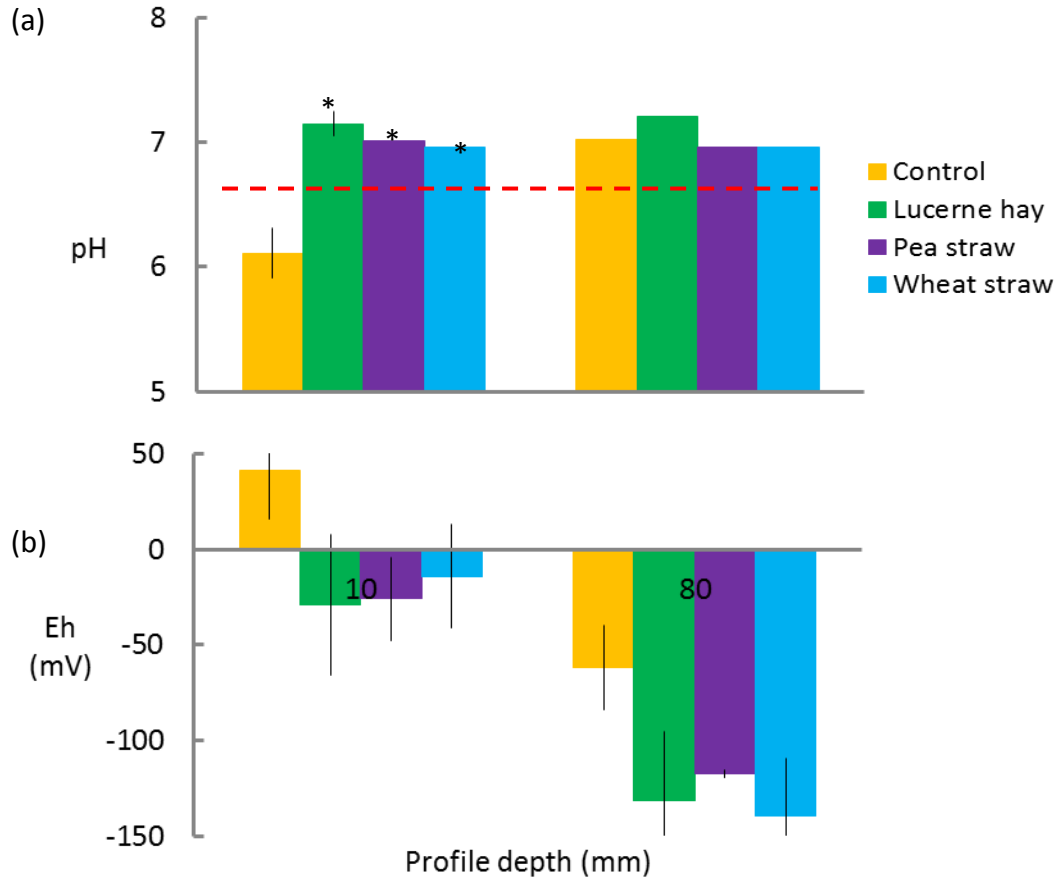

Figure 5.2. Effects of incorporated organic matter on (a) pH and (b) redox of sulfidic soil maintained under anaerobic conditions for 6 months. The red dotted line is the initial pH. Values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

Under anaerobic conditions, the pH of all of the amended soils was higher than the initial pH but unlike under aerobic conditions, it remained lower than 8 and similar to the pH of the control soil, except at the surface where the pH decreased to near 6 (Fig. 5.a). The influences of varying nitrogen content of the organic matter were not really evident, with the dominant effect being the low Eh produced by all treatments (Fig. 5.b).

5.2.3 Effects of simple carbon compounds on oxidation of sulfidic soil

In this study ([MI, Table 3.7), simple carbon compounds in the form of sodium acetate, glucose and molasses were applied to soil under flooded conditions to assess their effects on sulfidic soil over 6 months. Comparison was also made with soil amended with supplemental sulfate.

Under flooded conditions, the control soil pH remained unchanged throughout the profiles as expected due to the stronger reducing conditions (Fig. 5.3a). Among the simple carbon compounds, only acetate maintained the pH near the control level, whereas glucose and molasses strongly acidified the soils. Addition of sulfate did not significantly alter the pH.

As shown in Fig. 5.3b, all the treatment soils were reduced throughout the profiles. In agreement with their effects on pH, glucose and molasses recorded the highest Eh values. The relationships between the changes in redox and pH are shown in Fig. 5.3c.

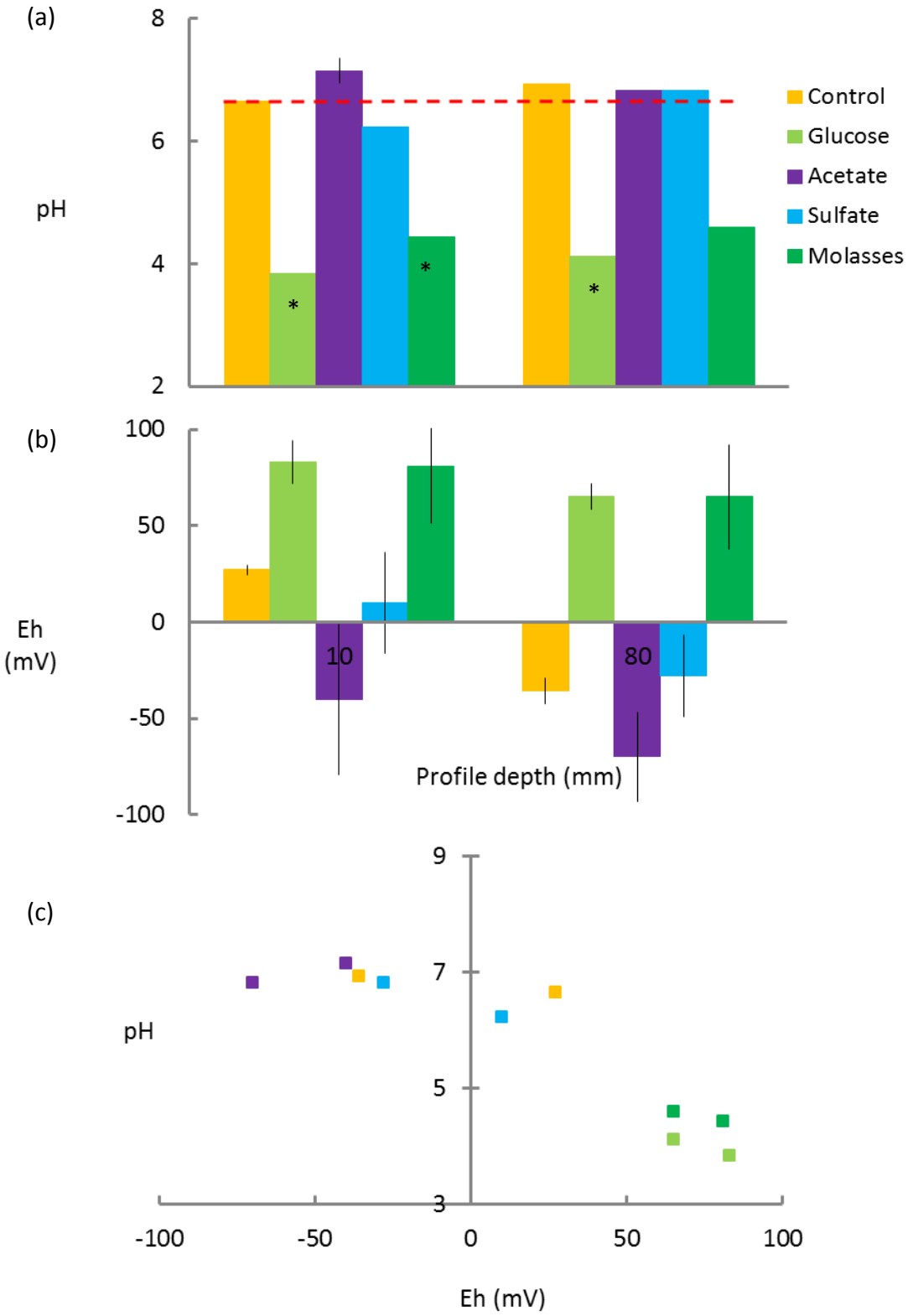

Figure 5.3. Effects of simple carbon compounds and sulfate on (a) pH and (b) redox of sulfidic soil maintained under anaerobic conditions for 6 months. The scatter plot shows the association between redox and pH. The red dotted line is the initial pH. The values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p < 0.05$) between treatment and control at the same depth.

A more detailed investigation of the effects of simple carbon compounds was undertaken by conducting a time-course study over 12 weeks for both aerobic and anaerobic conditions, and comparing the changes with those induced by complex organic matter in the form of lucerne hay.

Under aerobic conditions, acetate stabilised the pH while lucerne hay increased it (Fig. 5.4a-c). These changes occurred within the first 3 weeks then remained stable. In the control and glucose treatments, the pH gradually decreased over 12 weeks.

The changes in pH were generally correlated with changes in redox potential, with lucerne hay and acetate maintaining an Eh in the range of 0 to 200 mV at the surface (Fig. 5.4d-f). The Eh of the control soil fell to around 100 mV in the first 3 weeks, but then became quite aerobic, which corresponded to more rapid reductions in soil pH. In the glucose treatment, the Eh remained high throughout.

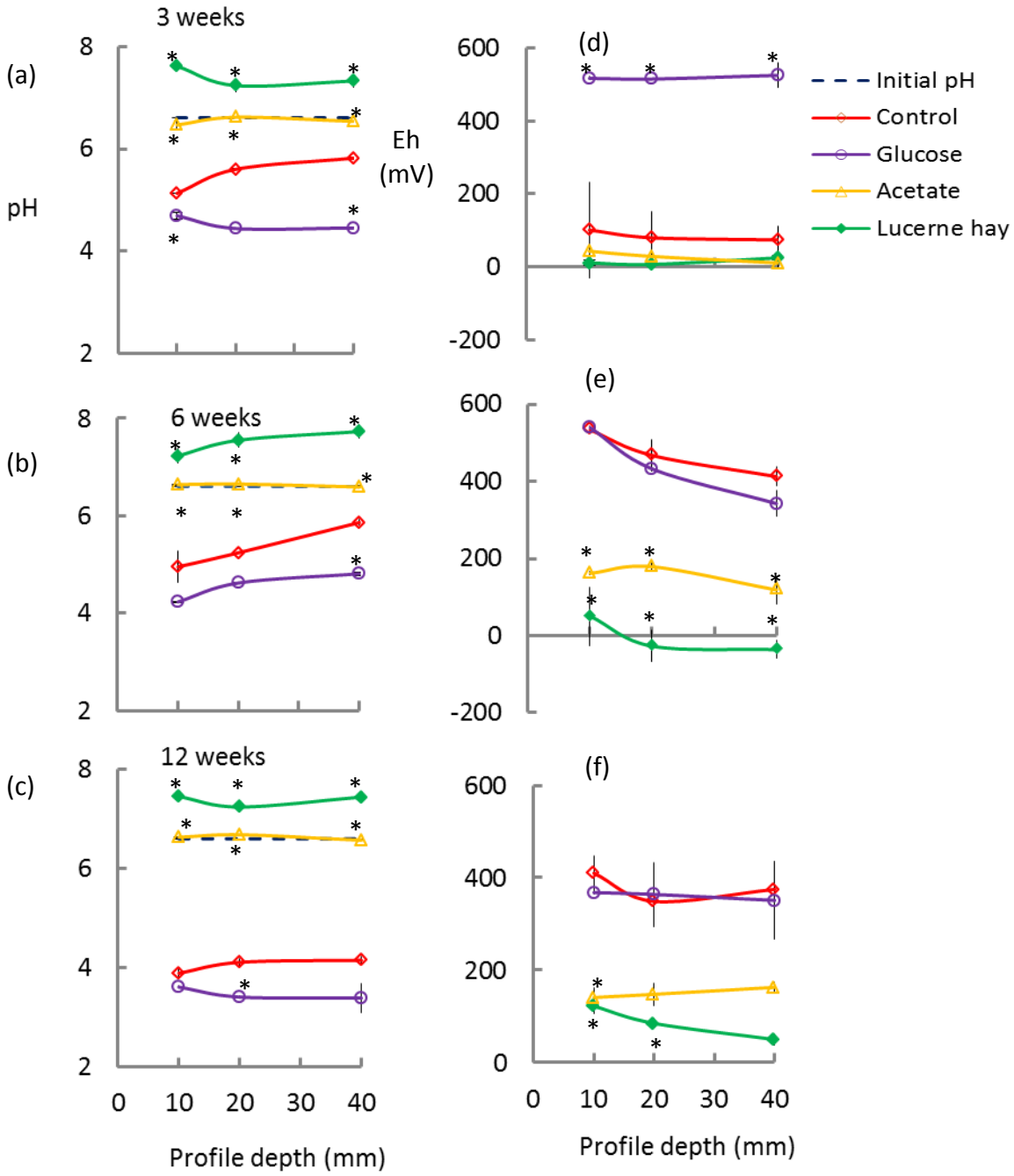

Figure 5.4. Time-course of the effects of simple carbon compounds and complex organic matter on (a-c) pH and (d-f) redox of sulfidic soil maintained under aerobic conditions for 3, 6 and 12 weeks. The values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

Under anaerobic conditions, the pH of the control soil remained stable, and there were no significant effects of lucerne hay or acetate (Fig. 5.5). However, glucose induced a strong acidification in the first 3 weeks after which the pH remained relatively stable between 4 and 5. The Eh in all treatments except glucose was around -60 mV at all

sampling times. In the glucose treatment, Eh was also reduced but was mostly in the range 60 to 100 mV.

During the processing of measurement in the soils amended with glucose, it was noted that there was strong smell of butyric acid, which may have been the end product of a specific bacterial metabolism.

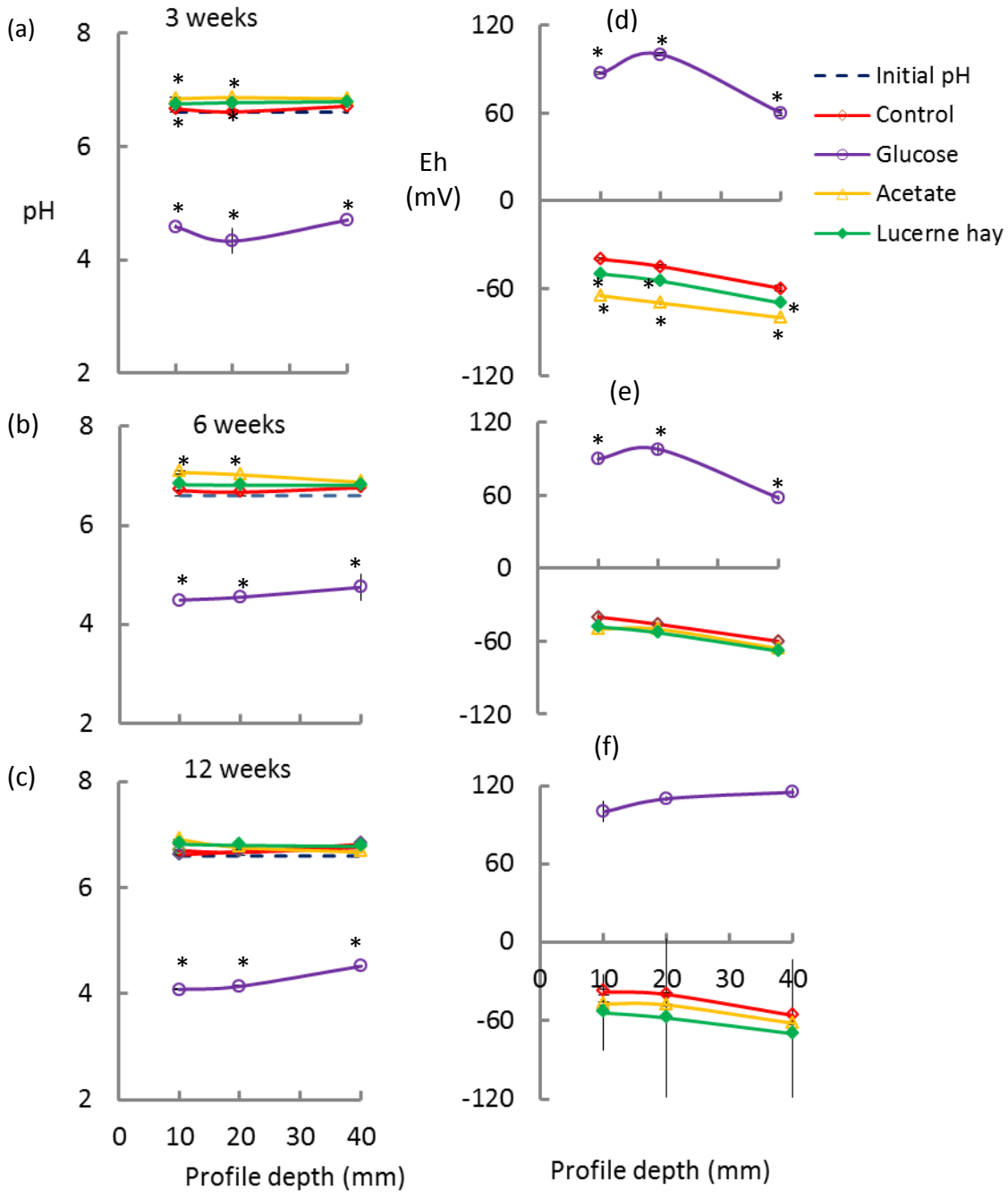

Figure 5.5. Time-course of the effects of organic matter and simple carbon compounds on (a-c) pH and (d-f) redox of sulfidic soil maintained under anaerobic conditions for 3, 6 and 12 weeks. Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

As was seen with sulfuric soil in Chapter 4, there was a close correlation between changes in pH and Eh in sulfidic soil (Fig. 5.6).

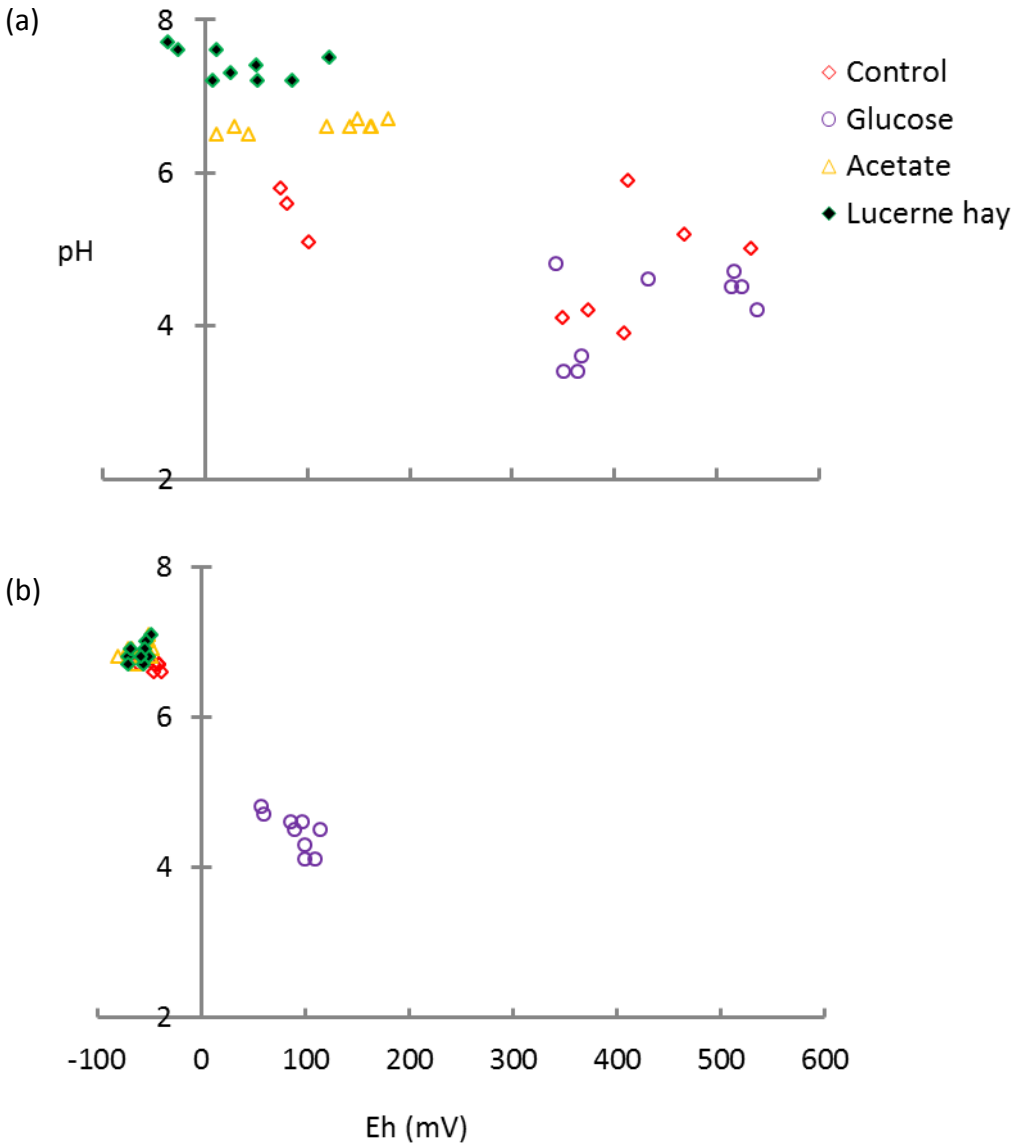

Figure 5.6. Scatter plots showing the association between Eh and pH under (a) aerobic and (b) anaerobic conditions. The values are means ± s.e. of three measurements (n=3).

5.2.4 Effects of simple nitrogen compounds on oxidation of sulfidic soil

The changes in sulfidic soil chemistry following the addition of simple nitrogen compounds under two moisture levels were examined throughout studies [XV] and [XVI] (Table 3.3).

Under aerobic conditions, the control soil pH was stable at the surface but increased slightly at depth (Fig. 5.7a). Urea addition strongly increased the pH, reaching

nearly 8. The pH of the soils mixed with nitrate and ammonium were lower, ranging between 6 at the surface and 7 at depth.

The control soil was generally reduced throughout (Fig. 5.7b), Eh ranging between 4 mV and -81 mV. All the amended soils were reduced in a similar manner. The sulfate content inversely correlated with changes in pH but did not mirror the changes in Eh (Fig. 5.7c).

The changes in pH and Eh measured under flooded conditions are shown in Fig. 5.8. In the control soil, the pH declined slightly to 6 at the surface but was constant at depth (Fig. 5.8a). In the amended soils, urea increased the pH to 7.8 throughout; nitrate moderately acidified the surface soil to 5.1 but was unchanged at depth; ammonium had a slight acidifying effect. In this experiment, Eh values were quite low and there was no obvious relationship between Eh and pH.

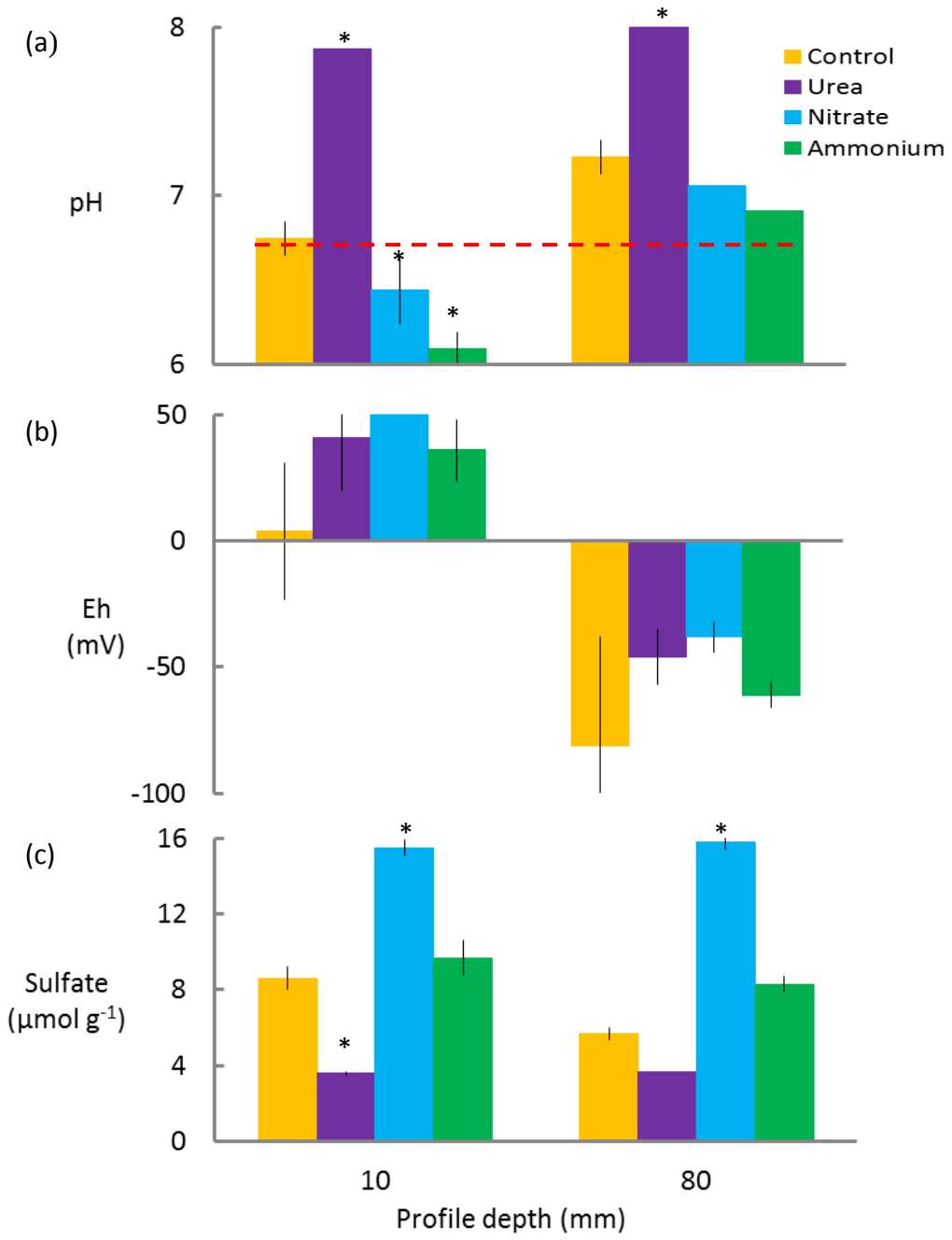

Figure 5.7. Effects of simple nitrogen on (a) pH, (b) redox and (c) sulfate content of sulfidic soil maintained under aerobic conditions for 6 month. The red dotted line is the initial pH. The initial sulfate content ranged from between 12 to 16 µmol g^{-1} soil. The values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

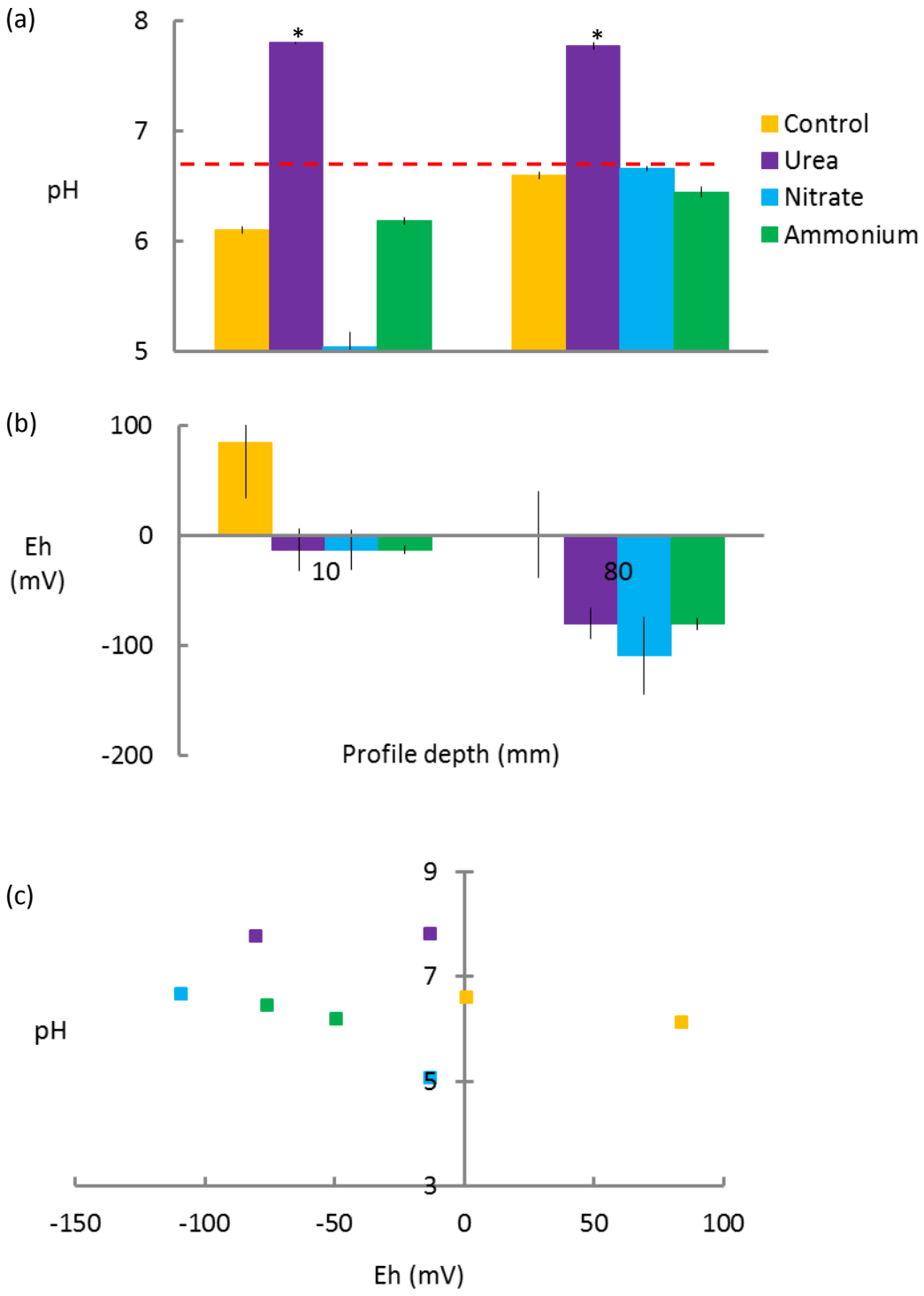

Figure 5.8. Effects of simple nitrogen compounds on (a) pH and (b) redox of sulfidic soil maintained under anaerobic conditions for 6 months. The association of redox with pH is shown by the scatter plot (c). The red dotted line is the initial pH. The values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

5.3 Discussion

5.3.1 Moisture and oxidation of sulfidic soil

The exposure of previously submerged sulfidic soil from Finniss River by falling water levels allows the surface minerals to interact with oxygen to produce sulfuric acid (Fig. 3.3c). The degree of dryness for this to happen has not been extensively investigated. This study has shown that oxidation of dry sulfidic soil is slow, so allowing exposed sulfidic soil to dry might be an important initial step in minimising oxidation. Increasing the moisture content to 25% field capacity accelerated the oxidation, which reached a maximum between 50 and 150% field capacity, with a slight tendency for the pH to increase under more flooded conditions (Table 5.1). Oxidation was lower (more likely slower) at low temperature and intermediate moisture conditions. However, surface drying would be expected to take longer under cooler conditions possibly leading to a greater eventual oxidation.

5.3.2 Complex organic matter

Under most soil use and management conditions, such as for farming, flooding is undesirable, except in rice production. Although lime is widely used, its application is commonly for the management of actual soil acidity already produced and acidified water bodies (Baldwin and Fraser, 2009; Borg et al., 1995; Fraser and Britt, 1982; Indraratna et al., 2006; Powell and Martens, 2005). The application of lime, however, can present undesirable effects on certain terrestrial soils and biotic forms (Buckton and Ormerod, 1997; Hindar et al., 1996; Matzner et al., 1985; Persson et al., 1989; Shore and Mackenzie, 1993) and in wetlands with sensitive aquatic species (Farmer, 1992; Henrikcson et al., 1995).

The practicality of using complex organic matter was extended from the studies on sulfuric soil in Chapter 4 to investigate the short and long-term effects on sulfidic soil oxidation. In exposed sulfidic soils, addition of organic matter on the surface would conserve moisture by acting as surface mulch and lower the redox of the surface soil as a result of microbial depletion of oxygen. Incorporation of organic matter would add a range of nutrients for the soil microbes, whose metabolism could generate biogenic

alkalinity. Addition of organic matter under anaerobic conditions would serve similar purposes, but in this case, the lower Eh would favour the activity of a different range of microbes.

Under aerobic conditions, the sulfidic soils progressively acidified over time in the absence of organic matter when the changes in soil properties as a function of time were studied. In contrast, incorporation of lucerne hay significantly prevented oxidation and increased the pH. The effects induced by both lucerne and acetate appeared to be very rapid, with little further change occurring between 3 and 12 weeks. The speed of the protective effect of organic matter may be an attractive feature in exposed floodplains to prevent mobilisation of toxic soil components such as heavy metals. In an agricultural setting the stabilisation of pH would be important but may be complicated by the lowering of the Eh that accompanies the breakdown of the organic matter, which could be undesirable for plant growth.

Under flooded conditions, the control pH was stable and neither lucerne hay nor acetate produced any change in pH. On the other hand, glucose caused the pH to drop by more than 2 units within the first 3 weeks and slightly more after 12 weeks (Fig. 5.5). Glucose was the only treatment in which Eh remained above 0 mV; in all other treatments the Eh was around -40 to -60 mV.

The positive effects of lucerne hay were maintained for at least 6 months under aerobic conditions (Fig. 5.1). The importance of nitrogen was demonstrated by comparison of the effects of organic matter with differing nitrogen contents. The increases in pH, and reductions in Eh and sulfate contents of the sulfidic soil by lucerne hay, pea straw and wheat straw were directly related to their measured nitrogen contents (Fig. 5.1). Under flooded conditions, the stability of pH is mainly due to the anaerobic conditions, and added organic matter only had small effects on pH and Eh.

Sulfate reducing bacteria are anaerobic in nature and need significantly reduced soil conditions to function (Muyzer and Stams, 2008). The observed reductions in Eh most likely involved aerobic bacteria acting on the organic matter and depleting oxygen. This would have created favourable conditions for anaerobic bacteria, including sulfate-reducing bacteria, which explains the large changes in both sulfate content and pH. What is not clear is whether the apparent nitrogen requirement, as evidenced by the difference

in effectiveness of organic matter with different nitrogen content, is for the metabolism of the aerobic or anaerobic bacteria.

5.3.3 Simple carbon compounds

Soil microbes use a range of carbon compounds (e.g. glucose and acetate) for cellular respiration and is well established that the supply is the main limitation for microbial activities (Kuzyakov et al., 2000; Neff et al., 2002). However there was a clear difference in the effects of glucose and acetate on soil pH, under both aerobic and anaerobic conditions. Under aerobic conditions, acetate was able to stabilise the pH of sulfidic soil, and rapidly induce moderate reductions in Eh, whereas glucose maintained a high Eh throughout and caused strong acidification of the soil (Fig. 5.4). These effects were also observed under anaerobic conditions with both glucose and molasses (Fig. 5.3). The obvious odour of butyric acid suggests that the acidification with glucose was mediated by fermentative microbes producing acidic metabolic end products.

The effects of acetate were interesting in relation to the apparent requirement for nitrogen when complex organic matter is added. The changes in pH and Eh following addition of acetate under aerobic conditions were almost as great as those of lucerne hay, and equally rapid (Fig. 5.4). Since acetate can be utilised as an energy source by some groups of bacteria under aerobic conditions and by sulfate and iron reducing bacteria under anaerobic conditions (Kamura et al., 1963; Thauer et al., 1989), the observed ameliorative effects may have resulted from the biogenic alkalinity generated by a range of microbes.

5.3.4 Simple nitrogen compounds

The need for nitrogen by soil microbes in the form of amino acids and amines for general growth and development is well established. Results from the previous studies confirmed that nitrogen can be a limiting factor in preventing sulfidic soil oxidation (Fig. 5.1). Addition of either nitrate or ammonium failed to increase the pH over the control, and in some treatments caused moderate acidification. The acidifying effects of ammonium in soils other than ASS have been reported (Martikainen, 1985). Urea however, had a strong alkalising effect under both aerobic and anaerobic conditions,

perhaps through metabolism by microbes requiring both organic carbon and a nitrogen source. The large decrease in sulfate content of the soil amended with urea suggests that sulfate-reducing bacteria were at least partially responsible for the increase in pH. Increase in pH of forest soils following urea addition has also been reported (Martikainen, 1985).

5.4 Conclusions

⇒ The rate and extent of oxidation of sulfidic material in ASS is strongly dependent on the moisture content and on temperature. Dry sulfidic soil oxidises slowly, moist sulfidic soils oxidises more rapidly and flooded soils much less.

⇒ Incorporation of complex organic matter or acetate into sulfidic soil caused the pH to rise under aerobic conditions, while glucose strongly acidified the sulfidic soil. These changes were largely complete within 3 weeks of incubation.

⇒ Under flooded conditions, the unamended sulfidic soil did not acidify and the pH was similar to sulfidic soil amended with either complex organic matter or acetate. However, glucose acidified the sulfidic soil.

⇒ Addition of nitrogen in the absence of organic carbon was ineffective in raising sulfidic soil pH but urea had a strong alkalising effect.

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Chapter 6

Effects of Plants on Acid Sulfate Soil Chemistry

6.0 Introduction

In inland soils, plants use oxygen surrounding roots for respiration (Tinh et al., 2001). In soils of limited oxygen, parenchymatous structures are used to transport oxygen from the leaves to support root respiration (Armstrong, 1979). In ASS, we know that unlimited oxygen leads to sulfidic soil oxidation and produces sulfuric soils. Under anaerobic conditions, release of oxygen into the rhizosphere by plant roots would lead to aeration of sediments and oxidation of reduced sulfide-bearing minerals, which upon rehydration would generate sulfuric acidity.

Plants also have the capacity to alter the physical and chemical compositions of the rhizosphere by turnover of organic matter and secretion of organic substances, which in turn would influence microbial activity around the roots (Reid and Butcher, 2011). While some of these processes have been well described for forest and agricultural soils of neutral pH (Foy, 1992; Haling et al., 2010; Haling et al., 2011; Tinh et al., 2001), little information is available for the effects of live plants on ASS. Recently, Reid and Butcher (2011) conducted a small scale investigation of the effects of several types of plants on ASS and found that the response varied depending on the plant and its depth of penetration into the soil. However, the results were limited to pH and other aspects of soil chemistry were not investigated.

In this research, three common inland and wetland plants (*Melaleuca*, *Typha* and *Phragmites*), which are globally distributed and known to grow under a range of soil conditions have been used to investigate the effects of plants on ASS chemistry. *Melaleuca* is moderate to deep rooting on inland soils and is tolerant to extremely acidic and occasionally flooded soils (Boland et al., 2006). *Typha* is most commonly found in wetlands but can grow under aerobic conditions if the soil moisture content is adequate (Selbo and Snow, 2004), whereas *Phragmites* grows in both inland and wetland soils (Marks et al., 1994).

While *Melaleuca* resembles a typical inland plant, *Typha* and *Phragmites* present an interesting scenario as they possess aerenchymous and parenchymatous tissues, which

would transport oxygen into sulfidic materials/sediments (Armstrong and Armstrong, 1991; Armstrong et al., 1996; Bendix et al., 1994; Tornberg et al., 1994) and potentially oxidise them producing sulfuric acidity. These plants are quite successful in colonising wastelands because of the extensive underground rooting (rhizomes) systems and self-mulching effects due to rapid turnover of organic matter, making them ideal plants to assess their effects on sulfidic soil oxidation and their potentials to rehabilitate sulfuric soils.

In this chapter, the findings of several studies conducted to investigate the effects of several common inland and wetland plants on ASS chemistry are presented.

6.1 Methodologies

All the studies were conducted in 50 cm tall stormwater tubes whose bottom ends were tightly capped. In all the tubes, the bottom 22 cm of the tubes was filled with sand and the top 22 cm with either sulfidic or sulfuric soil derived from sulfidic soil of Finniss River. Measurements were only made from the top 22 cm containing the ASS. Although the 'aerobic treatments' were regularly watered, it is probable that the moisture was unevenly distributed over time, with the upper parts being aerobic and the lower parts of the profile becoming waterlogged.

Redox potential (redox/Eh) and pH were measured as described previously under Sections 3.4 and 3.5. Sulfate content was quantified using soil samples from the surface (20 mm), middle (100 mm) and deep (200 mm) profiles, and root biomass was quantified as described in Fig. 3.10i-l. Data from the surface, middle and deep profiles are presented to clearly show the changes in ASS chemistry that occurred.

6.2 Results

6.2.1 Effects of *Melaleuca* and *Typha* plants

Melaleuca was established in studies [PIV] and [PVII] and *Typha* in [PV] and [PVIII] (Table 3.4). Figures 3.9 and C1 (Appendix) show the type of plants established. The effects of these plants on soil chemistry are shown in Fig. 6.1a. Despite the high acidity, both plants produced good amounts of both above ground and below ground biomass. In the

Melaleuca treatment, the root biomass decreased with depth, while for *Typha* biomass was highest in the middle of the profile but still reasonably abundant at depth. Consistent with the reducing conditions (Eh < 0), pH of the sulfuric soil without plants increased from an initial pH of 3.2 to 5.5 at the surface and 6.5 at depth. In the planted treatment, there were striking differences in both Eh and pH compared to the control.

In these treatments, Eh remained between 400 and 600 mV and the increases in pH were quite small, especially at depth (Fig. 6.1b, c). The changes in sulfate content mirrored the changes in pH, and correlated with the Eh, with low sulfate content associated with higher pH and lower Eh (Fig. 6.1d).

In the sulfidic soil, *Melaleuca* grew better than in the sulfuric soil, whereas for *Typha* the opposite was the case, especially at depth (Fig. 6.1e). These differences in biomass between the species allowed for comparisons between the treatments based on how much root was present in different parts of the profile. For example, the root biomass for *Melaleuca* was relatively constant across the profile, and the Eh remained highly oxidised (Fig. 6.1e, g). However, for *Typha*, most of the biomass was concentrated in the upper part of the profile where the Eh was also highly oxidised, but at depth where there were fewer roots, the Eh was similar to that of the control. As with the sulfuric soils, this translated into proportional changes in pH and sulfate content (Fig. 6.1f, h).

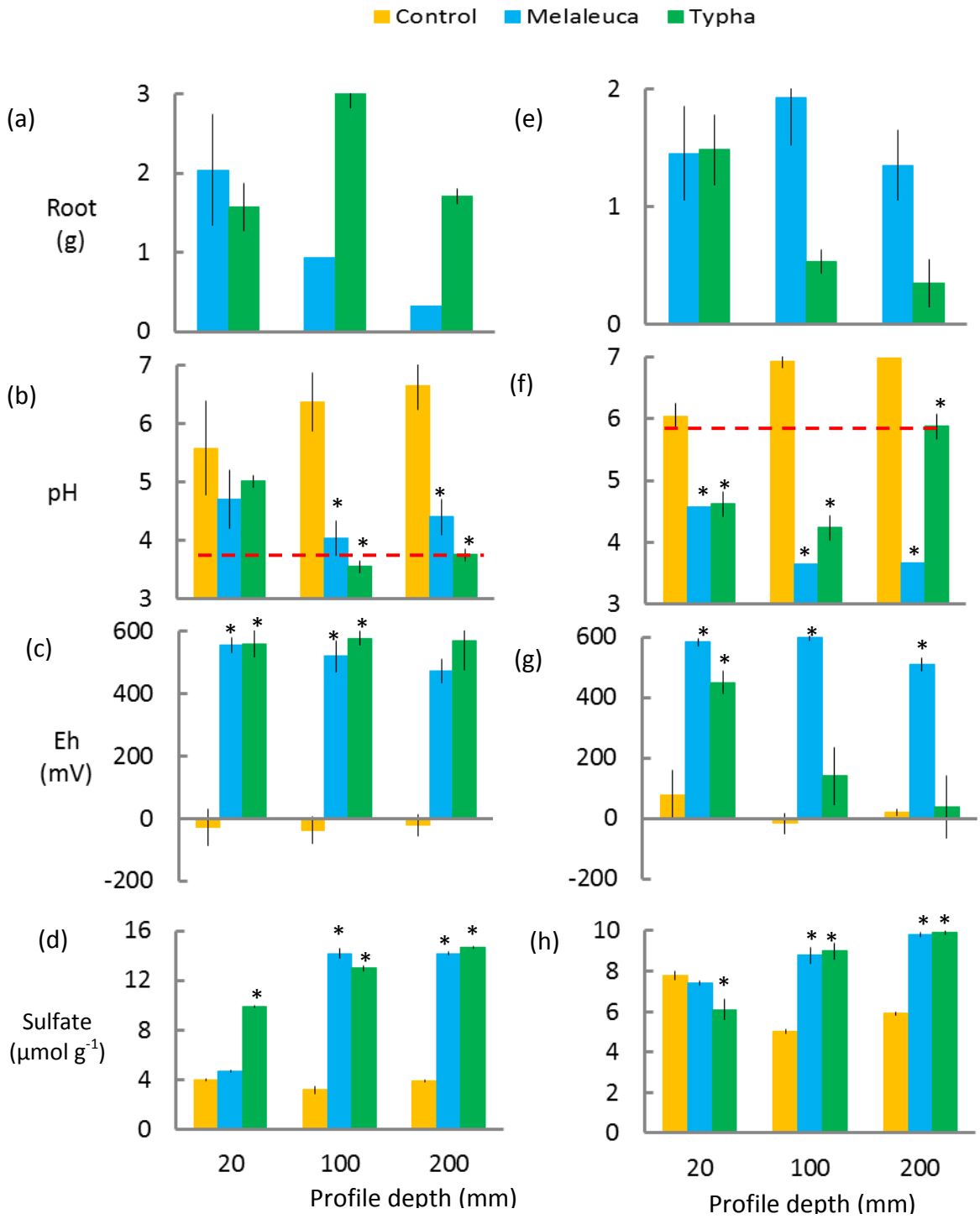

Figure 6.1. The effects of (a) *Melaleuca* and (e) *Typha* roots on (b & f) pH, (c & g) redox and (d & h) sulfate contents of (a-d) sulfuric and (e-h) sulfidic soil maintained by regular watering. Values are means ± s.e. of three measurements (n=3). The red dotted lines are the initial pH. The initial sulfate content of the sulfuric and sulfidic soils respectively ranged between 21-32 and 12-16 µmol g^{-1} soil. An asterisk indicates significant differences ($p<0.05$) between treatment and control at that depth.

6.2.2 Effects of *Phragmites* plants

The four interrelated studies conducted using *Phragmites* plants were described as [PIII], [PVI], [PIX] and [PX] under Section 3.3.4 and in Table 3.4. Studies [PIII] and [PXI] were conducted on sulfidic and [PVI] and [PIX] on sulfuric soils, respectively. Figures 3.7j and 3.9a respectively show the types of plants established under flooded and aerobic soil conditions. Additional photos are shown in Fig. C2 under Appendix section.

The pH of the control sulfuric soil under anaerobic conditions increased from 3.7 to near 7 (Fig. 6.2b), and the redox was below 0 mV (Fig. 6.2c) with little change across the profiles. Under anaerobic conditions, root biomass in the sulfuric soil decreased with depth (Fig. 6.2a). In the *Phragmites* treatment the pH also increased but less than the control but the redox, particularly near the surface was much higher than the control soil (Fig. 6.2c). The sulfate content of all treatments was substantially reduced (in comparison to the initial values), and roughly correlated with changes in Eh. The smallest change in sulfate was observed in the *Phragmites* treatment with the highest biomass and the most positive Eh.

The overall root biomass in the sulfidic soil was similar to that in the sulfuric soil, indicating that pH is not a major factor in colonisation of the soil. However, under sulfuric conditions, the roots were slightly more concentrated at the surface soil, whereas in the sulfidic soil root distribution was more even (Fig. 6.2e). pH of the control soil was largely unchanged, except for a small decrease at the surface (Fig. 6.2f), and the Eh remained below 0 mV throughout the profile. In the *Phragmites* treatment, the pH decreased by more than 1 unit, and the Eh was noticeably higher, remaining around 400 mV at the surface but decreasing with depth to 70 mV. The sulfate content of all treatments was lower, but less so in the *Phragmites* treatment where the soil acidification was highest.

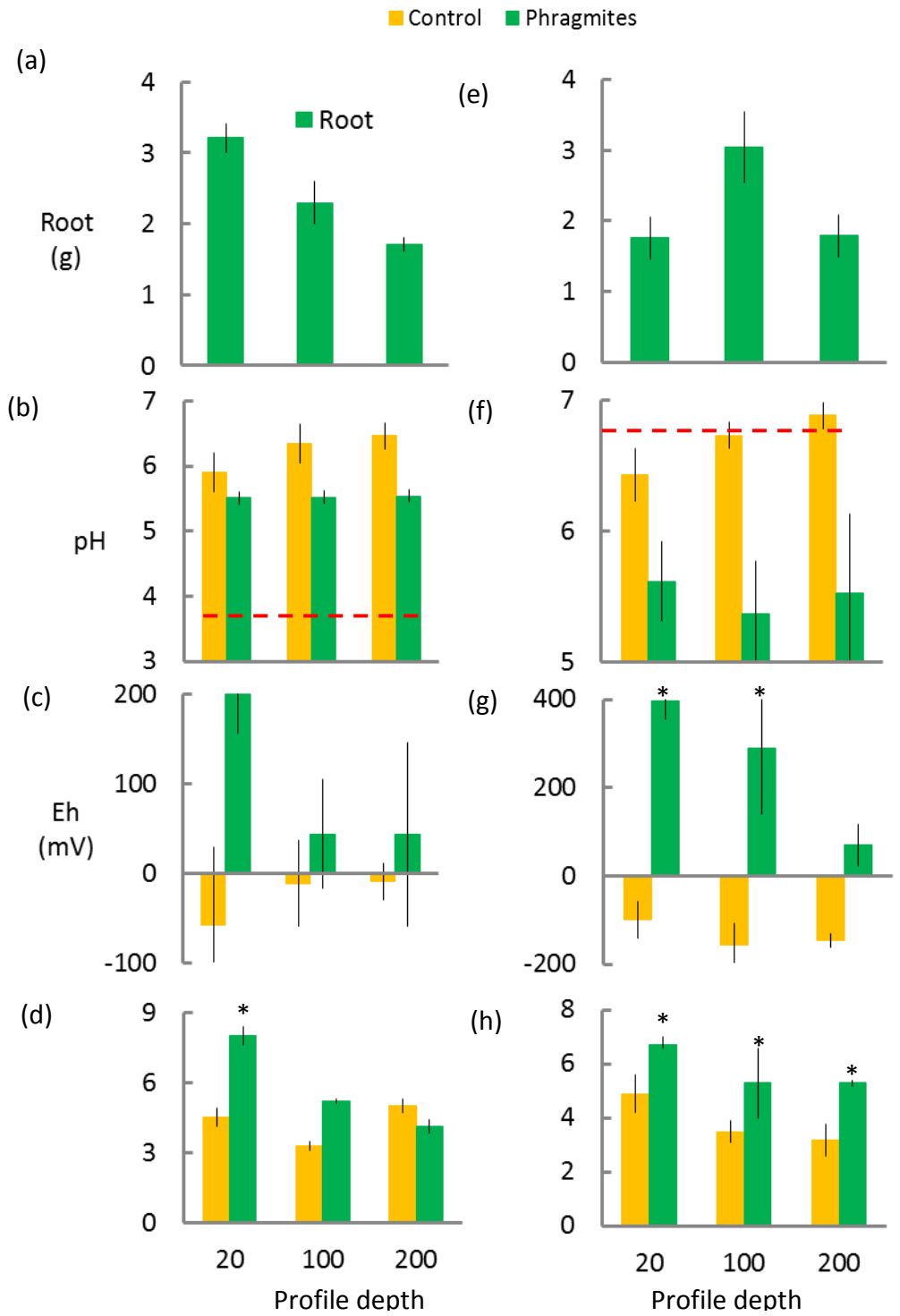

Figure 6.2. The effects of (a & e) *Phragmites* roots on (b & f) pH, (c & g) redox and (d & h) sulfate contents of (a-d) sulfuric and (e-h) sulfidic soils maintained under flooded (anaerobic) conditions for 12 months. Values are means ± s.e. of three measurements (n=3). The red dotted lines are the initial pH. The initial sulfate content of the sulfuric and sulfidic soils respectively ranged between 21-32 and 12-16 µmol g^{-1} soil. An asterisk indicates significant differences ($p<0.05$) between treatment and control at that depth.

Figure 6.3 shows the effect of *Phragmites* on sulfuric soil chemistry under aerobic conditions. As mentioned previously, in tubes receiving regular watering, it was not possible to ensure that the lower parts of the profiles received oxygen from the atmosphere. However, the relatively high Eh of the control suggests that near the surface at least, oxygen penetration was significant, and the surface pH remained close to the initial pH, whereas at depth the pH increased by more than 2.5 units. In the *Phragmites* treatment, the pH increased by 1.2 units at the surface but at depth remained unchanged (Fig. 6.3b), corresponding to the high Eh, which remained above 400 mV (Fig. 6.3c). The sulfate content of all the treatments decreased relative to the initial levels, but less so in the *Phragmites* treatment except in the surface but at depth remained unchanged (Fig. 6.3b), corresponding to the high Eh.

Under the aerobic soil conditions, soil pH and Eh decreased as root biomass increased at depth. Contrastingly, the sulfate content of the *Phragmites* treatment was higher at depth but the overall content was lower compared to the initial levels (Fig. 6.3c).

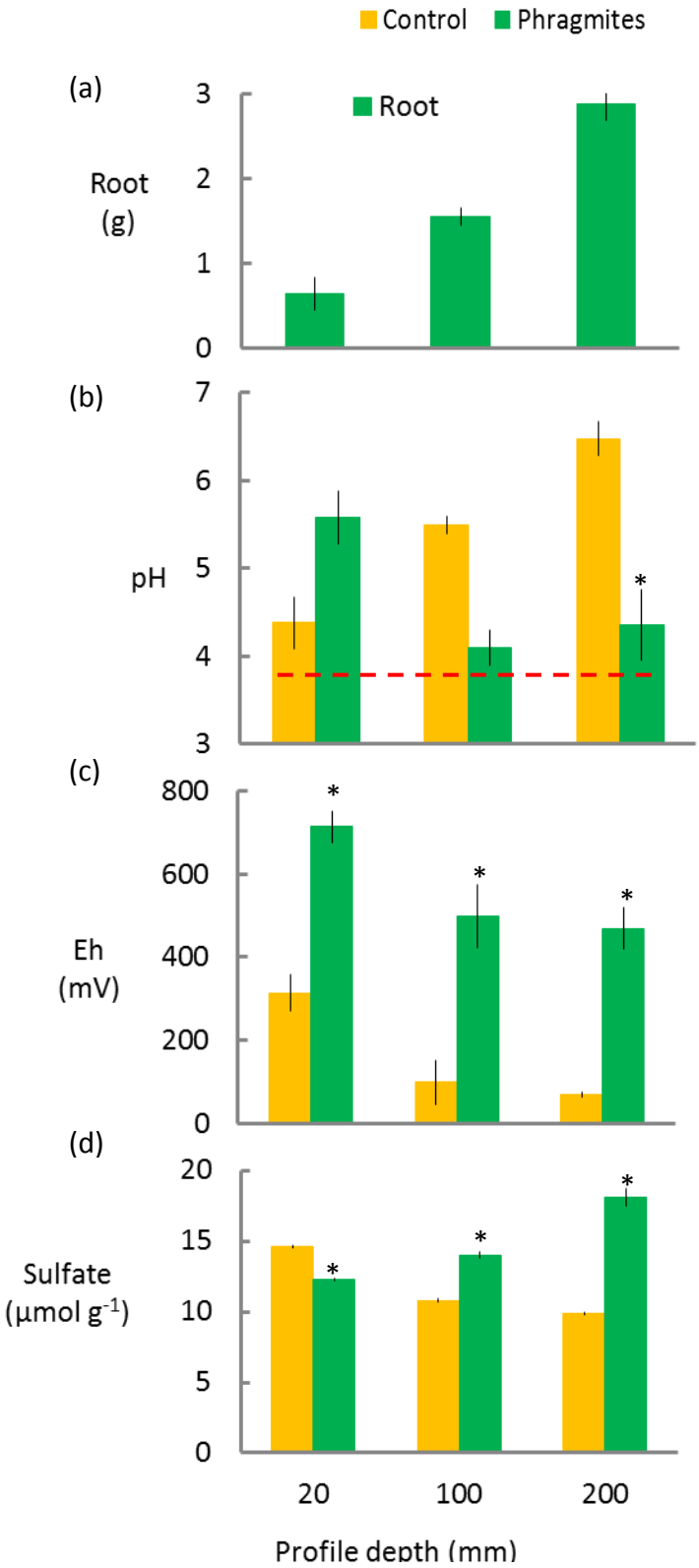

Figure 6.3. The effects of (a) *Phragmites* roots on (b) pH, (c) redox and (d) sulfate content of sulfuric soil maintained under aerobic conditions. Values are means ± s.e. of three measurements (n=3). The red dotted line is the initial pH. The initial sulfate content of the sulfuric soil ranged between 21-32 µmol g^{-1} soil. An asterisk indicates significant differences ($p<0.05$) between treatment and control at that depth.

6.2.3 Combined effects of *Phragmites* plants and organic matter

The general trend that has emerged from the previous chapters is that incorporation of dead plant material has a positive effect on the pH of sulfuric soil (less acid) whereas the results of this chapter show live plants tend to enhance the acidification. Study [PX]* (Table 3.4) was conducted to investigate the combined effects of plants and complex organic matter on sulfuric soil under aerobic conditions. A component of this study as well as a sulfidic set ([PIII, Table 3.4) were kept under flooded (anaerobic) conditions in a pond with all the treatment soils fully covered in water to compare the results. Figure 3.8 f & g shows photos of sample plants growing under water in a pond and aerobic conditions in a glasshouse respectively.

Figure 6.4 shows the changes in sulfuric soil chemistry for *Phragmites* growing in soil amended with organic matter under aerobic conditions. The types of plants established are shown in Fig. C2b. Comparison with the same experiment without added organic matter (Fig. 6.3) shows some similarities and differences. Firstly, the pattern of root growth was the same, with increasing root density with increasing depth. Secondly, except at the surface (where there were fewer roots), the pH was less acidic when organic matter was added and the Eh higher. Changes in sulfate content could be predicted from relative changes in pH and Eh of the treatments.

Under anaerobic conditions (Fig. 6.5), the changes in sulfuric soil chemistry induced by *Phragmites* and added organic matter virtually mirrored the changes for live plants alone suggesting that the dominant effect was exerted by the plant roots. Presence of plants increased the pH by 1.4 units from an initial pH of 4.2 throughout the profiles (Fig. 6.5b), even if the root biomass was decreasing with depth. Corresponding to the changes in pH, Eh decreased from 150 mV at the surface to 9 mV at depth, with the sulfate content at the surface being higher and significantly reduced at depth.

The changes in the sulfidic soil under anaerobic conditions are shown in Fig. 6.5 f-h. The types of plants established are shown in Fig. C2a. Except in the surface soil, the control soil pH was slightly higher at depth as the Eh was below 0 mV throughout and the sulfate content was lower relative to the initial levels. In the *Phragmites* and organic matter treatment where an equally distributed mass of roots were produced, the pH remained unchanged relative to the initial pH and Eh declined from to -28 mV at depth

(Fig. 6.5g). There was no clear relationship between the changes in pH and the sulfate content, which was high at depth (Fig. 6.5h).

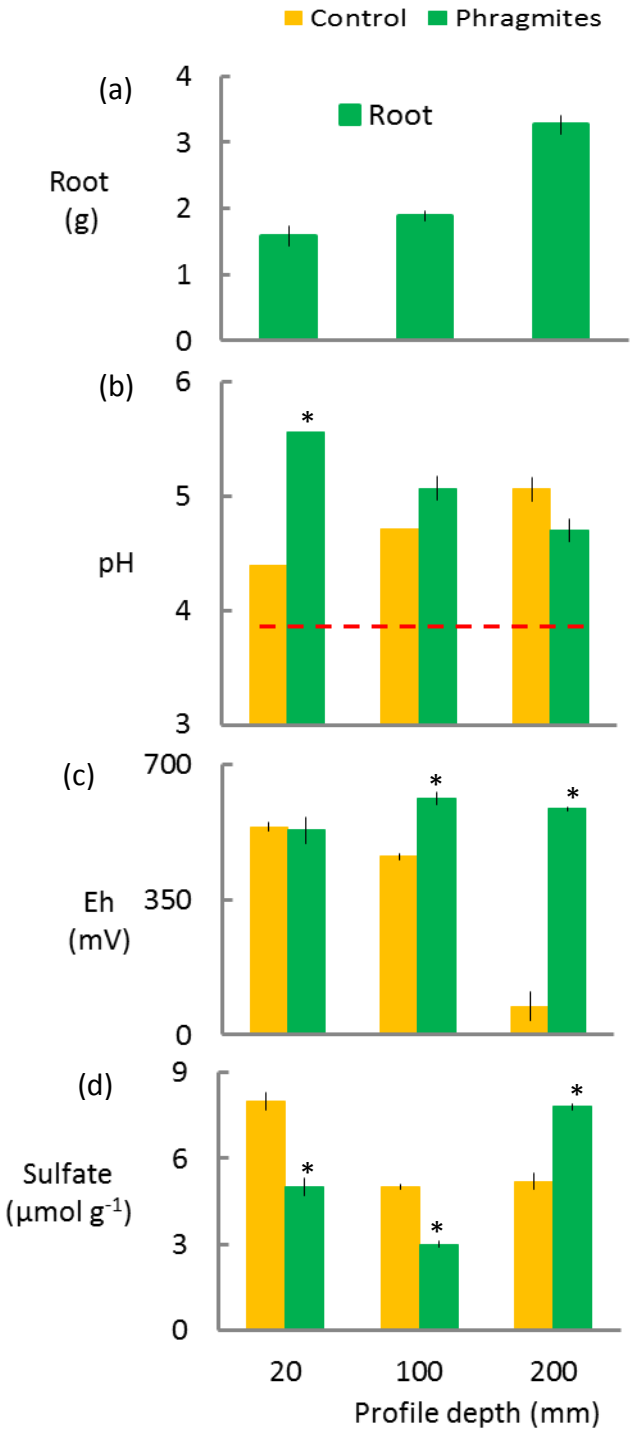

Figure 6.4. The effects of (a) *Phragmites* roots on (b) pH, (c) redox and (d) sulfate content of sulfuric soil with incorporated organic matter maintained under aerobic conditions. Values are means ± s.e. of three measurements (n=3). The red dotted lines are the initial pH. The initial sulfate contents of the sulfuric soil range between 21-32 µmol g^{-1} soil. An asterisk indicates significant differences ($p<0.05$) between treatment and control at that depth.

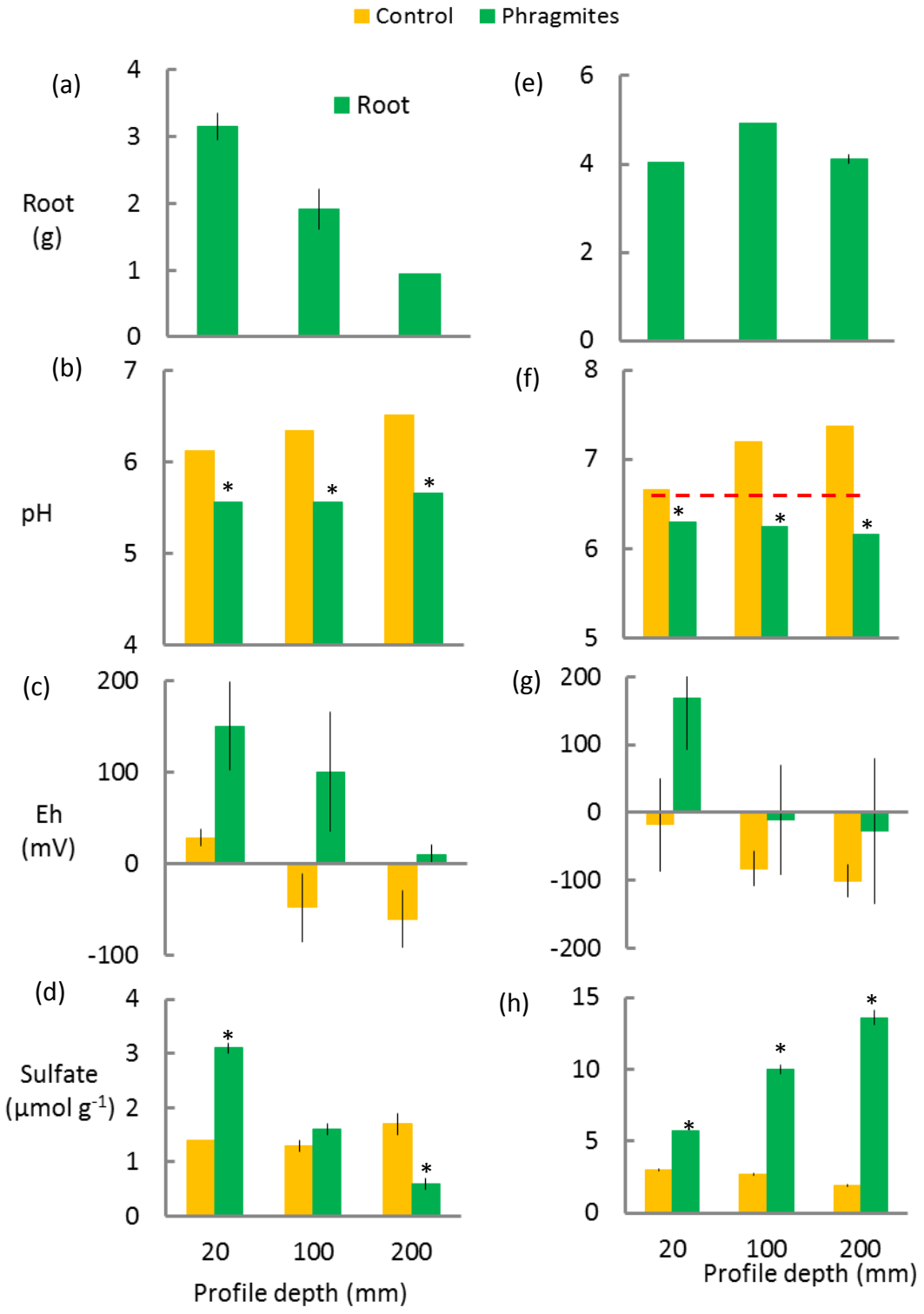

Figure 6.5. The effects of (a & e) *Phragmites* roots on (b & f) pH, (c & g) redox and (d & h) sulfate contents of (a-d) sulfuric and (e-h) sulfidic soils with incorporated organic matter maintained under flooded conditions for 12 months. Values are means ± s.e. of three measurements (n=3). The red dotted lines are the initial pH. The initial sulfate contents of the sulfuric and sulfidic soil respectively ranged between 21-32 and 12-16 µmol g^{-1} soil. An asterisk indicates significant differences ($p<0.05$) between treatment and control at that depth.

6.2.4 Effects of dead roots on ASS chemistry

As opposed to the effects of aboveground biomass and live roots, the effects of dead roots on ASS chemistry were investigated through the studies described as [DI] and [DII] under Section 3.3.3 and in Table 3.5. An image showing dead barley plants is shown in Fig. 3.10f. The soil texture was varied by incorporating sand at ratios of 1:0 (no sand) up to 1:4 to promote deeper root penetration. In these studies, barley was planted as seed and maintained under aerobic conditions by regular watering twice a day for 6 months. The plants were allowed to dry for another 3 months whilst keeping the soil moist and data collected. Generally, a good number of seeds germinated and grew to maturity, even in the unmixed soil as shown in bold in Figs. 6.6 & 6.7.

In the base soil (1:0), the pH remained acidic at the surface in both the control and planted treatments (Fig. 6.6a), but increased by 1-2 units in both treatments at depth. Increasing proportions of sand caused the pH near the surface of the planted treatments to rise more than the unplanted treatments but differences were not obvious at depth, partly due to the variability in values, most likely due to the heterogeneity of root distribution. Eh was generally in the oxidised range for both planted and unplanted treatments and for all sand proportions, but again variability was high (Fig. 6.6).

More detailed profiles of the changes in pH and redox measured in the sulfidic soil are shown in Fig. 6.7. In both the control and planted treatments, soils within the top 40 mm of the 1:0, 1:1 and 1:2 compositions were strongly acidified (Fig. 6.8 a-c). At depth, pH of all the soils increased to moderate levels except in the control soil of the 1:1 compositions which remained acidic throughout the profile (Fig. 6.7b).

In the 1:4 compositions, the pH of the control soil acidified to around 5 whereas in the planted treatment, the pH fell to between 3 and 4 (Fig. 6.7d). Generally, as expected due to the presence of sand, all the soils were within the oxidised range except in the unimproved 1:0 and 1:2 soils at 20 mm and at depth where Eh was reduced to -71 and -117 mV, respectively (Fig. 6.7 e-h).

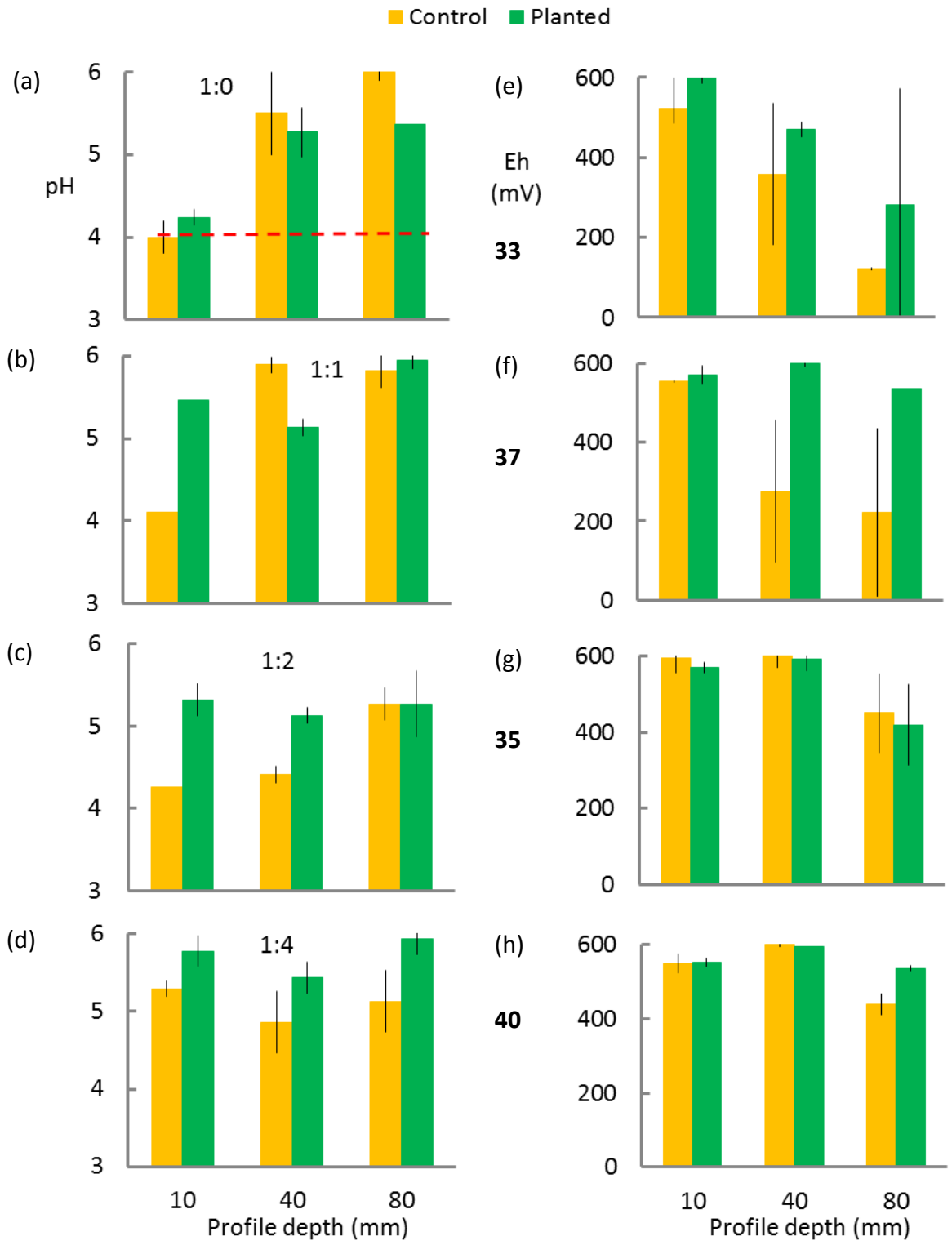

Figure 6.6. The effects of dead barley roots on (a-d) pH and (e-h) redox of sulfuric soil maintained by regular watering. The ratios indicate the proportions of sulfuric soil and sand. The bolded numbers indicate the number of plants that established. The values are means ± s.e. of three measurements (n=3).

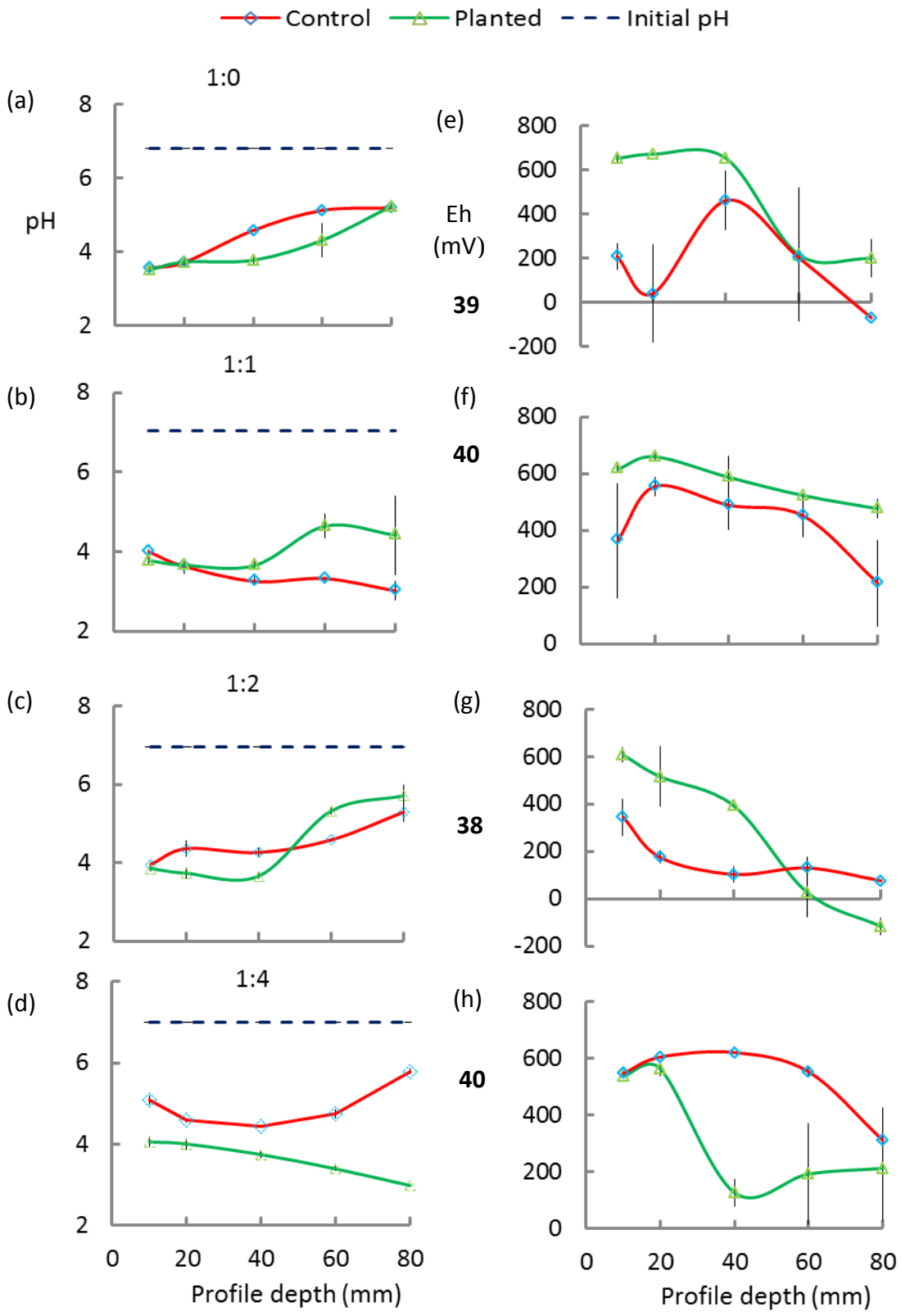

Figure 6.7. The effects of dead barley roots on (a-d) pH and (e-h) redox of sulfidic soil maintained by regular watering. The ratios indicate the proportion of sulfidic soil and sand. The bolded numbers indicate the number of plants established. The values are means ± s.e. of three measurements (n=3).

6.3 Discussion

All three of the plant species used in this research grew reasonably well under flooded conditions on neutral sulfidic soil, but also on sulfuric soil with an initial pH of 3.3 – 6.7. It was therefore possible to make some comments on the effect of live roots on the chemistry of these soils.

Root distribution varied depending on species, soil type and moisture regime. For *Melaleuca* and *Phragmites*, root biomass was more concentrated in the surface of sulfuric soil, and *Typha* in the middle of the profile under aerobic conditions. Under anaerobic conditions, *Phragmites* roots concentrated at depth. In sulfidic soil, *Melaleuca* and *Phragmites* roots were more abundant in the middle of the profile and *Typha* towards the top. There was also some accumulation of organic matter especially on the surface of the sulfuric soil in the presence of *Melaleuca* and *Typha* plants, which would have contributed to a mulching effect.

When comparing the results of the live plant trials to those using dead plants as organic matter, it should be noted that the live plants were grown in much deeper pots (300 mm instead of 80 mm tubes) and were grown for 12 months rather than 6 months or less. In general, the changes in pH, Eh and sulfate content of the control treatments were consistent with the shorter term experiments in tubes. Under flooded conditions, the control pH rose by 2-3 units in sulfuric soil and was either stable or increased slightly in the sulfidic soils (Figs. 6.1 & 6.2) which may be due to microbial use of the residual carbon (10.6%) of the unamended soil (Johnson et al., 2014). However, in all cases, when compared to the control soil at the same depth, the pH of the planted treatments was more acid, in some cases by more than 3 units. This applied to both the sulfuric and sulfidic soils.

Perhaps the most striking feature of the flooded soils was the difference in Eh between the planted and control soils. In the sulfuric soil, the Eh after 12 months was less than 0 mV but when planted, the Eh was quite aerobic, especially in the presence of *Melaleuca* and *Typha* where it remained between 400 and 600 mV (Fig. 6.1c). For *Typha*, there were more roots in the upper profile which corresponded to higher Eh and lower pH, whereas at the bottom of the profile where there were fewer roots, both Eh and pH were more similar to the control (Fig. 6.1 e, f, g).

In flooded soils there was a clear relationship between the changes in pH, Eh and the sulfate content, generally following the pattern of high pH corresponding to low Eh and low sulfate content.

Figure 6.3 shows the effects of *Phragmites* on sulfuric soil under 'aerobic' conditions. Interpretation of the experiments under non-flooded conditions is more complicated because of the effects of plant transpiration on soil moisture content. For the control soil, the surface alternated between dry and wet, and at depth would most probably wetter. Comparison of Figs. 6.2c and 6.3c shows that near the surface the Eh of the non-flooded control soil was significantly higher and the pH was significantly lower compared to flooded soil, but at depth the differences are much smaller. In the planted treatments there are several notable differences between the two moisture regimes. Firstly under flooded conditions, more roots grew near the surface whereas under non-flooded conditions, roots grew deep into the soil (Figs. 6.2a & 6.3a). Secondly, the differences in pH between control and planted treatments were much greater under the more aerobic conditions, and this may in part be attributed to oxygen transport down the profile via aerenchyma (as in the flooded treatment) but also to replenishment of water transpired from the roots to the shoots. Regular watering would tend to transport oxygen down the profile.

The tendency for live roots to enhance acidification seems to be linked to the maintenance of a higher Eh under both anaerobic and aerobic conditions. In addition to the possible increased transport of water down the soil column as a result of water being sucked out by plant roots to support transpiration, greater oxygen penetration into the soil could be mediated by either movement downwards in aerenchyma, which is pressurised in some species (Bendix et al., 1994; Tornberg et al., 1994), or by loosening of the soil by growth of the roots.

It was clear from the mulching experiments in Chapters 4 and 5 that incorporation of organic matter quickly generates reducing conditions in the soil that favours reduction of sulfate and increases pH, which is opposite to that of the live plants. It was therefore interesting to see how the combined effects of live and dead plant material would influence the pH and Eh. The results showed that under anaerobic conditions, incorporated organic matter had a positive effect on planted sulfuric soils, but that the pH remained significantly more acid than the control soil. Under aerobic conditions, it was

difficult to attribute any changes in pH to the organic matter since the Eh, pH and sulfate contents were quite similar in soils planted with *Phragmites* with or without organic matter.

In cropping situations, once the plant tops are harvested, the roots remain in the soil and may have some ameliorative effects on ASS. However, the effects on soil pH of residual root material of barley in this research were generally quite small, with some variation with depth and when the texture was improved by incorporation of sand.

6.4 Conclusions

⇒ Growth of live plants into both sulfuric and sulfidic soils enhanced rather than ameliorated soil acidification.

⇒ Plant roots tended to raise the redox potential of the soils. Several potential mechanisms were considered including greater oxygen penetration via aerenchyma, soil loosening by plant roots, and the sucking of water down the soil profile by transpiring plant roots.

⇒ Incorporation of organic matter prior to planting was partially effective in reducing the acidification by plant roots under anaerobic conditions but under aerobic conditions the effect was small or absent.

⇒ Residual plant roots remaining after harvesting of barley plants did not have any significant effect on soil pH, either positive or negative.

6.5 References

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Chapter 7

Neutralisation of sulfuric acidity using alkaline sandy loam and plants

7.1 Introduction

Many acidic soils have heavy textures that do not allow penetration of neutralising agents such as lime, and are not conducive to plant growth. Improvement of the texture of these soils can be achieved by the incorporation of sandy loam, which allows better penetration of water to facilitate leaching of excess salts, and better penetration of oxygen for plant growth, but this may sustain oxidation of sulfides in the soil. In this research, alkaline sandy loam was used to simultaneously improve soil texture and to increase pH. To improve penetration of oxygen and water to aid plant growth, while at the same time increasing soil pH.

In a short incubation study lasting 2 weeks, it was seen that addition of alkaline sandy loam buffered acidification in a sulfuric soil and prevented sulfidic soil from acidifying (Michael et al., 2012). This short-term study was extended to assess the long-term stability when an alkaline sandy loam was added to sulfuric soils and the pH raised to neutral level (near 7) and either organic matter was added or vegetation (plants) established.

7.2 Methodology

Preparation of the “neutralised sulfuric soil” is described under Section 3.1.4 and the proportions of mixing are appended in Table B1 (Appendix). The practical strategy to neutralise sulfuric soil acidity with alkaline sandy loam and the long-term stability of the neutralised sulfuric soil following organic matter amendment and establishment of plants are respectively given in Tables 3.2 ([I]-[IV]) and 3.4 ([PI]-[PII]. Natural sulfidic soil of Finniss River (see Fig. 7.4) was used in [IV] to compare the results.

The data collected are presented as described under Section 3.7 in Chapter 3.

7.3 Results

7.3.1 Effect of organic matter on neutralised sulfuric soil chemistry

The changes in soil chemistry measured following incorporation of organic matter and maintained under aerobic conditions are shown in Fig. 7.1. During 6 months of incubation, the pH of the unamended control soil was stable at the surface but decreased sharply to 4.5 at depth (Fig. 7.1a). Incorporation of organic matter in the form of chopped *Phragmites* leaves sustained the pH between 5 and 6 across the profile, but still more acid than the initial pH but 1 unit. The redox changes were hard to interpret. In all cases, the soils remained moderately to highly oxidised with similar values recorded for the control soil at pH 6.6 (surface) and pH 4.4 (80 mm depth) (Fig. 7.1b). Similar inconsistencies were observed for the sulfate content of the soil (Fig. 7.1c).

Figure 7.2 shows the changes in soil properties measured following incorporation of organic matter and maintained under flooded conditions. Compared to the initial pH, the unamended control soil acidified to near 5 at the surface but increased slightly to 7 at depth (Fig. 7.2a). Organic matter caused the pH to increase by 0.6 – 1 units across the profile. The pH changes broadly corresponded with the reciprocal changes in Eh and sulfate contents (Fig. 7.2b, c).

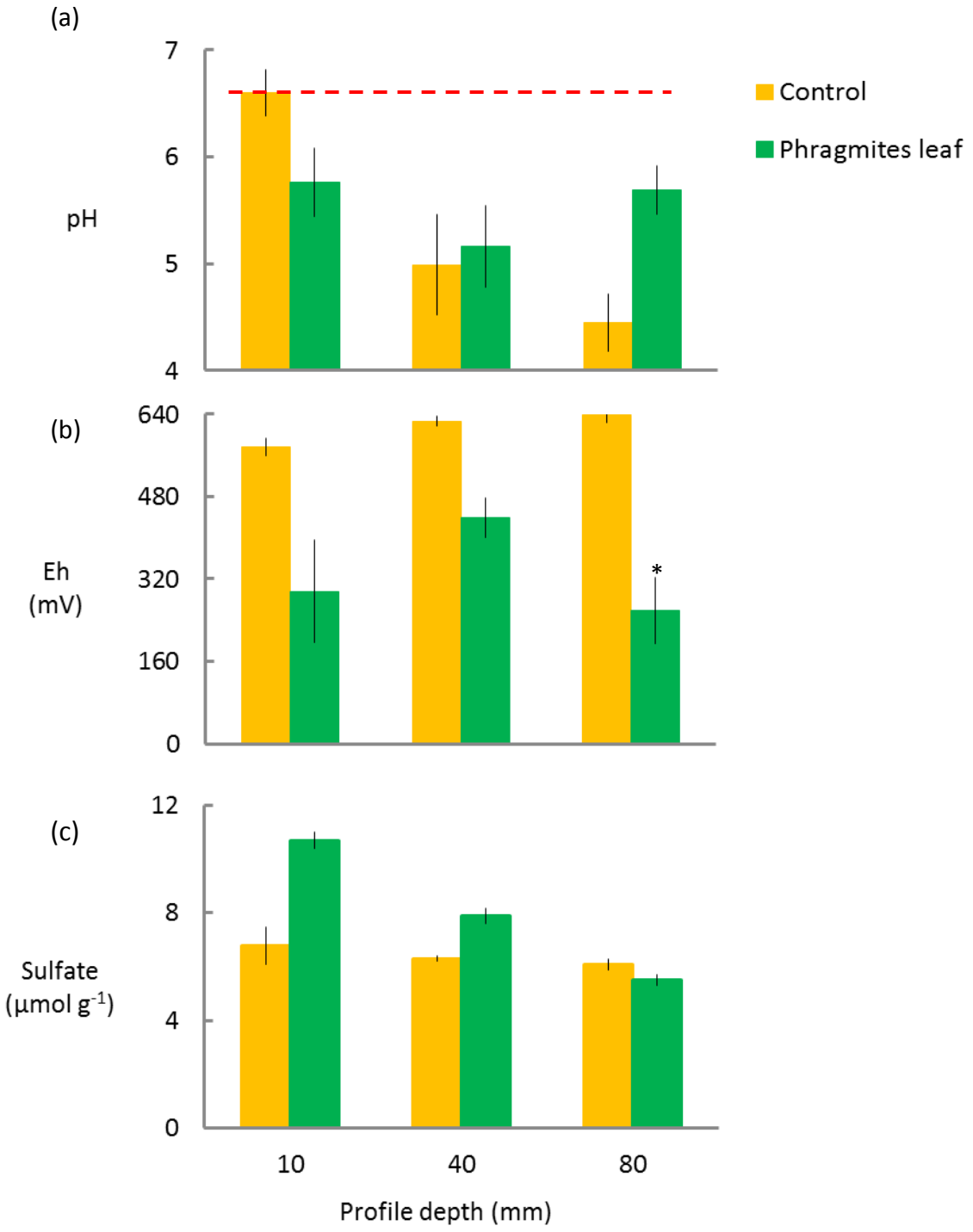

Figure 7.1. Effects of incorporated organic matter on (a) pH, (b) redox and (c) sulfate content of neutralised sulfuric soil maintained under aerobic conditions for 6 months. The red dotted line is the initial pH. The initial sulfate content of the neutralised sulfuric soil is 23 µmol g^{-1} soil. The values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

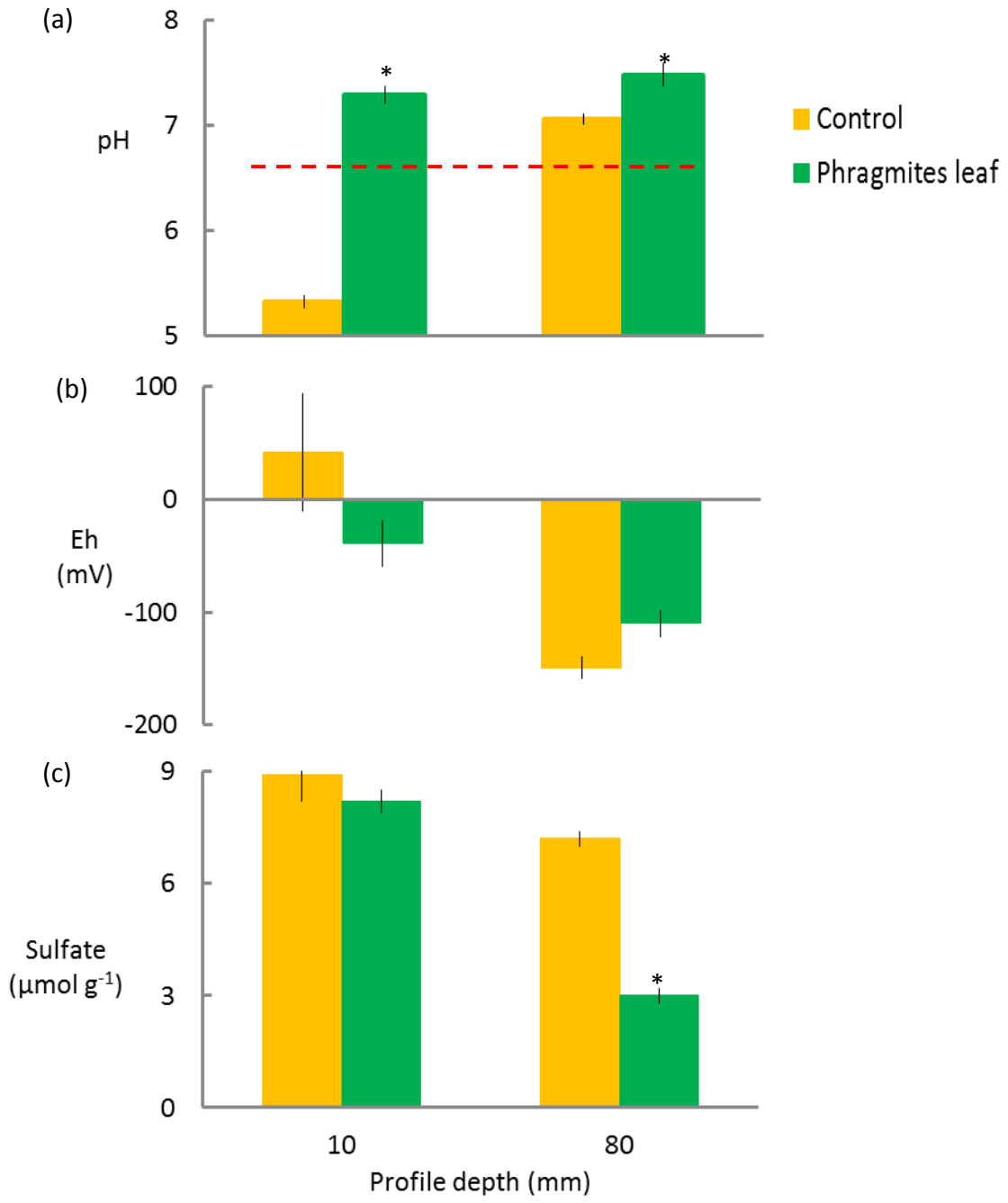

Figure 7.2. Effects of incorporated organic matter on (a) pH, (b) redox and (c) sulfate content of neutralised sulfuric soil maintained under anaerobic conditions for 6 months. The red dotted line is the initial pH. The initial sulfate content of the neutralised sulfuric soil is 23 µmol g^{-1} soil. Values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

When organic matter was overlaid and maintained under aerobic conditions, the pH of the control treatment remained stable around 6.8 at the surface, but decreased

strongly to around 4.3 at depth (Fig. 7.3a). Addition of organic matter to the surface caused a moderate acidification, which increased slightly with depth. Despite the changes in pH, the Eh and sulfate contents of all treatments varied very little (Fig. 7.3b, c). However, the sulfate contents after 6 months were much lower than the measured initial content, indicating the disappearance (most likely by reduction) of a significant amount of sulfate.

Under flooded conditions, the pH of the control sulfidic soil of Finniss River decreased slightly to near 6 at the surface and to 5.3 at depth, whereas in the *Phragmites* treatment, the pH rose sharply at the surface to more than 8 but was similar to the control soil at depth (around 5.7) (Fig. 7.4a). Despite the flooded soil condition, the Eh of the control soil oddly remained fairly highly oxidised maybe because of the presence of sandy loam soil which facilitated oxygen into the soil, whereas the *Phragmites* treatment was quite reduced (Fig. 7.4b). There was only a very weak correlation between the pH changes and the sulfate content; the soils with higher pH values tended to have low sulfate contents (Fig. 7.4a, c).

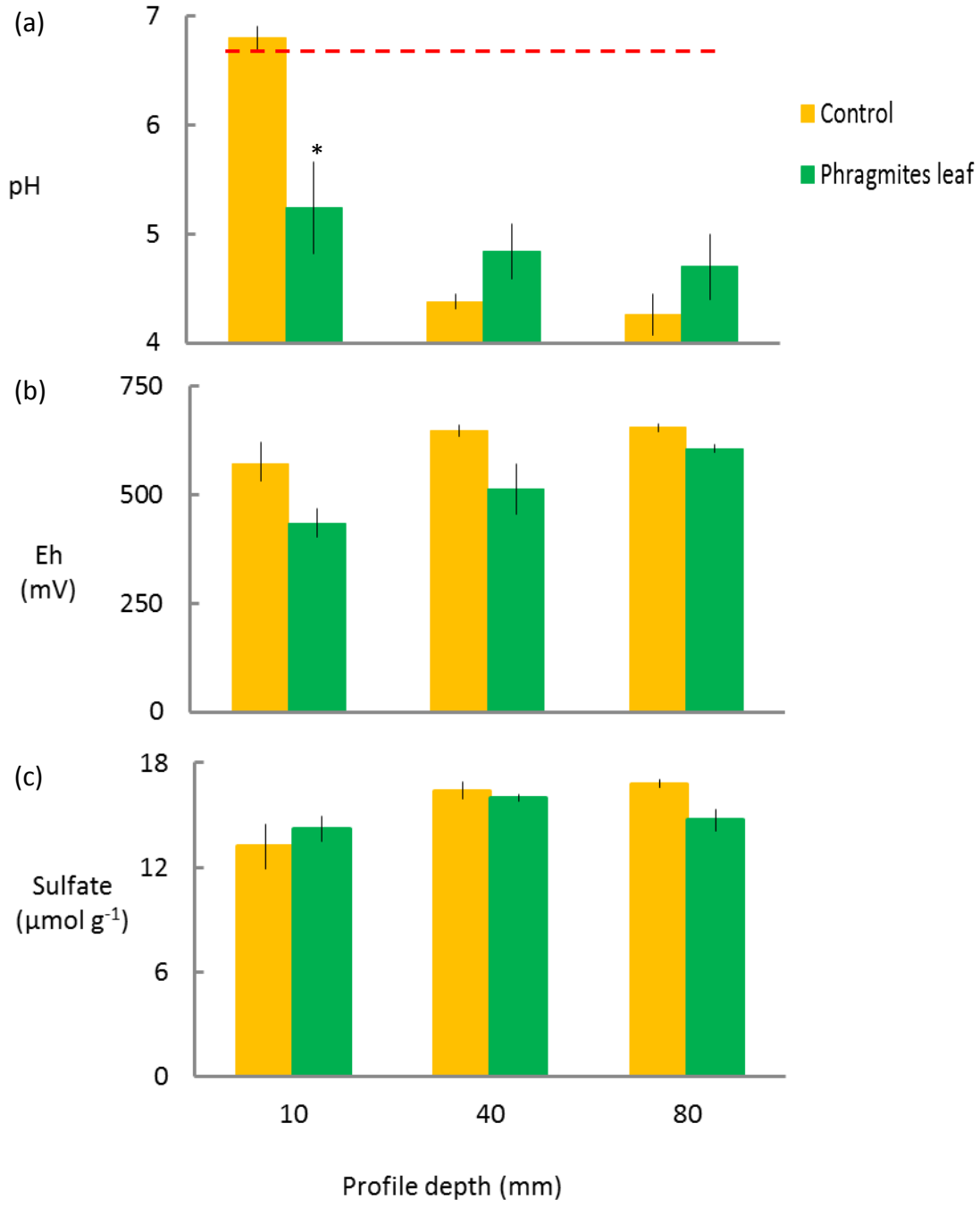

Figure 7.3. Effects of overlaid organic matter on (a) pH, (b) redox and (c) sulfate content of neutralised sulfuric soil maintained under aerobic conditions for 6 months. The red dotted line is the initial pH. The initial sulfate content of the neutralised sulfuric soil is 23 µmol g^{-1} soil. The values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

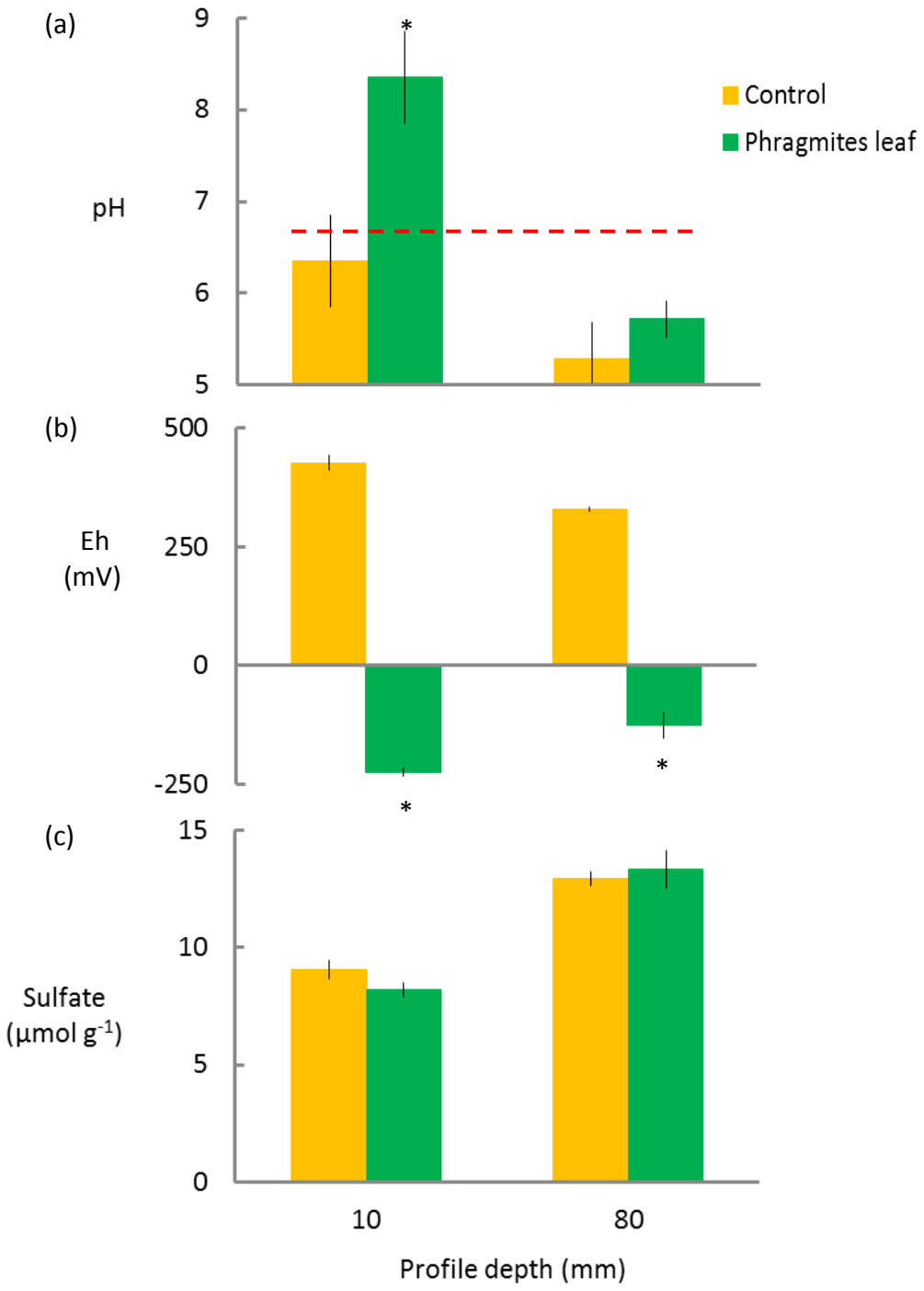

Figure 7.4. Effects of overlaid organic matter on (a) pH, (b) redox and (c) sulfate content of sulfidic soil maintained under anaerobic conditions for 6 months. The red dotted line is the initial pH. The initial sulfate content of the unamended sulfidic soil is 16 µmol g^{-1} soil. Values are means ± s.e. of three measurements (n=3). Asterisks indicate significant differences ($p<0.05$) between treatment and control at the same depth.

7.3.2. The impact of vegetation on neutralised sulfuric soil

The mixing of alkaline sandy loam with sulfuric soil has the combined advantage of raising the pH into the range where most plants grow optimally, and create a more open texture for root growth. In this section, a range of plants were grown with or without supplemental fertiliser in the neutralised soil to examine their impact of soil chemistry. Figs. 3.9d and 3.9e respectively show the types of smaller and larger plants established.

To assess whether fertilising alone had an effect, unplanted soils were fertilised with the same amount of Hoagland solution ([DIII], Table 3.5). Fertilising had small to no effect on soil pH and the Eh remained within the oxidised ranged as in the other treatments. Fertilisation greatly increased the growth of both lucerne and barley. The root biomasses obtained from the 0-20 mm (20 mm), 50-100 mm (100 mm), 150-200 mm (200 mm) and 250-300 mm (300 mm) profiles of the smaller and larger plants are respectively shown in Figs. 7.5a and 7.6a.

Near the surface, the pH remained relatively stable for all treatments (Fig. 7.5b). Lower in the profile, the control soil acidified to around pH 5, with similar values recorded for most of the treatments. There was no clear pattern connecting pH to Eh, the only noticeable feature being that Eh of the fertilised lucerne treatment tended to be lower than the other treatments (Fig. 7.5c). The apparent lack of effect of the planted treatments may be due to the relatively small amount of biomass contributed by the roots. There was also a high degree of variability in the measurements.

The effects of larger plants, *Allocasuarina*, *Eucalyptus* and *Melaleuca*, were highly variable and no clear trend could be discerned. There appeared to be no predictable relationship between root biomass, pH and Eh (Fig. 7.6). However, in all cases the final pH was higher than the initial pH, which is more likely due to the stabilising effect of alkaline components of the sandy loam as well as to the fact that the neutralised sulfuric soil may contain fewer oxidisable sulfides that natural sulfidic soils, than to the influence of plants.

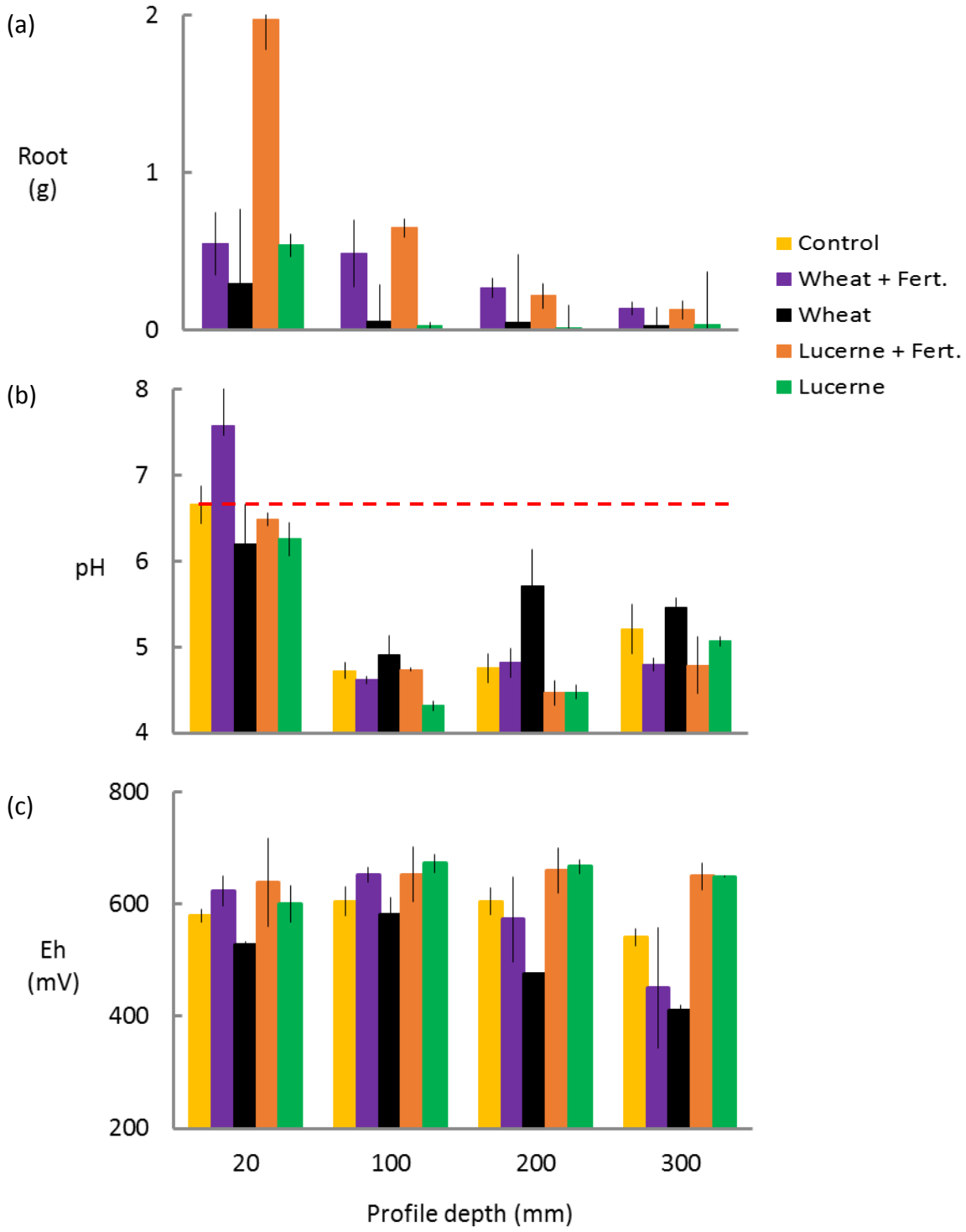

Figure 7.5. Effects of (a) roots of wheat and lucerne plants on (b) pH and (c) redox of neutralised sulfuric soil maintained under aerobic conditions for 12 months. The red dotted line is the initial pH. Values are means ± s.e. of three measurements (n=3). The profile was sampled at 0-20 mm (20 mm), 50-100 mm (100 mm), 150-200 mm (200 mm) and 250-300 mm (300 mm). No significant differences ($p<0.05$) between treatment and control soil properties were observed at the same depth.

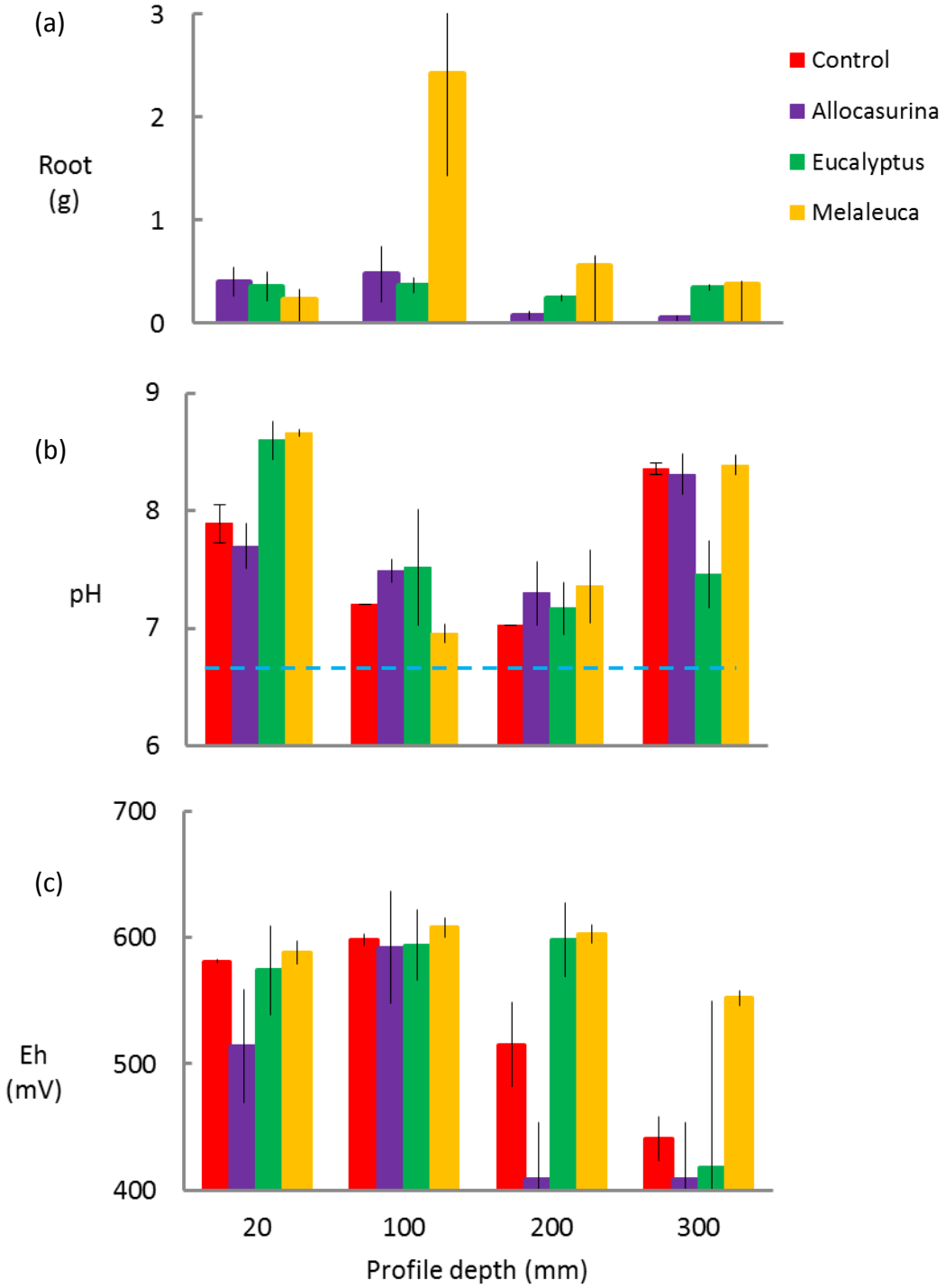

Figure 7.6. Effects of (a) roots of trees on (b) pH and (c) redox of neutralised sulfuric soil maintained under aerobic conditions for 12 months. The values are means ± s.e. of three measurements (n=3). The blue dotted line is the initial pH. The profile was sampled at 0-20 mm (20 mm), 50-100 mm (100 mm), 150-200 mm (200 mm) and 250-300 mm (300 mm). No significant differences ($p<0.05$) between treatment and control soil properties were observed at the same depth.

7.4 Discussion

In many developing countries (e.g. PNG), farming is an important component of the daily lives of the majority of the people and many households depend entirely on farm produce. In such areas, availability of arable land is limited due to high population densities coupled with urbanisation, and more wastelands (e.g. wetlands) are converted to farm lands and plantations. Where ASS is present (see Fig. 2.1), this is a serious problem as a result of soil acidification due to oxidation of reduced sulfide minerals, impacting on crop and food productivity and socio-economic securities and livelihood of the people.

Although application of lime is quite effective in treating acidic soils and is the most established soil acidity management strategy, availability and the need for larger quantities are major issues in the developing countries. In addition, application of lime under certain soil use conditions, such as after flooding for cultivation of crops like rice (*O. sativa*) and taro (*C. esculenta*) are not feasible. Additionally, remediation of acidic soils by lime is not permitted in certain environmentally sensitive areas such as Ramsar wetlands. In the experiments described in this chapter, the use of alkaline sandy loam as an alternative strategy for neutralising sulfuric acidity and preventing sulfidic soil oxidation was investigated.

Under aerobic conditions, the pH of the neutralised sulfuric soil remained stable near the surface at around 6.7 which would be quite good for establishing plants. However, at 80 mm depth, the pH had decreased to 4.4 which may be too low for many crop plants to extend roots into the subsoil (Fig. 6.1). Addition of *Phragmites* leaf into the profile improved the pH to between 5 and 6 which would allow the growth of many crop species. A further positive aspect of the neutralised sulfuric soil was that the Eh remained quite aerobic in the control soil, and also moderately aerobic in the soil with *Phragmites*.

Under flooded conditions, low Eh values were associated with increased pH, and changes in sulfate content suggested that this was mostly due to sulfate reduction. The addition of *Phragmites* leaves would not make a large difference to the pH of neutralised soil when the conditions are already anaerobic and oxidation of sulfides is inhibited.

One noticeable feature of *Phragmites* in the field is the accumulation of dead leaves on the surface of soil, sometimes leading to several centimetres of organic material at various stages of decay. In this study, these natural conditions were emulated by applying

mulched *Phragmites* leaves to the surface of neutralised soil. Under aerobic conditions the effects appeared to be little different to incorporation of this material into the profile (compare Figs. 6.1 & 6.3. Under anaerobic conditions there was a clear increase in pH in the mulched treatment, but only at the surface. The practical benefits of surface mulching have therefore not been proven, either under flooded or non-flooded conditions.

Growth of the wheat, lucerne and tree species had little effect on the neutralised soil pH and the changes that occurred were consistently similar to the unplanted controls. The neutralised soil pH was stable on the surface soils irrespective of the plant type established but the pH deteriorated at depths (Figs. 6.5 and 7.6).

As expected, all the soils were oxidised due to the presence of sand and plant roots but no evidence of roots having a clear and direct effect on soil pH and redox was found. Fertilising increased root growth of wheat and lucerne, without having any consistent impact on soil properties. The trees were not fertilised and root biomass distribution down the profile was strongly species dependent, but again there was no clear relationship between root biomass and soil properties.

7.5 Conclusions

⇒ Neutralised sulfuric soil is expected to contain fewer sulfides compared to the equivalent sulfidic soil before oxidation. Nevertheless, these soils still acidified at depth, but less than the sulfidic soils investigated in Chapter 5.

⇒ Incorporation of organic matter stabilised the pH but did not prevent oxidation under aerobic conditions. Under flooded conditions, the pH was more stable and increased when organic matter was incorporated.

⇒ Application of organic matter to the surface was only effective under flooded conditions.

⇒ In contrast to the effects of plants on sulfidic soil and sulfuric soils where the tendency was for plants to increase acidity (Chapter 6), growth of plants on neutralised sulfuric soil had little influence on pH.

This publication is a refereed conference paper that includes some of the data from Chapter 7

NOTE:
This appendix is included on pages 171-174 of the print copy of the thesis held in the University of Adelaide Library.

In: L.L. Burkitt, L.A. Sparrow (Eds.), Proceedings of the 5th Joint Australian and New Zealand Soil Science Conference: Soil solutions for diverse landscapes, pp. 146-149.

7.6 Reference

Michael, P.S., Reid, R., Fitzpatrick, R.W., 2012. Amelioration of slowly permeable hypersaline peaty-clayey sulfuric and sulfidic materials in acid sulfate soils by mixing with friable sandy loam soil. In: L.L. Burkitt, L.A. Sparrow (Eds.), Proceedings of the 5th Joint Australian and New Zealand Soil Science Conference: Soil solutions for diverse landscapes, pp. 146-149.

Chapter 8

General Discussion

8.1 Introduction

There is a range of management options for treating acid sulfate soils such as those discussed in Melville and White (2013) but none of these approaches involve plants. The work described in this thesis aimed to identify practical strategies for treating acid sulfate soils using live or dead plants. An associated aim was to try to understand how plants impacted on the chemistry of the soils, other than just the pH, including microbial activity that produces or consumes acidity. Drawing on the results of the four experimental chapters, it is possible to make some informed comment on the suitability of plants for treating acid sulfate soils in different scenarios.

8.2 Alternative strategies for management of acid sulfate soils

Scenario 1. *Treatment of sulfidic soil exposed by lowering of the water table*

If the surface of a sulfidic soil dries rapidly, there may be less of a tendency to acidify because of the need for water in the oxidation process. This was demonstrated in the experiments in which soils were wetted to different extents. Clearly the best strategy for treatment of exposed sulfidic soil is reflooding, which on its own prevented acidification. However, this is often not possible, especially in prolonged drought so alternative strategies need to be employed. Incorporation of organic matter such as *Phragmites* leaves, lucerne hay or pea straw was shown to be very effective in stabilising or even increasing the soil pH. This scenario is likely to arise along river beds and in wetlands where *Phragmites* commonly grows abundantly. The main requirement for mulching material appears to be a high nitrogen content. For this reason, plant material such as wheat straw, which has a low nitrogen content was found to be much less effective. If mechanical incorporation is not practical because of the unstable nature of the soil, an alternative treatment recognised by this research is the application of urea, which can be applied directly to the surface as a solution. Revegetating exposed sulfidic soils does not appear to be advisable since in most of the experiments in which plants

were established, the pH decreased, in some cases to quite a large extent; in the other experiments the pH did not change relative to the unplanted control soil but there were no instances in which the pH was increased by live plants. Acidification by plants was always associated with increases in Eh, which suggests that plants increase penetration of oxygen into soil, thereby increasing oxidation of soil sulfides.

Scenario 2. *Treatment of sulfuric soil to prevent mobilisation of acidity and toxic elements*

Reflooding of exposed sulfuric soil allows a gradual reversal of the oxidation process, but this can be greatly accelerated either by incorporation of organic matter such as *Phragmites,* or simply applying it to the surface. Under non-flooded conditions, applying organic matter to the surface was shown to increase pH by 1 to 1.5 units but when incorporated the pH increased by up to 4 units. Even though application just to the surface was not as effective as incorporation, the results from the flooded treatment suggests that surface organic matter on exposed sulfuric soils would cause the pH to increase much faster upon submerging. As for sulfidic soils, vegetating sulfuric soils can enhance acidification and therefore is not recommended.

Scenario 3. *Treatment of sulfidic soil for use in crop production*

The treatments mentioned for Scenario 1 are also applicable to soils targeted for agricultural use. Where water is available, keeping the soils under a layer of water would maintain neutral pH and be suitable for wet crops such as rice and taro. For dryland agriculture though, the incorporation of plant mulches would have the beneficial effect of increasing and stabilising the pH but at the same time would lower the Eh due to the oxygen demand created by the breakdown of the organic matter. The impact of this treatment needs to be investigated under field conditions. Under both flooded and non-flooded conditions, urea was shown to cause large increases in pH. If used as the nitrogen fertiliser, this could have beneficial effects on both plant growth and soil pH.

Scenario 4. *Treatment of sulfuric soil for use in crop production*

The treatment options for sulfidic soils (Scenario 3) carry the same advantages and drawbacks when applied to sulfuric soils. Organic mulches, especially those high in

nitrogen, as well as urea, all cause substantial reductions in Eh that may inhibit root growth into the soil.

8.3 Plant organic matter versus lime

It is clear from the total of the experiments conducted, that application of organic matter has a beneficial effect in stabilising or increasing the pH of acid sulfate soils, in both sulfuric and sulfidic soils. This is beneficial not only when applied the soil surface but also when incorporated at depth in the soil profile. Under some circumstances, stabilisation may be sufficient. For example, application of surface mulch to sulfuric soils under low moisture conditions resulted in a moderate increase in pH. Perhaps this is due to the lack of surface moisture for microbial activity, which in itself would inhibit oxidation. However, without excess water, mobilisation of acidity and toxic elements is less likely. The problem arises with heavy rain or reflooding, which can transport acids and toxins away from the site. As noted above, surface organic matter, can when flooded, lead to large increases in pH, which would inhibit such transport. The question is, how does this compare to lime? Economically, plant organic matter may be much less expensive, especially in areas with low labour costs for harvesting and distributing the plant material. Lime, unless highly purified and therefore more expensive, can contain contaminant materials and is therefore not permitted for treatment of environmentally important wetlands. The vast lower lakes system in South Australia comprises Ramsar-listed wetlands of Lake Alexandrina and Lake Albert in the lower Murray–Darling Basin, which underwent severe acidification during Australia's Millennium Drought, which lasted for 5 years. However, it was deemed necessary to apply agricultural lime even though it was not allowed. Part of the problem was that there appeared to be no alternatives because little was known about the effectiveness of applying plant organic matter, especially in the over 20,000 ha of the dried-out and cracked sulfuric soils, which was previously submerged lake beds and wetlands that became exposed (Fitzpatrick et al., 2009). As noted above, generation of alkalinity by microbial degradation of plant material causes changes in redox conditions that may be detrimental to plant growth, and therefore less appropriate for crop production except under flooded conditions. A compromise may be to apply amounts of organic matter that maintain a moderate Eh and lessen the amount of lime that needs to be used.

8.4 Mechanisms of plant effects on acid sulfate soil chemistry

Most literature on acid sulfate soils mainly consider pH changes in terms of oxidation or reduction of sulfur compounds, and to a lesser extent, oxidation and reduction of iron and nitrate. Decaying plants contribute carbon, nitrogen and other nutrients that can act as both energy supplies and electron acceptors for a range of microbes. This microbial activity can also influence the oxygen status of the soil and change the redox conditions to suit different groups of the microbial ecology. In these studies, breakdown of complex organic matter that led to increases in pH was associated with loss of sulfate from the soil and reduction in Eh. This suggests that the principal, but not necessarily only, process that contributed alkalinity was sulfate reduction.

Addition of carbon alone did not result in significant changes in soil sulfate and the pH changes caused by these compounds must have been due to microbes other than sulfate reducing bacteria. Although both simple carbohydrates, acetate and glucose had quite different effects most likely because of different metabolic pathways. Acetate caused the pH to increase and Eh to decrease, whereas glucose reduced the pH and Eh remained high.

The requirement for nitrogen was not clearly established. Simple nitrogen compounds in the form of nitrate or ammonia did not induce either large increases or decreases in pH or Eh, which may indicate that the main limitation was carbon. When nitrogen was applied in combination with carbon in the form of urea or molasses, pH increased and redox decreased, but there was little evidence of sulfate reduction that accompanied these changes. However, the strong correlation between nitrogen content of plant material and the effectiveness in ameliorating both sulfidic and sulfuric soils suggests that microbial breakdown of complex organic molecules requires nitrogen as a nutrient.

The general observation was that live plants acidified soil, most likely by increasing oxygen penetration. There are various possible mechanisms for this including oxygen transport down the profile in aerenchyma tissues in the root and loosing of soil as plant roots grow. Alternatively, some acid may be generated by microbial activity metabolising compounds excreted by plant roots, which include sugars, organic acids and amino acids.

8.5 Limitations of the research

One of the main limitations of the results obtained is that the effects of treatments on soil chemistry were only a snapshot at one point in time. The few time course experiments showed that soil responses can vary quite markedly depending on when they were measured. Some treatments may cause changes quite rapidly while others are more gradual and may be sustained for longer. One experiment in the paper submitted to *Soil Use and Management* shows a large effect of organic matter after 6 months, but after 12 months the conditions were similar to the untreated control.

In relation to the point just mentioned, the results were all obtained under laboratory and glasshouse conditions and need to be validated under field conditions and using a wide variety of ASS. Most of the findings presented in the thesis are from studies conducted in 70 ml tubes, 1.1 L small pots or 45 cm high stormwater tubes. The added organic matter was also chopped up to suit the experimental conditions. On a field scale, the quantity of organic matter that would be applied (estimated for acre-furrow-slice weighing 1000 tonnes) is between 29.8 (80:1, soil: organic matter) to 33.5 (90:1) tonnes per ha. Therefore, the practicality of adding coarse plant materials and their beneficial effects on acid sulfate soil chemistry in real-time situations, such as in farm or Ramsar-listed wetland soils, need to be tested.

Another significant gap relates to the limited insight that was obtained regarding the mechanisms of the changes in pH, in particular the microbial systems that must have been mainly responsible. It was originally intended to investigate this more thoroughly but it became clear that the complexity of the system would require another PhD. Progress that was made in identifying and quantifying sulfur reducing bacteria in soil following addition of organic matter is described in Appendix 1.

8.6 References

Fitzpatrick, R.W., Grealish, G., Shand, P., Simpson, S.L., Merry, R.H., Raven, M.D., 2009. Acid Sulfate Soil Assessment in Finniss River, Currency Creek, Black Swamp, and Goolwa Channel, South Australia. CSIRO Land and Water. Adelaide. Science Report 26/09. p. 213.

Melville, M.D., White, I., 2013. Acid Sulfate Soils: Management. Encyclopedia of Environmental Management DOI: 10.1081/E-EEM-120046329 Taylor & Francis. p. 6.

Appendices

Appendix 1. Molecular analysis of sulfur reducing bacteria in acid sulfate soils

As part of the description of changes in the chemistry of acid sulfate soils in response to addition of organic matter, it was originally intended to attempt to identify the types of bacteria that contributed to these changes, at least to confirm a major involvement of sulfur reducing bacteria (SRBs) in the changes. Some good progress was made in this area, but not sufficient to justify a chapter in the thesis. Therefore, the results obtained to date will be described, and hopefully can be expanded when time permits to form the basis of a future publication.

The aims of the experiments were:

1. To identify which SRBs were present in the ASS
2. To quantify the numbers of SRBs in different soil treatments using total DNA and RNA
3. To use Real Time PCR to quantify enzymes involved in sulfate reduction.

The following was achieved:

1. DNA and RNA were successfully isolated from soil treatments amended with organic mulches.
2. PCR probes were used to identify which SRBs or groups of SRBs were present. From this it was established that only one of the 6 groups of SRBs was present, and that the main type was *Desulfovibrio*.
3. Measurement of total DNA and RNA showed that the amounts of these polynucleotides increased strongly following addition of organic matter.
4. Primer pairs for measuring the expression of two genes involved in dissimilatory sulfate reduction were designed from published sequences for *dsr* AB genes from *Desulfovibrio vulgaris*. cDNA was prepared from the RNA isolated from the soil treatments. Standard curves for these genes were generated.

Methods

Isolation of RNA and DNA from soil

The total RNA and DNA were isolated using a RNA PowerSoil® Total RNA Isolation Kit obtained from MO BIO Laboratories Inc., CA, USA. Only the six major steps in the isolation procedure are described.

1. Cell lysis

Total RNA and DNA were isolated using 2 g soil samples by placing in a 15 ml bead tube. A 2.5 ml of a buffer (Bead Solution) was added to the bead tube containing the soil to disperse cells and soil particles and mixed by vortex, followed by addition of 0.25 ml of a cell lysis agent (Solution SR1) containing sodium dodecyl sulfate (SDS, an anionic detergent to break down fatty acids and lipids), and vortex again to mix.

Non-DNA organic and inorganic materials including proteins, cell debris and humic acids were removed by adding 0.8 ml of a precipitation reagent (Solution SR2). The mixture was homogenised by placing the bead tube horizontally on a vortex adapter at maximum speed for 5 min. The cell lysis efficiency and yield, trapping lysed cell components and denaturing protein were maximized by adding 3.5 ml of phenol: chloroform: isoamyl alcohol (pH 6.5-8.0), leaving the nucleic acids in the solution. This mixture was vortex until a biphasic layer that formed disappeared.

2. Phase separation

The final mixture was vortexed at maximum speed on the vortex adapter for 10 min to further facilitate homogenisation and lysis of the cell. After mixing, the mixture was phase separated by centrifugation at 2500 x g for 10 min at room temperature. The lower organic phase of the mixture contained proteins and cellular debris, the interphase contained humic, organic and inorganic materials and the upper aqueous phase contained the nucleic acids.

3. Precipitation

The upper aqueous phase was carefully transferred to a clean 15 ml collection tube, and a secondary precipitation step was performed to remove proteins and cellular debris by adding 1.5 ml of Solution SR3. This was then vortexed to mix and incubated at 4°C for 10 min. The tubes containing the precipitates were centrifuged at 2500 x g for 10 min at room temperature, and the supernatants were transferred to a new 15 ml tubes by decanting, without disturbing the pellets.

The nucleic acids in the supernatants were precipitated by adding 5 ml of 100% isopropanol (Solution SR4), vortexed to mix and incubated at -20°C for 30 min, followed by centrifugation at 2500 x g for 30 min at room temperature. After centrifuging, the supernatants were decanted and the pellets air-dried by inverting the 15 ml collection tubes on a paper towel for 5 min. The pellets were resuspended and the nucleic acids further precipitated by adding 1 ml of a salt solution (Solution SR5). To capture the RNA, a RNA capture column was prepared by placing a RNA capture column inside a 15 ml collection tube and a 2 ml of Solution SR5 was added to the RNA capture column to remove unbound contaminants. This was allowed to gravity flow through the column and collect in the 15 ml collection tube.

4. RNA elution and isolation

The RNA isolated was added to a RNA capture column and allowed to gravity flow through the column and collect in a 15 ml collection tube. The column was washed with 1 ml of Solution SR5 and allowed to gravity flow and collect in the 15 ml collection tube. The RNA capture column was then transferred to a new 15 ml collection tube and 1 ml of an elution buffer (Solution SR6) was added to the RNA capture column to elute and release the bound RNA into the 15 ml collection tube, leaving behind residual debris and inhibiting substances in RNA capture column.

Solution SR6 was allowed to gravity flow into the collection tube and the eluted RNA was transferred to a 2.2 ml collection tube. A 1 ml of Solution SR4 were added to the tube to precipitate the eluted RNA, the lids closed and inverted at least once to mix and incubated at -20^0C for 10 min. After incubating, RNA in the 2.2 ml collection tube was pelleted by centrifuging at 13 000 x g for 15 min, and the RNA pellets collected by decanting the supernatant and air-drying by inverting the 2.2 ml collection tube on a paper towel for 10 min. The RNA pellet was resuspended in 100 µl of RNase/DNase-free water (Solution SR7) and kept frozen at -80^0C.

5. DNA elution and isolation

After the RNA was eluted from the RNA capture column, the column was placed in a new 15 ml tube and 1 ml of elution buffer (Solution SR8) was added to elute the bound DNA into the 15 ml tube. The elution buffer was allowed to gravity flow into the tube at this stage. After that, the eluted DNA was transferred to a 2.2 ml collection tube and 1 ml

of Solution SR4 was added to precipitate the DNA. The tube was inverted once to mix and incubated at -20^{0}C for 10 min.

The collection tube containing the eluted DNA was pelleted by centrifuging at 13 000 x g for 15 min. The supernatant was decanted and the pelleted DNA air-dried on a clean paper towel by inverting the 2.2 ml collection tube. The pelleted DNA was resuspended in 100 µl of RNase/DNase-free water (Solution SR7). The pure DNA in the collection tube was kept frozen (-80^{0}C) prior to downstream applications.

6. Quantitation

The concentrations of DNA and RNA in the soil extracts were quantified using Quant-iT DNA and RNA kits in a Qubit fluorometer (Invitrogen Ltd, Paisley UK).

Results

Identification of sulfur reducing bacteria

Daly *et al*. (2000) published a phylogenetic analysis of SRBs based on 16s ribosomal DNA, which they divided into six groups based on similarity (Fig. A1). They then developed 16s rDNA primers to differentiate the groups (Table A1). These primers were used to probe DNA extracted from soil before and after addition of organic matter. Only one of the six groups was amplified by the primer sequences. This was Group 6 which includes the most common SRB, *Desulfovibrio*.

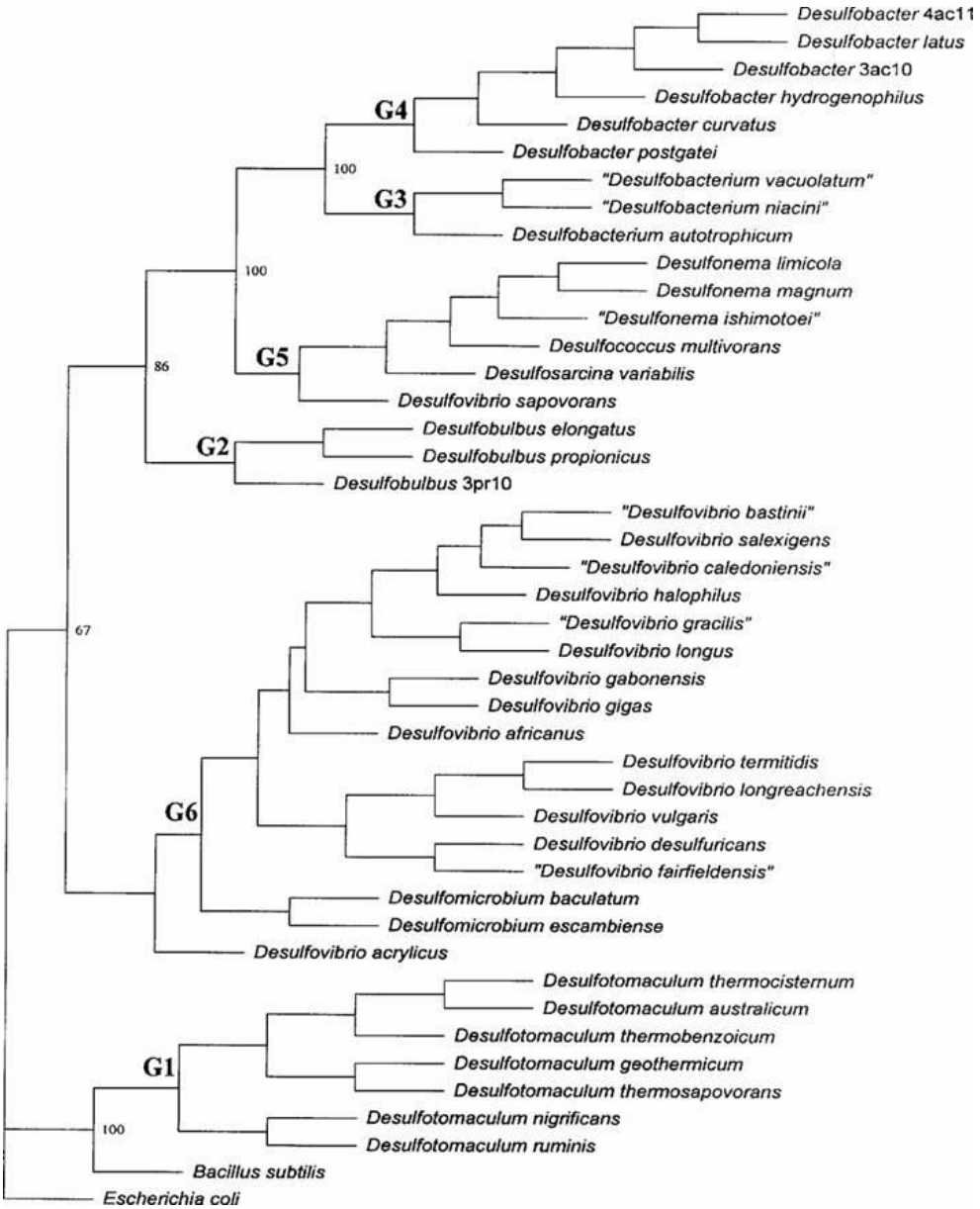

Figure A1. 16s rDNA showing the linkages of the six main groups of SRB.

Table A1. 16s rDNA-targeted PCR primer sequences for SRB subgroups (Daly *et al*., 2000)

Primer	Target site*	Sequence 5′–3′†	Specificity	Annealing temp. (°C)	Expected size product (bp
DFM140	140–158	TAG MCY GGG ATA ACR SYK G	Group 1	58	700
DFM842	842–823	ATA CCC SCW WCW CCT AGC AC			
DBB121‡	121–142	CGC GTA GAT AAC CTG TCY TCA TG	Group 2	66	1120
DBB1237‡	1237–1215	GTA GKA CGT GTG TAG CCC TGG TC			
DBM169	169–183	CTA ATR CCG GAT RAA GTC AG	Group 3	64	840
DBM1006	1006–986	ATT CTC ARG ATG TCA AGT CTG			
DSB127‡§	127–148	GAT AAT CTG CCT TCA AGC CTG G	Group 4	60	1150
DSB1273‡	1273–1252	CYY YYY GCR RAG TCG STG CCC T			
DCC305	305–327	GAT CAG CCA CAC TGG RAC TGA CA	Group 5	65	860
DCC1165	1165–1144	GGG GCA GTA TCT TYA GAG TYC			
DSV230‡	230–248	GRG YCY GCG TYY CAT TAG C	Group 6	61	610
DSV838	838–818	SYC CGR CAY CTA GYR TYC ATC			

Total RNA and DNA

The total RNA and DNA extracted from soil before and 4 and 6 weeks after incorporation of organic matter in the form of lucerne hay is shown in Fig. A2. There was approximately a 3-fold increase in RNA and 10-fold increase in DNA when measured 4 weeks after addition of organic matter but there was a sharp decline in these values between 4 and 6 weeks.

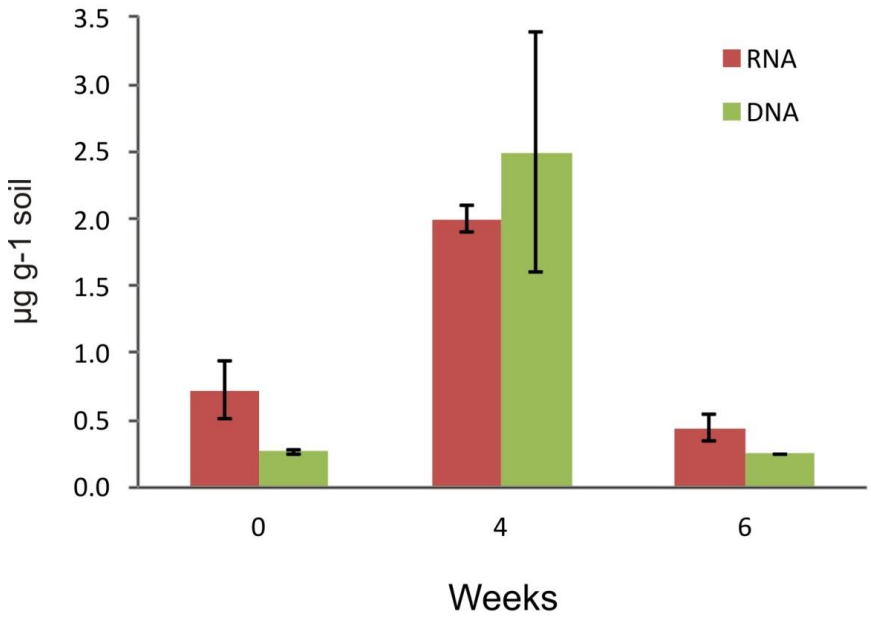

Figure A2. Changes in total RNA and DNA following addition of 1g of chopped lucerne hay to 40g of sulfuric soil, maintained at 75% field capacity. The error bars indicated the range of two replicate samples.

Expression analysis of genes for sulfate reduction

Dissimilatory sulfite reductase (DSR) is the enzyme responsible for the transfer of electrons to sulfite to form sulphide. A measurement of its expression would provide a much more accurate estimate of the sulfate reduction activity of a soil. Real-time PCR primers were designed based on published sequences of the DSR A and B subunits. These primers were used to develop standard curves for quantitation of cDNA reverse transcribed for the corresponding sequences on the RNA extracted from the soil. Typical Real-Time amplification traces are shown in Fig. A3. The threshold values (C_T) obtained were used to

Table A2. Primer sequences for Real Time PCR expression analysis of sulfite reductase genes in *Desulfovibrio.*

Primer pair	Sequence	Amplicon
dsrAB gene		
DSR1F DSR5R	ACCCACTGGAAGCACG TGCCGAGGAGAACGATGTC	223 bp
DSRp2060F DSR4R	CAACATCGTYCAYACCCAGGG GTGTAGCAGTTACCGCA	377 bp
16s rRNA 27F 534R	AGAGTTTGATCCTGGCTCAG ATT ACC GCG GCT GCT GG	526 bp

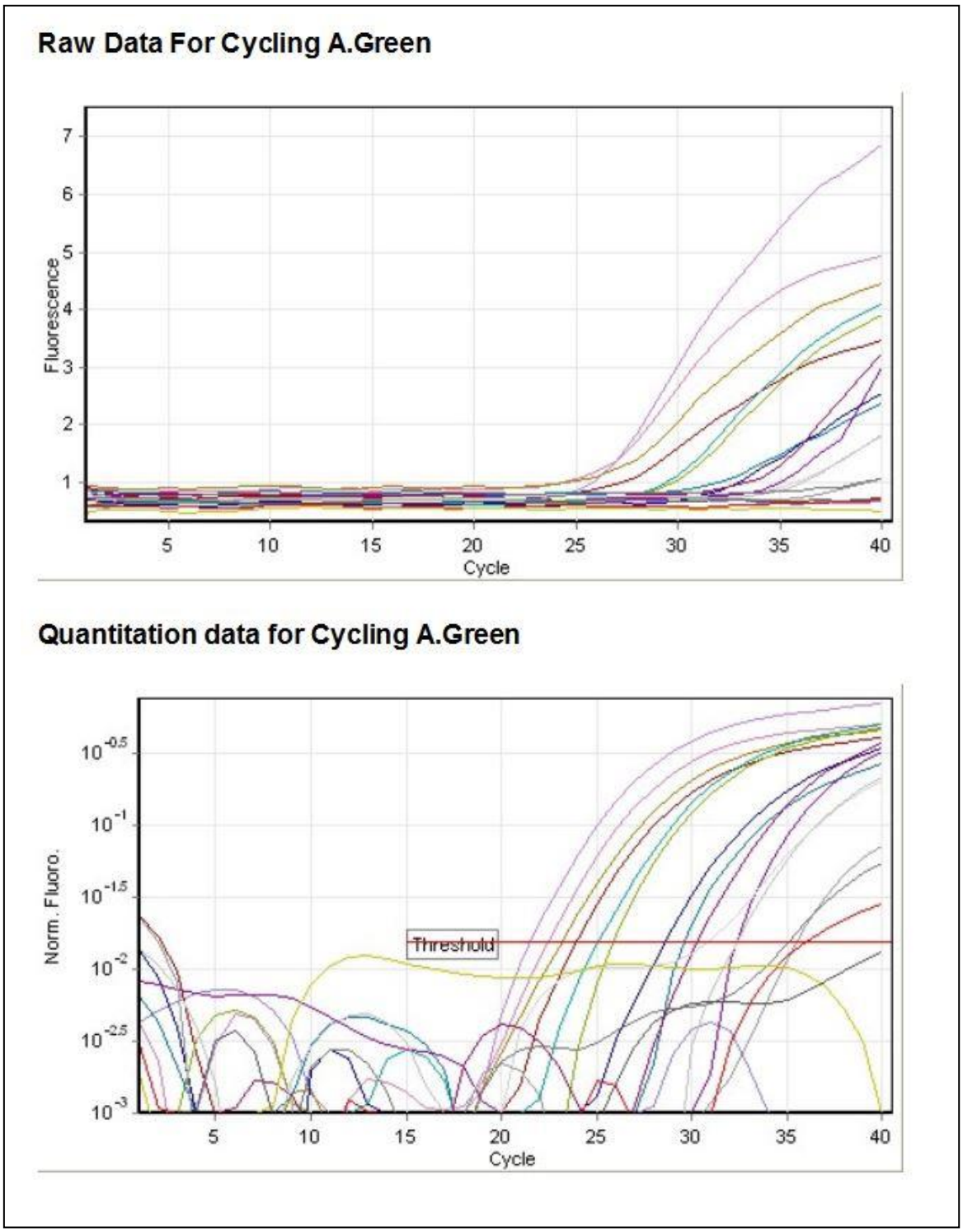

Figure A3. Typical Real Time PCR traces used to construct standard curves for expression of *dsr* genes. The Threshold value represents the number of amplication cycles needed to achieve a designated number of gene copies. The threshold values for a range of dilutions of the original cDNA are used to construct standard curves for analysing gene copies in sample extracts, as shown in Fig. A4.

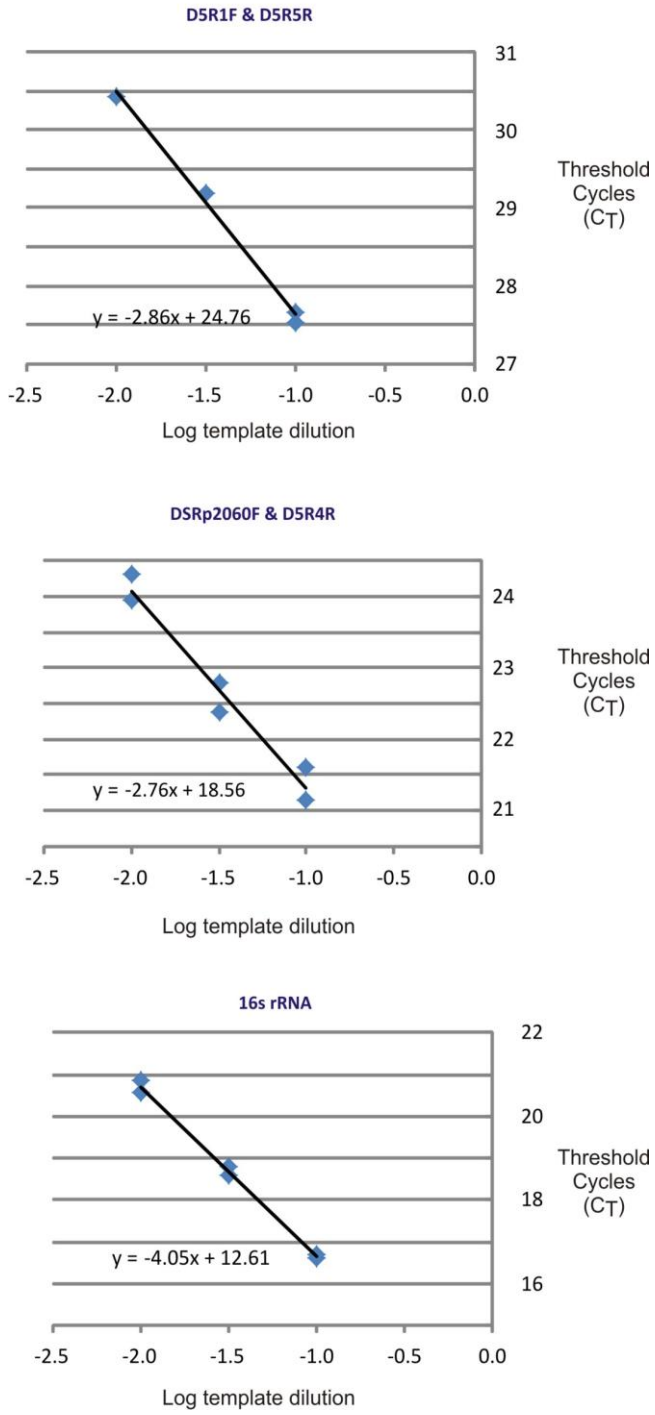

Figure A4. Standard curves for expression analysis of gene sequences of *dsrAB* genes from *Desulfovibrio* and for 16s rRNA

Reference

Daly, K., Sharp, R.J., McCarthy, A.J., 2000. Development of oligonucleotide probes and PCR primers for detecting phylogenetic subgroups of sulfate-reducing bacteria. Microbiology 146, 1693-1705.

Appendix B

The process of mixing Gillman sulfuric soil with alkaline sandy loam to obtain the "neutralised sulfuric soil" used in the studies presented in Chapter 7 are given in Table B1.

Table B1. The changes in pH_w and pH_{ox} of sulfuric soil following mixing with alkaline sandy at different proportions.

Ratio	pH_w	pH_{ox}	Sulfate content	Field capacity (%)
1:0	2.3	1.1	-	-
1:3	3.7	2.5	-	-
1:5	3.8	2.7	23.8 µmol g^{-1} soil	69
1:7	6.7	2.8	23 µmol g^{-1} soil	28

Explaination: Ratio is the proportion of mixing, sulfuric soil:alkaline sandy loam soil. The amounts are in spaceful of each substrates mixed using a cement mixture as described previously. The notation (–) means not determined.

Appendix C

Sample photos showing the types of plants established in the sulfuric and sulfidic soils of the plant-based studies (Chapter 6) are shown in Figs. C1 and C2.

Figure C1. *Melaleuca* and *Typha* plants of study presented under Section 6.2.1.

Figure C2. Sample photos showing *Phragmites* plants established under (a) anaerobic and (b) aerobic soil conditions. The shots were taken during harvest after 12 months of growth. The storm water tubes without plants are controls. Notice too that the soils under anaerobic conditions were fully covered in water and under aerobic fully conditions exposed. The studies are presented under Section 6.2.2.